Effect of Tannic Acid and Quercetin Antioxidants on Bond Strength of Resin Cement to Dentin after Internal Bleaching

Keywords

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ABSTRACT

Purpose: To evaluate the influence of tannic acid and quercetin on the immediate microshear bond strength (μSBS) of two resin cements to dentin after internal bleaching. Materials and Methods: The access cavity in eighty extracted maxillary incisors was internally bleached except the control groups. The labial surfaces were ground to expose a flat dentin surface. The specimens were randomly divided into eight groups (n=10) as follows: 1) Control/Dou; Doulink without bleaching, 2) BI/Dou; Bleaching+Doulink, 3) BI+Ta/Dou; Bleaching+Tannic acid+Doulink, 4) BI+Qu/Dou;Bleaching+Quercetin+Doulink, 5) Control/PSA; Panavia SA without bleaching, 6) BI/PSA;Bleaching+Panavia SA, 7) BI+Ta/PSA; Bleaching+Tannic acid+Panavia SA, 8) BI+Qu+PSA; Bleaching+Quercetin+Panavia SA. After 24 hours, the μSBS of the specimens was tested and the obtained data were analyzed with two-way ANOVA and Tamhane test (p<0.05). Results: The μSBS of Doulink groups were higher than the PSA groups (p<0.001). The μSBS of BI+Ta/Dou and BI+Qu/Dou groups did not differ from BI/Dou (p>0.05). The μSBS of BI+Ta/PSA group was statistically significantly higher than the BI/PSA group (p<0.001). There was no significant difference between μSBS of BI+Qu/PSA and BI/PSA groups (p=0.39). Conclusions: TA partially reversed the negative influence of internal bleaching on the μSBS of Panavia SA while quercetin did not increase the bond strength of both resin cements.

INTRODUCTION

Tooth discoloration is observed when pigments are accumulated in the tooth structure (intrinsic factors) or on the tooth surface (extrinsic factors).[1,2] Discoloration of nonvital teeth is originated from various factors such as pulp necrosis, the presence of filling materials and sealer , insufficient irrigation and debridement, pulp tissue remnant in access cavity, and intrapulpal hemorrhage product.[3] Dental bleaching is a simple yet effective treatment that may be recommended to enhance the appearance of discolored teeth before restoration with bonded restorative materials.[4,5] However, the etiology of discoloration should be evaluated by the clinicians to select the most appropriate bleaching approach.[4,5]

The most suitable approach for removing the intrinsic staining of nonvital teeth is internal bleaching.[6] In this approach, the bleaching agent, which can be either hydrogen peroxide, sodium perborate, sodium percarbonate, and carbamide peroxide, is employed to penetrate enamel and dentin[7] and

produce reactive oxygen molecules, free radicals, and anions.[5] The oxidation breaks the double bonds in the chromogens leading to teeth whitening.[1,8] Since the outcome of the bleaching process are not always esthetically acceptable, additional esthetic resin-bonded treatments, such as ceramic veneer or crown, may be required.[7] Some studies have revealed that applying adhesive restorations immediately after bleaching may decrease the bond strength to enamel/dentine.[9-11] One of the main reported reasons for this adverse effect is the residual oxygen released from the bleaching material which may intervene with the resin infiltration and prevent the resin polymerization.[11-13]

Various approaches have been presented to resolve the clinical problem related to the negative effect of bleaching on the bond strength, including removing the superficial layer of enamel, applying the alcohol on the bleached enamel before restoration, and using the adhesives containing organic solvents.[14] However, the most common clinical technique is to postpone the bonding procedure after bleaching as some studies have shown that tooth bonding is insufficient within this interval and the decrease in the bond strength is temporary.[14,15] This delay interval has been reported to change from 24 hr to 4 weeks.[11,16] However, this delay is not desirable in many clinical cases and clinicians often prefer to apply adhesive restorations immediately after bleaching. Hence, efficient solutions are required for immediate reversing the adverse effect of bleaching on the bond strength of adhesives to tooth structures.[17]

