Original Article

# Effect of Vitamin C Solution on Microtensile Bond Strength and Fracture Resistance of Non-vital Bleached Tooth Restored with Resin Composite

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## Abstract

The purpose of this study was to evaluate the effect of vitamin C containing sodium ascorbate prepared solution on the dentin bond strength of a resin composite to a non-vital bleached tooth and on the fracture resistance of a restored non-vital bleached tooth. Sixty (30 pairs, left and right) extracted sound human maxillary premolar teeth were collected from 30 patients. All the teeth were endodontically treated and each pair was randomly assigned into microtensile bond strength and fracture resistance tests. The teeth of each test were divided into three groups which were 1) non-bleached tooth, 2) bleached with 35% hydrogen peroxide and immediately restored with resin composite, and 3) bleached with 35% hydrogen peroxide, followed by an application of 10% vitamin C prepared solution and immediately restored with a resin composite. Samples of the microtensile bond strength test were cut to obtain stick-shaped specimens and tested with a universal testing machine. Samples of the fracture resistance test were embedded in acrylic resin with a simulated periodontal ligament before being subjected to an axial compression test with the universal testing machine. Results showed that the bleached tooth followed by 10% vitamin C solution application group had the highest microtensile bond strength while the bleached group had the significant lowest bond strength. The non-bleached group showed the highest fracture strength, and the bleached group had the significantly least strength. There was no significant difference between the non-bleached group and vitamin C solution application group in both microtensile bond strength and fracture strength tests. The most failure mode for all groups was adhesive failure in the microtensile bond strength test and was favorable failure in the fracture resistance test. The microtensile bond strength was positively correlated to the fracture resistance. In conclusion, the use of 10 % vitamin C containing sodium ascorbate prepared solution could reverse the microtensile bond strength and fracture strength of a non–vital bleached tooth.

**Keywords:** Antioxidant agent, Bleaching, Fracture resistance, Microtensile bond strength, Sodium ascorbate, Vitamin C

**Received Date:** May 28, 2021 **Revised Date:** Jun 2, 2021 **Accepted Date:** Jun 17, 2021

**doi:** 10.14456/jdat.2022.8

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## Introduction

Non-vital bleaching is a well-known procedure to correct a discolored tooth after endodontic treatment, and that such a bleached tooth requires adhesive restoration as soon as possible. Several studies showed that restoring the bleached tooth immediately with a bonding system and resin composite earned lower bond strength[1,2] and fracture strength[3,4] compared to that of a non-bleached tooth. The reduction in the bond strength and fracture strength was affected by the remnant of bleaching agents[5-7] and tooth structure alteration.[8,9] So, delaying the bonding procedure for at least one to three weeks was recommended.[1,10] However, delaying the bonding procedure in a non–vital bleached tooth may increase the risk of tooth fracture due to the usual loses of its structure because of the carious lesion and existing restorations, and the loss of structural integrity during access opening and canal preparation.[11] Therefore, appropriate and prompt restoration is highly recommended after the completion of endodontic treatment. In a questionable prognostic tooth that needs further follow-up prior to having final restoration, intermediate adhesive restoration can strengthen the tooth better than a non–adhesive one.[12] Hence, resin composite is the material of choice to reduce the risk of fracture because it is able to adhere to the cavity wall allowing partial recovery of the strength lost during access opening.

To minimize the undesired effect of the remnant bleaching agents on the bonding quality and fracture strength of the tooth, the use of antioxidants to neutralize free radicals before the restoration was proposed. Many researchers reported that the use of antioxidant agents, sodium ascorbate, could reverse the bond strength of a resin composite restoration without delaying the bonding procedure.[2,6,13] The sodium ascorbate application in the non-vital bleached tooth before a resin composite restoration also significantly gave higher fracture resistance compared to an immediately restored non-vital bleached tooth.[3,4] Pure sodium ascorbate is not a daily commercial product and hardly used for dental purposes. However, it is a component of a vitamin C tablet which is an over-the-counter product.[14,15] Regarding the statements mentioned above, this study aimed to evaluate the effect of vitamin C containing sodium ascorbate prepared into a solution on the bond strength of a resin composite to a non–vital bleached tooth and the fracture resistance of a non–vital bleached tooth after restoring with a resin composite. In addition, the correlation between the bond strength and fracture resistance was also assessed if there was any significant differences between groups for both the bond strength and the fracture resistance tests. The research proposal was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2020-045).

