# Efficacy of quercetin flavonoid in recovering the postbleaching bond strength of orthodontic brackets: A preliminary study

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# ABSTRACT

**Objectives:** To evaluate comparatively the effect of quercetin on postbleaching shear bond strength (SBS) and adhesive remnant index (ARI).

**Materials and Methods:** Intact maxillary premolars were divided randomly into 12 groups of 10 each: (1) bonding the bracket immediately after bleaching, (2) bonding 1 week after bleaching, (3–8) application of three experimental concentrations of quercetin (0.1%, 0.5%, and 1%) at two time durations (5 and 10 min), (9–10) application of the solvent of quercetin at two time periods (5 and 10 min), (11) application of 10% sodium ascorbate for 10 min, and (12) bonding the brackets on nonbleached teeth. Bleaching was performed using 15% carbamide peroxide gel for 5 days (6 h daily). After incubation and thermocycling, the SBS of brackets was measured. The ARI too was recorded at ×20. The data were analyzed statistically ( $\alpha$  =0.05).

**Results:** Bleaching reduced the SBS below 10 Megapascal (MPa) level (P < 0.05) while all the postbleaching treatments (except the application of the solvent of quercetin) recovered the SBS back to values greater than 10 MPa (P < 0.05) and also back to nonbleached SBS levels (P > 0.01). All eight postbleaching treatments had rather similar efficacies (P = 0.1396). The concentration of quercetin (beta = 0.259, P = 0.042) but not its duration (beta = 0.213, P = 0.093) significantly improved its efficacy.

**Conclusion:** Bleaching can weaken the bond strength of orthodontic brackets below acceptable levels. The application of quercetin or Vitamin C or delaying the bracket bonding improved the postbleaching SBS.

Key words: Antioxidants, ascorbic acid, delayed bonding, orthodontic brackets, quercetin, shear bond strength, tooth bleaching

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# INTRODUCTION

Bleaching is an effective and safe method for whitening discolored teeth by oxidation of molecules responsible for absorbing the light and darkening the tooth;<sup>[1]</sup> however, residuals of bleaching agents can discharge oxygen radicals, disrupting the bonding of composite materials to bleached teeth which this reduces the bond strength.<sup>[1-4]</sup> To

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avoid this, different strategies have been proposed; these include postponing the bleaching until after the finishing of the orthodontic treatment, delaying the bonding of brackets about 24 h to 4 weeks after bleaching, pumicing the bleached enamel, using resin-modified glass ionomer cements for bonding, and finally scavenging the oxidative residuals of bleaching agents with antioxidants.<sup>[1-13]</sup>

Antioxidant application seems to be one of the fastest, most convenient, and most effective methods in this regard. Previous studies have used sodium ascorbate (Vitamin C) on bleached teeth and showed its efficacy while being used at different doses and for different durations (up to 3 h)<sup>[2-4,9-14]</sup> except in few studies where 10% sodium ascorbate did not recover the shear bond strength (SBS) back to normal.<sup>[13]</sup> Tocopherol is another antioxidant agent derived from Vitamin E and proved successful in improving the bond strength of orthodontic brackets after bleaching.<sup>[13,14]</sup>

Another antioxidant material is quercetin which is a flavonoid available in many vegetables, fruits, and foods.<sup>[15-17]</sup> It is also available as food supplements and can be potentially used abundantly for reversing the effect of bleaching on SBS (if its SBS-improving effect is verified). Nevertheless, it has not been evaluated in the field of dentistry, adhesive materials, or orthodontics.

Therefore, this study was conducted for the first time to evaluate comparatively the effect of quercetin on postbleaching SBS and adhesive remnant index (ARI). The null hypotheses were (1) bleaching does not change the SBS; (2) delayed bonding does not change the postbleaching SBS; (3–5) quercetin, Vitamin C does, or delayed bonding do not recover postbleaching SBS to nonbleached SBS levels; (6) the effect of quercetin on the postbleaching SBS (if any) is not different from the effect of its solvent dimethyl sulfoxide (DMSO); (7) duration of quercetin application does not affect its efficacy; (8) concentration of quercetin does not influence its efficacy.

#### Table 1: An overview of the whole study procedures

# MATERIALS AND METHODS

This *in vitro* experimental research was carried out on 120 intact human maxillary first premolars extracted prospectively for treatment purposes only. The specimens were sequentially inspected for the exclusion criteria being hypoplasia, hypocalcification, caries, or fractures. In addition, patients needed to be 11–17 years old, with no history of previous bleaching or chemical agent application.

