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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ผลของสารละลายวิตามินซีต่อความแข็งแรงการยึดติดและความต้านทานต่อ
การแตกหักของฟันที่ผ่านการฟอกสีฟันแบบไม่มีชีวิตและบูรณะด้วยเรซินคอมโพสิต

น.ส.พิมพ์เดือน ศิวาวงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	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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พิมพ์เดือน ศิวาวงศ์ : ผลของสารละลายวิตามินซีต่อความแข็งแรงการยึดติดและความต้านทานต่อการแตกหักของฟันที่ผ่านการฟอกสีฟันแบบไม่มีชีวิตและบูรณะด้วยเรซินคอมโพสิต. (EFFECT OF VITAMIN C SOLUTION ON MICROTENSILE BOND STRENGTH AND FRACTURE RESISTANCE OF NON-VITAL BLEACHED TOOTH RESTORED WITH RESIN COMPOSITE) อ.ที่ปรึกษาหลัก : รศ. ทพ. ดร.ชัยวัฒน์ มณีนุษย์

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของการสารละลายวิตามินซีที่มีโซเดียมแอสคอร์แบทเป็นองค์ประกอบต่อความแข็งแรงยึดแบบดึงระดับจุลภาคและความต้านทานต่อการแตกหักของฟันที่ผ่านการฟอกสีฟันแบบไม่มีชีวิตและบูรณะด้วยเรซินคอมโพสิต ฟันกรามน้อยบน 60 ซี่ (30 คู่ ซ้ายและขวา) ที่ได้รับการถอนจากผู้ป่วย 30 รายถูกนำมารักษาราก จากนั้นฟันแต่ละคู่จะถูกสุ่มแบ่งไปเพื่อการทดสอบความแข็งแรงยึดแบบดึงระดับจุลภาคและความต้านทานต่อการแตกหักของฟัน ฟันในแต่ละการทดสอบจะถูกแบ่งออกเป็น 3 กลุ่ม ได้แก่ กลุ่มที่ 1 ฟันที่ไม่ผ่านการฟอกสีฟัน กลุ่มที่ 2 ฟันที่ผ่านการฟอกสีฟันด้วยไฮโดรเจนเปอร์ออกไซด์ความเข้มข้นร้อยละ 35 และทำการบูรณะด้วยเรซินคอมโพสิตโดยทันที กลุ่มที่ 3 ฟันที่ผ่านการฟอกสีฟันด้วยไฮโดรเจนเปอร์ออกไซด์ความเข้มข้นร้อยละ 35 ใช้สารละลายวิตามินซี และทำการบูรณะด้วยเรซินคอมโพสิตโดยทันที นำชิ้นตัวอย่างสำหรับการทดสอบความแข็งแรงยึดแบบดึงระดับจุลภาคมาตัดให้มีลักษณะเป็นแท่งขนาดพื้นที่หน้าตัดประมาณ 1x1 ตารางมิลลิเมตร และทดสอบด้วยเครื่องทดสอบอเนกประสงค์ ส่วนชิ้นงานสำหรับการทดสอบความต้านทานต่อการแตกหักถูกนำมาฝังในเรซินอะคริลิก และจำลองเอ็นยึดปริทันต์ ก่อนทดสอบด้วยเครื่องทดสอบอเนกประสงค์โดยใช้แรงกด ผลการศึกษาพบว่ากลุ่มที่ผ่านการฟอกสีฟันและใช้สารละลายวิตามินซีความเข้มข้นร้อยละ 10 ให้ค่าความแข็งแรงยึดแบบดึงระดับจุลภาคสูงที่สุด (55.566 ± 3.514 MPa) ในขณะที่กลุ่มที่ผ่านการฟอกสีฟันและทำการบูรณะด้วยเรซินคอมโพสิตโดยทันที ให้ค่าความแข็งแรงยึดแบบดึงระดับจุลภาคต่ำที่สุด (36.571± 2.609 MPa) นอกจากนี้กลุ่มที่ไม่ผ่านการฟอกสีฟันให้ค่าความต้านทานต่อการแตกหักสูงที่สุด (1053.44 ± 183.65 N) ในขณะที่กลุ่มที่ผ่านการฟอกสีฟันและทำการบูรณะด้วยเรซินคอมโพสิตโดยทันที ให้ค่าความต้านทานต่อการแตกหักต่ำที่สุด (616.98 ± 97.07 N) อย่างไรก็ตามค่าความแข็งแรงยึดแบบดึงระดับจุลภาค และค่าความต้านทานต่อการแตกหักของกลุ่มที่ไม่ผ่านการฟอกสีฟันและกลุ่มที่ผ่านการฟอกสีฟันและใช้สารละลายวิตามินซีความเข้มข้นร้อยละ 10 ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ยังพบว่า ความแข็งแรงยึดแบบดึงระดับจุลภาคและความต้านทานต่อการแตกหักนั้นมีความสัมพันธ์เชิงบวก กล่าวโดยสรุปคือ การใช้สารละลายวิตามินซีที่มีองค์ประกอบของโซเดียมแอสคอร์แบทความเข้มข้นร้อยละ 10 สามารถทำให้ค่าความแข็งแรงยึดแบบดึงระดับจุลภาค และค่าความต้านทานต่อการแตกหักของฟันที่ผ่านการฟอกสีฟันแบบไม่มีชีวิตสูงขึ้นเทียบเท่าฟันที่ไม่ผ่านการฟอกสีฟัน

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KEYWORD: Antioxidant agent, Bleaching, Fracture resistance, Microtensile bond strength, Vitamin C

Pimduean Sivavong : EFFECT OF VITAMIN C SOLUTION ON MICROTENSILE BOND STRENGTH AND FRACTURE RESISTANCE OF NON-VITAL BLEACHED TOOTH RESTORED WITH RESIN COMPOSITE. Advisor: Assoc. Prof. Chaiwat Maneenut, D.D.S., M.D.Sc., Ph.D.

The purpose of this study was to evaluate the effect of vitamin C containing sodium ascorbate prepared solution on dentin bond strength of resin composite to non-vital bleached tooth and on fracture resistance of restored non-vital bleached tooth. Sixty (30 pairs, left and right) extracted sound human maxillary premolar teeth were collected from 30 patients. All teeth were endodontically treated and each pair was randomized assigned into microtensile bond strength and fracture resistance tests. The teeth were divided into 3 groups for each test which were 1) non-bleach tooth, 2) bleached with 35% hydrogen peroxide and immediately restored with resin composite, and 3) bleached with 35% hydrogen peroxide, followed by application of 10% vitamin C prepared solution and immediately restored with resin composite. Samples of microtensile bond strength test were cut to obtain stick-shaped specimens and tested with a universal testing machine. Samples of fracture resistance test were embedded in acrylic resin with simulated periodontal ligament before subjecting to an axial compression test in the universal testing machine. Results showed that the bleached tooth followed by 10% vitamin C solution application group had the highest microtensile bond strength (55.566 ± 3.514 MPa) while the bleached group had the significant lowest bond strength (36.571± 2.609 MPa). The non-bleached group showed the highest fracture strength (1053.44 ± 183.65 N) and the bleached group had significant less strength (616.98 ± 97.07 N). 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 ($p>0.05$). The most failure mode in the microtensile bond strength test for all groups was adhesive failure and was favorable failure in the fracture resistance test. The microtensile bond strength was positively correlated to the fracture resistance ($r=0.639$, $p<0.001$). In conclusion, the use of 10% vitamin C containing sodium ascorbate prepared solution could increase the microtensile bond strength and fracture strength of non–vital bleached tooth comparable to non-bleached tooth.

Field of Study:	Operative Dentistry	Student's Signature .....
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Chapter I Introduction

Rationale and significance of the problem

Tooth can be discolored by either intrinsic or extrinsic causes or both. Pulpal hemorrhage and necrotic pulp are the most common intrinsic causes while the dietary sources such as coffee, tea, and wine are main extrinsic causes. Management options for discolored tooth depend on its causes and severity. Starting with minimally invasive procedures such as polishing, vital / non – vital tooth bleaching, microabrasion and macroabrasion is a concept that beneficially preserves the natural tooth structure. If these logical orders of treatment cannot eliminate or improve the discoloration, restoring the tooth with direct or indirect restoration might take into consideration. Currently, tooth bleaching is a popular treatment to correct the discolored tooth because of its conservative and less cost. (1, 2) Nevertheless, bleaching agents could affect both organic and inorganic components of tooth structure. (3-9)

In clinical situations, non–vital bleached tooth requires adhesive restoration as soon as possible. However, several studies showed that restoring the bleached tooth immediately with a bonding system and resin composite earned lower bond strength (10-13) and fracture strength (14, 15) compared to that of non-bleached tooth. The reduction of the bond strength and fracture strength was affected by the remnant of

bleaching agents (16-18) and tooth structure alteration. (5, 10, 13) Therefore, delaying the bonding restoration at least 1–3 weeks is recommended. (10, 16, 19-21)

Nevertheless, delay bonding restoration in non–vital bleached tooth may increase the risk of tooth fracture upon use due to the loss of structural integrity during access opening and canal preparation. Moreover, endodontically treated tooth usually loses its structure because of caries and existing restorations. (22) A significant reduction in strength occurs when the tooth lost the marginal ridge. (23) Therefore, appropriate and prompt restoration is highly recommended after the completion of endodontic treatment. (24) In a questionable prognostic tooth that needs further follow-up prior to having final restoration, proper intermediate adhesive restoration can strengthen the tooth better than non–adhesive one. (25) Resin composite is a material of choice to reduce the risk of tooth fracture and also provides a low number of unfavorable fractures because it is able to adhere to the cavity wall allowing partial recovery of the strength that lost during access opening and bleaching treatment. Approximately 87% of the strength was gained back which was higher than using glass ionomer material. (23) Several studies had revealed that the bond strength of resin composite to tooth structure was decreased after bleaching treatment (10-13), but there is still controversy about the fracture resistance of non – vital bleached tooth. (14, 15, 26, 27) However, most of the studies showed that the bleaching agent lower the fracture

resistance of the non-vital tooth. (14, 15, 26) Moreover, there are no studies in the current literature that conduct both bond strength and fracture resistance of non–vital bleached tooth to assess the relationship between these properties.

To minimize the undesired effect of the remnant bleaching agent on the bonding quality and fracture strength of the tooth, using of antioxidants to neutralize free radicals before restoring was purposed. Lai and colleagues discovered that the use of antioxidant agents such as sodium ascorbate which was a salt form of ascorbic acid could reverse the bond strength of resin composite restoration without delaying the bonding procedure. (17, 28) Khoroushi and colleagues also reported that sodium ascorbate hydrogel application in non-vital bleached tooth before resin composite restoring significantly gave higher fracture resistance compared to immediately restored non-vital bleached tooth. (15) Pure sodium ascorbate is not a daily commercial product and hardly found for dental use. However, the active antioxidant ingredient of sodium ascorbate is ascorbic acid which is a component of vitamin C tablet, an over-the-counter product. (29, 30) Regarding the statements mentioned above, this study aimed to evaluate the effect of vitamin C containing sodium ascorbate prepared solution on the bond strength of resin composite to non–vital bleached tooth and the fracture resistance of non–vital bleached tooth after restoring with resin composite. In addition, the correlation between the bond strength and fracture resistance was also assessed if

there was any significant difference between groups for both bond strength and fracture resistance tests.

Research question

Can vitamin C containing sodium ascorbate prepared solution increase the bond strength and fracture resistance of non–vital bleached tooth that immediately restored with resin composite?

Research objectives

1. To evaluate the effect of vitamin C containing sodium ascorbate prepared solution on the dentin bond strength of resin composite to non–vital bleached tooth
2. To evaluate the effect of vitamin C containing sodium ascorbate prepared solution on the fracture resistance of non–vital bleached tooth after restoring with resin composite
3. To evaluate the correlation between the dentin bond strength and the fracture resistance of non–vital tooth restored with resin composite

Statement of hypothesis

Null hypothesis

1. The bond strengths of resin composite to dentin of non–vital bleached tooth with and without vitamin C containing sodium ascorbate prepared solution application are not different.

2. The fracture resistances of non–vital bleached tooth restored with resin composite with and without vitamin C containing sodium ascorbate prepared solution application are not different.
3. The dentin bond strength of non-vital tooth restored with resin composite does not correlate with the fracture resistance.

Alternative hypothesis

1. The bond strengths of resin composite to dentin of non–vital bleached tooth with and without vitamin C containing sodium ascorbate prepared solution application are different.
2. The fracture resistances of non–vital bleached tooth restored with resin composite with and without vitamin C containing sodium ascorbate prepared solution application are different.
3. The dentin bond strength of non-vital tooth restored with resin composite correlates with the fracture resistance.

Scope of the study

Experimental Design: In vitro study

Study limitation

The clinical situation could not be completely simulated including oral environment and chewing force direction.

Basic assumptions

1. All procedures were performed under well–controlled condition.
2. All procedures were prepared and evaluated by a well-practiced operator.
3. All the materials and equipment used in the study were strictly followed the manufacturer's recommendation.
4. There were no financial or other conflicts of interest relevant to any subject of the study.

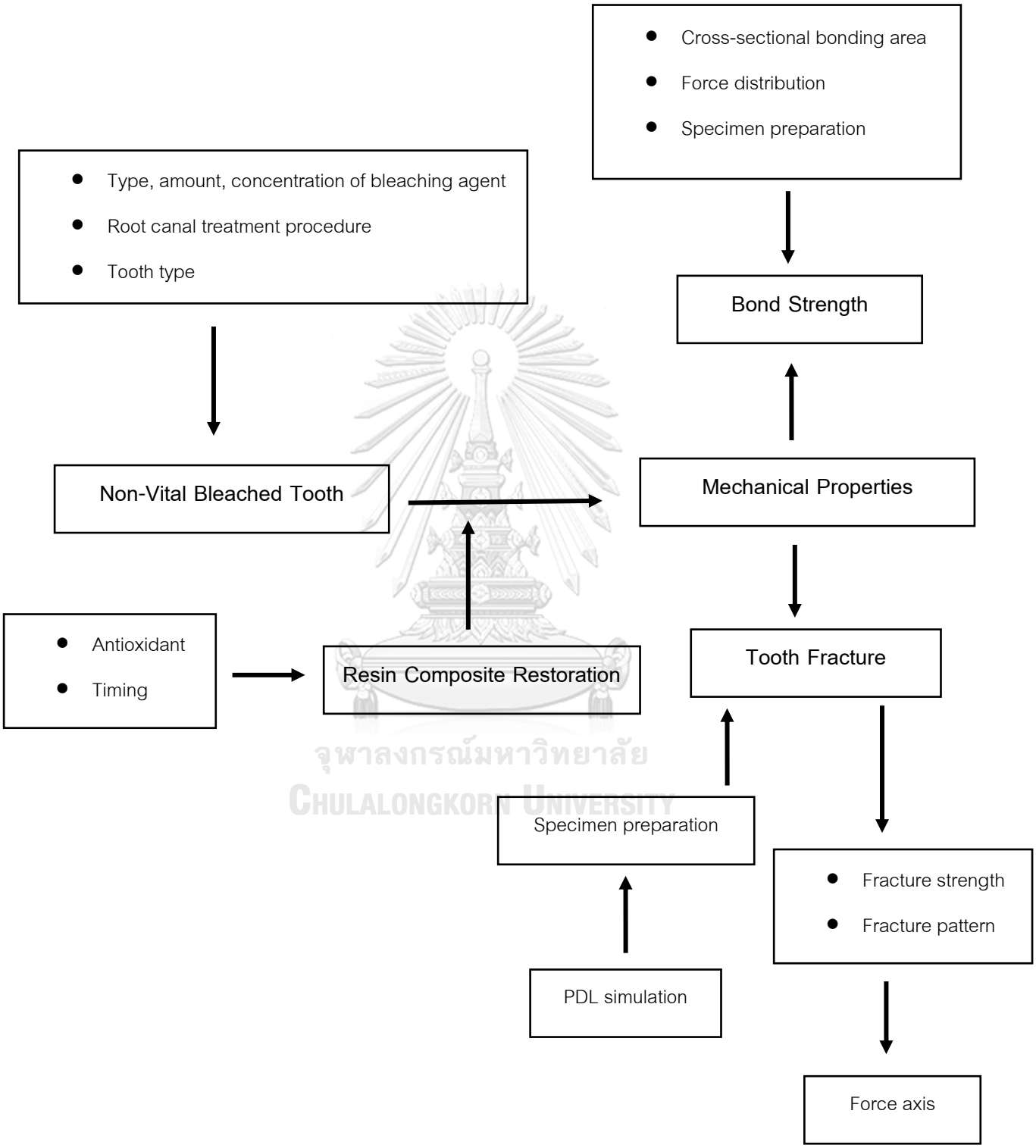
Key words

Antioxidant agent, Bleaching, Fracture resistance, Microtensile bond strength, Vitamin C

The expected benefits

The result of the study provides more information whether a vitamin C containing sodium ascorbate prepared solution can be used instead of pure sodium ascorbate as an antioxidant before the placement of resin composite restoration to the tooth immediately after bleaching procedure.