## Materials and Methods

Sixty (30 pairs, left and right) extracted sound human maxillary premolar teeth with similar width and length, single root with two root canals, and mature apices were collected from 30 patients, aged between 16–40 years old, according to ISO technical specification 11405. The teeth were extracted from each patient due to orthodontic reasons. The teeth were free of fracture/ crack line or carious lesions. All teeth were cleaned and stored in 1% chloramine-T trihydrate solution for one week at room temperature (24-26°C). Then, they were washed with tap water and stored in distilled water at 4°C. Collected teeth were used within six months after extraction.

The access opening was performed in all sixty premolar teeth with a dimension of 3 mm mesial-distal width and 4 mm buccal-lingual width. Working length was determined by subtracting 1 mm from the root length using a K-file. Instrumentation was carried out using a Ni-Ti rotary instrument (ProTaper™ Next, Dentsply Sirona, USA) from X1 to X3 at 300 rpm, 2N/cm by pecking motion. The root canals were finally flushed with 3 ml of 17% EDTA (ethylenediaminetetraacetic acid) for one minute followed by 5 ml of 2.5% sodium hypochlorite. Root canal obturation was done by single cone technique using match-tapered cone X3 (ProTaperTM Next, Dentsply

Sirona, USA) with resin-based sealer (AHTM plus, Dentsply Sirona, USA). The gutta-percha was cut to the level of 2 mm below the cemento-enamel junction. The pulp chamber was cleaned, dried, and temporarily filled with a cotton pellet and zinc oxide temporary filling (Cavit G, 3M ESPE, St. Paul, MN, USA). The teeth were immersed in distilled water at 37°C. After 24 hours, tooth access was reopened and resin-modified glass ionomer cement (Vitrebond, 3M ESPE, St. Paul, MN, USA) was applied in the cavity using a dycal carrier to gain the final depth of 6 mm measuring from the tip of the buccal cusp. The radiographic image was taken to reassure the adaptability of the resin-modified glass ionomer cement. Both left and right maxillary premolars of each pair were randomized and assigned into microtensile bond strength test and fracture resistance test groups. Teeth in each test were divided into three subgroups which were 1) non-bleached, 2) bleached and immediately bonded and restored with resin composite and 3) bleached, followed by application of 10% vitamin C solution, and immediately bonded and restored with resin composite.

The samples in groups 2 and 3 were treated with 35% hydrogen peroxide bleaching gel (Opalescence ENDO, Ultradent Products, South Jordan, UT, USA). The bleaching agent was filled in the cavity leaving 2 mm of space for a cotton pellet and zinc oxide temporary filling (Cavit G, 3M ESPE, St. Paul, MN, USA). The samples were stored in an incubator at 37°C and 100% humidity for four days as recommended by the manufacturer. After four days, the bleaching agent was washed out with distilled water for 60 seconds and the cavity was dried with oil-free air using a triple syringe.

A ten percent concentration by weight/volume of vitamin C solution was freshly prepared for each tooth using two vitamin C tablets (Hicee Sweetlets 500 mg, Bangkok, Thailand) containing ascorbic acid and sodium ascorbate. The vitamin C tablets (1000 mg) were ground for five minutes into fine powder using a mortar and pestle and dissolved in 10 ml distilled water. The pulp chamber of samples in group 3 was applied with 10 ml of vitamin C containing sodium ascorbate prepared solution at the rate of 1 ml/min, using the syringe, for ten minutes. The solution was agitated with a micro brush during the application.[16] The solution was washed out with distilled water and dried using the triple syringe.