It has been reported that applying an antioxidant may reverse the compromised bond strength of the bleached dentine[17,18] and enamel.[19] Antioxidants accelerate the removal of the remaining oxygen in the tooth structure after the bleaching gel application.[20] Sodium ascorbate is the widely studied antioxidant that has shown its efficiency to restore the side effect of hydrogen peroxide on the bond strength of bleached teeth.[21-23] However, it has been shown to have a short shelf-life as well as being mutagenic for mammalian somatic cells.[21,24] Other antioxidants such as alpha-tocopherol, grape seed extract, green tea extract, lycopene, and pine bark extract, have been recently applied in different *in vitro* studies to investigate their benefits as a post-bleaching treatment.[25] It is notable that the main influencing factors in applying antioxidants for improving the bond strength to the bleached teeth are the concentration, form (solution or gel), and application time, and type of the antioxidant agent.[25,26]

Tannic acid (TA) and quercetin are two antioxidants used in dentistry applications.[27,28] TA is a commercial form of tannin, a type of polyphenol with weak acidity.[29] TA has shown the bonding capabilities to an amide of collagen through hydrogen bonds.[30] Such bonding properties improve the stability of the collagen fibers and increase their robustness against dentine matrix metalloproteinases.[30,31] TA also enhances the surface porosity of dentine surface and partially removes the smear layer.[31]

The other antioxidant is quercetin (3,3' ,4' ,5,7- pentahydroxyflavone), a bioactive compound and one of the most common flavonols in nature that has attracted the attention of many researchers because of its biological and pharmaceutical properties.[32] It shows various other properties than antioxidative including anticarcinogenic, anti-inflammatory, anti-aggregator, and vasodilating effects.[33] A previous study showed that the bonding properties of a quercetin-doped dental adhesive may be preserved against collagenase aging and such adhseives preventd the growth of *S.mutans* biofilm.[34]

Although several researchers studied the efficiency of various antioxidants in the restoring of compromised bond strength of resin composite to the bleached teeth,[14,20,21] it is not clear whether applying TA and quercetin to the bleached dentine is effective for increasing the immediate bond strength of resin cement or not. The null hypothesis of this study was that applying antioxidants on after internal bleaching would not affect the immediate microshear bond strength of two resin cements to the bleached dentine.

MATERIALS AND METHODS

TOOTH PREPARATION

Following approval of the study design by the Research Ethics Committee of Shiraz University of Medical Sciences (Protocol # R.SUMS.DENTAL.REC.17927), eighty extracted maxillary central incisors were collected. The teeth with fractured or cracked crowns were discarded based on the stereomicroscopy examinations (Carl Ziess, Oberkochen, Germany) with 20 ×magnification. The teeth were stored in 0.5% chloramine solution at 4 for at most 2 weeks before use.

After cleaning the teeth, the endodontic access cavities were prepared from the lingual aspect using a diamond bur (Dia. Tessin, Gordevio, Switzerland) at high speed, with water cooling. The pulp was removed with endodontic instruments and the roots were sectioned 4 mm below to the cementoenamel junction (CEJ). The root orifice was filled with composite resin (Filtek Z250, 3M ESPE; St Paul, MN, USA) to a level of 2 mm below the CEJ.

EXPERIMENTAL DESIGN

The diagram of the experimental design is shown in Figure 1. The specimens were randomly categrorized into eight groups (n = 10), two unbleached control groups, and six bleached groups. The bleached groups were formed based on two factors: the antioxidants which were immediately applied after bleaching and the resin cements used for cementation *(Table 1)*. Two applied antioxidants were tannic acid (Sigma–Aldrich, St. Louis, MO, USA) and quercetin (Sigma–Aldrich, St. Louis, MO, USA), and the two resin cements were Doulink (Bisco, Schaumburg IL USA) and Panavia SA Luting Plus, (Kuraray Noritake, Japan). As shown in Table 1, groups 1 and 5 are the control groups in which different resin cements were applied to the unbleached teeth. In groups 2, 3, and 4, Doulink