Conceptual framework



Chapter II Review of Literatures

Tooth color is varied depending primarily on dentin, but it is also influenced by the color, translucency, and degree of enamel calcification as well as enamel thickness. Therefore, the color of the tooth is determined by the enamel shade reinforced by the underneath dentin shade. The light absorption, reflection and transmission also affect the appearance of the tooth. Any changes in tooth structures during the development or post-eruption can alter the light transmission properties and hence discoloration. (31)

Tooth discoloration

Knowing the etiology of tooth discoloration is essential in order to give the appropriate treatment. The causes of tooth discoloration are classified as either extrinsic or intrinsic discoloration or both.

Extrinsic discoloration

The affinity of materials is an important role in stain deposition. Types of forces associated with stain deposition are long-range interactions; electrostatic and van der Waals forces and short-range interactions; hydration forces, hydrophobic interactions, dipole-dipole forces, and hydrogen bonds. These forces allow the chromogen and tooth structure getting closer, so the force is one of the factors in the determination of chromogen adhesion. (32) Extrinsic stain can be classified into two categories: direct and indirect staining.

Direct staining

Direct staining is caused by dietary source derivatives such as coffee, tea, smoking or tobacco chewing, red wine, and plaque accumulation on the tooth surface. The polyphenolic compounds in food are responsible for the tooth stain. The mechanism is not clarified but certainly involved with the pellicle's protein reacting with the chromogen which is able to penetrate the dentin easier than the enamel since the dentin is more porous both in the intertubular dentin and the tubules.

Indirect staining

Indirect staining is associated with the chemical interaction at the tooth surface with another compound producing the stain. Cationic antiseptic mouthwash such as chlorhexidine, hexetidine and cetylpyridinium chloride (CPC) can give rise to the stain after prolonged uses due to the cationic ion of the mouthwash interacting with anion ion of the compounds derived from dietary sources turning the tooth to brown or black discoloration. The green discoloration has also resulted from the copper salts in mouthwash or violet to black discoloration caused by permanganate containing mouthwash. Moreover, polyvalent metal salts could also alter the tooth color of iron foundry workers into black color. (31)

Generally, the management of extrinsic stain is simply by polishing with prophylactic pastes or a combination of abrasive and surface-active agents such as toothpaste.

Intrinsic discoloration

Intrinsic discoloration occurs following the alteration in the composition of the tooth structure. The chromogen can absorb into the tooth structure before or after tooth eruption. Intrinsic discoloration is classified into systemic and local causes.

Systemic causes are 1) drug-related (tetracycline, minocycline); 2) metabolic: alkaptonuria, congenital erythropoietic porphyria, hyperbilirubinemia; and 3) genetic: amelogenesis imperfecta, dentinogenesis imperfecta (32)

1. Drug-related

Systemic administration of the drug including tetracycline and minocycline could cause teeth staining. The chelation of the drug and calcium ions on the surface of hydroxyapatite crystals, mainly in dentin, resulted in the formation of tetracycline-calcium orthophosphate complexes. These complexes are affected in dentin because of the larger surface area of the dentin apatite crystals than the enamel. The severity of staining depends on the type and amount of the tetracycline, the period and duration of the drug administration, and the dosage. The staining ranges from light yellow to blue or brown. (1, 31)

2. Metabolic causes

- Alkaptonuria is an error of metabolism in infants. It causes brown discoloration of permanent dentition.

- Congenital erythropoietic porphyria is an autosomal metabolic disorder caused by the deposition of the porphyrin pigments in dentin and bone resulting in red/purple–brown staining.

- Hyperbilirubinemia is characterized by yellow–green discoloration caused by the accumulation of bile pigments in dentin.

3. Genetic causes

Amelogenesis imperfecta is a hereditary condition during enamel mineralization and matrix formation. The appearance can be shown with hypoplastic thin yellow to yellow-brown enamel. In the hypomineralization condition, the enamel is snow-capped. When the enamel is impaired in crystallites maturation, it is known as hypomaturation. On the other hand, dentinogenesis imperfecta is an inherited disorder of dentin. (31)

Local causes are 1) pulp necrosis; 2) intrapulpal hemorrhage; 3) pulp tissue remnants after endodontic therapy; 4) endodontic materials; 5) coronal filling materials; 6) tooth resorption; 7) aging; 8) caries; and 9) enamel hypoplasia. (32)

1. Pulp necrosis

Bacterial, mechanical, and chemical irritation could damage pulpal tissue by releasing noxious products that can penetrate the dentin leading to discoloration of surrounding tooth structure. The degree of tooth discoloration depends on the duration since pulpal necrosis has occurred. (32)

2. Intrapulpal hemorrhage

Hemorrhage in the pulp chamber could result from pulp extirpation or severe trauma. The ruptures of blood vessels allow the blood component flowing into the dentinal tubule causing tooth discoloration. The tooth might initially turn to pink followed by hemolysis of the red blood cells due to the hemolysis resulted in the release of heme which reacted with pulpal tissue to form iron. Iron will convert the bacterial by-products, hydrogen sulfide, to form iron sulfide which discolors the tooth into grey. These products will penetrate dentinal tubule deeper and finally discolor the entire tooth color. (1, 2, 32)

The discoloration of disinfected or trauma teeth is caused by hemoglobin and hematin accumulation. Without the infection, iron does not release from the protoporphyrin ring since there is no reaction from bacterial by–products. Therefore, the discoloration of the tooth becomes more severe over time in pulpal necrosis tooth caused by trauma. On the other hand, the discolored pinkish tooth initially disappears

within 2-3 months because of revascularization of the pulp tissue if the trauma does not cause pulpal necrosis. (32)

3. Pulp tissue remnants after endodontic therapy

Pulpal extirpation and failure to remove all pulpal remnants causing the pulp tissue gradually degenerate are the reasons for discolored teeth. This situation often occurs because of an inadequate access opening, resulting in pulpal tissue remnants especially at the pulpal horn. Hence, the complete removal of pulpal tissue is crucial. (2, 32)

4. Endodontic materials

The incomplete removal of root canal filling, sealer and medication containing tetracycline from the pulp chamber can cause tooth discoloration. The prolonged contact of the material allows the penetration into the dentinal tubules. Despite the inability to penetrate the enamel, the change in tooth color is still possible. (2, 32)

5. Coronal filling materials

Microleakage of resin composite restoration may cause the marginal discoloration and eventually discolor the tooth. Amalgam is also the main cause of dentin discoloration into dark grey due to the penetration of tin into the dentinal tubules.

Moreover, metal posts can release metallic ion into the dentinal tubules so that the dark–pigmented dentin can be observed after restoration removal. (2, 32) Besides, the use of eugenol containing medication can cause an orange / yellow stain. (31)

6. Tooth resorption

Internal or external root resorption occasionally appears as a pink appearance or pink spot initially at cemento-enamel junction. (2, 32)

7. Aging

Enamel is thinner and changes in texture with aging. This results in less light transmission leading to a darker appearance. The deposition of secondary and tertiary dentin also influences darker tooth color due to the narrower pulp space, meaning the increase in the tooth structure that affects the opacity of the tooth. Furthermore, the chemical component in tooth structure also changes over time. (2, 32)

8. Caries

The appearance of the initial carious lesion is a white spot because of demineralization increasing the porosity in tooth structure which affects the refractive index of the tooth. (2)

9. Enamel hypoplasia

The disturbance during tooth development resulting from trauma, infection or other environmental factors causes incompletely form of the tooth. The clinical appearance exhibits as pits, grooves or total to partial absence of the enamel. The opacity of the enamel is also related to the degree of mineralization which affects its translucency. (1, 2)

Non–vital bleaching

Discoloration of non-vital tooth resulted from pulpal necrosis, intrapulpal hemorrhage, remnant of dental pulp tissue, endodontic or restorative materials often perceives as an esthetic problem. Bleaching is considered as one of the conservative treatments to improve the appearance. Although the non–vital bleaching was introduced by Garretson in 1895 using chlorine as the bleaching agent, it was until 1951 that hydrogen peroxide was used to bleach the non-vital tooth. (33) In 1960, Nutting and Poe purposed a walking bleaching technique by using superoxol, hydrogen peroxide, and sealed it within the pulp chamber without any heat application. A year later, Spasser introduced the use of sodium perborate mixing with water decomposing into sodium metaborate and hydrogen peroxide. Until 1963, Nutting and Poe modified the non-vital bleaching method using the combination of hydrogen peroxide and sodium perborate expecting a synergistic effect. They believed that hydrogen peroxide gave an immediate

bleaching effect while sodium perborate would gradually provide a long term effect. (34) It was subsequently shown that there was no significant difference between sodium perborate mixing with water and a combination of sodium perborate and hydrogen peroxide at the end of the experiment period. (35-37)

Bleaching agent

Hydrogen peroxide

Hydrogen peroxide is a colorless liquid that is slightly more viscous than water. Its molecular weight is 34.01 g/mol. Due to its low molecular weight, hydrogen peroxide could penetrate the dentinal tubules and break double bond conjugation in organic and inorganic compounds. The concentration usually uses in dentistry is between 5% to 35% hydrogen peroxide. It acts as a strong oxidizing agent and gives rise to reactive oxygen molecules and hydrogen peroxide anions. Hydrogen peroxide is naturally produced, controlled and eliminated by the normal function of the human body. The products of hydrogen peroxide degradation are hydroxyl free radical, perhydroxyl free radical and superoxide anion. The equations of the reaction are shown below. (38)

$$H_2O_2 \longrightarrow 2HO\bullet$$

$$HO\bullet + H_2O_2 \longrightarrow H_2O + HO_2\bullet$$

$$HO_2\bullet \longleftrightarrow H^+ + O_2\bullet^-$$

Carbamide peroxide

Carbamide peroxide decomposes into hydrogen peroxide and urea. The concentration commonly uses in dentistry is between 10% to 35%. A 10% carbamide peroxide breaks down into 3% hydrogen peroxide and 7% urea. Hydrogen peroxide eventually degrades into oxygen and water while urea degrades into carbon dioxide and ammonia resulting in higher pH of the solution. (21) The urea also has proteolytic properties that may affect bleaching efficiency. (39)

Sodium perborate

Sodium perborate is a white, odorless, insoluble powder. It is stable in the dry environment but in the acidic condition, warm air, and water, it breaks down to form sodium metaborate, hydrogen peroxide, and oxygen. (40) There are various forms of sodium perborate including monohydrate, trihydrate, and tetrahydrate which differ in oxygen content and bleaching efficiency. Monohydrate form contains the highest percentage of oxygen, 16%, while tetrahydrate and trihydrate contain 11.8% and 10.4% oxygen, respectively. (41) The combination of sodium perborate and distilled water affects equally to 16.3% hydrogen peroxide. (42) The equations of the reaction are shown below. (40)

$2Na^{+}[(HO)_2B(-O-O-)_2B(OH)_2]^{2-} + 2H_2O$
Sodium peroxoborate

$2Na^{+} = 2[H_2BO_3]^{-} + 2H_2O_2$
Sodium hydrogenborate

H•, O•, OH•, HO_2• (radicals)

OH^{-}, OOH^{-} (ions)

The two most common bleaching agents in non–vital teeth are hydrogen peroxide and sodium perborate. However, the active gradient in the bleaching mechanism is the hydrogen peroxide and its derivatives. Many studies showed that 35% of hydrogen peroxide was more effective than sodium perborate in non-vital bleaching regarding the equal bleaching times. (43, 44) Therefore, hydrogen peroxide was selected as the intracoronal bleaching agent in this current study.

Bleaching mechanism

Color producing stains often comprise organic compounds containing double bonds in the tooth structure that can absorb the light resulting in discoloration. To lighten up the teeth color is to break the double bonds of chromophore molecules. Therefore, the theory of teeth bleaching is by oxidizing the chromophore into smaller and lightening molecules. The bleaching mechanism consists of 3 phases as follow. (45)

1. Movement of the whitening agent into the tooth structure

The dental hard tissues are highly permeable to fluids. The enamel is highly permeable in the interprismatic space, while the dentin is at dentinal tubules. (45) Therefore, both enamel and dentin act as a semipermeable membrane allowing hydrogen peroxide to penetrate according to Fick's second law of diffusion which explains that the surface area, diffusion coefficient, and concentration are proportional to the diffusion rate. On the contrary, it is inversely proportional to the diffusion distance. Moreover, the higher hydrogen peroxide concentration, prolonged application, increased temperature, light activation, and the larger size of dentinal tubules can enhance the diffusion rate of hydrogen peroxide. (46)

2. Interaction of the whitening agent with the stain molecules

The bleaching mechanism has been represented by the chromophore theory involving in the oxidation reaction of hydrogen peroxide and organic chromophores within the tooth structure. Reactive oxygen species convert the conjugated molecules into a simpler structure or alter the optical properties resulting in lightening color. The by-products of the reactions are both more polar and lower molecular weight than the original stain molecule. (45)

The decomposition rate of the bleaching agent and the type of reactive oxygen species depend on the temperature, the co-catalyst, the concentration, and the pH of

peroxide. Hydroxyl radical, hydroperoxyl radical, hydroperoxyl radical anion, superoxide radical anion and superoxide radical cation are the reactive oxygen species that are the products of hydrogen peroxide oxidation reaction. (38) However, it remains unable to detect chromophore in tooth structure and to locate the specific site of the chromophore and bleaching agent reaction.

3. Alteration of the tooth structure surface

Hydrogen peroxide can penetrate through the enamel and react with tooth structure causing the change in morphological and optical properties which consequently resulted in the alteration of the tooth appearance.