The prepared cavities in all teeth were etched with 37.5% phosphoric acid for 15 seconds, washed with a water spray for 15 seconds, and gently dried for three seconds with the triple syringe but did not desiccate. The primer (Optibond FL, Kerr, Orange, CA, USA) was applied for 15 seconds with a gentle agitating motion and air-dried to evaporate the solvent. The adhesive (Optibond FL, Kerr, Orange, CA, USA) was applied with a light brushing motion for 15 seconds and light-cured for 20 seconds (~1,000 mW/cm$^2$) with a light-emitting diode (LED) light-curing unit (Demi™ LED light-curing system, Kerr, Orange, CA, USA). Bulk-fill resin composite (Filtek™ One Bulk Fill Restorative capsule; 3M ESPE, St. Paul, MN, USA) was placed into the cavity. The first layer was a 4 mm increment and light cured for 40 seconds. A dental probe was used to measure the depth of the increment. The second layer was a 2 mm increment which was adapted to occlusal margin and light cured for 40 seconds. A radio graphic image was taken to reassure that there was no gap at the bonded interface. The teeth were stored in 37°C distilled water for 24 hours before the microtensile bond strength and fracture resistance tests.

For the microtensile bond strength test, the occlusal portion of the sample was horizontally cut at 2 mm from the buccal cusp tip to expose the dentin. The coronal part was vertically cut with 1 mm width in a mesial-distal direction to the cemento-enamel junction and horizontally cut with 1 mm thickness in a buccal-lingual direction to obtain stick-shaped specimens. Four specimens were obtained from two levels of the mid-dentin of each tooth. (Fig. 1)

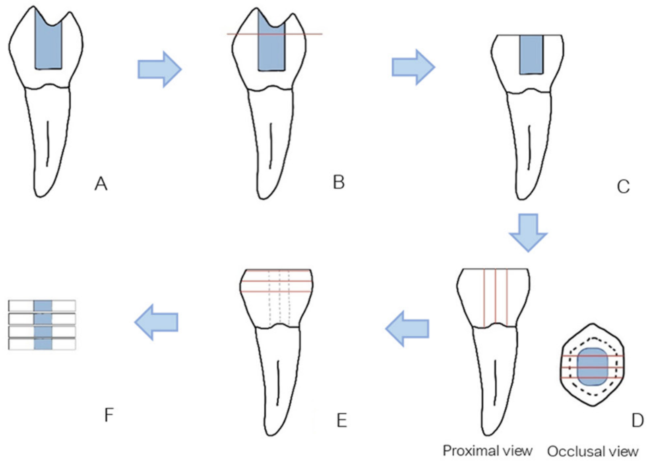


*Figure 1* *Diagrammatic presentation of specimens' preparation for microtensile bond strength test*
*A) The tooth was restored with resin composite*
*B) The occlusal portion was horizontally cut at 2 mm from the buccal cusp tip to expose the dentin*
*C) The tooth with a flat occlusal surface*
*D) The tooth was vertically cut with 1 mm width in mesial-distal direction to the cemento-enamel junction*
*E) The tooth was horizontally cut with 1 mm thickness in buccal-lingual direction at mid-dentin*
*F) Four specimens were obtained from two levels of mid-dentin of each tooth*

The size of the specimens was reassured with the range of 1.0 ± 0.1 mm in thickness by a digital caliper. The specimen was attached to the universal testing machine (EZ-S, Shimadzu, Japan) with cyanoacrylate adhesive and subjected to a tensile force at 500 N and crosshead speed at 1 mm/min until the specimen was fractured. The bond strength expressed in megapascal (MPa = 1 N/mm$^2$) was calculated using an equation as the least force breaking the bond tension (N) divided by the cross-sectional area (mm$^2$). The mean bond strength of four stick-shaped specimens derived from each tooth represented the microtensile bond strength of that tooth. All fractured specimens were examined by a stereomicroscope at x45 magnification (ML 9300®, MEIJI, Japan) to define the location of the bonded failure. The bonded failure mode was modified from Turkun and his colleague[17] which was classified as adhesive failure (>75% of failure along the dentin and resin composite interface), cohesive failure in dentin (>75% of failure within the dentin), cohesive failure in resin composite (>75% of failure within the resin composite), and mixed failure (combined at least two of the failure types from above).