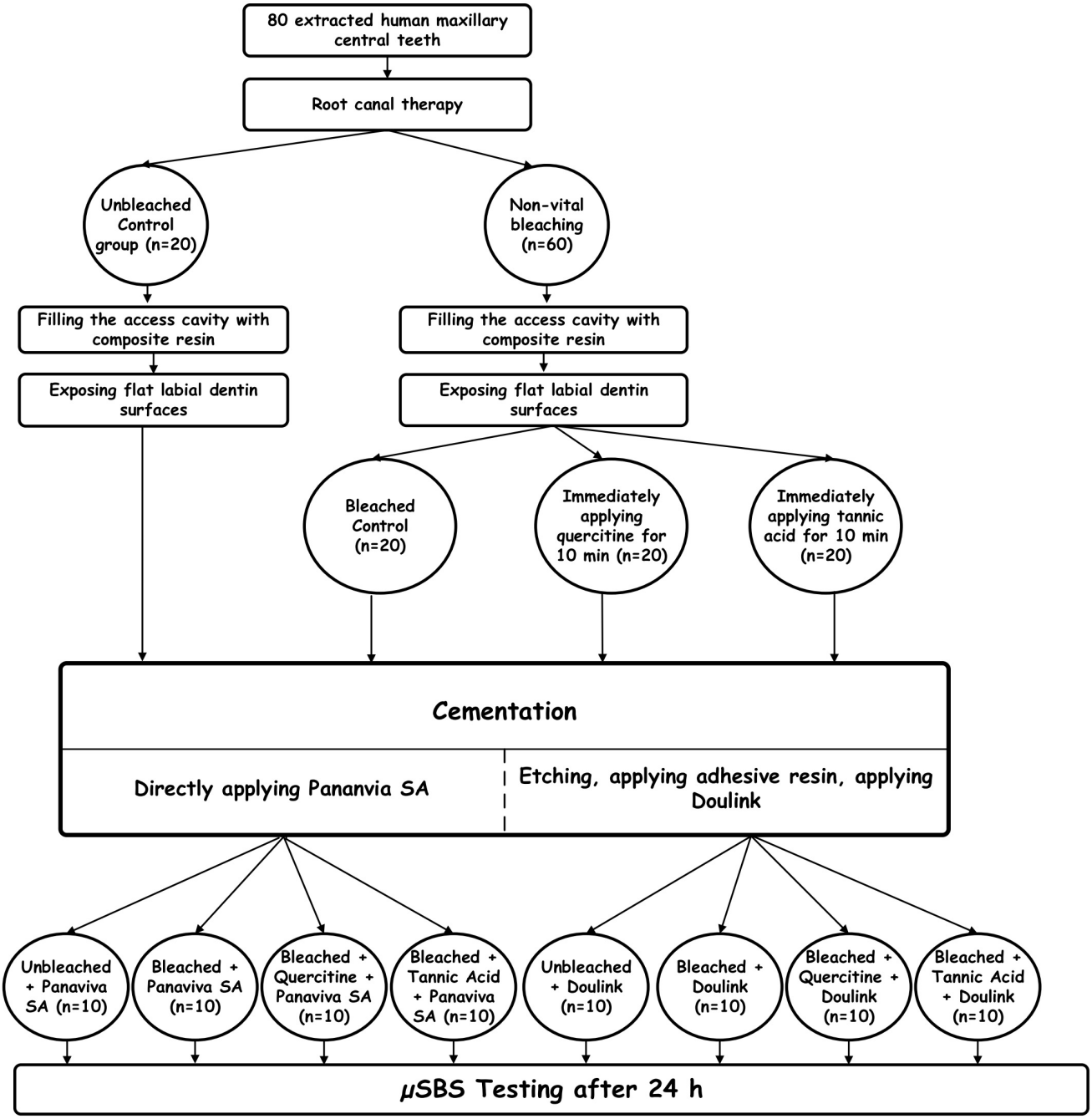

Figure 1: The diagram of the experimental design and groups

cement was bonded to the bleached teeth, respectively, after applying no antioxidant, tannic acid, and quercetin. Groups 6, 7, and 8 included the bleached teeth which were bonded by Panavia SA cement after applying no antioxidant, tannic acid, and quercetin, respectively. The materials used in the current study are presented in Table 2.

