EFFECT OF DESENSITIZING TOOTHPASTE CONTAINING CALCIUM SODIUM PHOSPHOSILICATE ON MICROTENSILE BOND STRENGTH OF ADHESIVE SYSTEMS

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ผลของยาสีฟันลดการเสียวฟันที่มีส่วนประกอบของแคลเซียมโซเดียมฟอสโฟซิลิเกตต่อความแข็งแรงดึงระดับจุลภาคของสารยึดติดระบบต่างๆ

น.ส.วาริน สิทธิไวทยาภรณ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม ไม่สังกัดภาควิชา/เทียบเท่า
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Thesis Title	EFFECT OF DESENSITIZING TOOTHPASTE CONTAINING CALCIUM SODIUM PHOSPHOSILICATE ON MICROTENSILE BOND STRENGTH OF ADHESIVE SYSTEMS
By	Miss Warin Sittiwaitayaporn
Field of Study	Esthetic Restorative and Implant Dentistry
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วาริน สิทธิไวทยาภรณ์ : ผลของยาสีฟันลดการเสียวฟันที่มีส่วนประกอบของแคลเซียมโซเดียมฟอสโฟซิลิเกตต่อความ
แข็งแรงดึงระดับจุลภาคของสารยึดติดระบบต่างๆ. (EFFECT OF DESENSITIZING TOOTHPASTE CONTAINING
CALCIUM SODIUM PHOSPHOSILICATE ON MICROTENSILE BOND STRENGTH OF ADHESIVE SYSTEMS) อ.
ที่ปรึกษาหลัก : รศ.ทพญ. ดร.ศิริวิมล ศรีสวัสดิ์

วัตถุประสงค์: เพื่อศึกษาผลของยาสีฟันลดการเสียวฟันที่มีส่วนประกอบของแคลเซียมโซเดียมฟอสโฟซิลิเกตต่อความแข็งแรงดึงระดับจุลภาคในสารยึดติดระบบต่างๆ วิธีการศึกษา: ฟันกรามซี่ที่สามจากมนุษย์ 52 ซี่ถูกตัดเพื่อเผยเนื้อฟัน จากนั้นถูกแบ่งเป็นสองกลุ่ม กลุ่มแรกไม่แปรงฟัน และกลุ่มที่สอง แปรงด้วยยาสีฟันเซ็นโซดายน์ รีแพร์แอนด์โพรเทคท์ ด้วยเครื่องแปรงฟันอัตโนมัติ (V-8 cross brushing machine, Sabri Dental Enterprise, Inc., USA) 10,000 รอบ หลังจากนั้นทั้งสองกลุ่ม ได้ถูกแบ่งกลุ่มย่อย 3 กลุ่มตามชนิดของสารยึดติด ได้แก่ ออพติบอนด์ เอฟแอล เคลียร์ฟิลเอสอีบอนด์ และซิงเกิลบอนด์ ยูนิเวอร์แซล เพื่อนำไปยึดติดกับคอมโพสิตชนิดบ่มด้วยแสง นำฟันตัวอย่างที่เสร็จแล้วมาตัดเป็นชิ้นตัวอย่าง โดยชิ้นตัวอย่างจากฟันซี่เดียวกัน ได้ถูกแบ่งเป็นสองกลุ่มย่อย กลุ่มย่อยแรกแช่น้ำกลั่น อุณหภูมิ 37 องศาเซลเซียส 24 ชั่วโมง และนำไปทดสอบหาค่าความแข็งแรงดึงระดับจุลภาค และกลุ่มย่อยที่สองหลังจากแช่น้ำกลั่น อุณหภูมิ 37 องศาเซลเซียส 24 ชั่วโมง นำไปจำลองการใช้งานด้วยการทำเทอร์โมไซคลิงจำนวน 10,000 รอบ และนำไปทดสอบหาค่าความแข็งแรงดึงระดับจุลภาค