For the fracture resistance test, the tooth-samples were subjected to periodontal ligament simulation preparation using a polyvinyl siloxane-based material (DMG Silagum Light, Hamburg, Germany).[18] The samples were embedded in self-cured acrylic resin at 2 mm underneath the cemento-enamel junction to simulate alveolar bone level. All specimens were subjected to an axial compression test in a universal testing machine (LR10K, LLOYD Instruments, England) using a 4-mm steel sphere head positioned at the middle of the occlusal surface in which the lingual incline of the buccal cusp and the buccal incline of the lingual cusp were touched. (Fig. 2)

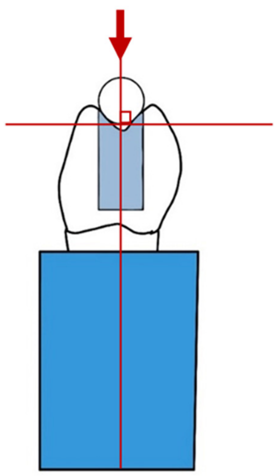

*Figure 2* *Diagrammatic presentation of the load application in the axial compression test*

The load was applied with a crosshead speed of 0.5 mm/min. A reduction of the force by 30 % of the maximum load indicating the presence of failure so the maximum force up to this point was recorded as the force at fracture.[19] The specimen was removed from the acrylic resin mold to assess the mode of fracture by visual inspection. The fracture above the simulated bone level was classified as a favorable failure while the fracture below the simulated bone level was classified as an unfavorable failure.[19]

The microtensile bond strength and fracture resistance tests were analyzed by one-way ANOVA with Tukey post-hoc analysis. The correlation between microtensile bond strength and fracture resistance test was determined by Pearson's correlation. The failure mode after the microtensile bond strength and fracture resistance test was analyzed using the Chi-square test. The significance was set at $p<0.05$.

## Results

The study revealed that group 3 (bleached and vitamin C solution application) had the highest microtensile bond strength (55.566 ± 3.514 MPa) followed by group 1 (non-bleached) (54.949 ± 7.541 MPa), and group 2 (bleached) (36.571± 2.609 MPa). (Table 1)

*Table 1 Mean microtensile bond strength, standard deviation, standard error, maximum, and minimum values*

| Group | n | Mean (MPa) | Standard deviation (MPa) | Standard error (MPa) | Minimum (MPa) | Maximum (MPa) |
|---|---|---|---|---|---|---|
| Non-bleached | 10 | 54.949[a] | 7.541 | 2.385 | 41.830 | 64.927 |
| Bleached | 10 | 36.571[b] | 2.609 | 0.825 | 32.792 | 39.747 |
| Bleached and Vitamin C | 10 | 55.566[a] | 3.514 | 1.111 | 51.282 | 62.361 |

**Same abbreviation letter means no statistically significant difference*

The microtensile bond strength of group 3 was not significantly different from group 1 ($p>0.05$). The most common failure mode was adhesive failure followed by mixed failure. (Fig. 3) There was no statistical difference in failure mode among the groups ($p>0.05$).