Effect of bleaching agents to the tooth structure

Both organic and inorganic components in tooth structure are altered after the bleaching process. (47)

Effect to the organic component

Kawamoto and Tsujimoto revealed that the higher the concentration of hydrogen peroxide, the more hydroxyl radical (· OH) was detected. They also suggested that the inorganic component was not influenced by hydrogen peroxide and hydroxyl radicals but the organic component in dentin was. Besides, the structure of polypeptide chains in dentin was changed due to the destruction of proline and alanine while glycine was not affected. (3) Bleaching agents also induced collagen degradation by activation of

both cathepsin B and matrix metalloproteinase (MMPs) enzymes so these proteolytic activities contributed to the reduction of dentin mechanical properties. (4)

Effect to the inorganic component

Several studies presented that the inorganic component of enamel and dentin was not influenced by bleaching agents. McCracken and Haywood indicated that there was only 1.06 μg/mm^2 of calcium loss when the teeth exposed to 10% carbamide peroxide. This amount of calcium loss was not significantly different from teeth immersed in cola beverages for 2.5 minutes. (48) The result was correlated with the study investigating the mineral loss of tooth after bleaching with 10% carbamide peroxide by Goo and his colleagues. (49) Moreover, Lee and others also revealed that the mineral loss of teeth exposed to 30% hydrogen peroxide was slightly less than the non-bleached teeth and was not different from the teeth that exposed to a soft drink or juice for a few minutes. Therefore, they concluded that the mineral loss caused by the bleaching procedure may not be harmful to the tooth structure. (50)

On the contrary, Rotstein and others found that calcium level was reduced while phosphate level was increased resulting in a reduction of calcium and phosphate ratio following treatment with hydrogen peroxide in both enamel and dentin. (5) Al-Salehi and others discovered that the higher the concentration of hydrogen peroxide, the more the amount of calcium and phosphorus ions released from enamel and dentin. (51) Hence,

the treatment time and concentration of hydrogen peroxide used were proportional to the surface alteration of tooth structure. (8) Although it was found that the mineral lost in enamel after bleaching with 10% carbamide peroxide without any fluoride or calcium composition for 14 days, there was no significant difference in the mineral loss in enamel bleached with 10% carbamide peroxide which contained fluoride or calcium group. Cavalli and others also revealed that the mineral lost at the surface up to 60 μm from the outer enamel layer but the treated enamel could be recovered at the depth of 80 to 200 μm. Therefore, the addition of fluoride and calcium in 10% carbamide peroxide could reduce enamel mineral loss. (52) Furthermore, fluoride application following bleaching treatment was also recommended because the fluoride enhances remineralization and inhibits the demineralization of dental hard tissue. (53, 54) However, highly concentrated fluoride product was suspected of blocking the surface of initial caries resulting in remineralization barrier. (55)

Considering surface morphology after bleaching treatment, many studies suggested that the enamel surface was not changed after the bleaching process. Joiner and others found that 6% hydrogen peroxide did not affect enamel and dentin microhardness. (56) Scherer and others also studied the surface of enamel and dentin after the bleaching procedure at 5, 15, and 30 days. Scanning electron microscope

(SEM) analyses showed no obvious morphologic difference between the control and bleached groups. (57)

Nevertheless, several studies showed that the tooth surface was affected by bleaching agents. Zalkind and others presented that the bleaching agents caused porosity on the enamel surface, roughening and etching like appearance on dentin surface, and extensive morphological changes in cementum. The reason that the cementum was more affected by bleaching agents might attribute to its high proportion of organic component. (58) Moreover, perikymata was more apparent following bleaching treatment. Pits in the groove and some prisms were also exposed. (59) However, Turkun and others indicated that the enamel surface was immediately changed after bleaching with 10% carbamide peroxide but it could revert to almost normal within 3 months. (60)

Effect of bleaching agents to resin composite restoration

Various studies revealed that the reduction of resin composite bond strength was associated with bleaching treatment if the restoration was performed immediately. The reduction of the bond strength was ranged from 30% to 50% compared with the non–bleached teeth. (10-13) The causes of this problem can be concluded as follow.

1. Influence of residual peroxide after bleaching treatment

Residual peroxide in a collagen matrix and dentinal tubule interrupted the resin penetration into the dentin. This residual peroxide would eventually break down into oxygen and water. (17)

Scanning electron microscope illustrated that resin tags were uniform, consistency, and non-fragmented in non–bleached tooth. (Figure1) (16)

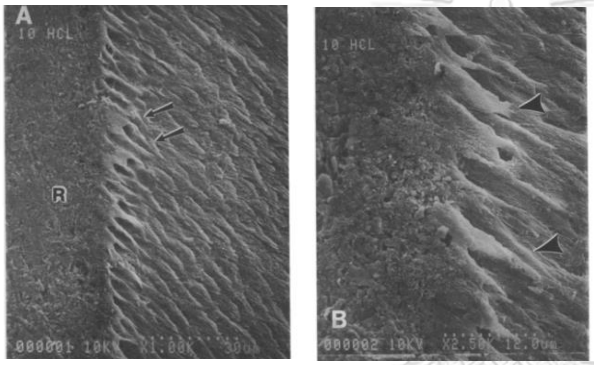


Figure 1 Scanning electron micrograph of resin tags in non-bleached enamel

Resin tags were uniform, consistency, and non-fragmented in non–bleached tooth.

Reference: Titley and others, 1991 (16)

In peroxide–treated etched enamel, the resin was covered the enamel surface, but the tags were irregular and sparse. (Figure 2) The porosities might be the result of the gas which was the oxidation reaction by-products and the remaining peroxide underneath the enamel surface. The quality of the resin tags and their depth of penetration consequently contribute to the bond strength of resin composite and tooth structure. (16)

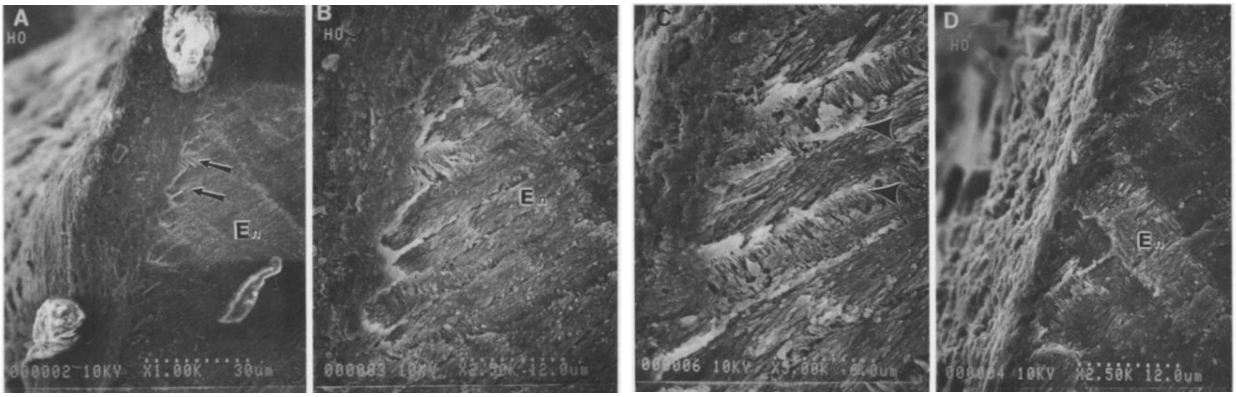


Figure 2 Scanning electron micrograph of resin tags in bleached enamel

a) The resin covered the enamel surface, but the tags were irregular and sparse.

b) Higher power scanning electron micrograph of area identified by arrows in A. Acid-treated enamel rods are prominent. Irregularity in tag frequency and consistency can be noted.

c) Resin tags (arrowheads) in another area of the specimen displayed granularity and porosity.

d) Resin-free enamel in another area of the specimen showed that the rods arranged in different directions.

Reference: Titley and others, 1991 (16)

The high concentration of oxygen remaining in enamel porosities after bleaching was the cause of incomplete resin polymerization and inhibited resin penetration into tooth structure. The exact depth of oxygen penetration was unknown, but it was greater than 5 to 10 μm; otherwise, acid etching could remove it. (18) Perdigao and others demonstrated that the enamel surface at the depth of 1 to 5 μm was not changed after bleaching with 10% carbamide peroxide. (61) The results were also similar to the study by Ruse and others. (62) Therefore, the reduction in bond strength was not associated with oxygen accumulation in enamel but in dentin. (63)

2. Oxygen reservoir in dentin

Dentin and dentinal fluid acted as an oxygen reservoir after bleaching. (63) The dissolved oxygen was eliminated by pulpal microcirculation and diffused through the external surface. (64) Hydrogen peroxide could penetrate the enamel and reach to the dental pulp because of its low molecular weight. The study showed that the bleaching agent could penetrate the pulp chamber of the restored tooth more than the sound tooth. (65) However, the remaining hydrogen peroxide in the tooth structure leached continuously. (66)

A dental adhesive is polymerized by a free radical polymerization mechanism which generates from light-activated redox initiators. Double bond conjugation of a carbon atom at both ends of the methacrylate molecule breaks down into a single bond and the polymer chain is created. When hydrogen peroxide breaks down, it releases oxygen causing a deleterious effect on free-radically cured products. It was reported that the steady-state concentration of oxygen allowing the polymerization was approximately 4.2×10^{-6} mol/L. Moreover, the rate of oxygen concentration about 5×10^{8} to 10^{9} L/mol/s could quench the initiating and propagating reaction. Consequently, the chain transfer reaction in the propagation stage could not be performed due to the high affinity of oxygen and the polymerization was not completed. (67)

Hydrogen peroxide reacted with enamel less than dentin since enamel contained fewer organic compounds, approximately 2%. The hydrogen peroxide diffused through the dentin and reacted within dentin which contained more organic compounds, approximately 38%. This resulted in the higher concentration of hydrogen peroxide accumulation at outer dentin comparing to the inner dentin because the organic compounds were presented more at the dentin-enamel junction area. (Figure 3) (68)

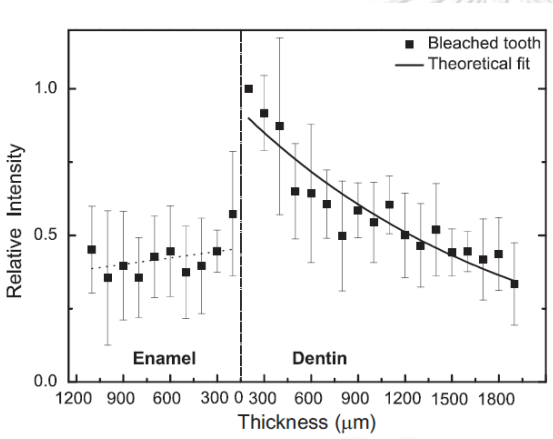


Figure 3 Micro-Raman Spectroscopy showed that hydrogen peroxide penetrate enamel and dentin The higher concentration was found at dentin-enamel junction.

Reference: Ubaldini and others, 2013 (68)

During the penetration of hydrogen peroxide through the interprismatic space of enamel, it did not attach itself to the enamel compounds. On the other hand, the accumulation of hydrogen peroxide occurred in the dentin. It was found approximately 63% hydrogen peroxide at 2 mm into dentin and 37% hydrogen peroxide at 3 mm into dentin. This brought to the conclusion that hydrogen peroxide could attach to the organic compounds in the dentin more than the enamel. Moreover, the size of dentinal tubules also affected the concentration of hydrogen peroxide accumulation since the

dentinal tubules near the dentin-enamel junction were smaller than the tubules near the dental pulp. This could be another reason supporting the assumption of a higher concentration of hydrogen peroxide at outer dentin. (68)

3. Alteration in organic substances

When the deproteinization of enamel occured following bleaching treatment, the superficial matrix of enamel was removed allowing the surrounding water to occupy the space left by the protein. (69)

Carbamide peroxide decomposed into urea which was a deproteinizing agent specific for enamel. (39, 70) Urea could penetrate up to 50 to 60 μm into enamel resulting in enamel porosities. (71) Although the enamel was more porous at the inter-prismatic area comparing to the intra-prismatic area (39), urea preferentially attacked the intra-prismatic area (71) and removed the enamel's protein in the intercrystallite spaces. (70) The morphology of the enamel after 5 M urea application was similar to artificially induced caries-like lesions. (39)

The reduction in bond strength was associated with the removal of nonfibrous organic content of tooth structure after the bleaching procedure. (72) Hydrogen peroxide had been suspected of causing protein denaturation in the organic compound of enamel and dentin, affecting the ratio of organic to inorganic compounds with an increasing inorganic component. (73)

4. Enamel surface alteration

There were more porous and precipitation on the enamel surface after immersing in hydrogen peroxide solution. Titley and others demonstrated that enamel immersed in 35% hydrogen peroxide for 30 minutes and etched with 37% phosphoric acid for 60 seconds showed more porosity and precipitation on the surface (Figure 4) while the enamel immersed in 35% hydrogen peroxide for 60 minutes and etched with 37% phosphoric acid for 60 seconds showed dense precipitation covering almost the entire surface. (Figure 5) (13)

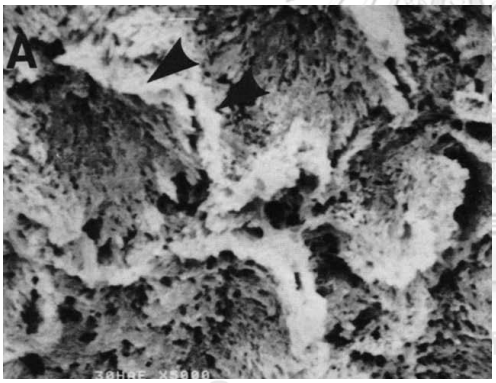


Figure 4 Enamel surface alteration after immersion in hydrogen peroxide for 30 minutes and etched with 37% phosphoric acid for 60 seconds. The enamel showed more porous and precipitation on the surface.

Reference: Titley and others, 1988 (13)

Figure 5 Enamel surface alteration after immersion in hydrogen peroxide for 60 minutes and etched with 37% phosphoric acid for 60 seconds. The dense precipitation covered almost the entire enamel surface.

Reference: Titley and others, 1988 (13)

It was not only the precipitation on the enamel surface but also the loss of mineral (10) and reduction of enamel and dentin microhardness that affected the bond strength of resin composite following bleaching treatment. (74)

Method to improve bond strength after bleaching treatment

Immediately restore the tooth with resin composite after bleaching treatment would reduce the bond strength of resin composite to tooth structure. Several studies showed that the bond strength of resin composite to bleached tooth was less than non–bleached tooth (10-13) due to the remnant of hydrogen peroxide (16-18) and alteration of tooth structure following bleaching process. (5, 10, 13) Therefore, many researchers have attempted to improve the bond strength of resin composite after bleaching treatment as follows.

1. Delay bonding procedure after bleaching treatment

The bond strength reduction was time-dependent. (18) Titley and others found that prolonged water storage of hydrogen peroxide treated enamel specimens before bonding procedure could reverse the deleterious effect of the bleaching process completely. (16)

Dishman and others demonstrated that bonding with enamel after 25% hydrogen peroxide bleaching for 24 hours and 1 week was not significantly different in the bond strength comparing to the non–bleached group. This data indicated that the

effect of the bleaching agent to the bond strength lasted less than 24 hours. (18) Elorza and others also revealed that the chemical composition of enamel was changed after bleaching with 38% hydrogen peroxide. There was approximately 9.25% calcium reduction and 9.43% oxygen weight increase compared with non–bleached enamel, and after 2 weeks oxygen weight decreased by only 2.5%. This indicated that 2 weeks were not enough time for releasing all the residual oxygen from the enamel surface. (75)

A large amount of hydrogen peroxide released immediately after dentin bleaching particularly in the first 24 hours, but it was declined dramatically toward zero after 48 hours. (Figure 6) However, the remaining amount of hydrogen peroxide was not different from the non-bleached dentin. It was concluded that hydrogen peroxide remnant in dentin was time-dependent. (76) The result of previous study was correlated with the studied by Camp and colleagues who found that the releasing rate of hydroxyl ions during the bleaching process increased in the first 24 hours and decreased after 48 hours passed. The lowest hydroxyl ions were at 120 hours after bleaching. (77)

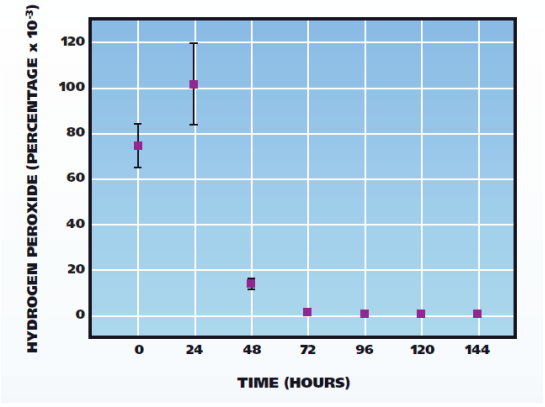


Figure 6 Graph of the amount of residual hydrogen peroxide in the dentin samples The hydrogen peroxide remnants after bleaching 144 hours (6 days).

