Effect of an Antioxidizing Agent - containing Citric Acid on the Shear Bond Strength of Adhesive Bonded to Bleached Human Enamel

ผลของสารผสมระหว่างสารต้านอนุมูลอิสระและกรดซิตริกต่อความแข็งแรงยึดเฉือนของสารยึดติดกับฟันมนุษย์ที่ถูกฟอกสีฟัน

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ABSTRACT

The purpose of this study was to evaluate the effect of ascorbic acid combining with citric acid on the shear bond strength of brackets bonded with composite resin to human teeth after bleaching. For shear bond strength test, fifteen maxillary premolar teeth were divided to 3 groups (n=5). In control group, 37% phosphoric acid (PA) was applied on unbleached teeth 15 secs. For group 1, 37% PA was applied on bleached teeth 15 secs. In group 2, 50% ascorbic acid-50%citric acid formulation (50AA50CA) was applied instead of PA on bleached teeth 5 mins. Data were analyzed by one-way ANOVA. The result was that the 50AA50CA had significantly higher shear bond strength than PA group ($p<0.02$) but lower strength than control group ($p>0.05$). In conclusion, the 50AA50CA improved adequate shear bond strength for etching in the orthodontic bracket bonding to the bleached teeth.

บทคัดย่อ

วัตถุประสงค์ของการศึกษาครั้งนี้คือการประเมินผลของกรดแอสคอร์บิกรวมกับกรดซิตริกบนความแข็งแรงยึดเฉือนของแบร็กเกตที่ถูกยึดติดด้วยเรซินคอมโพสิตกับฟันมนุษย์หลังจากการฟอกสีฟัน โดยงานวิจัยในครั้งนี้จะใช้ฟันกรามน้อยบน 15 ซี่แบ่งออกเป็น 3 กลุ่ม (5 ซี่ต่อกลุ่ม) ในกลุ่มควบคุมจะใช้กรดฟอสฟอริกความเข้มข้นร้อยละ 37 กัดบนผิวเคลือบฟันที่ไม่ได้ฟอกสีฟัน 15 วินาที สำหรับกลุ่มทดลองที่ 1 จะใช้กรดฟอสฟอริกความเข้มข้นร้อยละ 37% กัดบนผิวฟันที่ถูกฟอกสีฟัน 15 วินาที กลุ่มทดลองที่ 2 นั้นจะใช้กรดที่ผสมด้วยกรดแอสคอร์บิกและกรดซิตริกที่ความเข้มข้นร้อยละ 50 กัดบนผิวฟันที่ถูกฟอกสีฟัน 5 นาที โดยใช้สถิติการวิเคราะห์ความแปรปรวนแบบทางเดียวในการวิเคราะห์ การศึกษานี้พบว่ากลุ่มทดลองที่ 2 มีความแข็งแรงของยึดเฉือนของสูงกว่ากลุ่มทดลองที่ 1 อย่างมีนัยสำคัญ แต่มีความแข็งแรงต่ำกว่ากลุ่มควบคุม สรุปได้ว่า กรดแอสคอร์บิกและกรดซิตริกที่ความเข้มข้นร้อยละ 50 สามารถเพิ่มความแข็งแรงยึดเฉือนที่เพียงพอสำหรับแบร็กเกตที่ถูกยึดติดด้วยเรซินคอมโพสิตกับฟันที่ถูกฟอกสีฟัน

Keywords: Tooth bleaching, Antioxidants, Shear bond strength

คำสำคัญ: การฟอกสีฟัน สารต้านอนุมูลอิสระ ความแข็งแรงยึดเฉือน

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Introduction

Due to a growing trend towards aesthetics dentistry, vital tooth bleaching had currently become one of the most popular cosmetic dental procedures especially for adult patients. Whitening was requested more frequently, especially by orthodontic patients.

In addition to its general purpose, tooth whitening was necessary in patients with teeth discoloration from nonvital pulp, particularly in the anterior region. However, many studies showed that the bond strength of resin composite and enamel was reduced when the tooth had been bleached with an in-office or at-home technique (Dishman et al., 1994; Sung et al., 1999; Cavalli et al., 2001).