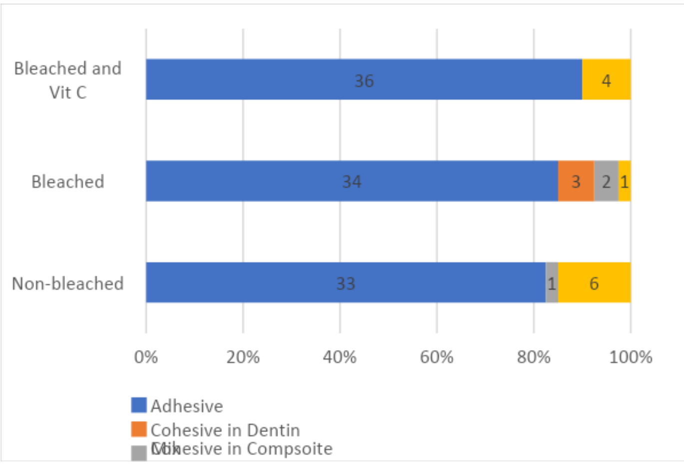


*Figure 3 The percentage of the failure mode in microtensile bond strength test*

Fracture resistance results demonstrated that group 1 (non-bleached) had the highest strength (1053.44 ± 183.65 N), followed by group 3 (bleached and vitamin C solution application) (972.39 ± 164.39 N) while group 2 (bleached) had the significant lowest strength (616.98 ± 97.07 N). (Table 2)

*Table 2* Mean fracture resistance, standard deviation, standard error, maximum, and minimum values

| Group | n | Mean (MPa) | Standard deviation (MPa) | Standard error (MPa) | Minimum (MPa) | Maximum (MPa) |
|---|---|---|---|---|---|---|
| Non-bleached | 10 | 1053.44[a] | 183.65 | 58.08 | 792.77 | 1326.00 |
| Bleached | 10 | 616.98[b] | 97.07 | 30.70 | 473.26 | 778.80 |
| Bleached and Vitamin C | 10 | 972.39[a] | 164.39 | 51.98 | 724.90 | 1175.40 |

**Same abbreviation letter means no statistically significant difference*

The fracture strength of group 1 was not significantly different from group 3 ($p>0.05$). The most common failure mode was favorable failure. (Fig. 4) There was no statistical difference of failure mode among the groups ($p>0.05$). All the fractures occurred at the lingual cusp where the restoration remained in the cavity. (Fig. 5)