ผลการศึกษา: จากการวิเคราะห์ความแปรปรวนสองทาง (Two-way ANOVA) พบว่า การแปรงฟันด้วยยาสีฟันลดการเสียวฟันที่มีส่วนประกอบของแคลเซียมโซเดียมฟอสโฟซิลิเกต ไม่ส่งผลกระทบต่อค่าความแข็งแรงดึงระดับจุลภาคของสารยึดติดระบบต่างๆ ทั้งหลังการแช่น้ำกลั่น 24 ชั่วโมง และหลังการจำลองการใช้งานด้วยการทำเทอร์โมไซคลิงจำนวน 10,000 รอบ โดยสารยึดติดออพติบอนด์ เอฟแอลมีค่าความแข็งแรงดึงระดับจุลภาคที่วัดค่าทันทีมากกว่าสารยึดติดเคลียร์ฟิลเอสอีบอนด์ และซิงเกิลบอนด์ ยูนิเวอร์แซล อย่างมีนัยสำคัญ ในขณะที่ค่าความแข็งแรงดึงระดับจุลภาคที่วัดค่าทันทีของสารยึดติด เคลียร์ฟิลเอสอีบอนด์ และซิงเกิลบอนด์ ยูนิเวอร์แซล ไม่แตกต่างกันอย่างมีนัยสำคัญ หลังจากจำลองการใช้งานด้วยการทำเทอร์โมไซคลิงจำนวน 10,000 รอบ พบว่า สารยึดติดออพติบอนด์ เอฟแอลมีค่าความแข็งแรงดึงระดับจุลภาคมากกว่าสารยึดติดเคลียร์ฟิลเอสอีบอนด์ และซิงเกิลบอนด์ ยูนิเวอร์แซล อย่างมีนัยสำคัญ และสารยึดติดเคลียร์ฟิลเอสอีบอนด์มีค่าค่าความแข็งแรงดึงระดับจุลภาคมากกว่าซิงเกิลบอนด์ ยูนิเวอร์แซล อย่างมีนัยสำคัญเช่นเดียวกัน นอกจากนี้ยังพบว่า การจำลองการใช้งานด้วยการทำเทอร์โมไซคลิงจำนวน 10,000 รอบ ไม่ได้ส่งผลกระทบต่อค่าความแข็งแรงดึงระดับจุลภาคของสารยึดติดออพติบอนด์ เอฟแอล และ เคลียร์ฟิลเอสอีบอนด์ แต่ส่งผลกระทบต่อค่าดังกล่าวในสารยึดติดซิงเกิลบอนด์ ยูนิเวอร์แซล

สรุป: ยาสีฟันลดการเสียวฟันที่มีส่วนประกอบของแคลเซียมโซเดียมฟอสโฟซิลิเกตไม่มีผลกระทบต่อค่าความแข็งแรงดึงระดับจุลภาคในสารยึดติดออพติบอนด์ เอฟแอล เคลียร์ฟิลเอสอีบอนด์ และ ซิงเกิลบอนด์ ยูนิเวอร์แซล ทั้งค่าที่วัดทันทีและหลังการจำลองการใช้งานด้วยการทำเทอร์โมไซคลิงจำนวน 10,000 รอบ และการบูรณะฟันด้วยสารยึดติดซิงเกิลบอนด์ ยูนิเวอร์แซล หลังแปรงฟันด้วยยาสีฟันลดการเสียวฟันที่มีส่วนประกอบของแคลเซียมโซเดียมฟอสโฟซิลิเกต ค่าความแข็งแรงดึงระดับจุลภาคลดลงอย่างมีนัยสำคัญหลังจากจำลองการใช้งานด้วยการทำเทอร์โมไซคลิงจำนวน 10,000 รอบ

สาขาวิชา ทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม
ลายมือชื่อนิสิต ....
ปีการศึกษา 2563
ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

6075833532 : MAJOR ESTHETIC RESTORATIVE AND IMPLANT DENTISTRY

KEYWORD:

Warin Sittiwaitayaporn : EFFECT OF DESENSITIZING TOOTHPASTE CONTAINING CALCIUM SODIUM PHOSPHOSILICATE ON MICROTENSILE BOND STRENGTH OF ADHESIVE SYSTEMS. Advisor: Assoc. Prof. SIRIVIMOL SRISAWASDI, D.D.S., M.S., Ph.D

Purpose: To evaluate the effect of a desensitizing toothpaste, containing calcium sodium phosphosilicate, on microtensile bond strength of adhesive systems treated to dentine.

Methods: Fifty-two human third molars were embedded into acrylic resin, and cut to expose flat dentin surface. The specimens were randomly divided into two groups, 1) no brushing, and 2) brushing with Sensodyne Repair&Protect (GSK, London, UK) for 10,000 cycles with a V-8 cross brushing machine (Sabri Dental Enterprise, Inc., USA). Subsequently, both groups were divided into three groups for resin composite build-up using different adhesive agents (OptiBond FL® (Kerr, Orange, CA, USA), Clearfil SE Bond® (Kuraray Medical Inc, Japan), Single Bond Universal® (3M ESPE, USA)). All samples were subsequently sectioned to obtain microtensile test specimen, after which the sectioned sticks in the same tooth were divided into two subgroups: 1) microtensile bond strength test, and 2) thermocycling for 10,000 cycles, followed by microtensile bond strength test.