Reference: Freire and others, 2011 (76)

Shinohara and others studied the bond strength of both enamel and dentin after sodium perborate and 37% carbamide peroxide application. They found that 2 weeks after enamel bleaching could reverse the bond strength to the level that was not different from the non–bleached enamel but it took only 1 week after dentin bleaching to reverse the bond strength. (19)

Unlu and others demonstrated that the bond strength at 24 hours after bleaching with 10% carbamide peroxide was not significantly different from non–bleached enamel while it took 1 week after bleaching with 35% hydrogen peroxide to reverse the bond strength. (10) Nevertheless, Cavalli and others concluded that the bond strength of resin composite returned to the condition of non–bleached enamel after delaying the procedure for 3 weeks. (21)

Table 1 The results of various studies on the delay bonding procedure after bleaching treatment.

Study	Bleaching type	Bleaching agent	Tooth structure	Delayed period*
Dishman and others, 1994 (18)	In-office	25% HP (10min x 2 cycles)	Enamel	24 hours
Elorza and others, 2013 (75)	In-office	38% HP (8min x 3 cycles)	Enamel	> 2 weeks
Freire and others, 2011 (76)	In-office	35% HP (15min x 3 cycles)	Dentin	72 hours
Unlu and others, 2008 (10)	In-office	35% HP (10min x 3 cycles)	Enamel	1 week
	home	10% CP (8 hours per day for 14 days)	Enamel	24 hours
Cavalli and others, 2001 (21)	home	10, 16, 20% CP (6 hours per day for 10 days)	Enamel	3 weeks
Shinohara and others, 2005 (19)	Internal	SP	Enamel	2 weeks
			Dentin	2 weeks
		37% CP	Enamel	1 week
			Dentin	1 week

Remark abbreviation: 1. HP = hydrogen peroxide, 2. CP = carbamide peroxide, 3. SP = sodium perborate

* The delay bonding period presented in the table was the period that gave the bond strength which was not significantly different from the non-bleached tooth.

2. Superficial layer removal

The assumptions that could affect the bond strength were the alteration of tooth composition and the formation of acid-etch-resistant layer after bleaching. (62) Therefore, the superficial layer removal of enamel approximately 0.5–1.0 mm before resin composite restoration would give the bond strength values similar to the non–bleached enamel. In contrast, this suggestion contradicts to minimal intervention concept. (78)

3. Alcohol/acetone based adhesive system application

Barghi and Godwin introduced the use of water displacement solution and dentin bonding agent comprising of acetone on enamel surface after bleaching to increase the bond strength of resin composite to enamel because of water chaser property of acetone so that the resin was able to penetrate the enamel after acid etching application. (61, 79) Acetone had been recommended as the best solvent for introducing resin into the tooth structure while water-based adhesive produced the space in the enamel hybridization area and the penetration of the resin could not completely impregnate the enamel crystallites. (61)

Alcohol application on bleached enamel surface decreased the surface water and higher enamel bond strength. (79) Kalili and others also introduced the use of the alcohol-based adhesive system to minimize the influence of bleaching procedure since

the alcohol could interact with residual oxygen and counteract residual water and oxygen from the bleaching agent. (80) However, Shinohara and others demonstrated that the use of water/alcohol and acetone–based adhesive systems could not reverse the bond strength after the bleaching procedure. (81) This conclusion was also similar to the study performed by Kimyai and Valizadeh. (82)

4. Antioxidant

Antioxidants are substances that react with free radicals generated by hydrogen peroxide and neutralizing them. Antioxidants are classified into two categories: enzymatic and non – enzymatic. (83)

1. Enzymatic antioxidants

Enzymatic antioxidants involve in eliminating reactive oxygen species including superoxidase dismutase, catalase, and glutathione peroxidase. (84) These enzymes catalyze a reaction of hydrogen peroxide molecules resulting in oxygen and water formation. (85, 86)

I. Superoxide dismutase (SOD)

Superoxide dismutase, a free radical scavenger enzyme, catalyzes the dismutation of superoxide resulting in the formation of hydrogen peroxide and oxygen. (83)

$$2O_2\bullet^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

II. Catalase

Catalase or hydrogen peroxide oxidoreductase is a tetramer of 4 polypeptide chains. Each chain is approximately 500 amino acids long. The polypeptide chain contains 4 porphyrin heme groups allowing the enzyme to react with hydrogen peroxide. One molecule of catalase can react with millions of hydrogen peroxide molecules, so it is considered to be one of the highest turnover rate enzymes. (87) The reaction of catalase and hydrogen peroxide generates the formation of two molecules of water and one molecule of oxygen. (88)

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

The reaction rate depends on hydrogen peroxide concentration (87) and consumes only a little energy. Catalase can accelerate hydrogen peroxide decomposition over the 100,000,000-fold because the reaction does not depend on the temperature. Therefore, catalase is essential in the metabolism process of the human body to decompose hydrogen peroxide into water and oxygen. (88)

Lai and others reported that the use of catalase on bleached enamel and dentin immediately after the bleaching procedure could

reduce waiting periods before performing resin composite restoration. (17, 28) Another study conducted by Torres and his colleagues demonstrated that the application of catalase on bleached enamel significantly gained higher bond strength compared to bleached enamel without catalase application. (89) Rotstein also recommended that 3 minutes of catalase application could eliminate the residual hydrogen peroxide. (88)

2. Non–enzymatic antioxidants

Non–enzymatic antioxidants include ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), and polyphenol compounds such as flavonoid which enriches in vegetables and fruits. (84)

Ascorbic acid

Ascorbic acid or vitamin C can exert its antioxidant activity both directly and also in concert with vitamin E. In dietary supplements, ascorbic acid is usually combined with ascorbate salts such as sodium ascorbate which is a more stable form. Ascorbic acid and ascorbate are both naturally present in the body but the forms interconvert according to the pH. Ascorbic acid has a pH approximately of 4 while the ascorbate salt, sodium ascorbate, has a pH of approximately 5.6 – 7.0 in an

aqueous solution. The reducing agents can convert the oxidized forms of the molecule such as dehydroascorbic acid to ascorbic acid. (90)

Sodium ascorbate is formed by dissolving the ascorbic acid into the water and reacts with sodium bicarbonate. The sodium ascorbate is precipitated by the addition of isopropanol after the cessation of the effervescence. A 1000 mg sodium ascorbate contains 889 mg of ascorbic acid and 111 mg of sodium. (83) Since the active ingredient of sodium ascorbate is predominantly ascorbic acid and is more stable in the ascorbate salt form, it has been widely used in many studies. (17, 82, 91, 92) The study by Lai and colleagues reported that the use of sodium ascorbate could reverse the bond strength of resin composite restoration without delaying the bonding procedure. (17, 28) The contributing factors for sodium ascorbate to reverse the bond strength of resin composite after bleaching treatment are as follows.

1) Sodium ascorbate concentration (82, 93)

Turkun and others demonstrated that less than 10% concentration of sodium ascorbate had no effect on neutralization ability. (91) Several studies also showed that there was no significantly different among sodium ascorbate at a concentration above 10%. (92, 94, 95)

Hence, it was expected that 10% of sodium ascorbate might be effective in reversing the bond strength and neutralizing the oxidation effects on tooth structure after bleaching treatment. (96)

Briso and others claimed that sodium ascorbate was more effective as an antioxidant after bleaching with carbamide peroxide since it generated a lower amount of oxygen compared with hydrogen peroxide. (97) 10% carbamide peroxide decomposed into only 3% hydrogen peroxide which was 10 times lower than 35% hydrogen peroxide so this probably influenced the different amounts of sodium ascorbate required to neutralize the effect. (21)

The residual amount of oxygen after bleaching also correlated to the resin tag formation resulting in the reduction of resin composite bond strength. (98, 99) Moreover, it was suggested that the continuously refreshed sodium ascorbate and agitatedly applied on bleached enamel surface could enhance the antioxidant effect. (100)

2) Sodium ascorbate form (82, 93)

A hydrogel is a network of insoluble polymer chains. It was speculated that hydrogel form should have been applied longer because of its low penetration capability comparing to the solution. One

disadvantage of sodium ascorbate in solution form is its higher flowability so it is more difficult to control the solution and it is needed to apply the solution several times before the bonding procedure. (93, 94)

However, Kimyai and Valizadeh proved that the form of sodium ascorbate did not affect the efficacy of the antioxidant effect. They found that both the solution and hydrogel form could reverse the bond strength of resin composite after bleaching treatment. Furthermore, the concentration at 10% might be as effective as 20% concentration of sodium ascorbate hydrogel in neutralizing the oxidizing effect. Thus, they recommended placing the sodium ascorbate hydrogel in the bleaching tray for 3 hours prior to the bonding procedure. (94)

3) Duration of sodium ascorbate application (82, 93)

Freire and others showed that the amount of sodium ascorbate used strongly correlated with the reaction and the amount of hydrogen peroxide reduced by sodium ascorbate. Additionally, the longer application of sodium ascorbate on the bleached tooth did not result in a higher neutralization of the oxidizing effect. Therefore, they suggested applying 25% sodium ascorbate for 5 minutes when bleaching with 35%

hydrogen peroxide because the reaction of sodium ascorbate and hydrogen peroxide was initially rapid. (101)

The application of 35% sodium ascorbate immediately after bleaching treatment showed an immediate reduction in residual hydrogen peroxide. There was no significant difference in hydrogen peroxide release between 60 minutes and 10 minutes application. The result also represented that the residual hydrogen peroxide was unable to detect if sodium ascorbate was applied on the dentin more than once, irrespective of the duration of application. This study indicated that the number of antioxidant applications was more crucial than the contact time. (76) On the contrary, Lai and others suggested applying sodium ascorbate on the bleached tooth at least 3 hours or one-third of the bleaching time to reverse the bond strength. (28) A possible explanation for the difference in the results might be the longer the sodium ascorbate in contact with dentin, the longer reaction time between the oxidizing and antioxidant agents. However, the highest rate of action was within the first minute, after which the reaction decreased substantially. (76)

Moreover, the dentinal characteristics such as chemical composition, thickness, depth, and sclerosis of dentinal tubules might

influence the bond strength value so that the adhesive systems should be considered. (102) Khoroushi and Aghelinejad studied on bleached enamel with 20% carbamide peroxide for 6 hours per day for 5 consecutive days and then restored with resin composite immediately, 1 week after the bleaching treatment or after 10% sodium ascorbate application. They discovered that 3 step-etch and rinse system gave the highest bond strength in every experimental group compared to 2 step-etch and rinse and 1 step-self etch adhesive system. (103)

Lai and others demonstrated that the bond strength of bleached dentin was significantly decreased but applying sodium ascorbate immediately after bleaching treatment increased the bond strength to the value that was not significantly different from the non-bleached teeth. According to the scanning electron microscope, hydrogen peroxide bleached dentin showed fractured hybrid layer contained incompletely infiltrated collagen fibrils and most of the resin tags were dislodged from the dentinal tubules. (Figure 7) On the other hand, hydrogen peroxide bleached dentin followed by sodium ascorbate application prior to bonding procedure showed partially dislodged of hybrid layer indicating the retention of fractured resin tags. (Figure 8) (17)

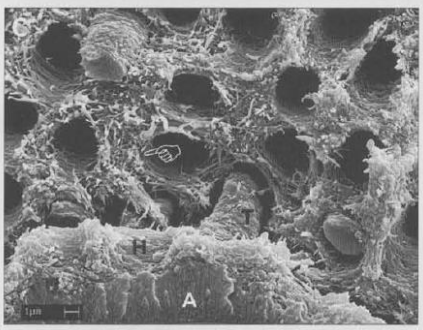


Figure 7 Scanning electron micrograph of bleached dentin prior to resin composite restoration

Reference: Lai and others, 2001 (17)

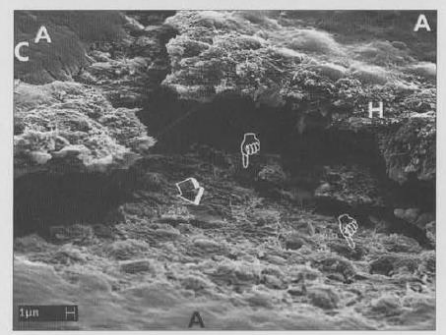


Figure 8 Scanning electron micrograph of bleached dentin after sodium ascorbate application and resin composite restoration

Reference: Lai and others, 2001 (17)

Sodium ascorbate can neutralize and reverse the oxidizing effect of hydrogen peroxide because it allows the propagation stage in the polymerization process to proceed without premature termination. (17) Rotstein reported that complete elimination of the residual hydrogen peroxide could not be performed by simply rinsing with water but it required many rinses for several hours. Therefore, water rinses immediately after bleaching might not be effective in rapidly removed residual hydrogen peroxide. The hydrogen peroxide remnant gradually reduced due to its dilution and natural decomposition. Three cycles of 5-minute water rinse and 1-hour teeth immersion in water after bleaching with 30% hydrogen peroxide significantly reduced the residual hydrogen peroxide. (88)

According to a case report with one year follow up,10% sodium ascorbate was applied inside a home bleaching tray for 1 hour after bleaching treatment. After that, the

direct resin composite restoration was performed on the lateral incisor. A one–year follow up showed no pulpal and periodontal disease and the restoration remained in good condition. (104)

Furthermore, alpha-tocopherol or vitamin E is the most powerful antioxidant in the lipid phase of the human body. It can protect the body against free radical reactions so some authors believed that the antioxidant properties of alpha-tocopherol should be more effective than sodium ascorbate. Sasaki and others revealed that a 10% alpha-tocopherol solution enhanced the bond strength of bleached enamel. (86) Considering the composition of the alpha-tocopherol solution, there was the presence of alcohol which might be one of the contributing factors that increased the bond strength of bleached enamel.

Moreover, other antioxidants are extracting from fruits and vegetables such as grape seed, guava seed, green tea, aloe vera, and sweet potato extract. However, the results of these studies are still controversial.

Effect of bleaching to the fracture strength of endodontically treated tooth

Bleaching agents seems to influence the fracture strength of endodontically treated tooth. The fracture resistance of sodium perborate and superoxol bleached teeth was 45% lower compared to intact teeth and 20% lower compared to non-bleached teeth with coronal opening. (14) Pobbe and others found that the fracture resistance of

endodontically treated tooth submitted to bleaching with 38% hydrogen peroxide more than two sessions was reduced. (26) Khoroushi and others discovered that combination bleaching with 38% and 9.5% hydrogen peroxide gel as in-office and at-home bleaching method before restoring with resin composite in endodontically treated tooth decreased the fracture resistance when compared with the non-bleached group. (15) The reduction in fracture resistance could be attributed to the action of hydrogen peroxide on dentin (3) and the duration that the teeth were exposed to the bleaching agent.