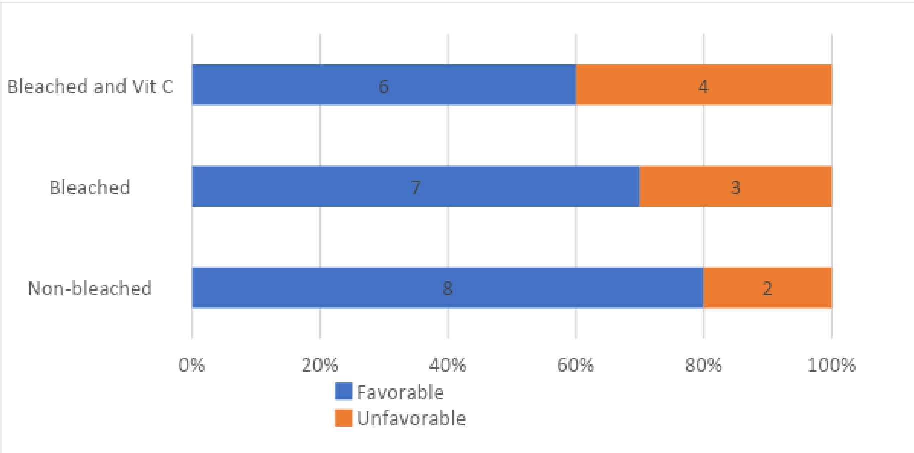


***Figure 4*** *The percentage of the failure mode in fracture resistance test*

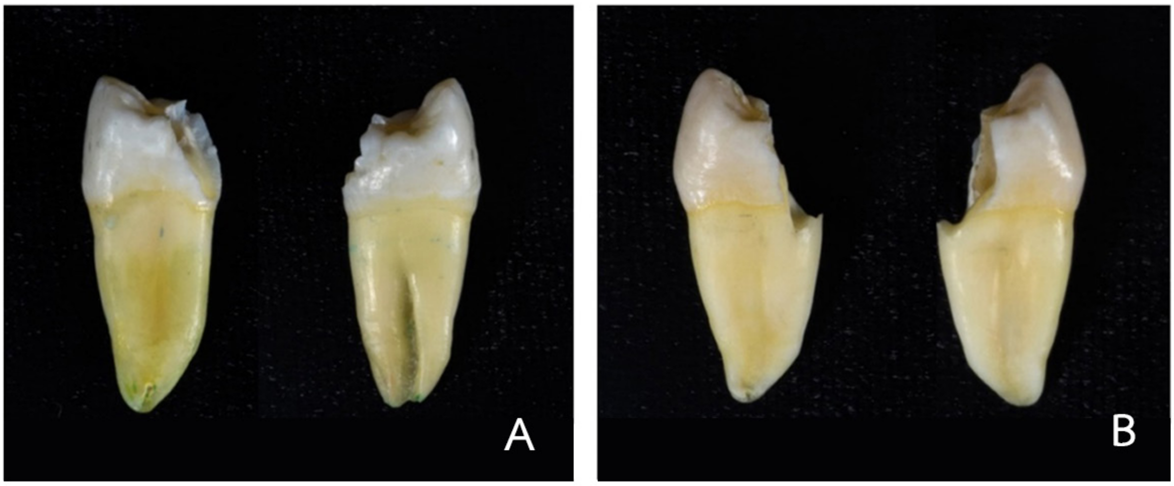


***Figure 5*** *Fracture pattern; A) Favorable failure, B) Unfavorable failure*

Pearson's correlation analysis of all specimens from all groups showed positively correlated between microtensile bond strength and the fracture strength (r=0.639, $p<0.001$).

## Discussion

In this study, the factors that could affect the microtensile bond strength and fracture strength were controlled. The width and length of selected premolar teeth were not statistically different between groups ($p>0.05$). All the access cavities were prepared with the same dimension and those of the root canal were prepared by the Ni-Ti rotary instrument from X1 to X3. To simulate the occlusal force distribution of the natural teeth, all specimens subjected to a fracture resistance test were simulated periodontal ligament with a 0.2-0.3 mm thickness which was approximately equal to the average thickness of the periodontal ligament. The polyvinyl siloxane-based material was used because its elastic modulus resembles a human periodontal ligament.[20]

In terms of the bond strength test, the microtensile bond strength test was used in this study because it provides a more uniform stress distribution due to its smaller specimens compared to either a tensile or a shear bond strength test.[21] All teeth were cut to obtain stick-shaped specimens with almost the same dimensions. Four specimens were obtained from two levels of the mid-dentin because the bonding interface at those levels was straight in which the applying force would be perpendicular to the bonded interface. (Fig. 1) The mean of the bond strength from all four specimens would represent the bond strength of the cavity. However, the size and density of the dentinal tubules are different in each dentin depth which affects the bonding efficiency. The outer dentin is less sensitive to the bonding quality than the inner dentin.[22] Therefore, the mean bond strength at superficial and deep dentin may be different from the presented bond strength in this study.