#### **Sample Preparation**

Each tooth was first stored in 0.2% thymol solution for 24 h, and then placed in deionized distilled water until the completion of sample size collection (which took <6 months). The 120 teeth were divided randomly into 12 groups of 10 each, including four different control groups, a delayed bonding group, a Vitamin C group, and six quercetin groups [Table 1].

#### Group 1 (control)

In this group, brackets were bonded immediately after bleaching, without any treatments taken place before bonding.

#### Group 2 (delayed bonding)

Bracket bonding was postponed for 1 week after bleaching.

#### Groups 3-8 (quercetin application)

After bleaching, 3 experimental concentrations of quercetin (0.1%, 0.5%, and 1%) diluted within a 10% DMSO solvent were applied to the bleached surface, for either 5 or 10 min [Table 1].

#### Groups 9 and 10 (control)

The solvent of the quercetin (10% DMSO) was itself applied to the bleached surface (for 5 and 10 min) after bleaching.

#### Group 11

After bleaching, 10% ascorbic acid was applied for 10 min.

#### Group 12 (control)

No bleaching was performed. Brackets were bonded to normal teeth.

Group	Bleaching Treatment		Bracket bonding	Incubation and thermocycling	SBS and ARI	
1 (C)	•	-	•	٠	•	
2	•	Waiting for 1 week	•	•	•	
3	•	Q 0.1% for 5 min	•	•	•	
4	•	Q 0.1% for 10 min	•	•	•	
5	•	Q 0.5% for 5 min	•	•	•	
6	•	Q 0.5% for 10 min	•	•	•	
7	•	Q 1% for 5 min	•	•	•	
8	•	Q 1% for 10 min	•	•	•	
9 (C)	•	DMSO for 5 min	•	•	•	
10 (C)	•	DMSO for 10 min	•	•	•	
11	•	Vitamin C 10% for 10 min	•	•	•	
12 (C)	-	-	•	•	•	

C - Control; Bullet - Present; Hyphen - Absent/none; DMSO - Dimethyl sulfoxide; SBS - Shear bond strength; ARI - Adhesive remnant index

#### **Bleaching**

First, the buccal surfaces were cleansed using water and pumice applied for 10 s with a low-speed rubber cup. After water spraying for 30 s, they were air dried for 15 s. Bleaching was carried out (according to the manufacturer instructions) using a 15% carbamide peroxide gel (Opalescence, Ultradent Products, South Jordan, UT, USA) applied for 6 h a day for 5 subsequent days. Each day, 0.02 mL of the bleaching gel was applied with the graduated syringe of the bleaching kit to the buccal surface. Then, the bleached tooth was immersed in artificial saliva at 37°C for the rest of the day, with its buccal surface and the gel placed over it staying out of saliva. After daily bleaching, the tooth would be rinsed with the spray of water for 60 s, and then would be immersed completely in the artificial saliva for the rest of the day. The specimens in the group 12 (no bleaching) were kept in the artificial saliva at 37°C for the whole period. The saliva was changed every day.

#### **Postbleaching Treatments**

#### Delayed bleaching

The teeth were kept in artificial saliva at 37°C for another 7 days. Then, they were bracket-bonded.

#### Quercetin application

Since there was no previous study on quercetin, three concentrations were determined for its application by a pharmacologist. The solvent of quercetin solution was first prepared by diluting DMSO (Sigma-Aldrich, St. Louis, MO, USA) 1:10 in distilled water. For preparing three concentrations of quercetin, 0.1, 0.5, and 1 g of quercetin powder (Sigma-Aldrich) was added to 100 mL of the above solution. The prepared solutions were applied for either 5 or 10 min to the buccal surface. The durations and concentrations depended on the group numbers as detailed in Table 1. Afterward, the enamel surface was rinsed with distilled water for 60 s and was air dried.

#### Vitamin C application

The 10% ascorbic acid solution was prepared by adding 10 g powder (Sigma-Aldrich) to 100 mL distilled water. The solution was applied to the bleached surface for 10 min. Then, the tooth was rinsed for 60 s and air dried.

#### Dimethyl sulfoxide application

To control for the potential effect of the solvent of quercetin, 10% DMSO too was applied for two durations (5 and 10 min) to the bleached surface.

#### **Bracket Bonding**

The used brackets were stainless steel 0.022 inch edgewise upper premolar brackets (American Orthodontics, Sheboygan, WI, USA). The composite resin used to bond the brackets was light curable (3M Unitek Transbond, Maplewood, MN, USA). All the procedures were performed at room temperature (24°C). First, a 38% phosphoric acid gel (Etch-Rite, Pulpdent, Watertown, USA) was applied according to the instructions of its manufacturer (30 s application, 30 s water spraying, followed by complete air drying).