Although Bonfante and others revealed that internal bleaching with 37% carbamide peroxide did not significantly weaken the tooth, the group of bleached teeth and the pulp chamber seal with zinc oxide eugenol cement showed lower fracture strength than the non-bleached group and the pulp chamber seal with zinc oxide eugenol cement. They discussed that the reduction in dentin microhardness after bleaching procedure might be beneficial because it increased the coefficient of elasticity which made the tooth less friable and more resistant to fracture. Moreover, bleached teeth restored with resin composite at 14 days after bleaching showed higher fracture resistance than bleached teeth with a temporary restoration. The fracture mode was also analyzed in this study, it demonstrated that the most favorable failure pattern was found in bleached teeth that restored with resin composite and bleached teeth

restored with the prefabricated post while leaving the bleached teeth with non–adhesive temporary restoration could cause fracture of the entire crown. (27)

As described above that antioxidant agent has been introduced to eliminate reactive oxygen species resulted from bleaching procedure. Khoroushi and others also studied the effect of sodium ascorbate after bleaching with 9.5% hydrogen peroxide home bleaching for 2 hours every day for 3 weeks and 3 sessions of in-office bleaching with 38% hydrogen peroxide every week for 3 weeks on the fracture resistance of endodontically treated tooth. They discovered that a bleached tooth that used 10% sodium ascorbate hydrogel for 24 hours before performing resin composite restoration demonstrated higher fracture resistance than immediately restored bleached tooth. The sodium ascorbate group also showed higher favorable fracture compared to the 1-week delayed bonding group.(15)

Bond strength test

One of the most well-known analyses to evaluate the properties of the material is bond strength testing. The measurement of bond strength is influenced by the concentration of defects within or between materials, specimen size and geometry, materials properties, and loading application method. The two most common bond strength tests are shear bond strength and tensile bond strength test. Shear bond strength is one of the most prevalent bond tests. The specimen preparation of the shear

bond strength test is simple, but the drawback of this method is that the stress distribution is non-uniform in the substrate. Mostly, the failure does not initiate at the weakest point of the specimens and usually occurs in the material or tooth specimen. The measured bond strength found to be underestimated the actual stress that the specimen resisted at fracture. On the contrary, the loading application in the tensile strength test is perpendicular to the adhesive bond interface and the stress distribution is more uniform in the cross-sectional bonded area. A smaller the test specimens, a larger amount of bond strength due to a lower chance of the critical-sized defect presence because the bond strength value at failure depends on both fracture strength and the presence of defects. (105, 106)

Sano and others demonstrated that tensile bond strength depended on the bonded surface area. They discovered that the smaller surface area provided higher tensile bond strength. The larger specimens offered more probability of more defect compared to the smaller specimens. The failure of the tensile strength usually initiated at the circumference of specimens, coincident with the position of stress concentration and the failure propagate in the direction toward the center. Therefore, the larger surface area probably gives lower tensile bond strength than the actual bond strength because of the non–uniform stress distribution especially at the edge of the interface dominantly resulted in adhesive failure. According to this study, Sano and others suggested using a

small surface area approximately 1.6 – 1.8 mm^2 for microtensile bond strength test. (107)

The advantages of microtensile bond strength testing are as follows. First, it provides minimum variable results and produces adhesive failure in most of the specimens. Second, higher interfacial bond strengths can be measured. Third, the means and variances can be calculated for single teeth due to the small area of each specimen. Because of the small size of specimens, it allows measuring regional bond strength. It is also able to measure the bond strength on irregular surfaces. Finally, the mode of failure can be evaluated by a scanning electron microscope after the failure. However, the limitations of microtensile bond strength testing are labor-intensive, technically demanding, and difficult in measuring the bond strengths of less than 5 MPa. The technique also requires special equipment. Moreover, the specimens dehydrate rapidly since they are very small. (106) Therefore, the specimens should be prepared carefully to minimize these defects.

Fracture resistance test

Fracture resistance was tested by universal testing machine using compressive force until initiating the crack in the materials. The maximum force (F_{max}) initiating the failure was recorded as fracture resistance. A crosshead speed also influences the fracture resistance. The crosshead speed is generally used at 0.5 – 1.5 mm/min.(108-

110) The lower the crosshead speed, the more plastic deformation of the materials, means an increase in strength values. (111)

The previous study demonstrated that acrylic resin could not simulate the periodontal ligament, so the tooth fracture pattern was not similar to the clinical situation and the fracture resistance value was higher than the one with periodontal ligament simulation because acrylic resin acted like a ferrule effect. Therefore, it was recommended to simulate the periodontal ligament by using polyvinyl siloxane- or polyether-based material. (112, 113) The elastic modulus of human periodontal ligament approximately ranges from 0.12 to 0.96 MPa depending on loading magnitude (114) while the elastic modulus of polyvinyl siloxane- and polyether-based material ranges from 1-2 MPa and 4-5 MPa, respectively. (115)

Chapter III Materials and Methods

Experimental design

Laboratory research approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University Study Code: HREC-DCU 2020-045

Sample description

Sample size for microtensile bond strength test was calculated by G*Power 3.1 (F-test family for one-way ANOVA) with effect size = 0.88 (8.5), power β = 80% and α = 5% according to the study of Lai and colleagues. (17) Total sample size was at least 18 which were 6 samples for each group. Therefore, this study designed to totally use 30 samples (10 samples for each group).

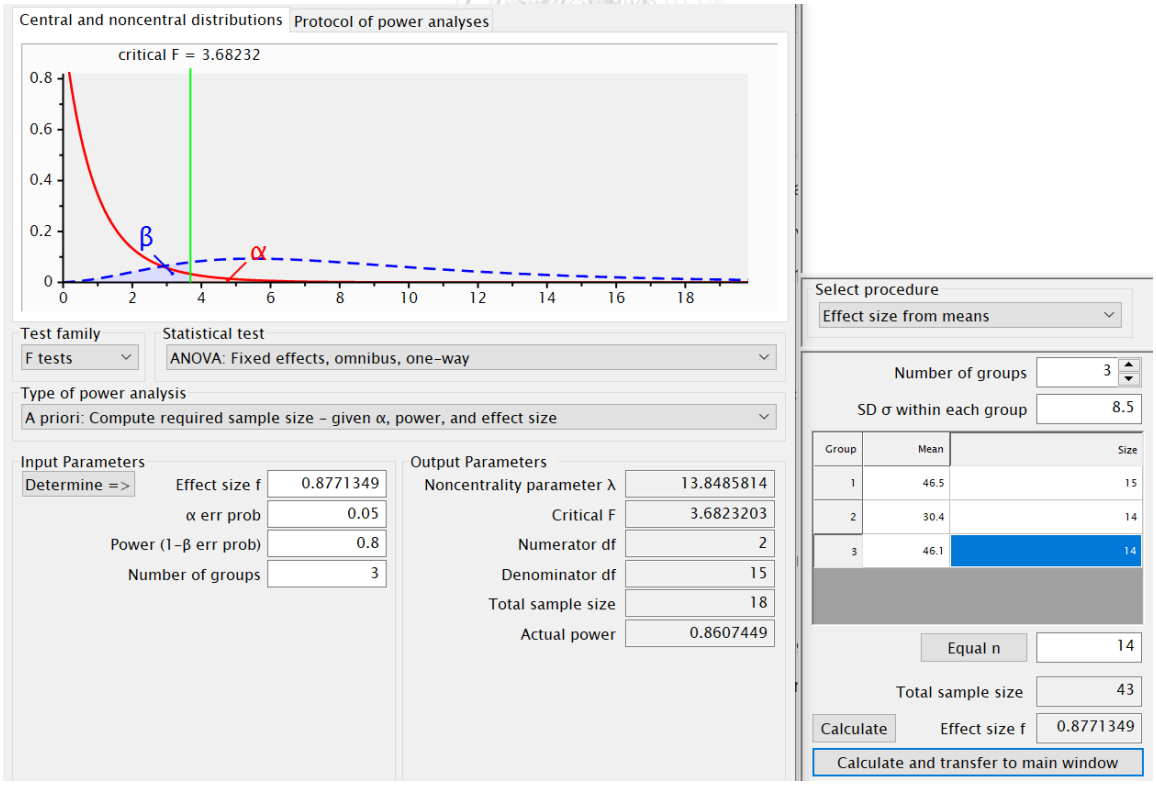


*Figure 9 Sample size calculation by G*Power program for microtensile bond strength test*

Sample size for fracture resistance test was calculated by G*Power 3.1 (F-test family for one-way ANOVA) with effect size = 0.7 (0.34), power β = 80% and α = 5% according to the study of Jordão-Basso and colleagues. (110) Total sample size was at least 24, which were 8 samples for each group. Therefore, this study designed to totally use 30 samples (10 samples for each group).

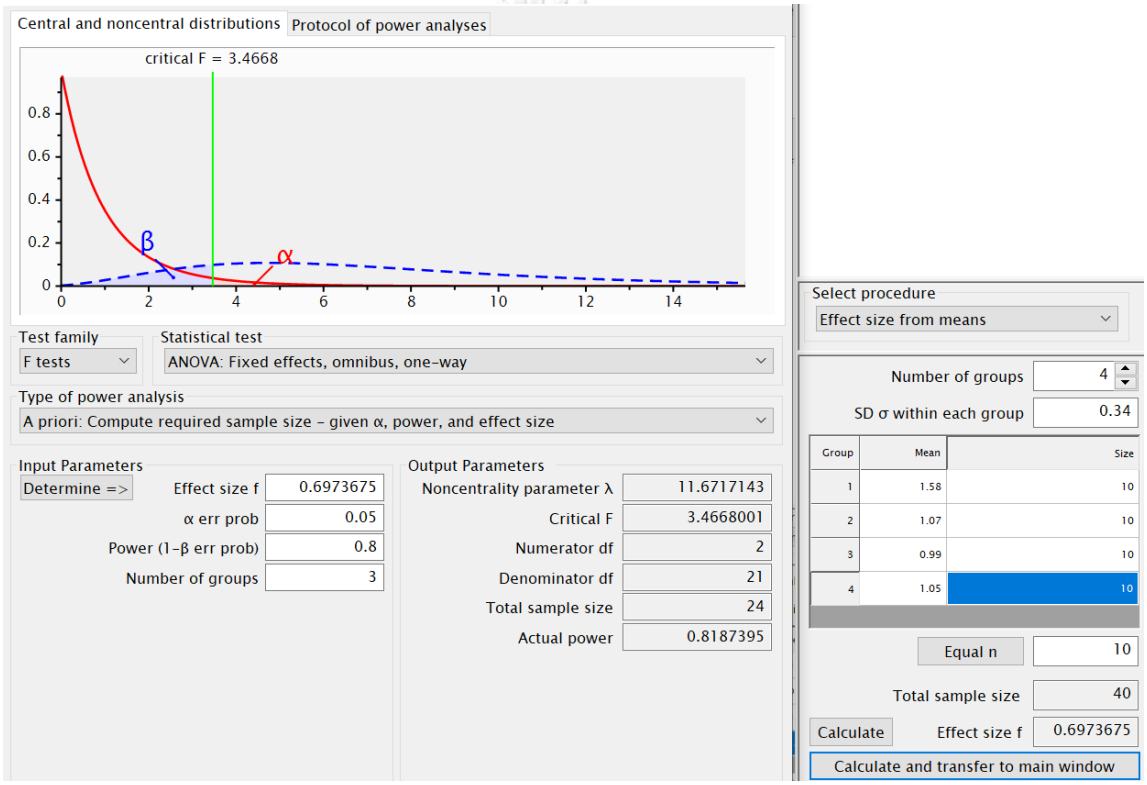


*Figure 10 Sample size calculation by G*Power program for fracture resistance test*

Materials

1. Extracted human maxillary premolar (60 teeth)
2. 1% Chloramine-T trihydrate solution (Loba Chemie, Mumbai, India)
3. Self-cured acrylic resin (Formatray; Kerr, Orange, CA, USA)
4. Occlusal indicator wax (Kerr, Orange, CA, USA)
5. Polyvinyl siloxane impression material, light type (DMG Silagum Light, Hamburg, Germany)
6. Polyvinyl siloxane impression material, putty type (DMG Silagum Putty, Hamburg, Germany)
7. Silicon carbide disc (National Keystone Products, USA)
8. Polyvinyl chloride (PVC) tube 2 cm in diameter
9. Stainless steel rod 1 mm in diameter and 1 inch in length
10. Barbed broach (Dentsply Sirona, USA)
11. Diamond cylindrical bur ISO 012 (Intensiv, Switzerland)
12. Long shank diamond round bur ISO 009 (Intensiv, Switzerland)
13. Diamond pointed–taper bur ISO 012 (Intensiv, Switzerland)
14. # 8-40 K file 25 cm in length (Dentsply Sirona, USA)
15. Nickel-titanium rotary file (ProTaper™ Next, Dentsply Sirona, USA)
16. 2.5% Sodium hypochlorite (NaOCl, Faculty of Dentistry Chulalongkorn University, Thailand)

17. 17% Ethylenediaminetetraacetic acid (EDTA, Faculty of Dentistry Chulalongkorn University, Thailand)
18. Match-tapered cone X3 (ProTaper™ Next, Dentsply Sirona, USA)
19. Resin-based sealer (AH™ plus, Dentsply Sirona, USA)
20. Cotton pellet
21. Paper point
22. Zinc oxide temporary filling (Cavit G, 3M ESPE, St. Paul, MN, USA)
23. Distilled water (Oral Biology Research Center, Faculty of Dentistry, Chulalongkorn University)
24. Resin-modified glass ionomer cement (Vitrebond, 3M ESPE, St. Paul, MN, USA)
25. Bleaching agent (Opalescence ENDO, Ultradent Products, South Jordan, UT, USA)
26. Vitamin C tablets (Hicee Sweetlets 500 mg, Bangkok, Thailand)
27. 37.5% phosphoric acid (Kerr, Orange, CA, USA)
28. Bonding agent (Optibond FL, Kerr, Orange, CA, USA)
29. Bulk-fill resin composite (Filtek™ One Bulk Fill Restorative (capsule) 3M ESPE, St. Paul, MN, USA)
30. Cyanoacrylate glue: Model repair II Blue (Dentsply, Japan)

Equipment

1. Stereomicroscope (ML 9300®, MEIJI, Japan)
2. Low speed cutting machine (IsoMet® 1000, Buehler, USA)
3. Resin composite carver (Hu-Friedy, USA)
4. Spoon excavator (Hu-Friedy, USA)
5. LED light-curing unit (Demi™ LED light-curing system, Kerr, Orange, CA, USA)
6. Radiometer (Demetron L.E.D. Radiometer, Kerr, USA)
7. Universal testing machine (EZ-S, Shimadzu, Japan)
8. Universal testing machine (LR10K, LLOYD Instruments, England)
9. Incubator (Contherm 1200, Contherm, New Zealand)
10. Nickel-titanium rotary instrument (ProTaper™ Next, Dentsply Sirona, USA)
11. Digital caliper (Mitutoyo, Japan)

Methods of data collection

Teeth collection

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, age between 16–40 years old, according to ISO technical specification 11405. The age of the patient was noted during teeth collection. Both left and right maxillary premolars were extracted from each patient due to orthodontic reasons. One was assigned to microtensile bond strength test while the other was assigned to fracture resistance test.

Calculus, plaque, and tissue remnants were removed with scaling instruments and polishing with pumice using a rubber cup. The teeth were stored in 1% chloramine-T trihydrate solution for 1 week at room temperature (24-26 °C) for antimicrobial effect following ISO technical specification 3696. The teeth were washed with tapped water and stored in distilled water at 4 °C. The teeth were examined under the stereomicroscope (ML 9300®, MEIJI, Japan) to ensure that there was no presence of fracture/ crack line or carious lesions. Collected teeth were used within six months after extraction.

Root canal treatment procedure

The access opening was performed in all sixty premolar teeth using diamond cylindrical bur 1.2 mm in diameter (Intensiv, Switzerland) to obtain a 3 mm mesial-distal

width and 4 mm buccal-lingual width with the depth reaching to the pulp chamber. (Figure 11) Pulpal remnant was removed by barbed broach and washed with distilled water for 15 seconds. The teeth were dried with oil-free air using a triple syringe and paper point.