According to Uysal et al. (2009), the shear bond strength was reduced after intracoronal bleaching. To overcome this problem, an antioxidant could be applied before application of the adhesive system. In the majority of the studies, sodium ascorbate that was a sodium salt of ascorbic acid with a neutral pH was applied due to higher antioxidant activity of SA in comparison to several evaluated substances.

However, to simplify adhesive procedures according to the current trend of dental adhesive development, considering that both sodium ascorbate and etchant needed to be removed. The incorporation of antioxidant in etchant was an interesting option.

So, in this study, we investigated ascorbic acid's efficacy as an antioxidant in the presence of citric acid to create the etchant formulations, which could improve bond strength of brackets to bleached enamel.

Objectives of the study

The purpose of this study is to evaluate the effect of ascorbic acid combining with citric acid on the shear bond strength of metallic brackets bonded with composite resin to human teeth after bleaching.

Methodology

All study protocols were approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2020-113).

Tooth sample preparation

Fifteen human maxillary premolars indicated for orthodontic extraction were collected from patients aged between 17 and 30 years (Hobson et al., 2001), cleaned of debris and disinfected in 10% formalin solution for two weeks.

Teeth with caries, enamel defects, restorations and pretreatments of chemical agents such as derivatives of peroxide, acid or any other form of bleaching were excluded.

After that, the roots of teeth were cut below CEJ 2 mm. and the crowns were stored in fluoride-free artificial saliva at 37°C. All specimens were stored in artificial saliva for 1 week before bonding. The artificial saliva was changed twice per day during the 1-week period.

The fluoride-free artificial saliva was prepared from Department of Pharmacology, Faculty of Dentistry, Chulalongkorn University.

The teeth were randomly divided into 3 groups as follows:

Control group (n = 5)**:** 37% phosphoric acid on unbleached teeth 15 seconds

Group 1 (n = 5)**:** 37% phosphoric acid on bleached teeth 15 seconds

Group 2 (n = 5)**:** 50% ethyl ascorbic acid + 50% citric acid on bleached teeth 5 minutes

Control group

After the specimens had been removed from the artificial saliva, the enamel surfaces were rinsed (10 seconds) and dried (10 seconds) with a triple syringe. Then, buccal surfaces of teeth were etched with 37% phosphoric acid (Ormco®) for 15 seconds. The etching area was wider than bracket base about 1 mm for each margin. The solution was applied with a disposable applicator with continuous agitation, followed by rinsing for 30 seconds and drying for 10 seconds with oil-free compressed air.

In the experimental groups, before bleaching procedure, the enamel surfaces were polished with fluoride-free pumice powder and water by using a brush and a slow-speed handpiece, rinsed 10 seconds and dried with a triple syringe for 10 seconds.

Group 1

35% HP bleaching gels (Pola Office®) were applied in four applications for 8 minutes each, for a total of 32 minutes of bleaching according to the manufacturers' instructions. After each bleaching session, the bleaching agents were removed with high power suction. When bleaching procedure ends, enamel surfaces were cleaned with water (30 seconds) and air-dried (10 seconds). For the rest of the day, the teeth were stored in artificial saliva at 37 °C. The procedure was continued for 1 week.

After the specimens had been removed from the artificial saliva, the enamel surfaces were rinsed (10 seconds) and dried (10 seconds) with a triple syringe. Then, buccal surfaces of teeth were etched with 37% phosphoric acid (Ormco®) for 15 seconds. The etching area was wider than bracket base about 1 mm for each margin. The solution was applied with a disposable applicator with continuous agitation, followed by rinsing for 30 seconds and drying for 10 seconds with oil-free compressed air.

Group 2

35% HP bleaching gels (Pola Office®) were applied in four applications for 8 minutes each, for a total of 32 minutes of bleaching according to the manufacturers' instructions. After each bleaching session, the bleaching agents were removed with high power suction. When bleaching procedure ended, enamel surfaces were cleaned with water (30 seconds) and air-dried (10 seconds). For the rest of the day, the teeth were stored in artificial saliva at 37 °C. The procedure was continued for 1 week.

After the specimens had been removed from the artificial saliva, the enamel surfaces were rinsed (10 seconds) and dried (10 seconds) with a triple syringe. Then, buccal surfaces of teeth were etched with ascorbic acid-containing citric acid formulation (50% ascorbic acid +50% citric acid) for 5 minutes.