Results: Two-way ANOVA revealed that μTBS values of each adhesive system was not significantly affected by brushing with desensitizing toothpaste containing calcium sodium phosphosilicate. After brushing with desensitizing toothpaste containing calcium sodium phosphosilicate, OptiBond FL® had a significant highest μTBS value. Clearfil SE Bond® showed no significant different immediate μTBS value compared to Single Bond Universal®, but showed a significant higher μTBS value than Single Bond Universal® after 10,000-cycle thermocycling. In addition, 10,000-cycle thermocycling significantly decreased the μTBS value of Single Bond Universal® after brushing.

Conclusion: Desensitizing toothpaste containing calcium sodium phosphosilicate had no effect on OptiBond FL® Clearfil SE Bond® and Single Bond Universal® adhesive in both immediate μTBS or after 10,000-cycle thermocycling. In addition, 10,000-cycle thermocycling significantly reduced μTBS value of Single Bond Universal® brush group.

Field of Study:	Esthetic Restorative and Implant Dentistry	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....

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Warin Sittiwaitayaporn

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CHAPTER I INTRODUCTION

Rationale and Significance of the Problem

Tooth hypersensitivity is characterized by short sharp pain arising from exposed dentin in response to stimuli such as thermal, evaporative, tactile, osmotic pressure or chemicals(1, 2), usually found in a tooth where underlying dentin has been exposed.(3, 4) Using desensitizing toothpaste is one of the treatments for tooth hypersensitivity. The advantage of using desensitizing toothpastes was that they were immediately available for treatment when compared with agents applied by a professional.(5) Their function was either to block pulp nerve response or occlude opened dentine tubules.(6, 7) To block the nerve, some products contained potassium salts, which were thought to diffuse inside the dentinal tubules and lower the excitability of pulpal nerve fibers.(8) Occlusive therapies for the treatment of dentinal hypersensitivity are frequently used. It was believed that sealing dentinal surface subsided movement of fluid inside the tubules and reduced the sensitivity.(5) Strontium salt provided layers of deposited small particles to block the opened dentinal tubules.(9) However, several clinical trials failed to demonstrate the superior efficacy of strontium-based formulations containing silica over that of conventional fluoridated toothpaste.(10, 11) There was also a study reporting that arginine-calcium carbonate desensitizing paste provided complete occlusion of open dentinal tubules.(12) There were also in vitro and clinical studies showing that arginine-calcium carbonate toothpastes reduced sensitivity.(6, 12, 13) Recently, a component of calcium sodium phosphosilicate has been introduced. It has been used as a component in dentifrice to provide relief from dentine hypersensitivity. Several studies have shown that dentifrice containing calcium sodium phosphosilicate formed a deposit over dentine and in the tubules. When calcium sodium phosphosilicate was exposed to saliva, calcium and phosphate ions were released from particles, pH was increased to facilitate the precipitation of calcium and phosphate from the particles and from saliva to form a calcium phosphate layer on tooth surfaces, or into tubules. This layers crystalized into hydroxycarbonate apatite-like deposits, which were chemically and structurally similar to mineral found in tooth.(14, 15) The study demonstrated that deposition of calcium sodium phosphosilicate on dentine was more acid-resistant and showed better dentinal tubule occlusion and retention than the application of arginine-containing toothpaste.(16) However, topical desensitizing agent had a temporary effect on occluding the dentinal tubule. If sensitivity persisted or the lesion became more extensive, stronger and more adhesive materials were preferred for longer-lasting desensitization.(17) When extended to consider restorative strategies, resin-based composite restoration has been a preferable choice based on its excellent esthetic properties and good clinical performance in studies one year or more in duration.(3, 18) A study showed that using dentifrice was significantly less effective in reducing sensitivity than sealant and the restorative treatment, either in clinical or reported patients.(3) When long-term desensitization using toothpaste fails as the tooth surface loss becomes extensive, definitive restoration of the hypersensitive area using resin composite may be needed. Previous studies showed that the use of desensitizing toothpaste resulted in occlusion of the dentinal tubules, which might affect bonding performance of subsequent restoration.(18, 19) A study found that microtensile bond strength of adhesive to dentin specimens treated with arginine or strontium acetate desensitizing toothpaste was significantly lower than that of regular toothpaste when using a three-step etch-and-rinse and a self-etch bonding agent.(19) On the other hand, another study showed that prolonged use of desensitizing toothpaste containing 8% arginine/calcium carbonate, 8% strontium acetate and 5% calcium sodium phosphosilicate did not influence the bond strength of a self-etching adhesive system to dentin.(20) Even though there have been studies concerning the effect of desensitizing toothpaste on dental adhesives, focus on the effect of calcium sodium phosphosilicate remains scarce. Therefore, the objective of this study was to evaluate the effect of desensitizing toothpaste containing calcium sodium phosphosilicate on microtensile bond strengths of various adhesive systems treated to dentin.