The results of this study revealed that immediately bonded with resin composite in a non-vital bleached tooth significantly decreased the microtensile bond strength to the dentin by approximately 30 % and decreased the fracture strength by approximately 40 % compared to a non-bleached tooth. The scanning electron microscope illustrated that the immediately bonded the bleached tooth with resin composite showed a fractured hybrid layer contained incomplete infiltrated collagen fibrils and most of the resin tag were dislodged from the dentinal tubules.[2,6] Titley and others showed the porosities in the resin tag of peroxide-treated etched enamel. The porosities could occur from the gas which was a by-product of the oxidation reaction and remained underneath the enamel surface.[7] Although the hydrogen peroxide could affect both the enamel and dentin bonding, the impact on dentin was more than on the enamel due to its higher organic compound.[23] These factors could be the cause of the lowest microtensile bond strength and fracture strength found in bleached groups. Even though the fracture resistance of bleached teeth conducted by Bonfante and colleagues found that internal bleaching with 37% carbamide peroxide did not weaken the dental tissue.[24] This different result to our study could be the different amount of active hydrogen peroxide. About 37% carbamide peroxide used in their study was decomposed into only 11.1% hydrogen peroxide which was lower than 35% hydrogen peroxide used in this study.

Nevertheless, the remaining hydrogen peroxide from the bleaching procedure in the tooth structure could leach out continuously. To shorten the delaying time before performing the final bonded restoration, an antioxidant such as sodium ascorbate had been introduced.[2,6,13] Since sodium ascorbate is a component of vitamin C tablets which is easily available from a drug store or convenient store, a prepared vitamin C solution was used in this study. The result of this study demonstrated that the use of 10% vitamin C containing sodium ascorbate prepared solution could reverse the microtensile bond strength to dentin and improve the fracture strength of the bleached tooth. No significant difference was found in both microtensile bond strength and fracture strength of non-bleached and bleached followed by the vitamin C solution application groups ($p>0.05$). The result was consistent with the study performed by Niyatiwatchanchai and Maneenut who evaluated the effect of vitamin C suspension on microtensile bond strength of bleached dentin to resin composite. In their study, the vitamin C suspension at 10% concentration was used after internal bleaching with 35% hydrogen peroxide followed by immediately restored with resin composite. They found that vitamin C suspension could recover the compromised bond strength of the bleached dentin.[25] Another *in vitro* study was conducted by Khoroushi and colleagues to assess the fracture resistance of endodontically treated teeth undergoing a combination of 38% hydrogen peroxide in–office bleaching and 9.5% hydrogen peroxide home bleaching. The 10% sodium ascorbate hydrogel was applied in the access cavity and on the buccal surfaces for 24 hours before a resin composite restoration. The results also showed that the use of sodium ascorbate significantly increased the fracture resistance.[4] This reverse effect of both bond and fracture strengths may be due to the antioxidant capacity of the vitamin C containing sodium ascorbate which is able to quench reactive free radicals in biological systems. The improvement of bond and fracture strength may be explained by the ability of this reducing agent, ascorbate, in donating two high–energy electrons to scavenge the free radicals resulting from the bleaching treatment and turns into dehydroascorbate.[26] Hence, the resin polymerization could proceed without premature termination which resulted in reversing the negative effect of the bleaching agent.

Many studies have used sodium ascorbate since it has a neutral pH. Several studies revealed that there was no significant differences among sodium ascorbate above 10% concentration, so it was expected that the concentration of sodium ascorbate at 10% was effective in reversing the strength and neutralizing the oxidation effect after the bleaching procedure.[27,28] Kimyai and Valizadeh also discovered that there was no significant difference between hydrogel and solution form of sodium ascorbate in reversing the bond strength.[29] Freire and colleagues demonstrated that there was no significant difference in the release amount of hydrogen peroxide between a 60-minute and a 10-minute application time.[30] Moreover, Bulut and colleagues suggested that the continuously refreshed and agitatedly applied antioxidant could enhance the neutralizing effect.[16] Therefore, the 10% vitamin C containing sodium ascorbate prepared solution was freshly prepared before agitatedly applied into the pulp chamber for ten minutes at the rate of 1 ml/min for this study.