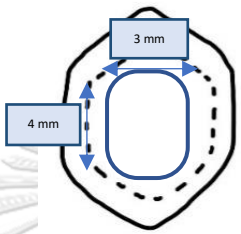


Figure 11 Diagrammatic presentation of cavity dimension for access opening

After access opening, long shank diamond round bur 0.8 mm in diameter (Intensiv, Switzerland) and diamond pointed–taper bur 1.2 mm in diameter (Intensiv, Switzerland) were used to obtain straight-line access. Working length was determined by subtracting 1 mm from the root length using K-file. The reference point for the buccal canal was the tip of buccal cusp and for the palatal canal was the tip of palatal cusp. Instrumentation was carried out in buccal and palatal canals using Ni-Ti rotary instrument (ProTaper™ Next, Dentsply Sirona, USA) X1-X3 at 300 rpm, 2N/cm by pecking motion to the working length under 2.5% sodium hypochlorite (NaOCl, Faculty of Dentistry Chulalongkorn University, Thailand) irrigation, and final flushed with 3 ml of 17% ethylenediaminetetraacetic acid (EDTA, Faculty of Dentistry Chulalongkorn University, Thailand) for 1 minute followed by 5 ml of 2.5% sodium hypochlorite. The

canals were dried using paper point. Root canal-obturation was done by single cone technique using match-tapered cone X3 (ProTaper™ Next, Dentsply Sirona, USA) with resin-based sealer (AH™ plus, Dentsply Sirona, USA). The radiographic image was taken to evaluate the root canal filling. The gutta-percha was cut to the level of 2 mm below the cemento-enamel junction. The pulp chamber was cleaned using alcohol-soaked cotton pellets, dried, and temporary filled with cotton pellet and zinc oxide temporary filling (Cavit G, 3M ESPE, St. Paul, MN, USA). The teeth were immediately immersed in distilled water at 37°C. After 24 hours, tooth access was re-opened and resin-modified glass ionomer cement (Vitrebond, 3M ESPE, St. Paul, MN, USA) was applied into the cavity to close the canal orifices using dycal carrier and gain the final depth of 6 mm measuring from the tip of buccal cusp. The radiographic image was taken to reassure the adaptability of resin-modified glass ionomer cement. (Figure 12)

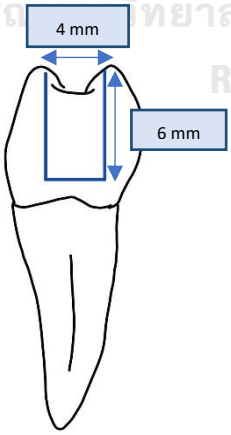


Figure 12 Diagrammatic presentation of cavity dimension in depth after root canal treatment

After root canal treatment procedure, both left and right maxillary premolar of each patient were randomize assigned into microtensile bond strength test and fracture resistance test groups. Teeth in each test were divided into 3 subgroups with an approximately equal mean of age.

- Group 1: Non-bleached group
- Group 2: Bleached and immediately bonded with resin composite group
- Group 3: Bleached, followed by application of vitamin C solution and immediately bonded with resin composite group

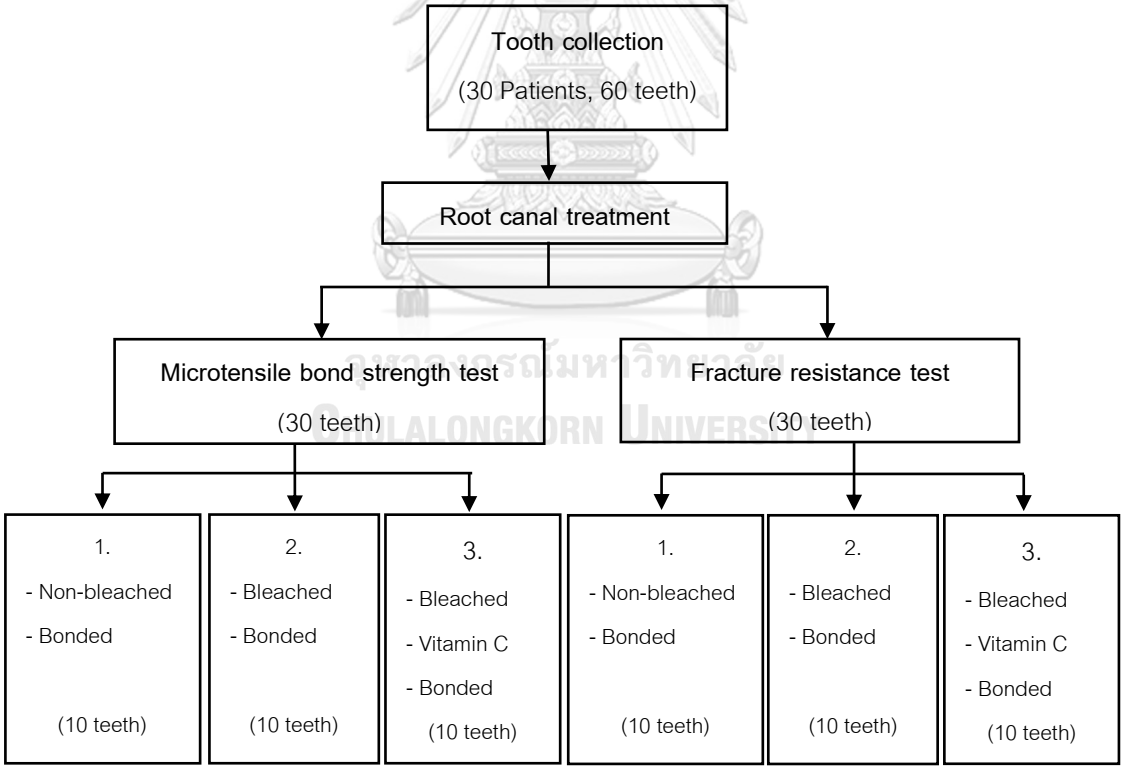


Figure 13 Diagram of study design

Bleaching treatment

The samples in group 2 and 3 were treated with 35% hydrogen peroxide bleaching gel (Opalescence ENDO, Ultradent Products, South Jordan, UT, USA) according to the manufacturer's instructions. The bleaching agent was filled in the cavity leaving 2 mm space for 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 4 days as recommended by the manufacturer. After 4 days, the zinc oxide temporary filling and cotton pellet were removed. The bleaching agent was washed out for 60 seconds with distilled water and the cavity was dried with oil-free air using a triple syringe.

Vitamin C solution preparation

Vitamin C solution was freshly prepared at 10% concentration by weight/volume according to previous studies' recommendation (82, 91, 92, 94, 95, 101) using vitamin C tablets (Hicee Sweetlets 500 mg, Bangkok, Thailand). The composition is shown in table 2.

Table 2 Composition of vitamin C tablet (Hicee Sweetlets 500 mg, Bangkok, Thailand)

Compositions	Amount
Vitamin C (ascorbic acid)	250 mg
Sodium ascorbate	281.2 mg (equivalent to ascorbic acid 250 mg)

In detail, two tablets of vitamin C (1000 mg or 1 g) were ground into a powder using mortar and pestle and dissolved in 10 ml distilled water to obtain the solution which was equal to 10% ascorbic acid by weight/ volume.

Vitamin C solution application

After rinsing the bleaching agent and drying, 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 10 minutes. The solution was agitated with micro brush during the application. (100, 116) The solution was washed out with distilled water for 30 seconds and the cavity was dried with oil-free air using triple syringe.

Bonding procedure

The prepared cavities in all teeth were bonded and restored. The Optibond FL (Kerr, Orange, CA, USA) bonding system was used according to the manufacturer's instructions. The cavity was etched with 37.5% phosphoric acid (Kerr, Orange, CA, USA) for 15 seconds, washed with a water spray from triple syringe for 15 seconds at 1 cm away from the cavity. The cavity was dried for 3 seconds by gentle air blow from the triple syringe but did not desiccate. The primer was applied for 15 seconds with gentle agitating motion and air-dried to evaporate the solvent by using the triple syringe at 1 cm away from the cavity. The adhesive was applied with a light brushing motion for 15

seconds and light-cured for 20 seconds (~1000 mW/cm^2) with a light-emitting diode (LED) light-curing unit (Demi™ LED light-curing system, Kerr, Orange, CA, USA). The tip of light guide was placed above the cavity as close as possible. The output of the light-curing unit was constantly measured by radiometer (Demetron L.E.D. Radiometer, Kerr, 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 4 mm increment and light cured for 40 seconds. Dental probe was used to measure the depth of the increment. Second layer was 2 mm increment which was adapted to occlusal margin and light cured for 40 seconds. (Figure 14) Radiographic image was taken to reassure that there was no gap. The teeth were stored in 37 °C distilled water for 24 hours before microtensile bond strength and fracture resistance tests.

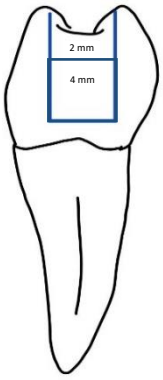


Figure 14 Diagrammatic presentation of bulk-fill resin composite restoration

Teeth preparation for microtensile bond strength test

The samples subjected to the microtensile bond strength test were cut by low speed cutting machine (IsoMet® 1000, Buchler, USA). Firstly, the occlusal portion of the sample was horizontally cut at 2 mm from the buccal cusp tip to expose dentin. The coronal part was vertically cut with 1.0 mm width in mesial-distal direction to the cemento-enamel junction and horizontally cut with 1.0 mm thickness in a buccal-lingual direction to obtain stick-shaped specimens. Four specimens were obtained from two levels from the middle third of the coronal part of each tooth. (Figure 15) At each level, one specimen was measured the bond strength at the mesial interface and the other was measured at the distal interface. Therefore, two values of the bond strength from the mesial and two values of the bond strength from the distal bonding interface were used to calculate the mean microtensile bond strength. The size of specimens was reassured with the range of 1.0 ± 0.1 mm in thickness by a digital caliper. The pre-test failure specimens including those with cracks and voids were excluded.

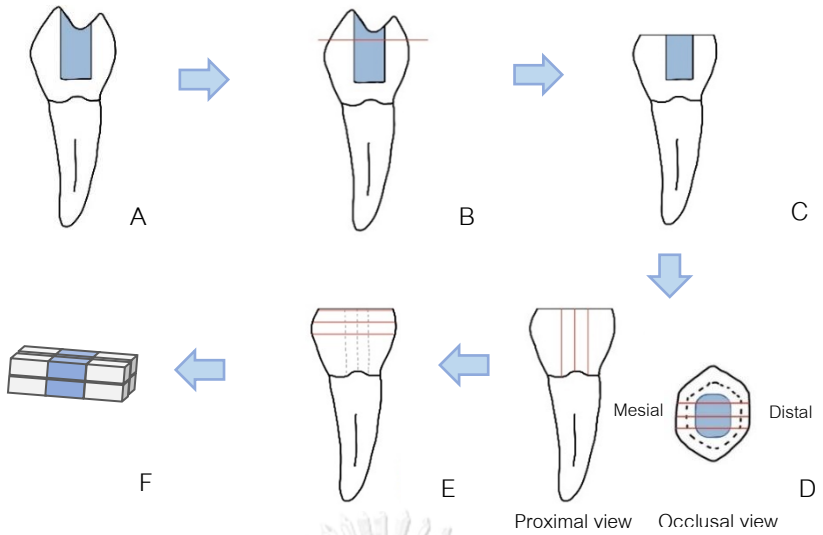


Figure 15 Diagrammatic presentation of specimens' preparation for microtensile bond strength test

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 flat occlusal surface.

D) The tooth was vertically cut with 1.0 mm width in mesial-distal direction to the cemento-enamel junction.

E) The tooth was horizontally cut with 1.0 mm thickness in buccal-lingual direction to obtain stick-shaped specimens.

F) Four specimens were obtained from the middle third of the coronal part of each tooth.

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 least amount of force causing fractured specimen was recorded. 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 4 stick-shaped specimens derived from each tooth represented the microtensile bond strength of that tooth, generating 10 values per group.

All fractured specimens were examined by a stereomicroscope at x45 magnification (ML 9300®, MEIJI, Japan) to define the location of the bond failure. The failure mode was modified from Turkun and his colleagues (91) classified into 4 types:

1. Adhesive failure, which was a failure more than 75% along the dentin and resin composite interface.
2. Cohesive failure in dentin, which was a failure more than 75% within the dentin.
3. Cohesive failure in resin composite, which was a failure more than 75% within the resin composite.
4. Mixed failure, which was a failure combining at least two of the failure types above.

Teeth preparation for fracture resistance test (Figure 16)

The tooth-samples were subjected to periodontal ligament simulation preparation which was modified from the study of Sirimai and his colleagues. (112) Occlusal indicator wax, with a thickness of 0.2–0.3 mm, approximately equal to the average thickness of the periodontal ligament, was covered the root at 2 mm

underneath the cemento-enamel junction. Self-cured acrylic resin was mixed and put into the 2 cm diameter polyvinyl chloride (PVC) tube. The tooth was placed at the center of the PVC tube. Stainless-steel rods with 1-mm diameter and 1-inch length were placed at 3 mm away from the interproximal surfaces of the tooth. The mold was immersed in the cooling water to reduce the heat occurring from the polymerization reaction. Base and catalyst of putty impression material (DMG Silagum Putty, Hamburg, Germany) were mixed and pressed over lingual half of the tooth and the stainless–steel rods to be a guide of re-positioning the tooth. After the putty impression material sets, removed the tooth from an acrylic resin mold, and hot water at 80–90 °C was rinsed to eliminate occlusal indicator wax. Light polyvinyl siloxane material (DMG Silagum Light, Hamburg, Germany) was placed into the acrylic resin mold followed by re-positioning the tooth using putty impression material guide. After the light polyvinyl siloxane was completely set, the stainless-steel rods were cut at the level of acrylic resin mold by silicon carbide disc (National Keystone Products, USA) and the excess polyvinyl siloxane was trimmed with a blade.

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 occlusal surface in which the lingual incline of the buccal cusp and the buccal incline of the lingual cusp were touched. The load was applied with

a crosshead speed at 0.5 mm/min. (Figure 17) 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. (109)

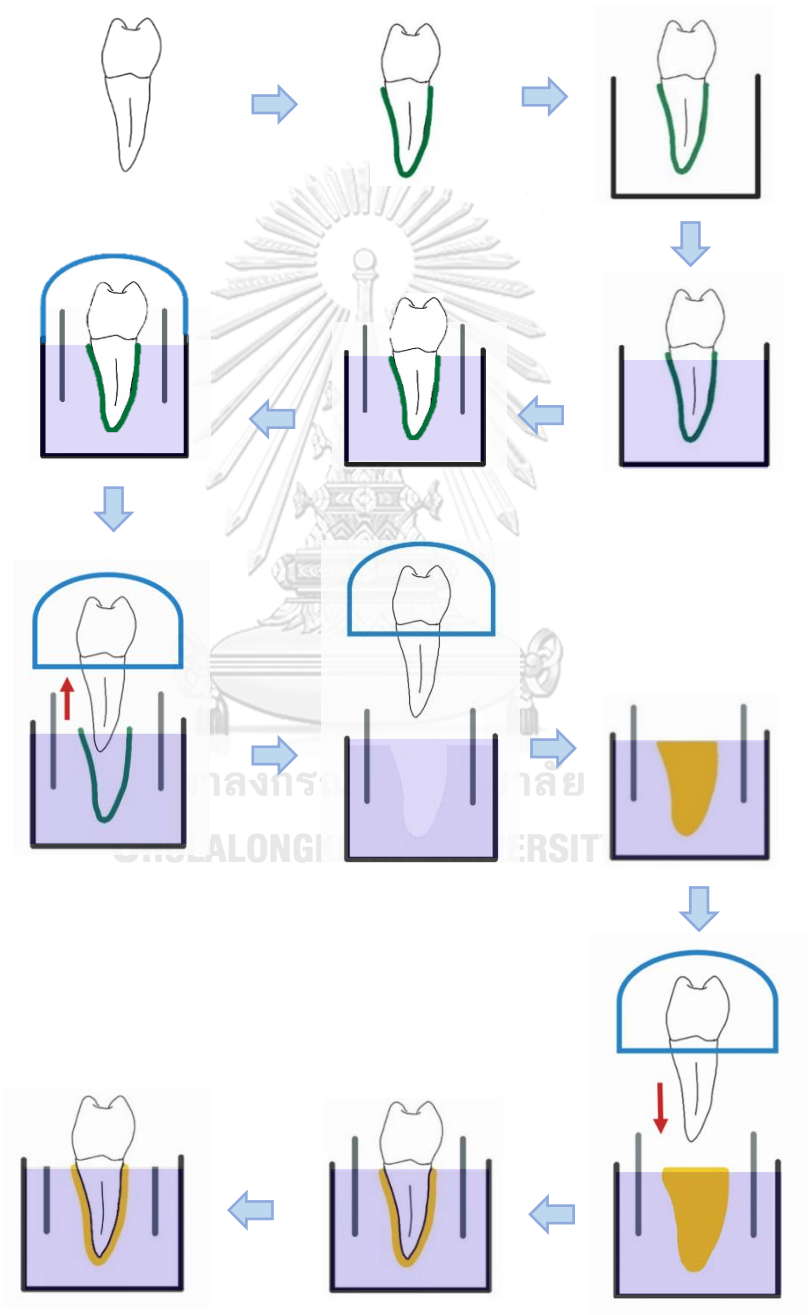


Figure 16 Diagrammatic presentation of periodontal ligament simulation

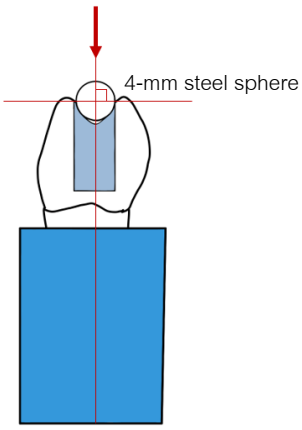


Figure 17 Diagrammatic presentation of the load application of the sphere used for the axial compression test

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. (Figure 18) (109)

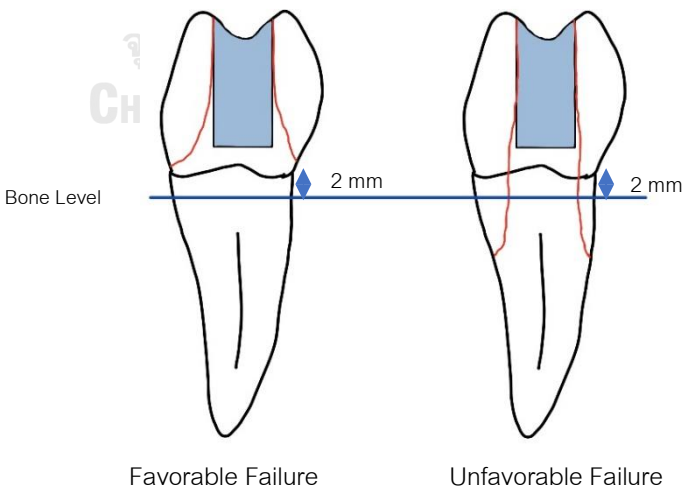


Figure 18 Diagrammatic presentation of fracture mode

Statistical analysis

SPSS for windows version 26.0 was used to analyze the data with a 95% confidence interval to indicate the significant difference.