NON-VITAL BLEACHING PROCEDURE

In all bleached subgroups, Opalescence Endo (Ultradent, Utah, USA) which is 35 % hydrogen peroxide, the ready-to-use bleaching agent was inserted into the access cavity with cotton pellets. Then, the access cavities were closed with resin-modified glass ionomer cement (Fuji II LC, GC Corporation, Tokyo, Japan). The teeth were individually stored in an aplastic vial at 37 °C and 100 % humidity. The bleaching agent was removed and rinsed from the access cavity and replaced with a fresh solution after 3 days. This exchange was repeated two times over a period of 9 days[19,35] After the last treatment, bleaching gel was removed by rinsing it off using distilled water. The access cavity of all teeth were etched with 37% phosphoric acid (Denfil/ Vericom Co., Ltd., Korea) for 15 seconds, rinsed with water for 15 seconds, and gently air-dried. Two coats of Adper Single Bond 2 (3M ESPE, St Paul, MN, USA) were applied to the dentin surface, thoroughly air-dried for 5 seconds to evaporate the solvent, and light-cured for 20 seconds. Then, the access cavity was incrementally filled with composite resin (Filtek Z250, 3M ESPE, St Paul, MN, USA) with 2-mm layer thickness. Each layer was cured for 20 seconds.

Table 1. Experimental design considering the antioxidant immediately applied after bleaching and resin cement before cementation for the experimental and control groups (bleached and unbleached)

Group No.	Group Name	Bleaching	Antioxidant	Resin cement	Number of specimens (n)
1	Unbleached + Doulink (control/Dou)	Unbleached	Without	Doulink	10
2	Bleaching + Doulink (Bl/Dou)	Bleached	Without	Doulink	10
3	Bleaching + Tannic acid + Doulink (Bl+TA/Dou)	Bleached	Tannic Acid	Doulink	10
4	Bleaching + Quercetin + Doulink (Bl+Qu/Dou)	Bleached	Quercetin	Doulink	10
5	Unbleached + Panavia SA (control/PSA)	Unbleached	Without	Panavia SA	10
6	Bleaching + Panavia SA (Bl/PSA)	Bleached	Without	Panavia SA	10
7	Bleaching + Tannic acid + Panavia (Bl+TA/PSA)	Bleached	Tannic Acid	Panavia SA	10
8	Bleaching + Quercetin + Panavia SA (Bl+Qu/PSA)	Bleached	Quercetin	Panavia SA	10

Table 2. The brand names, manufacture, chemical composition of the materials used in this study.

Brand/Manufacturer	Manufacturer	Chemical composition
Opalescence Endo 35%	Ultradent, Utah, USA	Hydrogen peroxide 35%, potassium nitrate, fluoride
Phosphoric acid etching	Denfil/ Vericom Co., Ltd., Korea	A 37% phosphoric acid gel etchant
Adper Single Bond 2	C 3M ESPE, St Paul, MN, USA	HEMA, water, ethanol, Bis-GMA, dimethacrylates, amines, metacrylate functional copolymer polyacrylic and polyitaconic acids, nanometer-diameter spherical silica particles
Duolink	Bisco, Schaumburg, IL, USA	Base:bis-GMA, triethyleneglycoldimethacrylate urethane dimethacrylate, glass filler Catalyst: bis-GMA, triethyleneglycoldimethacrylate, glass filler
Panavia SA plus	SA Luting Plus, Kuraray Noritake	A paste: Bis-GMA, TEGDMA, dimethacrylate, 10-MDP, silanized Ba glass filler, silanized colloidal silica, photo-initiator, chemicalinitiator B paste: Bis-GMA, dimethacrylate, silanized Ba glass filler, silanized colloidal silica, silanized NaF, chemical accelerator, pigment.
Filtek Z250	C 3M ESPE, St Paul, MN, USA	UDMA, Bis-GMA, Bis EMA, TEGDMA, Zirconia/Silica fillers
Resin-modified glass-ionomer cement	GC Corp. Tokyo, Japan	Fluroaluminosilicate glass, 2-hydroxylethyl methacrylate, polyacrylic acid , 2,2,4 trimethyl hexamethylene dicarbonate , proprietary ingredient

TOOTH PREPARATION

The enamel of the labial surface was removed using 80-, 320-, and 600-grit silicon carbide under running water until a flat dentin surface was exposed. Each tooth was placed in self-activated acrylic resin (Acropars, Marlik Co., Tehran, Iran) into a square-shaped mold (25 × 25 × 25 mm). The root of each tooth was vertically placed in the midle of the acrylic resin block with its longitudinal axis orienting the flattened dentine surface vertically such that the coronal portion was kept out of the block just above CEJ.