Research Question

Would using of desensitizing toothpaste containing calcium sodium phosphosilicate have any effects on microtensile bond strength of adhesive systems treated to dentin?

Research Objectives

To evaluate the effect of a desensitizing toothpaste containing calcium sodium phosphosilicate on microtensile bond strength of adhesive systems treated to dentine.

Hypotheses

Null Hypothesis

1. There was no significant difference in microtensile bond strength of adhesive systems treated to dentin between the groups using desensitizing toothpaste containing calcium sodium phosphosilicate compared to non-brushed group in each adhesive system.
2. There was no significant difference in microtensile bond strength between total etch adhesive systems, self-etch adhesive systems, and universal adhesive systems after treated with desensitizing toothpaste containing calcium sodium phosphosilicate.

3. Microtensile bond strength of adhesive-dentin bond after treated with desensitizing toothpaste was not affected by thermocycling

Alternative hypothesis

1. There was at least one significant difference in microtensile bond strength of adhesive systems treated to dentin between the groups using desensitizing toothpaste containing calcium sodium phosphosilicate compared to non-brushed group.
2. There was at least one significant difference in microtensile bond strength between total etch adhesive systems, self-etch adhesive systems, and universal adhesive systems after treated with desensitizing toothpaste containing calcium sodium phosphosilicate.
3. Microtensile bond strength of the adhesive-dentin bond of adhesive systems after treated by desensitizing toothpaste containing calcium sodium phosphosilicate was affected by thermocycling

Conceptual Framework

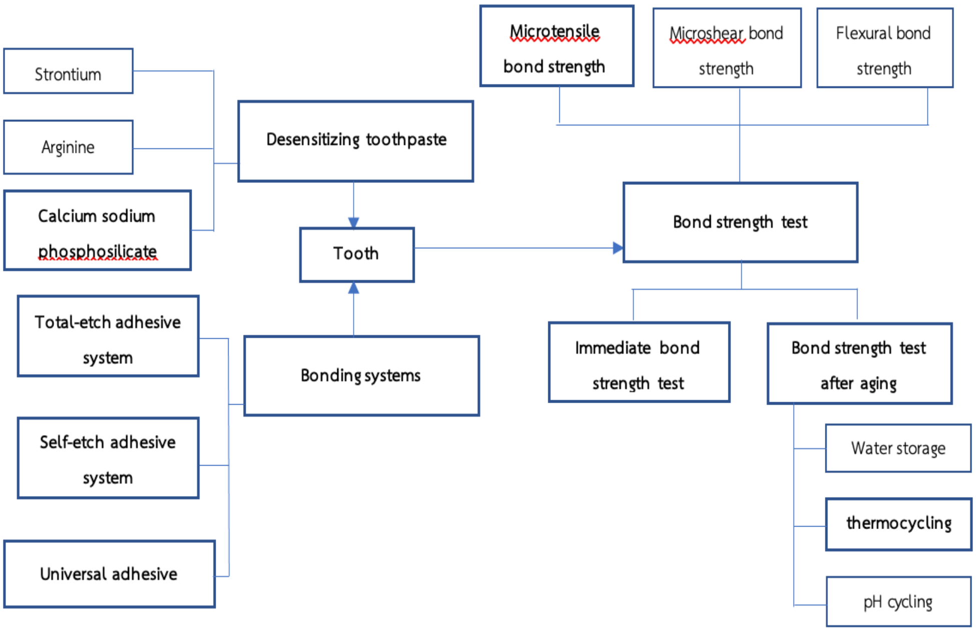


Figure 1 Diagram of Conceptual Framework

Keywords

Bonding agents, Calcium sodium phophosilicate, Desensitizing toothpaste, Microtensile bond strength, Thermocycling

Expected Benefit of the Study

Outcome of the present study may provide useful information concerning the use of dentine bonding agents in teeth that have been treated with dentifrice containing calcium sodium phosphosilicate.

CHAPTER II REVIEW OF THE LITERATURES

The literatures in these following topics have been reviewed.

- Dentin hypersensitivity
- Desensitizing toothpaste:
    - Calcium Sodium Phosphosilicate
- Adhesive systems:
    - Etch and rinse adhesives
    - Self-etch adhesive systems
    - Universal adhesives
- Microtensile Bond Strength Test
- Thermocycling

Dentin hypersensitivity

Dentin hypersensitivity in exposed dentin is a response to stimuli such as thermal, evaporative, tactile, osmotic or chemical. It occurs as a short sharp pain. (1, 2) Hydrodynamic theory is used to explain the dentin hypersensitivity. The theory had showed that when an appropriate stimulus was applied to exposed dentin, there was an increase in the rate of fluid flow in the dentinal tubules which associated with A-beta and A-delta mechano-receptor nerve responses and caused pain.(21, 22) It has been reported that dentin hypersensitivity progresses in two stages. The lesion is first characterized by the exposure of dentin, which is caused by enamel or cementum loss. Second, the smear layer or tubular plugs are removed, exposing the dentinal tubules' outer ends, which subsequently leads to sensitivity.(17, 23)