1. Microtensile bond strength and fracture resistance test data analysis

   The distribution of the data was determined using the Shapiro Wilk test, followed by one-way ANOVA with Tukey post-hoc analysis.

2. The correlation between microtensile bond strength and fracture resistance test

   The distribution of the data was determined using the Shapiro Wilk test, followed by Pearson's correlation.

3. The failure mode after the microtensile bond strength and fracture resistance test was analyzed using the Chi-square test.

Chapter IV Results

The data of both microtensile bond strength and fracture resistance tests were normal distribution with equal variance according to Shapiro Wilk and Levene's test ($p<0.05$), respectively. Therefore, one-way ANOVA with Tukey post-hoc analysis was used.

The mean microtensile bond strength of all groups is presented in Table 3. 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). The microtensile bond strength of the group 3 was not significantly different from group 1 ($p=0.959$). The most common failure mode was adhesive failure followed by mixed failure. (Table 4) There was no statistical difference in failure mode among the groups ($p=0.072$).

Table 3 Mean microtensile bond strength, standard deviation, standard error, minimum, and maximum 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

**Statistical differences ($p<0.05$) between groups are represented by lower case superscript letters (same letter denotes no statistical difference).*

Table 4 Failure mode of microtensile bond strength test specimens

Group	Failure types			
	Adhesive n (%)	Cohesive in dentin n (%)	Cohesive in composite n (%)	Mixed n (%)
Non-bleached	33 (82.5)	0 (0)	1 (2.5)	6 (15.0)
Bleached	34 (85.0)	3 (7.5)	2 (5.0)	1 (2.5)
Bleached and Vitamin C	36 (90.0)	0 (0)	0 (0)	4 (10.0)

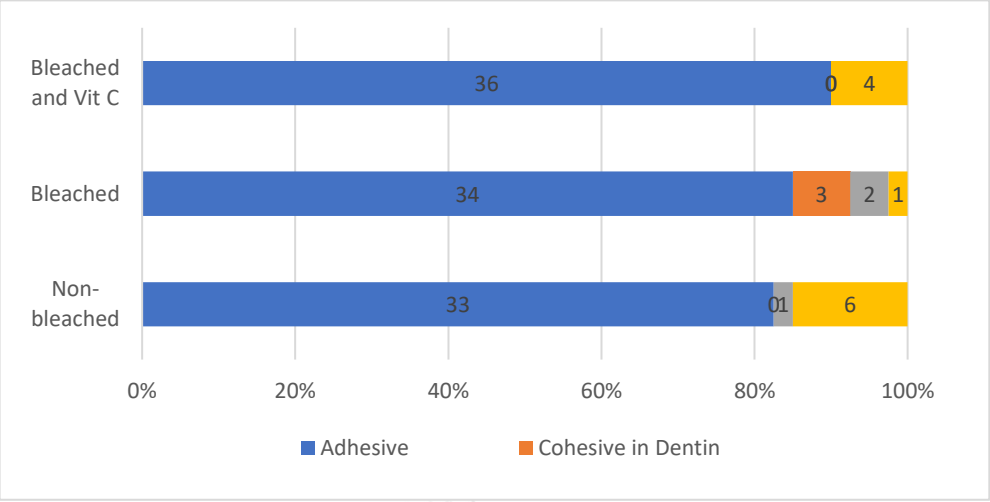


Figure 19 The percentage of the failure mode in microtensile bond strength test

The results of fracture strength are presented in table 5. 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 lowest strength (616.98 ± 97.07 N). The fracture strength of the group 1 was not significantly different from group 3 (p=0.472). The most common failure mode was favorable fracture. (Table 6) There was no statistical difference of failure mode among the groups (p=0.621). All the fractures occurred at the lingual cusp. (Figure 21)

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).

Table 5 Fracture resistance mean, standard deviation, standard error, maximum, and minimum values

Group	n	Mean (N)	Standard deviation (N)	Standard error (N)	Minimum (N)	Maximum (N)
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

**Statistical differences between groups are represented by lower case superscript letters (same letter denotes no statistical difference).*

Table 6 Fracture pattern of fracture resistance test specimens

Group	Failure types		Location of fracture	
	Favorable failure, n (%)	Unfavorable failure, n (%)	Buccal cusp, n (%)	Lingual cusp, n (%)
Non-bleached	8 (80.0)	2 (20.0)	0 (0)	10 (100)
Bleached	7 (70.0)	3 (30.0)	0 (0)	10 (100)
Bleached and Vitamin C	6 (60.0)	4 (40.0)	0 (0)	10 (100)

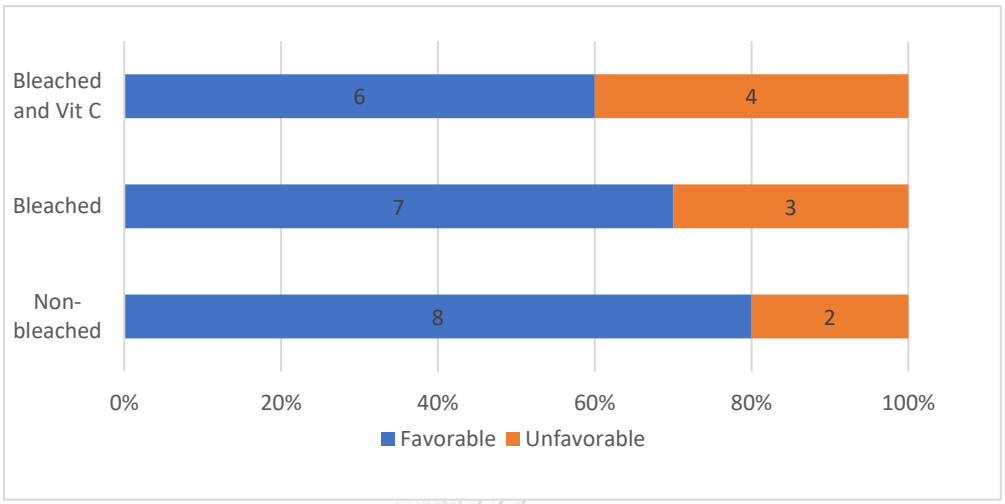


Figure 20 The percentage of the failure mode in fracture resistance test

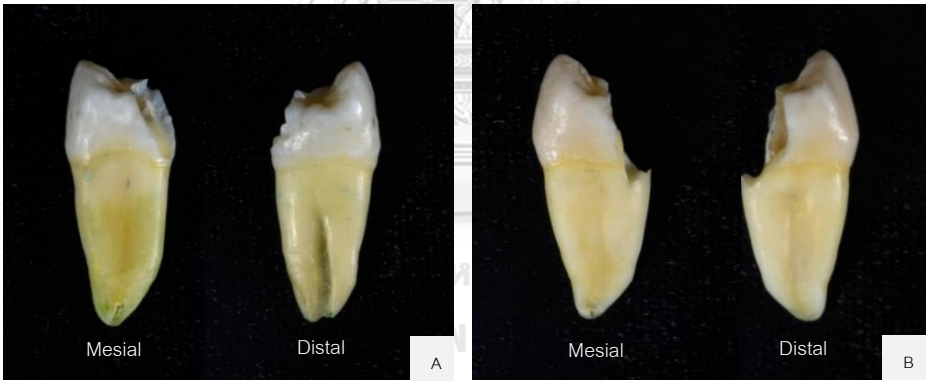


Figure 21 Fracture pattern; A) Favorable failure, B) Unfavorable failure

Chapter V 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 that subjected to fracture resistance test were simulated the periodontal ligament with the 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 human's periodontal ligament. (114, 115)

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 comparing to either tensile or shear bond strength test. (107) All teeth were cut to obtain stick-shaped specimens with almost the same dimension. Four specimens were obtained from two levels of the middle third of the coronal part of the tooth because the bonding interface at those levels was straight in which the applying force would be perpendicular to the bonded interface. (Figure 15) Mean of the bond strength from all four specimens would represent the bond strength of the cavity which were mainly the deep dentin.

The results of this study revealed that immediately bonded with resin composite in 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 the non-bleached tooth. The scanning electron microscope by Lai and others illustrated that immediately bonded the bleached tooth with resin composite showed fractured hybrid layer contained incompletely infiltrated collagen fibrils and most of the resin tag were dislodged from the dentinal tubules. (17) The hydrogen peroxide was not only lower the bond strength of the dentin but also the enamel. Titley and others showed the porosities in the resin tag of peroxide-treated etched enamel. The porosities could occur from the gas which was the by-product of the oxidation reaction and remained underneath the enamel surface. (16) 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. (68) Therefore, the alteration in organic substances may also lower the bond strength and fracture strength. These factors could be the cause of the lowest microtensile bond strength (36.571 ± 2.609 MPa) and fracture strength (616.98 ± 97.07 N) found in bleached groups of this study. 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. (27) This different result to our study could be the different amount of active hydrogen peroxide. 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, the antioxidant such as sodium ascorbate had been introduced to reverse the negative effect of bleaching agent. (17, 28) Since the active ingredient of sodium ascorbate is ascorbic acid, a component of vitamin C tablets which easily get from the drug or convenient stores, vitamin C containing sodium ascorbate prepared solution was used in this study. The result of this study demonstrated that the use of 10% vitamin C containing sodium ascorbate prepared solution (10% ascorbic acid) could increase 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 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 to irrigate the cavity for 10 minutes 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. (117) 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 home bleaching was applied 3 weeks daily for 2 hours and 3 sessions of in–office bleaching every 7 days. The 10% sodium ascorbate hydrogel was applied in the access cavity and on the buccal surfaces for 24 hours before resin composite restoration. The result also showed that the use of sodium ascorbate significantly increased the fracture resistance. (15)

The result of the present study implied that the remaining free radicals from the bleaching agent were the most influential factor affecting the bond and fracture strength since the use of vitamin C containing sodium ascorbate could reverse those strengths. This reverse effect of both bond and fracture strengths might be due to the antioxidant capacity of vitamin C containing sodium ascorbate which was able to quench reactive free radicals in biological systems. The improvement of bond and fracture strength might be explained by the ability of this reducing agent, ascorbic acid, in donating two high–energy electrons to scavenge the free radicals resulting from the bleaching treatment and turned into dehydroascorbate. (90) Hence, the resin polymerization could

proceed without premature termination resulted in reversing the negative effect of the bleaching agent.

Since sodium ascorbate is a more stable form, it had been widely used in many studies. (17, 82, 91, 93) However, the predominantly active ingredient that scavenged the free radicals resulting from the bleaching treatment was ascorbic acid. The 10% vitamin C containing sodium ascorbate prepared solution used in this study was equivalent to 10% ascorbic acid which was a slightly higher concentration compared to ascorbic acid deriving from 10% sodium ascorbate of previous studies. Since 1000 mg of sodium ascorbate contains ascorbic acid 889 mg so that 10% sodium ascorbate contains 8.89% of ascorbic acid. Many studies revealed that the concentration of sodium ascorbate at 10% equally to 8.89% ascorbic acid or above was effective in reversing the strength and neutralizing the oxidation effect after the bleaching procedure. (92, 94, 95) Kimyai and Valizadeh also discovered that there was no significant difference between hydrogel and solution form of sodium ascorbate in reversing the bond strength. (94) Freire and colleagues demonstrated that there was no significant difference in the release amount of hydrogen peroxide between 60 minutes and 10 minutes application time. (76) Moreover, Bulut and colleagues suggested that the continuously refreshed and agitatedly applied antioxidant could enhance the neutralizing effect. (100) Therefore, the 10% vitamin C containing sodium ascorbate

solution was freshly prepared before agitatedly applied into the pulp chamber for 10 minutes at the rate of 1 ml/min in this study.

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 which the stress uniformly distributed when the force was perpendicular to the interface dominantly. (105, 106)

In terms of fracture resistance test, mostly of the specimens showed favorable failures. (Figure 21) This might be explained by the quality of the bond strength at the tooth structure and resin composite interface. Mostly applied force could be absorbed by the resin composite and the tooth structure which were bonded, above the simulated bond level. The less remaining force was transferred to the tooth structure underneath the simulated bond level. Therefore, we assumed that the bond efficiency related to the failure mode of the fracture resistance test. Moreover, the cavity design in this study was an ideal access opening for maxillary premolar teeth in which both marginal ridges had remained. The difference in the remaining tooth structure might affect the fracture strength and its failure mode. Despite the lack of statistical difference among the study groups, unfavorable failures were presented in some of the specimens. This might

correlate with the cavity configuration of the access opening that probably created the stress concentration at the point angle of the base of the pulp chamber resulting in the fracture of the tooth structure below the simulated bond level. In addition, all specimens were fractured at the lingual cusp. This might related to the size of the lingual cusp of maxillary premolar which was smaller than the buccal cusp and the more concave at the cemento-enamel junction. (118) The fracture also located at the interface of resin composite and tooth structure in all specimens which revealed that the fracture strength in this study was the effect of bonded restoration. Nevertheless, 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. (15)

According to the result in this study, there was a relationship between the microtensile bond strength and fracture resistance of 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. Therefore, 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 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 from 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.

For clinical relevance, normally both vertical and lateral forces could affect the maxillary premolar. Although, the vertical force was the main application force using in this study, there might be a slight of the lateral force due to the position of the sphere head contacting with the lingual incline of the buccal cusp and the buccal incline of the lingual cusp. However, there is also a fracture resistance test simulating the eccentric movement or extreme slide movement contact by positioning the sphere head at an angle of 45° to the tooth axis to test the lateral force directly. (119) 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 laterally applied. (110) However, their study was performed in bovine incisors and there are no studies in the current literature that evaluate the lateral force on the bleached human maxillary premolar tooth. Furthermore,

the bond strength obtained from the present study was an immediate of both bond strength and fracture strength of the resin composite after bleaching treatment. Therefore, the long - term bond strength and fracture strength such as a 6 - month storage in water or aging process including thermocycling and fatigue test is required for further investigation.