ANTIOXIDANT APPLICATION

Quercetin powder with concentrations of 1.0 wt%. was achieved by directly dissolving it into pure ethanol.[28] Tannic acid powder with concentrations of 20 wt%. was achieved by directly dissolving it into distilled water.[31]

Immediately after bleaching, TA and quercetin solutions were applied on the dentine surfaces using a microbrush for 10 min. After applying the antioxidant, the dentine surfaces were thoroughly washed and dried gently.

LUTING PROCEDURES

In this study, two dual-cure resin cements were used; i.e. a conventional resin cement (Doulink) and a self-adhesive resin cement (Panavia SA). Equal amounts of base and catalyst of each cement were mixed, placed into a polyvinyl chloride microtube with an internal diameter of 0.7 mm and height of 0.5 mm, and bonded to the dentin surface. Then, a dental probe was used to remove the extra adhesive cement and the resin cement was polymerized with a light cure device (VIP Junior, Bisco, Schaumburg, IL, USA) at 600 mW/cm^2 for 40 seconds. One micro-cylinder was placed on each specimen. In Doulink groups and before cement placement, the exposed dentin surface was etched with 37% phosphoric acid for 15 seconds, rinsed with water for 15 seconds, and gently air-dried. Two coats of Adper Single Bond 2 were applied to the dentin surface and thoroughly air-dried for 5 seconds to evaporate the solvent and tnen, light-cured for 20 seconds. In Panavia SA groups, the mixed cement directly bonded to the dentin surface.

MICROSHEAR BOND STRENGTH TEST

After the cementation process, the specimens were kept at 100% humidity for 24 hours. Then, the micro-shear bond strength (μSBS) was measured using a universal testing machine (Instron, Z020, Zwick Roell, Germany) while a wire loop was positioned at the interfac of dentine/cement at a 1 mm/min constant speed. The μSBS was measured in newtons (N) and recorded in megapascals (MPa). One experienced operator performed the measurements for all eight groups at the same time and using the same device.

FAILURE MODE ANALYSIS

After μSBS testing, the failure mode was characterized using a stereomicroscope (Carl Ziess, Oberkochen, Germany) at 40× magnification. The failure modes were categorized as follows: A) adhesive failure along the interfacial region between the dentine and resin cement, B) cohesive failure in the dentine or resin cement, and C) mixed fracture (the combination of of a combination of two)

STATISTICAL ANALYSIS

The normal distribution of the μSBS values was verified using the Kolmogorov-Smirnov normality test. The mean microshear bond strength values of all groups were evaluated with the two-way ANOVA test. A one-way ANOVA test was used to analyze the relationships among four groups in each cement. As a post-hoc test, the Tamhane test was used for the pairwise comparisons ($p < 0.05$). SPSS software version 17 (SPSS Inc., Chicago, USA) was used for the data analysis.

RESULTS

The mean and standard deviations (SD) of μSBS values in MPa is shown in Table 3. Based on two-way ANOVA, the antioxidant and resin cement showed significant effects on the μSBS ($p < 0.001$), and also, the interaction between these two factors had a statistically significant effect ($p < 0.001$). Based on the one-way ANOVA, there was a statistically significant difference among the four groups for both types of cement ($p < 0.001$).

The mean μSBS values of the Doulink group were higher than the Panavia group in all subgroups ($p < 0.001$). The unbleached groups of both cements (control/Dou and control/PSA) showed a significantly higher bond strength than the other three groups ($p <0.05$). The mean μSBS values of the bleached groups in both cements (Bl/Dou and Bl/PSA) were lower than that of unbleached groups; the difference was statistically significant ($p < 0.001$).

There was not a statistical significant difference between the μSBS values of Bl/Dou and Bl+Ta/Dou groups ($p = 0.92$), and also Bl/Dou and Bl+Qu/Dou groups ($p = 0.94$). These results indicated a lack of effect of TA and quercetin on the bond strength of Doulink.