Managements of dentine hypersensitivity include removal or minimization of etiologic factors and provide the treatment of the sensitivity.(6) Two methods have been used in the treatment of sensitivity which utilized blockage of nerve activity and tubular occlusion. The nerve activity was blocked by direct ionic diffusion, increasing the concentration of potassium ions acting on the pulpal nerve sensorial activity. (5, 6, 21) There are several methods to stop or reduce the fluid flow by occluding the dentinal tubules; such methods are application of high concentration fluoride, oxalate materials, adhesive materials, and desensitizing toothpaste. (1, 6, 8)

Desensitizing toothpaste

Using desensitizing toothpaste is one of the treatments of dentine hypersensitivity. It functions by either blocking the pulp nerve responses or occluding opened dentine tubules. (6, 7) The toothpastes which block the pulp nerve response usually contain potassium salts. It is believed that potassium can diffuse into the dentine tubules and lower the excitability of the pulpal nerve fibers. (8, 24) However, the majority of desensitizing products function by occluding the dentine tubules. It contains a wide variety of active components such as oxalates, strontium-based compounds, citrate-based compounds, arginine-based compounds, and calcium sodium phosphocilicate. (8, 9, 24) Strontium salt-based desensitizing products can be incorporated into tooth. Its ability to be taken up by enamel and dentin has been described. The studies have shown that strontium chloride occluded dentinal tubules and reduced hypersensitivity of the tooth (9, 25) Arginine-based desensitizing product containing arginine and calcium which carbonate worked together in saliva to accelerate the natural mechanisms of occlusion by depositing dentin-like minerals, containing calcium and phosphate, within the dentinal tubules and formed protective layer on dentin surface.(12)

Calcium Sodium Phosphosilicate

Calcium sodium phosphosilicate is a bioactive glass, originally developed as bone-regenerative material which is biocompatible. It is reactive when exposed to body fluids and has desensitizing effect by creating hydroxycarbonate apatite, a mineral that is chemically similar to natural tooth mineral.(15, 26) The chemical reactions initiated by calcium sodium phosphosilicate to promote the formation of a hydroxycarbonate apatite layer for the treatment of dentinal hypersensitivity may also be useful in treating demineralized tooth structure and preventing further demineralization. Moreover, in vitro studies demonstrated that calcium sodium phosphosilicate, alone and in combination with fluoride, enhanced remineralization of enamel and dentin, and prevented demineralization from acid challenges.(15, 27) The toothpaste containing calcium sodium phosphosilicate relieved sensitivity by 18-50% with continuous use after two weeks and 37-72% after six to eight weeks of use.(28-30) In a 12-week clinical study of Sharma et al , twice-daily use of a toothpaste containing 7.5% calcium sodium phosphosilicate reduced 91% of pain from the baseline, and provided lower pain score than the toothpaste with 5% potassium nitrate and a gel containing 0.4% stannous fluoride.(31) Moreover, another study showed that application of a 5% calcium sodium phosphosilicate toothpaste to dentin provided better dentin tubule occlusion and retention than the application of 8% arginine containging toothpaste.(32)

Adhesive systems

Etch and rinse adhesives

Etch and rinse adhesives were begun with an initial acid etching step which demineralized dentin in order to remove the smear layer and unplug the tubules achieving a micro-porous surface with enhanced bonding capacity. (33) Nakabayashi was the first to demonstrate that resins could infiltrate into acid-etched dentin to form a hybrid layer.(34) Etch and rinse adhesive protocols can be either three or two steps depending on chemical composition design.

Three-step etch and rinse adhesive have been the most favorable and reliable for long-term usage.(33, 35) There are three essential steps. First step is acidic conditioning step or etching step with phosphoric acid and acid is totally rinsed off. Second step is to apply the primer ensuring sufficient wetting of resin monomer into the exposed collagen network and removes remaining water. A primer solution contains reactive bifunctional monomers dissolved in organic solvent such as acetone, ethanol or water. The last step is applying the adhesive resin which is essentially solvent-free hydrophobic monomers. The main function of this class of adhesive is to fill up the interfibrillar spaces that have been left between the collagen fibrils. After curing, a hybrid layer and resin tags were created providing micromechanical retention to the restoration. (33)

The two-step etch and rinse systems combine the hydrophilic and hydrophobic monomers with solvents in the same bottle, but still have a separate etching step. Since two-step etch and rinse adhesives have shown to be more hydrophilic in nature compared to three-step systems(36) , they exhibited greater permeability after polymerization, thus facilitating the presence of water-filled areas within hybrid layer.(37)