The present study was designed to simulate the clinical situation so both the sodium hypochlorite and EDTA had been used to irrigate the root canal. The previous study showed that the bond strength of dental adhesives was compromised using sodium hypochlorite on the root and dentinal crown due to the presence of reactive residual free – radicals in the reagent which tended to compete with propagating vinyl free–radicals generating light activation of dental adhesive resulting in the premature chain termination of the propagation stage and incomplete polymerization.[6,31] Moreover, the EDTA also causes the demineralization of the dentin, decreases the dentin hardness, increases the dentin permeability, and removes the inorganic component of the smear layer.[32] Hence, sodium hypochlorite may negatively affect the dentin bond strength while the use of EDTA probably improves not only the adaptability of the root canal sealer to the root canal wall but also the penetration of the dental adhesive into the dentinal tubule which possibly enhances the bond strength and fracture strength.

Considering the failure mode, adhesive bond failure was a desirable mode to assure the real bond strength between two substrates. In this study, adhesive bond failures were predominantly observed in all microtensile bond strength test groups. There were only a few cohesive and mixed failures. This result was due to the small surface area at the bonding interface where the stress is uniformly distributed when the force was perpendicular to the interface dominantly.[21] In terms of the fracture resistance test, most of the specimens showed favorable failures and occurred at the lingual cusp. (Fig. 5) This can be explained by the size of the lingual cusp of the maxillary premolar that was smaller than the buccal cusp and more concave at the cemento-enamel junction.[33] The fracture located at the interface of the resin composite and the tooth structure in all the specimens which revealed that the fracture strength in this study was the effect of bonded restoration. However, the fracture pattern was not significantly different among the groups ($p$>0.05). Khoroushi and colleagues also found that the favorable fracture was higher in the non-bleached group and 10% sodium ascorbate group while the immediately bonded after bleaching group significantly showed the highest unfavorable fracture. All the specimens in their study were thermocycling for 500 cycles before fracture resistance evaluation so the study also supported our results in the way that the non-vital bleached tooth could not withstand the bite force in the long term if they were immediately bonded after bleaching.[4]

According to the results in this study, there was a relationship between the microtensile bond strength and fracture resistance of a non-vital bleached tooth restored with resin composite (r=0.639, $p$<0.001). This could be implied that the higher bond strength can strengthen the entire tooth structure so that they can withstand the higher bite force. We could infer from the results of this present study that 10% vitamin C containing sodium ascorbate prepared solution could be used as an alternative substrate instead of pure sodium ascorbate prepared solution to treat the non–vital bleached tooth before being immediately restored with resin composite. The alteration in the oxidation reaction of the oxidized substrate, vitamin C, allowed the chain transfer reaction in the propagation stage of the free radical polymerization to proceed without premature termination and reversed the bond strength and fracture strength affected by hydrogen peroxide. The appropriate and prompt restoration is highly recommended after the tooth undergoes endodontic treatment due to the loss of structural integrity during access opening and root canal preparation.

In respect to the clinical relevance, normally both vertical and lateral forces could affect the maxillary premolar. Jordão-Basso and colleagues demonstrated that the non-vital bleached tooth had the lowest fracture strength while the 10% sodium ascorbate treated tooth significantly increased the fracture strength when the force was applied with an angle of 135° relative to the long axis of the root.[3] However, their study was performed in bovine incisors and there are no studies in the current literature that evaluate the lateral force on the human bleached maxillary premolar tooth. Therefore, further study is required for more information.

## Conclusion

Within the limitations of this study, it could be concluded that non–vital tooth bleaching decreased the microtensile bond strength of the dentin to resin composite and the fracture resistance. However, the use of 10% vitamin C containing sodium ascorbate prepared solution immediately after bleaching could reverse both the bond strength of resin composite to dentin and the fracture resistance of a non–vital bleached tooth which was not significantly different from the non-bleached tooth. Also, the microtensile bond strength was positively correlated to the fracture resistance of a non–vital bleached tooth.

## Declaration and Acknowledgement

The authors do not have any financial interest in the companies whose materials are discussed in this article. The authors gratefully acknowledge the Dental Material Research and Development Center , Faculty of Dentistry, Chulalongkorn University for their hospitality.

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