A layer of primer (Unitek Transbond XT Primer, 3M, St. Paul, MN, USA) was applied to the surface and light cured for 10 s using a calibrated unit (Woodpecker Medical Instrument, Guilin, China). Each bracket was placed 4 mm apically to the cusp tip, and perpendicular to the axis of the tooth. A tension and compression gauge (Dentaurum, Ispringen, Germany) was used to exert a 250-g compressive force and standardize and decrease the adhesive thickness. The excess resin was removed by a dental scaler. Light curing was performed for 20 s (10 s per mesial and distal sides).

#### Aging

After distiller water storage at 37°C for 24 h, the teeth were subjected to thermal cycling at 500 cycles (transfer time = 20 s, dwell time = 30 s,  $5^{\circ}C \pm 2^{\circ}C$  to  $55^{\circ}C \pm 2^{\circ}C$ ).

#### **Shear Bond Strength**

A straightened 0.019 × 0.025 inch archwire with both ends attached to fixed rods of same height (Ortho Technology, FL, USA) was placed in the slots of 6 brackets, to hold them at a standardized height from the ground. In this upright position, the teeth were mounted by their roots in self-cured acrylic resin blocks of 3 cm × 3 cm × 3 cm. This way, all brackets were mounted in a perfect vertical position.

A universal testing machine (ZO-50, Zwick Roell, UIm, Germany) exerted a vertical force to the occlusal sides of bracket wings, at 0.5 mm/min crosshead speed. The breakage force was recorded in Newton. The breakage force was divided by area of the bracket base (4.16 mm × 2.85 mm) to compute the SBS in Megapascal unit (MPa).<sup>[18]</sup>

#### **Adhesive Remnant Index**

A stereo microscope (Motic SMZ 143 Series, Micro-Optic, NJ, USA) was employed to examine the extent of remaining adhesive over the enamel under a  $\times 20$  magnification. The ARI scores were (1) the entire composite remained on the enamel; (2) more than 90% of the composite stayed on the enamel; (3) between 90% and 10% of the composite remained on the surface; (4) <10% of the composite remained; and (5) no composite existed on the enamel.<sup>[18]</sup>

#### **Statistical Analysis**

Descriptive statistics for SBS and ARI were calculated. SBS differences between experimental groups and their respective controls were evaluated using a Mann–Whitney U-test. The effects of three postbleaching treatments (delayed bonding, Vitamin C, and three concentrations and two durations of quercetin) were compared using the Kruskal–Wallis test. The effects of duration and concentration of quercetin application on SBS were evaluated using a multiple regression analysis. A clinically acceptable bracket bond is considered by different researches as *in vitro* SBS values above 6, 8, or

10 MPa.<sup>[18,19]</sup> The SBS of each group was compared with the value 10 MPa (as the ceiling of this range), using a one-sample Wilcoxon test of the SPSS program (version 20.0, IBM, Armonk, USA). The level of statistical significance was set at 0.05.

### RESULTS

The one-sample Wilcoxon test showed that bleaching reduced the SBS below the acceptable 10 MPa level. All the postbleaching treatments (except the DMSO) succeeded to recover the SBS back to values above 10 MPa. SBS of the group 12 (control, no bleaching) was as well above 10 MPa [Table 2 and Figure 1].

Compared to the results pertaining to the group 1 (control, bonding immediately after bleaching), all treatments (delayed bonding (P < 0.001), quercetin application at any concentration and for any time period (all the six P < 0.01), Vitamin C application (P < 0.001), and DMSO application (both P < 0.03) increased the SBS significantly, according to the Mann–Whitney U-test [Table 3].



**Figure I:** Mean and standard deviation of SBS values (Megapascal). Q: Quercetin; DMSO: Dimethyl sulfoxide; Green bars: Antioxidants; Yellow bar: Delayed bonding; Blue bars: control groups

Compared to the "no bleaching" control group, all the experimental groups (delayed bonding, quercetin, and Vitamin C) except for the two DMSO groups had SBS levels not significantly different than the "no bleaching" SBS (all eight Mann–Whitney P > 0.15). The SBS of DMSO groups did not reach the normal SBS of "no bleaching" group (both P < 0.04) [Table 3].

The Kruskal–Wallis test did not detect a significant difference between the efficacy of three treatments (delayed bonding, six groups of quercetin each separately and Vitamin C), in recovering the postbleaching SBS (P = 0.1396).