Self-etch adhesive systems

Self-etch adhesive systems have been established to simplify bonding procedures and utilize smear layer to be part of bonding interface in order not to increase dentin permeability as found in etch and rinse systems. They are considered simplified adhesive materials as they do not require a separate acid conditioning step and moist post-rinse control. This system contained acidic bifunctional monomers which solubilized and primed the tooth at the same time.(38, 39) Self-etch adhesive systems produced a hybridized complex comprising the residual smear layer and a thin, demineralized dentine collagen matrix (40) This system did not remove the smear layer from dentin completely and incorporated smear layer as part of hybrid layer.(41) Other claimed advantages of this system over conventional etch-and-rinse systems including less technical sensitivity and shorter application time.(38, 39, 42-44) However, some studies have shown that self-etch adhesive systems were not able to etch enamel as effectively as the phosphoric acids used in etch-and-rinse adhesive systems due to their lower acidity.(45-48)

Universal adhesives

The multi-mode universal adhesive, which was shown to be a single step self-etch adhesive in nature, has been designed to bond to tooth structures via etch-and-rinse technique or the self-etch technique using the same single bottle of adhesive solution. They may also be used for selective etching technique, which combined the advantages of the etch-and-rinse technique on enamel with the simplified self-etch mode on dentine with probable additional chemical bonding. (40, 49) Similar bond strength values were observed for the universal adhesives regardless of application mode, which made them reliable for working under different clinical conditions. (50) Studies showed that the multi-mode system also showed similar bonding potential when used in the self-etch or etch-and- rinse bonding approaches.(49-51)

Microtensile Bond Strength Test

The microtensile bond strength is calculated as the tensile force at failure divided by the cross-sectional area of bonded interface. Sano et al. introduced microtensile testing to dentistry to measure the ultimate tensile strength and modulus of elasticity of mineralized and demineralized dentin.(52-55) A smaller bonding area reduces the probability of sample internal defects and provides a more homogeneous distribution of stress during loading, thus fewer cohesive failures in substrates occur. Although this bond strength test is technically difficult to measure very low bond strength (<5 MPa), multiple specimens can be obtained from single tooth making it necessary to treat the respective bond strength values as repeated measurements.(54, 55) In the microtensile bond test, the occlusal surface of the tooth was ground flat. The entire surface would be bonded, and a large resin composite was built up. (54) Theoretically, it was not necessary to produce a flat surface using polishing devices. The bonded surfaces could be fractured, polished, or bur cut. (56) Specimens were sectioned into a stick with approximately thickness of

0.5-1.5 mm. Each stick composed of tooth structure and resin composite that bonded together in order to be tested. (53, 54)

Thermocycling

Thermocycling is a laboratory method that simulates aging in oral condition. This method involved subjecting specimens to cycles of temperature changes. High temperatures were known to weaken the composite restoration bonding interface.(57) The ISO TR11450 standard indicated that a thermocycling regimen comprising of 500 cycles in water between 5 and 55°C was an appropriate artificial aging test. Results of previous research showed that 500 thermocycles did not significantly affect the bond strength of composite to dentin surfaces. Literature review showed that 10,000 cycles conformed approximately 1 year of function. (58, 59) A study reported that thermocycling was more effective in degradation of the composite resin restorations than other aging methods, therefore it represented a more challenging condition for the material tested.(60)

CHAPTER III MATERIALS AND METHODS

Research Design

This study was an in vitro experimental study, which compared microtensile bond strengths between dentin that use desensitizing toothpaste containing calcium sodium phosphosilicate and dentin without using desensitizing toothpaste in three adhesive systems.