The pH of the ascorbic acid-containing citric acid formulations was measured by pH meter (Mettler Toledo®). The etching area was wider than bracket base about 1 mm for each margin. The solution was applied as an irrigating solution for 5 minutes with a flow rate of 1 mL per minute under continuous agitation, followed by rinsing for 30 seconds and drying for 10 seconds with oil-free compressed air.

For the adhesive procedure, the upper premolar brackets with slot 0.018” x 0.025” (Omi arch® Roth type, TOMY) were used in this study. Transbond™ XT (3M Unitek) adhesive primer was applied on the etched surfaces. Prior to seating on tooth surface, Transbond™ PLUS (3M Unitek) adhesive was applied to the bracket base according to the manufacturer’s instructions.

The brackets were fixed on the tooth surface with 300 grams of force for 5s using a Dontrix gauge (Orthopli) to standardize the amount of force used for all samples and positioned on FA point of buccal surface. Excess composite would be removed with fine explorer. Ortho curing light (Mini LED SATELEC®, Acteon) would be used for lightening 10 seconds at each margin from 5 mm distance (Jain et al., 2013) and the same curing light would be used throughout the procedure. A radiometer (Kerr Corporation) would be used to ensure the light is curing constant output of 1800 - 2200 mW/cm^2.

Afterwards, in order to minimize misalignment of the testing apparatus, a guiding index was made so that the buccal surface of the tooth would be parallel to the applied force during the shear bond strength test.

The guiding index is made by attaching two brackets (Upper premolar brackets, Omi arch® Roth type, TOMY) to the opposite rims of a PVC pipe (21 mm in diameter and 5 mm in length) and inserting guiding wire (0.018” x 0.025” rectangular stainless-steel wire) into slots of the guiding brackets (Fig. 1A).

The prepared crown was ligated to the guiding wire at the center with an elastomeric o-ring. Then, the crown attached to the guiding index was placed on a PVC pipe (21 mm in diameter and 25 mm in length), containing self-cured acrylic (Fig. 1B). The palatal half of the tooth was embedded in self-cured acrylic at the established position. After the acrylic was set, the elastomeric o-ring was removed, followed by the guiding index.