The regression analysis identified the concentration of quercetin as a factor significantly increasing the efficacy of quercetin in SBS recovery (beta = 0.259, P = 0.042). However, the effect of the duration of quercetin application did not reach the level of significance (beta = 0.213, P = 0.093).

The ARI scores mostly revolved around the Score 3 and 4 indicating 0% to 90% composite remnant on the debonded surface [Table 4].

# DISCUSSION

Similar to most other studies<sup>[1-4,14]</sup> (but unlike few ones),<sup>[18]</sup> this study showed that bleaching reduces the SBS below acceptable levels. The findings of this pilot study showed that all the evaluated concentrations and durations of quercetin application might succeed in recovering the bond strength back to normal prebleaching levels. The same also was seen for the delayed bonding and Vitamin C application, with no statistically detectable difference between the techniques. Since there is no study on quercetin effect (either in orthodontics or restorative dentistry etc.), we are limited to compare our findings with other methods of SBS recovery. Our findings in terms of the application of both quercetin and Vitamin C were in line with most of studies on orthodontic brackets. In orthodontics, Vitamin C application has been suggested as a successful method for

Table 2: Shear bond strength values (megapascal) in different groups, and the result of one-sample Wilcoxon test comparing each group with the value 10 megapascal

Group	Mean	SD	CV	Minimum	Q1	Median	Q3	Maximum	95%	% CI	Р
Immediate bonding	5.3	4.4	81.9	0.0	0.9	4.8	9.6	12.4	2.2	8.5	0.020
Delayed bonding	16.9	7.7	45.6	5.8	10.2	15.6	25.4	25.9	11.4	22.5	0.037
0.1% Q (5 min)	16.5	10.1	61.4	5.9	6.8	13.2	26.1	31.2	9.2	23.7	0.193
0.1% Q (10 min)	20.8	11.7	56.3	5.6	9.4	19.6	31.4	38.9	12.4	29.1	0.020
0.5% Q (5 min)	21.8	8.8	40.4	7.8	14.4	23.8	29.0	32.1	15.5	28.1	0.010
0.5% Q (10 min)	26.5	8.7	32.9	11.4	19.2	26.1	32.8	40.2	20.3	32.7	0.002
1% Q (5 min)	23.2	6.8	29.4	11.9	17.4	24.1	29.3	32.9	18.3	28.1	0.002
1% Q (10 min)	26.3	8.6	32.6	11.1	19.9	28.5	33.3	35.9	20.2	32.5	0.002
DMSO (5 min)	11.0	5.4	49.1	6.4	7.8	9.4	12.8	24.7	7.2	14.9	1.0
DMSO (10 min)	13.0	6.6	51.1	2.7	5.6	14.6	19.2	20.5	8.3	17.8	0.275
Vitamin C	20.9	8.9	42.6	5.7	12.9	22.4	27.8	34.1	14.6	27.3	0.010
No bleaching	21.3	7.1	33.4	10.8	15.0	22.2	28.3	28.9	16.2	26.3	0.002

SD - Standard deviation; CV - Coefficient of variation; Q1 - 25th percentile; Q3 - 75th percentile; CI Confidence interval for the mean; Q - Quercetin; DMSO - Dimethyl sulfoxide

Table 3: Mann-Whitney's P values calculate	ed by comparing
each of the groups with each of the 4 cont	rol groups

Group	Immediate bonding	DMSO (5 min)	DMSO (10 min)	No bleaching
Immediate bonding	-	0.0288	0.0115	<0.0001
Delayed bonding	0.0015	0.0630	0.3930	0.1655
0.1% Q (5 min)	0.0068	0.4359	0.5288	0.2176
0.1% Q (10 min)	0.0021	0.0753	0.1431	0.9705
0.5% Q (5 min)	0.0003	0.0232	0.0185	0.9118
0.5% Q (10 min)	<0.0001	0.0003	0.0029	0.1903
1% Q (5 min)	<0.0001	0.0011	0.0068	0.5288
1% Q (10 min)	<0.0001	0.0005	0.0039	0.1431
DMSO (5 min)	0.0288	-	0.4359	0.0011
DMSO (10 min)	0.0115	0.4359	-	0.0355
Vitamin C	0.0002	0.0115	0.0753	1.0
No bleaching	<0.0001	0.0011	0.0355	-