Research Methodology

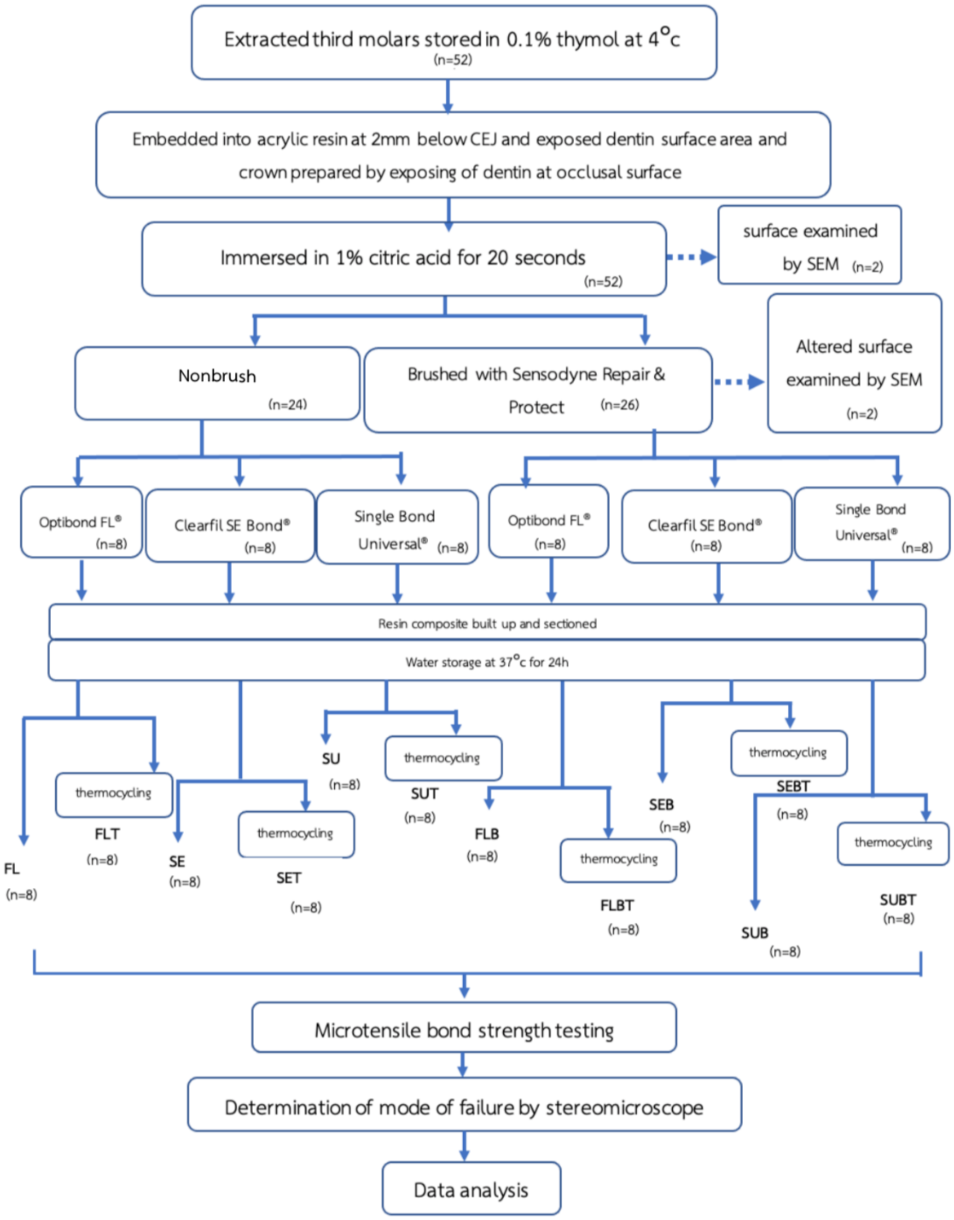


Figure 2 Diagram of study design

Abbreviations

FL= OptiBond FL® nonbrush group, FLT= OptiBond FL® nonbrush group with 10,000-cycle thermocycling, FLB= OptiBond FL® brush group, FLBT= OptiBond FL® brush group with 10,000-cycle thermocycling, SE= Clearfil SE Bond® nonbrush group, SET= Clearfil SE Bond® nonbrush group with 10,000-cycle thermocycling, SEB= Clearfil SE Bond® brush group, SEBT: Clearfil SE Bond® brush group with 10,000-cycle thermocycling, SU= Single Bond Universal® nonbrush group, SUT= Single Bond Universal® nonbrush group with 10,000-cycle thermocycling, SUB= Single Bond Universal® brush group, SUBT= Single Bond Universal® brush group with 10,000-cycle thermocycling.

Sample size description

The mean and standard deviation values for calculation were obtained from the pilot study. The highest number of specimen was calculated from this formula;

$$n = \frac{2\sigma^2 (Z_\alpha + Z_\beta)^2}{(\mu_1 - \mu_2)^2}$$

Where n was sample size estimation (per group)

σ was standard deviation of microtensile bond strength in each group.

σ^2 was calculated from this formula;

$$\sigma^2 = \frac{n_1S_1^2 + n_2S_2^2}{n_1 + n_2}$$

$$\sigma^2 = \frac{4(61.82) + 4(125.25)}{8}$$

$$\sigma^2 = 93.535$$

Z_α was the value of the standardized score cutting off α/2 proportion of each tail of a standard normal distribution (for a two-tailed hypothesis test) (Z=1.96 for **α** = 0.05).

Z_β was the value of the standardized score cutting off the upper proportion (Z_β = 0.84 for **β** = 0.2 = 80% power).