Table 3. Mean ± standard deviation of microshear bond strength values (MPa) for different groups

	Mean ± SD of μ SBS			
	Unbleached	**Bleached**	**Tannic acid**	**Quercetin**
Duolink	16.19 ± 1.6 ^{A,a}	12.52 ±1.54 ^{A,b}	13.27±1.8 ^{A,b}	11.84 ± 1.7 ^{A,b}
Panavia SA	11.8 ± 1.60 ^{B,a}	1.58 ± 0.62 ^{B,b}	6.12±1.23 ^{B,c}	2.13 ± 0.69 ^{B,b}

Different uppercase letters in columns and lowercase letters in rows indicate statistically significant difference ($p<0.05$).

The application of TA after the bleaching for Panavia SA (Bl+Ta/PSA) resulted in a statistically significantly higher mean μSBS compared to that of Bl/PSA group ($p < 0.001$). However, there was no significant difference between μSBS of Bl/PSA and BL+Qu/PSA groups ($p = 0.39$). These results demonstrated a beneficial effect of TA and no effect of quercetin on the bond strength of Panavia SA.

According to the failure mode analysis, the mixed failure was predominantly observed in all the groups (*Table 4*).

Table 4. Failure mode (%) of the groups, after the microshear test

Groups/	Duolink				Panavia SA			
failure mode	UD	BD	TD	QD	UP	BP	TP	QP
Adhesive	0	20	10	20	10	70	30	60
Cohesive	10	20	20	20	20	10	20	20
Mixed	90	60	70	60	70	20	50	20

DISCUSSION

The present research aimed to study the influence of TA and quercetin antioxidants on the bond strength of Doulink and Panavia SA resin cements to intracoronary bleached dentine. The obtained results showed that TA antioxidant increased the μSBS of Panavia SA resin cement to the bleached dentine but the bond strength of Doulink resin cement was not improved. The results also indicated that the bond strength of both resin cements to the bleached dentine was not affected by the quercetin antioxidant. So, the null hypothesis was partially rejected.

In this study, we used hydrogen peroxide at a concentration of 35% as it shows a high enamel/dentine penetration and the potential of changing the nature of pigment macromolecules.[11,36] Moreover, hydrogen peroxide provides a higher efficacy compared to carbamide peroxide with a similar concentration.[37]

Based on the obtained results, the bleaching procedure adversely affected the microshear bond strength of both Doulink and Panavia SA resin cements to intracoronary bleached dentine. This result is consistent with the findings of the previous studies.[37,38] There are several undesirable effects associated with the bleaching process including the morphological alteration and composition changes of dental hard tissues.[13,33] Free radicals (i.e. breakdowns of the bleaching process) or the oxygen released during the bleaching process alone or in combination may compromise the tooth adhesion.[39] These molecules might penetrate and trap into dentinal tubules for one or two weeks after bleaching.[40] This might restrict the resin infiltration into the dentine or prevent the complete polymerization process and decrease the bond strength of resin based materials.[40]

Notably, the bond strength reduction caused by bleaching is more in the Panavia SA group compared to the Doulink one. The bleaching procedure may change the surface free energy and collagen sequence, resulting in a reduction of the surface wettability and the penetration of the resin cement to the dentine.[41] Moreover, Panavia SA is a self-adhesive resin cement and the thickness of the hybrid layer may be reduced (even down to zero) due to the superficial interaction between the self-adhesive resin cement and dentine.[42,43] Such property is not observed for conventional resin cement placed after a pre-treatment (phosphoric acid and/or adhesive).[42,43] These two factors synergistically reduce the bond strength of the Panavia SA cement to bleached dentine.