Q - Quercetin; DMSO - Dimethyl sulfoxide

# Table 4: Frequency distribution of adhesive remnant index scores in each group

Group	Score (100%)	Score 2 (>90%)	Score 3 (10-90%)	Score 4 (<10%)	Score 5 (0%)
Immediate bonding	0	0	4 (40)	5 (50)	1 (10)
Delayed bonding	0	1 (10)	7 (70)	2 (20)	0
0.1% Q (5 min)	0	0	8 (80)	2 (20)	0
0.1% Q (10 min)	0	0	7 (70)	3 (30)	0
0.5% Q (5 min)	0	0	6 (60)	4 (40)	0
0.5% Q (10 min)	0	1 (10)	5 (50)	4 (40)	0
1% Q (5 min)	0	0	8 (80)	2 (20)	0
1% Q (10 min)	0	1 (10)	7 (70)	2 (20)	0
DMSO (5 min)	0	0	5 (50)	5 (50)	0
DMSO (10 min)	0	0	5 (50)	5 (50)	0
Vitamin C	0	0	7 (70)	3 (30)	0
No bleaching	0	0	8 (80)	2 (20)	0

Q - Quercetin; DMSO - Dimethyl sulfoxide

reversing the deteriorating effect of bleaching on SBS, by Lai et al.,<sup>[12]</sup> Bulut et al.,<sup>[3,4]</sup> Kaya et al.,<sup>[11]</sup> Gökçe et al.,<sup>[9]</sup> Lima et al.,<sup>[20]</sup> Khsoravanifard et al.,<sup>[18]</sup> and Yadav et al.<sup>[14]</sup> The only study failing to observe its proper effect was that of Sasaki et al.[13] In the present study, 10% Vitamin C was used for 10 min. Earlier studies have suggested different durations for Vitamin C to be effective. For example, according to Kaya et al.,[11] at least 1 h of Vitamin C application was needed, while according to Lima et al.,[20] only 1 min of VC application would suffice. We employed the duration used successfully by Khosravanifard et al.[18] and found it useful. The guercetin as well could be used for short durations of 5 or 10 min with similar efficacies. The mechanism responsible for SBS increase is likely to be the reduction of free oxygen radicals remaining after bleaching.<sup>[1-4]</sup> The oxygen inhibits polymerization of resin monomers and hence reduces the SBS.[1-4,21] Antioxidants reduce the amount of free oxygen radicals and allow proper polymerization of the bonding agent which accounts for the SBS increase.[3,4,9,11,12,14,18,20]

The findings of the present study in terms of the efficacy of delayed bonding was in line with the results of Bulut *et al.*,<sup>[3,4]</sup>

who found both Vitamin C application and delayed bonding similarly effective in reversing the effect of bleaching, while was in contrast to the study of Gökçe *et al.*,<sup>[9]</sup> who reported that only Vitamin C application and not delayed bonding was useful in neutralizing the negative effect of bleaching. These differences can be due to various factors involved such as the duration of postbleaching delay, the concentration of the evaluated Vitamin C, or its application duration. Although delayed bonding might be efficient in many cases, it is slow and time-consuming (not to mention cost increasing), and hence is less favorable clinically.

In this study, the duration of quercetin application did not have a significant effect on its efficacy. It was in contrast to a study showing that the SBS increases proportional to the application period of Vitamin C.<sup>[11]</sup> The difference could be attributable to confounding factors in this study, as well as methodological differences. Our result showed marginally significant effects, which could become significant if the two compared application times had a greater contrast and/or if better control was present over latent variables in this setup. The effect of quercetin concentration on the other hand reached the level of significance. Since it was the first study of its kind, these concentrations were suggested by a pharmacologist. It is possible that higher concentrations of this flavonoid can have even faster and greater effects. Future studies are warranted to find the optimum concentration of quercetin.

There were some limitations in this study. The findings of this *in vitro* study cannot be necessarily generalized to other brands of the used materials and are also not completely representing clinical conditions. Nevertheless, we tried to improve the reliability of results by employing four different control groups (including different durations of application of the solvent of quercetin), three different treatments, and three durations of application of two different concentrations of quercetin as well as using artificial saliva at body temperature. Moreover, we used the ceiling of the controversial range of suggested values for acceptable bond strengths (6–10 MPa<sup>[18,19]</sup>) to improve the confidence in the positive results. In addition, incubation and thermocycling were performed as a standard procedure to simulate the oral environment.

# CONCLUSION

Bleaching can weaken the bond strength of orthodontic brackets below acceptable levels. Delaying the bracket bonding or 10 min of 10% Vitamin C application can recover the SBS. It was showed for the first time that these experimental concentrations of quercetin (0.1%, 0.5%, and 1%) were capable of improving the SBS to normal levels, regardless of the duration of application.

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#### **Conflicts of Interest**

There are no conflicts of interest.

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