Conclusion

Within the limitation of this study, the following conclusions can be drawn:

1. Non–vital tooth bleaching decreased the microtensile bond strength of the resin composite to dentin and decreased the fracture resistance of restored tooth.
2. The use of 10% vitamin C containing sodium ascorbate prepared solution immediately after bleaching could increase both the bond strength of resin composite to dentin and fracture resistance of non–vital bleached tooth to the level of the non-bleached tooth.
3. The microtensile bond strength was positively correlated to the fracture resistance in restored endodontically tooth for no bleached, bleached and bleached with vitamin C solution conditions.

APPENDICES

Appendix A Copy of study protocol and consent form of approval (Study code HREC-DCU 2020-045)

No. 037/2020

Study Protocol and Consent Form Approval
Certificate of Exemption

The Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand has approved the following study to be carried out according to the protocol and patient/participant information sheet dated and/or amended as follows in compliance with the **ICH/GCP**

Study Title	: Effect of Vitamin C solution on microtensile bond strength and fracture resistance of non-vital bleached tooth restored with resin composite
Study Code	: HREC-DCU **2020-045**
Study Center	: Chulalongkorn University
Principle Investigator	: Ms. Pimduean Sivavong
Protocol Date	: May 7, 2020
Date of Approval	: June 10, 2020
Date of Expiration	: June 9, 2022

K. Bhalang

.....

(Associate Professor Dr. Kanokporn Bhalang)
Chairman of Ethics Committee
Associate Dean for Research

*A list of the Ethics Committee members (names and positions) present at the Ethics Committee meeting on the date of approval of this study has been attached (upon requested). This Study Protocol Approval Form will be forwarded to the Principal Investigator.

Approval is granted subject to the following conditions: (see back of the approval)

Appendix B The mean and standard deviation of tooth size for fracture resistance test

Descriptive Statistics

Experiment		N	Mean	Std. Deviation
Non-bleach	MDwidth	10	7.8210	.25497
	BLiwidth	10	10.0080	.34402
	Toothlength	10	21.2460	1.99350
	Valid N (listwise)	10		
Bleach	MDwidth	10	7.6150	.18295
	BLiwidth	10	9.8090	.29913
	Toothlength	10	21.1790	1.27738
	Valid N (listwise)	10		
Vitamin C	MDwidth	10	7.8260	.37390
	BLiwidth	10	9.9330	.53901
	Toothlength	10	21.4230	1.40120
	Valid N (listwise)	10		

Appendix C Data distribution of tooth size for fracture resistance test

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Experiment	Statistic	df	Sig.	Statistic	df	Sig.
MDwidth	Control	.255	10	.065	.911	10	.285
	Bleach	.235	10	.124	.905	10	.251
	VitC	.192	10	.200*	.935	10	.498
BLiwidth	Control	.210	10	.200*	.864	10	.085
	Bleach	.160	10	.200*	.946	10	.616
	VitC	.158	10	.200*	.939	10	.538
Toothlength	Control	.238	10	.113	.919	10	.348
	Bleach	.213	10	.200*	.909	10	.278
	VitC	.227	10	.156	.863	10	.082

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix D Test of homogeneity of variances of tooth size for fracture resistance test

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
MDwidth	Based on Mean	3.626	2	27	.040
	Based on Median	3.435	2	27	.047
	Based on Median and with adjusted df	3.435	2	22.383	.050
	Based on trimmed mean	3.608	2	27	.041
BLiwidth	Based on Mean	2.972	2	27	.068
	Based on Median	2.696	2	27	.086
	Based on Median and with adjusted df	2.696	2	22.706	.089
	Based on trimmed mean	2.962	2	27	.069
Toothlength	Based on Mean	1.527	2	27	.235
	Based on Median	.580	2	27	.566
	Based on Median and with adjusted df	.580	2	20.829	.568
	Based on trimmed mean	1.395	2	27	.265

Appendix E One-way ANOVA and Welch ANOVA analysis of tooth size for fracture resistance test

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MDwidth	Between Groups	.290	2	.145	1.825	.181
	Within Groups	2.145	27	.079		
	Total	2.435	29			
BLiwidth	Between Groups	.202	2	.101	.608	.552
	Within Groups	4.485	27	.166		
	Total	4.687	29			
Toothlength	Between Groups	.318	2	.159	.063	.939
	Within Groups	68.122	27	2.523		
	Total	68.440	29			

Robust Tests of Equality of Means

		Statistic[a]	df1	df2	Sig.
MDwidth	Welch	2.663	2	16.787	.099
BLiwidth	Welch	.938	2	17.252	.410
Toothlength	Welch	.082	2	17.518	.922

a. Asymptotically F distributed.

Appendix F The microtensile bond strength, bonded failure mode, fracture resistance, fracture mode, and location of fracture of all test groups

Failure mode of microtensile bond strength test is represented as following number

1: Adhesive failure

2: Cohesive failure in dentin

3: Cohesive failure in composite

4: Mixed failure

Fracture mode is represented as following number

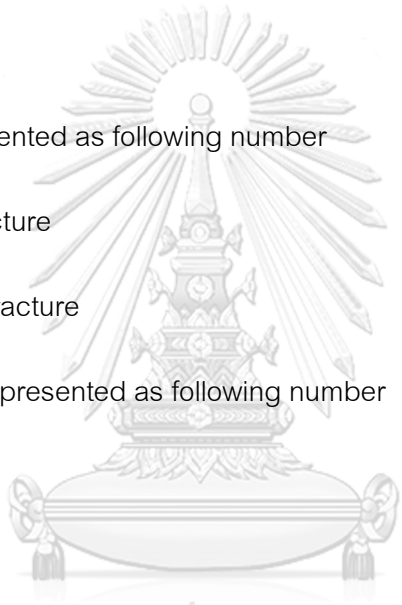
1: Favorable fracture

2: Unfavorable fracture

Location of fracture is represented as following number

1: Lingual cusp

2: Buccal cusp



Group	Microtensile bond strength test (M)		Fracture resistance test (F)		
	Bond strength (MPa)	Bonded failure mode	Fracture resistance (N)	Fracture mode	Location of fracture
A	54.011	1,1,1,1	1189.42	1	1
A	55.353	1,1,1,1	964.49	1	1
A	53.557	1,1,3,1	925.61	1	1
A	56.078	1,1,4,1	1326.00	1	1
A	58.878	1,1,1,1	792.77	1	1
A	60.706	1,1,4,1	1281.00	2	1
A	41.830	1,1,1,4	1074.00	1	1
A	64.927	1,1,1,1	1088.00	1	1
A	42.835	4,1,1,4	1092.00	2	1
A	61.313	4,1,1,1	801.10	1	1

Group	Microtensile bond strength test		Fracture resistance test		
	Bond strength (MPa)	Bonded failure mode	Fracture resistance (N)	Fracture mode	Location of fracture
B	37.524	1,1,1,1	650.00	1	1
B	33.797	4,1,1,3	750.75	1	1
B	33.651	2,1,1,1	530.79	1	1
B	36.213	1,1,2,1	473.26	2	1
B	39.448	1,1,1,2	602.30	2	1
B	39.747	3,1,1,1	536.60	1	1
B	38.911	1,1,1,1	581.10	2	1
B	35.115	1,1,1,1	778.8	1	1
B	38.513	1,1,1,1	593.90	1	1
B	32.792	1,1,1,1	672.30	1	1

Group	Microtensile bond strength test		Fracture resistance test		
	Bond strength (MPa)	Bonded failure mode	Fracture resistance (N)	Fracture mode	Location of fracture
C	62.361	1,1,1,1	1175.40	1	1
C	51.531	1,4,1,1	1111.41	1	1
C	55.549	1,1,1,1	966.37	1	1
C	53.353	1,1,1,1	1020.37	1	1
C	51.282	4,1,1,1	1122.00	2	1
C	59.360	4,1,1,1	753.80	2	1
C	54.300	1,1,1,1	724.90	1	1
C	57.802	1,1,1,1	945.40	2	1
C	53.656	1,4,1,1	1106.00	2	1
C	56.466	1,1,1,1	798.20	1	1

Appendix G The minimum, maximum, mean, and standard deviation of microtensile bond strength and fracture resistance tests

Descriptive Statistics

		Experiment		
		Non-bleach	Bleach	Vitamin C
Bondstrength	N	10	10	10
	Minimum	41.830	32.792	51.282
	Maximum	64.927	39.747	62.361
	Mean	54.94865	36.57103	55.56611
	Std. Deviation	7.540717	2.609174	3.514335
Fractureresistance	N	10	10	10
	Minimum	792.77	473.26	724.90
	Maximum	1326.00	778.80	1175.40
	Mean	1053.4390	616.9800	972.3850
	Std. Deviation	183.64942	97.07466	164.38986
Valid N (listwise)	N	10	10	10

Appendix H Data distribution of the microtensile bond strength and fracture resistance tests of all groups

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Experiment	Statistic	df	Sig.	Statistic	df	Sig.
Bondstrength	Non-bleach	.227	10	.156	.902	10	.232
	Bleach	.172	10	.200*	.909	10	.275
	Vitamin C	.141	10	.200*	.951	10	.678
Fractureresistance	Non-bleach	.145	10	.200*	.948	10	.650
	Bleach	.160	10	.200*	.961	10	.801
	Vitamin C	.192	10	.200*	.903	10	.235

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix I Test of homogeneity of variances of microtensile bond strength and fracture resistance tests

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Bondstrength	Based on Mean	3.276	2	27	.053
	Based on Median	2.883	2	27	.073
	Based on Median and with adjusted df	2.883	2	13.166	.092
	Based on trimmed mean	3.149	2	27	.059
Fractureresistance	Based on Mean	2.085	2	27	.144
	Based on Median	1.793	2	27	.186
	Based on Median and with adjusted df	1.793	2	22.533	.189
	Based on trimmed mean	2.104	2	27	.142

Appendix J One-way ANOVA and Tukey post hoc analysis of microtensile bond strength and fracture resistance tests

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Bondstrength	Between Groups	2329.771	2	1164.885	45.970	.000
	Within Groups	684.187	27	25.340		
	Total	3013.957	29			
Fractureresistance	Between Groups	1077929.745	2	538964.873	23.041	.000
	Within Groups	631571.624	27	23391.542		
	Total	1709501.370	29			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Experiment	(J) Experiment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bondstrength	Non-bleach	Bleach	18.377620*	2.251233	.000	12.79587	23.95937
		Vitamin C	-.617460	2.251233	.959	-6.19921	4.96429
	Bleach	Non-bleach	-18.377620*	2.251233	.000	-23.95937	-12.79587
		Vitamin C	-18.995080*	2.251233	.000	-24.57683	-13.41333
	Vitamin C	Non-bleach	.617460	2.251233	.959	-4.96429	6.19921
		Bleach	18.995080*	2.251233	.000	13.41333	24.57683
Fractureresistance	Non-bleach	Bleach	436.45900*	68.39816	.000	266.8714	606.0466
		Vitamin C	81.05400	68.39816	.472	-88.5336	250.6416
	Bleach	Non-bleach	-436.45900*	68.39816	.000	-606.0466	-266.8714
		Vitamin C	-355.40500*	68.39816	.000	-524.9926	-185.8174
	Vitamin C	Non-bleach	-81.05400	68.39816	.472	-250.6416	88.5336
		Bleach	355.40500*	68.39816	.000	185.8174	524.9926

*. The mean difference is significant at the 0.05 level.

Appendix K Chi-square test of bonded failure mode

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	11.590[a]	6	.072
Likelihood Ratio	13.507	6	.036
Linear-by-Linear Association	.959	1	.328
N of Valid Cases	120		

a. 9 cells (75.0%) have expected count less than 5. The minimum expected count is 1.00.

Appendix L Chi-square test of fracture failure mode

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.952[a]	2	.621
Likelihood Ratio	.966	2	.617
Linear-by-Linear Association	.921	1	.337
N of Valid Cases	30		

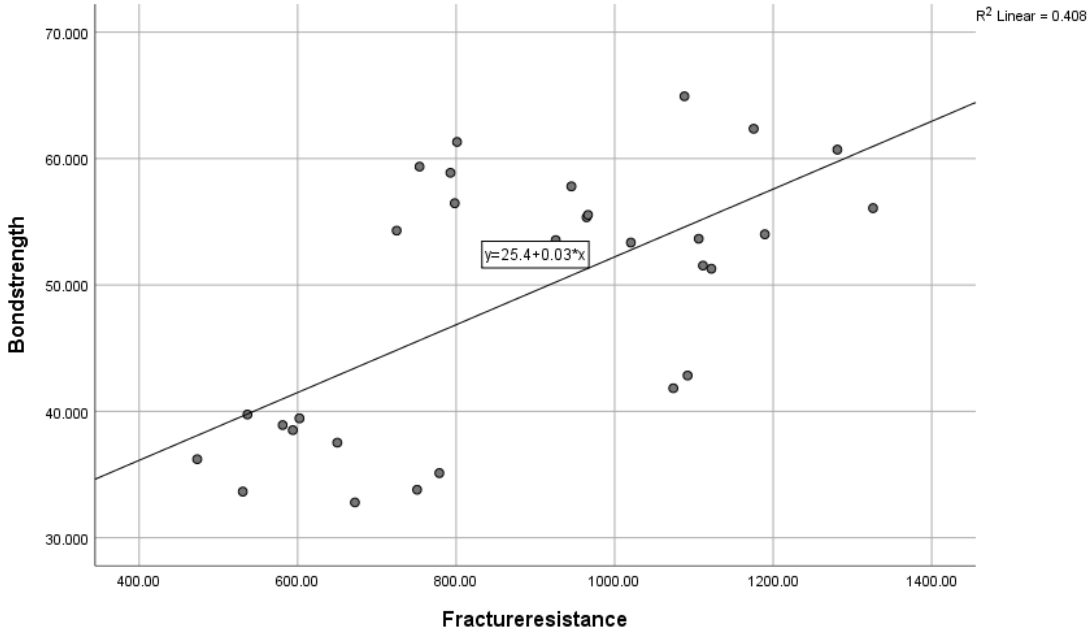
a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 3.00.

Appendix M Pearson's correlation test between microtensile bond strength and fracture resistance tests

Correlations

		Bondstrength	Fractureresistance
Bondstrength	Pearson Correlation	1	.639**
	Sig. (2-tailed)		.000
	N	30	30
Fractureresistance	Pearson Correlation	.639**	1
	Sig. (2-tailed)	.000	
	N	30	30

**. Correlation is significant at the 0.01 level (2-tailed).



Appendix N Factsheet of Hicee Vitamin C tablets

HICEE SWEETLETS

VITAMIN C

VITAMIN C TABLETS

- VITAMIN C is antioxidant that serves to protect the capillary basement membrane.
- VITAMIN C is necessary for the biosynthesis of hydroxyproline, a precursor of collagen, osteoid, and dentin.
- VITAMIN C has been helped to prevent and treat the common cold.

COMPOSITION

Hicee Sweetlets are easy-to-take yellow tablets, each contains ;

Vitamin C	250.0	mg.
Sodium ascorbate	281.2	mg.
(equivalent to vitamin C	250.0	mg.)

INDICATIONS

VITAMIN C deficiency

ADMINISTRATION AND DOSAGE

Usually 1-2 tablets daily. Allow tablet to dissolve slowly in the mouth. Dosage may be increased according to condition.

PRECAUTION

Stopper tightly after opening and store away from humidity and light.

Sight change in color of the tablets may occur but there is no change in the potency.

STORAGE CONDITION

Store below 30°C, away from humidity and light

PACKAGE

Tube of 10, 14, 15, 20 and 25 tablets.

Box of 120 tablets (Foil of 6 tablets)

Foil of 2 tablets

Manufactured by:

INTERTHAI PHARMACEUTICAL MANUFACTURING LTD. Bangkok, Thailand

for Takeda (Thailand), Ltd., Bangkok,Thailand

Licensed by :

TAKEDA PHARMACEUTICAL COMPANY LIMITED. OSAKA, JAPAN

REVISION DATE : April 2018

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