Various antioxidants have been used in restoring the bond strength of composite resin to the bleached enamel/dentine.[44,45] However, there are a few previous studies in which these agents were used to increase the bond strength of resin cement.[15,19,35] In a study by Gokce *et al.*, the influence of sodium ascorbate on the shear bond strength of resin cement to bleached enamel was investigated.[15] It was shown that sodium ascorbate may be suitable in restoring the compromised bond strength.[15] In another study by Kılınc *et al.*, it was concluded that cementation on enamel after internal bleaching was beneficial only with a 14-day delay, or with a one-week-day along with sodium ascorbate application.[19] A similar result was found by May *et al.*, which reported that a delay for one weak with sodium ascorbate application before adhesive cementation increased bond strength after bleaching.[35]

In this study, we investigate the effect of two antioxidants on restoring the bond strength of bleached dentine; i.e. quercetin and TA. TA reversed the adverse influence of bleaching on the bond strength of Panavia SA to some extent as the μSBS of the Bl+Ta/PSA group revealed a significantly higher bond strength when compared to Bl/PSA group. However, the value of Bl+Ta/PSA did not reach the level of the control and it was significantly lower than that of the unbleached control group. TA showed its ability in partial removal of the smear layer which enhances the surface porosity of TA-treated dentine. This effect was observed in scanning electron microscopic (SEM) images of surfaces reported in some previous studies.[31,46] In another study by Pavan *et al.*, it was shown that TA did not improve the microtensile bond strength of self-adhesive resin cements to dentine.[31] However, the dentine was bleached in this current study, and applying TA on the bleached dentine was useful in partial compensation of the adverse effect of bleaching on the bond strength. To the best of the authors' knowledge, there is no previous research about the TA effect on bleached teeth and thus, it was impossible to compare our results with the related studies of μSBS recovery of bleached teeth.

According to the obtained results for Doulink cement, TA application did not restore the bond strength reduction caused by the bleaching procedure. This effect is different from the one observed for Panavia SA where TA partially restored the negative effect of bleaching on the bond strength. Doulink is a

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conventional resin cement with an etching step that removes the smear layer completely while Panavia SA is a self-adhesive resin cement that interacts superficially with the dentine surface and cannot demineralize the dentine completely and therefore, cannot completely remove the smear layer.[47,48] TA can act as a smear layer removing agent because it has been previously proposed as a material for smear removal/modification to enhance the bond strength between glass ionomer cement and dentine.[15,49] It was assumed that the application of TA on the bleached dentine was capable of partially removing the smear layer along with the bleached dentine. This could facilitate the self-adhesive resin penetration and its interaction of the functional monomer (10-Methacryloyloxydecyl dihydrogen phosphate, MDP) contained in Panavia SA with the calcium content of the dentin. The achieved chemical bond might contribute to the beneficial effect of TA in improving the bond strength of bleached teeth.

Quercetin was another antioxidant that was investigated in this study. According to the obtained results, the mean μSBS was not statistically significantly different in both Doulink and Panavia SA groups when compared to the bleached one. These findings indicated that quercetin did not show any efficiency in restoring the negative influence of the used bleaching agent on bond strength to the dentine. This is in contrast with the findings of the study by Shamsedin *et al*., in which the effect of quercetin on SBS of orthodontic brackets on bleached enamel was investigated.[28] They showed that quercetin improved the SBS to normal levels. However, they studied the SBS of orthodontic brackets on bleached enamel while the current study investigated the μSBS of the resin cements to the internally bleached dentin.

The current study has a few limitations. Before the microshear bond strength test, the specimens were stored in distilled water only for 24 hours and thus, the impact of long-term storage and thermocycling on bond strength was not evaluated. So, this *in-vitro* study may not represent the long-term changes in bond strength under oral fluid conditions. Moreover, this investigation was not capable of simulating the static, chemical, and cyclic mechanical fatigue events in the interfaces of resin cement-tooth structure. Moreover, those results should also be compared with long-term clinical studies. Future studies could include evaluating the influence of different application times and concentrations of the quercetin and TA on bond strength of cements to bleached dentine. Furthermore, additional studies could evaluate other antioxidants, which have been shown to restore the adverse effect of bleaching on bond strength to tooth structures. Also, the impact of antioxidant on the bond strength of access cavity to the composite resin can be investigated in the future studies.

CONCLUSION

The non-vital internal bleaching revealed an adverse effect on the bond strength of Doulink and Panavia SA to the labial dentine. TA partially reversed the negative influence of the internal bleaching on the immediate μSBS of Panavia SA to the dentine while quercetin did not improve the immediate bond strength of both Panavia SA and Doulink resin cements.

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