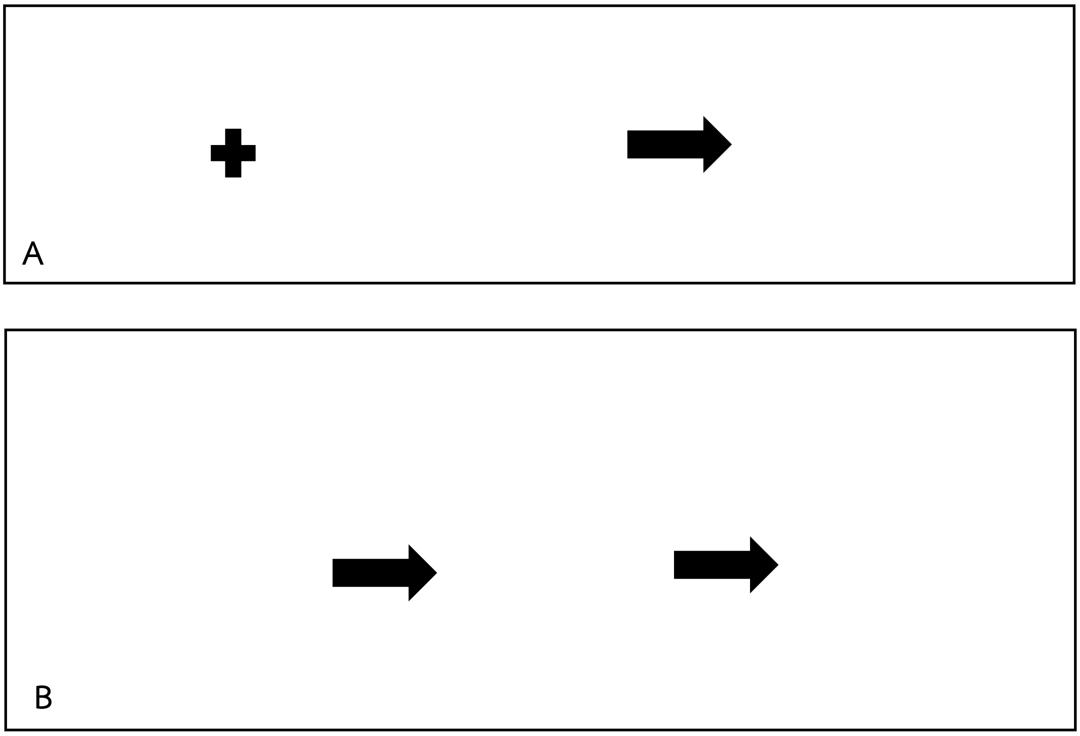

Figure 1 The illustration of guiding index **A**, the prepared crown was ligated to the guiding index with an elastomeric o-ring at the center of the guiding wire. **B**, the prepared crown attached to the guiding index as placed on a PVC pipe which contained self-cured acrylic. The palatal half of the tooth was embedded in self-cured acrylic at the established position.

The finished specimens were stored in artificial saliva at 37°C for 24 hours before bond strength testing.

Enamel shear bond strength test

A universal testing machine (EZ-S, SHIMADZU) was used for the shear bond strength test. The crosshead speed was set at 1 mm per minute until failure occurs. The direction of the blade was occluso-gingival to teeth and the shear force was applied parallel to the height of contour of the teeth. A blade was positioned as close as possible to the resin–enamel interface (Fig 2). The shear force was recorded in newton (N). The shear bond strength (MPa) was then calculated as the ratio of shear force to bracket base area (12.28 mm^2).

Statistical Analysis

Statistical analysis program (SPSS version 22, statistic software) was used for all the tests. The data were collected and shown as mean ± standard deviation (SD). Shear bond strength was analyzed by one-way analysis of variance (ANOVA). Since variance was not homogeneous, multiple comparisons of shear bond strength were evaluated by Dunnett's T3 Test. A *p*-value less than 0.05 was considered to be statistically significant.

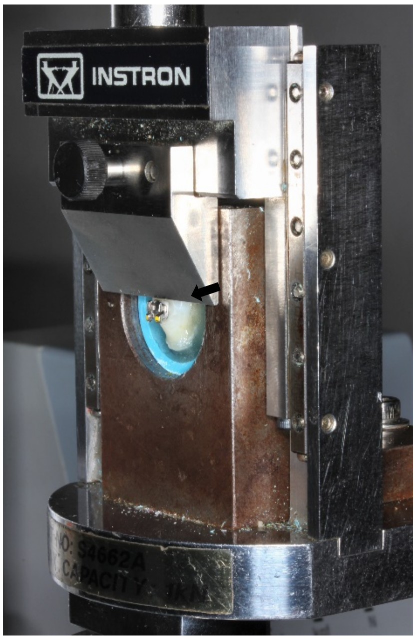

Figure 2 The universal testing machine (EZ-S, SHIMADZU) with a specimen in place. The edge of blade (arrow) was positioned as close as possible to the resin–enamel interface.

Results

The mean and standard deviation for each group were shown in Table 1. One-way ANOVA (Table 2) showed significant differences of shear bond strength among the tested groups (p<0.001).

A Dunnett's T3 test (Table 1) showed that shear bond strength of the brackets bonded immediately after bleaching enamel with 35% HP was significantly lower compared to that of the brackets bonded with the unbleached enamel (p<0.001).

For the bleaching groups, there was statistically significant difference with respect to the shear bond strength between ascorbic acid-citric acid formulation group and phosphoric acid group (p<0.02).

However, when the ascorbic acid-citric acid formulation group was compared with the control group, there was no statistically significant difference (p>0.05).

Accordingly, it showed that the etching formulation with 50% ascorbic acid combine with 50% citric acid was significantly effective in increasing the shear bond strength values of the bleached teeth that were statistically similar to those of the unbleached specimens.

Table 1 The mean, standard deviation of shear bond strength (MPa) for each treatment by group

Group	Mean	SD	Std error	95% Confidence interval		Min (MPa)	Max (MPa)
				Lower bound	*Upper bound*		
Control	27.16*a*	1.57	0.70	25.20	29.11	25.58	29.18
1	12.79*b*	2.64	1.18	9.52	16.07	10.22	16.48
2	20.95*a*	4.16	1.86	15.78	26.12	15.79	25.25

Note: Different letters indicate a significant difference using Dunnett's T3 Test for multiple comparisons ($p \leq 0.02$).

Table 2 One-Way ANOVA test for shear bond strength

	Sum of Squares	df	Mean Square	F-value	*P*-value
Between Groups	518.984	2	259.492	29.110	<0.001
Within Groups	106.971	12	8.914		
Total	625.955	14			

Discussion and Conclusions

There have been several studies regarding the interaction between bleaching agent and bond strength of composite resin to enamel. The recent reports found that peroxide in bleaching agents had negative impacts to physical and chemical properties in enamel and dentin. Their bond strength decreased as the compound not only restricted monomers from polymerization in hybrid layer but also inhibited resin tags' penetration and formation (Attin et al., 2004).

In order to prevent side effects of diminished bond strength after bleaching, several studies had used sodium ascorbate (SA) as an antioxidant to eliminate all residual oxygen generated by bleaching agents (Lai et al., 2001; Lai et al., 2002; Kaya et al., 2008; Briso et al., 2012). Ascorbic acid and its salts were common antioxidants that can reduce diverse oxidative compounds, especially free radicals (Rose, Bode, 1993).

From this study, the null hypothesis was rejected as there was a significant difference in shear bond strength among the tested groups. The data revealed that etching the bleached enamel with 50% ascorbic acid plus 50% citric acid before bonding was able to restore the lost shear bond strength of the metal brackets bonded by composite resin to the bleached enamel with 35% HP.

The results appeared to align with the recent studies (Torres et al., 2006; Briso et al., 2014) that antioxidant treatment effectively regained the bond strength of composite resin to bleached enamel. Furthermore, considering that both sodium ascorbate and etchant needed to be removed, the incorporation of sodium ascorbate in phosphoric acid was an interesting choice.

Nevertheless, due to high pH and short application time of phosphoric acid, it had many restrictions to combine with antioxidant. So, citrid acid was rather applied in this study.

Citric acid was a weak tricarboxylic acid that was naturally concentrated in citrus fruits (Best et al., 2019). In the fields of dentistry, the clinical efficiency of this solution had been reported previously (Newman et al., 1968; Pérez-Heredia et al., 2006).

However, from the review literature, citric acid was rarely used as the etchant for enamel surface preparation due to its long application time. However, after applying citric acid more than three minutes, the result of enamel bond strength to brackets was enough for clinically acceptable range (Reynolds, 1975; Lee et al., 1971).

However, using a concentration of ascorbic acid and citric acid at 50% and application time of 5 minutes was reasonable due to the findings from previous studies. It was showed that when hydrogen peroxide and carbamide peroxide with high concentrations were applied on bleached teeth, 10% sodium ascorbate was not sufficient to restore the original level of the unbleached group's bond strength despite substantial increase in the strength after its use (Kaya et al., 2008; Türkün, Kaya, 2004).

According to Coppla et al. (2019), 35% sodium ascorbate was adequate for all tooth whitening groups. In contrast, the results from 10% and 20% sodium ascorbate reported in previous studies (Torres et al., 2006; Briso et al., 2014) were satisfactory only in case of at-home bleaching.

Additionally, the results of the study by Freire et al. (2009) revealed the quick reaction of 35% hydrogen peroxide and sodium ascorbate. It was reported that 5-minute duration was already a sufficient period of time for manifesting its antioxidant potential. This was the minimum amount of time taken for sodium ascorbate's effects to be adequate.

Furthermore, from the study of H. Lee et al. (1971), the adhesive strength from etching enamel with 50% citric acid was more than 8 MPa, which met the minimum requirement of 5.9 to 7.8 MPa for clinical orthodontic treatment, in three-minute application time. Besides, the strength didn't decrease when the time was passed to five-minute. Consequently, the result of 50% citric acid for direct bonding in orthodontic dentistry might be satisfactory to create the optimal bond strength of brackets.

As a result of this study, the ascorbic acid and citric acid concentration of 50% and the application time of 5 minutes assisted in the enamel surface preparation for appropriate bonding and hence effectively improved the shear bond strength of brackets to bleached enamel. Due to the promising outcome, further studies might take less application time in order to reduce clinical chair time and thus increase patient satisfaction.

Conclusion

In conclusion, under the conditions of this *in vitro* study, the 50% ascorbic acid combining with 50% citric acid can increase adequate shear bond strength for etching in the orthodontic bracket bonding to the bleached teeth.

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