μ was mean microtensile bond strength in each group.

$$n = \frac{2\sigma^2(Z_\alpha+Z_\beta)^2}{(\mu_1-\mu_2)^2}$$

$$n = \frac{2(93.535)(1.96+0.84)^2}{(35.89-32.19)^2}$$

$$n = \frac{2(93.535)(7.84)}{13.69}$$

$$n = 107.13$$

Eight number of specimens in each group were selected for this study according to the study from Armstrong et al.(61)

In addition, two teeth were confirmed of the tubular opening by scanning electron microscope after mimic a dentine hypersensitivity. And two teeth from brushing group were confirmed of the tubular occlusion by a scanning electron microscope. Therefore, the total numbers of specimen were 52.

Table 1 Material, Manufacturer, and Component

Materials	Components
Sensodyne® Repair&Protect (GSK group, New Zealand) (LOT NO: 3120319, 3110719)	Glycerin, PEG-8, Silica, Calcium Sodium Phosphosilicate (NOVAMIN®), Cocamidopropyl Betaine, Sodium Methyl Cocoyl Taurate, Sodium Monofluorophosphate, Aroma, Titanium Dioxide, Carbomer, Saccharin Sodium, Limonene. Contains Sodium Monofluorophosphate 1.08 %w/w (1450ppm Fluoride)
OptiBond FL® (Kerr, Orange, CA, USA) (LOT NO: 7105544))	Etchant: 37.5% phosphoric acid Primer: HEMA, GPDM, PAMM, ethanol, water, photoinitiator Adhesive: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, filler (fumed SiO_2, barium aluminoborosilicat, Na_2SiF_6), coupling factor A174 (approximately 48 wt% filled) photoinitiator
Clearfil SE Bond® (Kuraray Medical Inc, Japan) (LOT NO: 7W0574))	Primer: MDP, HEMA, camphorquinone, hydrophilic dimethacrylate, N, N-diethanol P-toluidine and water. Bond: MDP, BIS-GMA, HEMA, hydrophobic aliphatic dimethacrylate, camphorquinone, N, N-diethanol-P-toluidine, silanized colloidal silica.
Single Bond Universal® (3M ESPE, USA) (LOT NO: 90521B)	Adhesive: 10-MDP, Bis-GMA, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane-treated silica
Premise® (Kerr, Orange, CA, USA) (LOT NO: 7115985)	Filler: Prepolymerized filler (PPF), 30 to 50 µm, Barium glass, 0.4 µm, Silica filler 0.02 µm Resin: Ethoxylated bis-phenol-A-dimethacrylate, TEGDMA, Light-cure initiators and stabilizers

Materials used in this study

1. Thymol 0.1% (M Dent, Bangkok, Thailand)
2. Self-curing resin (Suksapan, Bangkok, Thailand)
3. Citric acid (Chemipan corporation, Bangkok, Thailand)
4. Biotene® (GSK group, New Zealand) (LOT NO: U0C161)
5. Distrilled water (Faculty of Dentistry Chulalongkorn University, Thailand)
6. Model RepairII Blue (Dentsply-Sankin, Ohtawara, Japan) (LOT NO: K990C5)

Instruments used in this study

1. Low-Speed Cutting Machine (Isomet® 1000, Buehler, USA)
2. Universal Testing Machine (EZ-S,Shimadzu, Japan)
3. Stereomicroscope (ML 9300, Meiji Techno Co. Ltd., Japan)
4. Thermo Cycling Unit (KMITL, Bangkok, Thailand)
5. V-8 cross brushing machine (Sabri Dental Enterprise, Inc., USA)
6. Scanning electron microscope (JSM-5410LV, JEOL, Japan
7. LED Light-Curing System (Demi™ Plus, Kerr, USA)

Methods

This study was approved by the ethical committee of the Faculty of Dentistry, Chulalongkorn University, Thailand (approval number: HREC-DCU 2019-041).

Preparation of Specimens

Dentin samples were prepared from 52 permanent third molar teeth extracted with informed consent stored in a 0.1% thymol solution at 4°c and used within 3 months of extraction. Teeth were carefully inspected using a stereomicroscope (ML 9300; Meiji Techno Co. Ltd., Japan) at 40X magnification to ensure that they were free of caries, cracks or restoration. Teeth were embedded in a self-curing resin with their occlusal surfaces exposed parallel to a horizontal plane at 2 mm below the cemento-enamel junction. Occlusal one-third of crown was removed perpendicular to long axis of the tooth using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under running water until the enamel was completely removed. Each tooth was carefully inspected using a stereomicroscope to ensure that it was free of enamel. One percent citric acid solution was used to immerse specimens for 20s and rinsed with distilled water for 20s to open up dentinal tubules to mimic a dentine hypersensitivity scenario. Then, two teeth were confirmed for the tubular opening using a scanning electron microscope (Quanta 250, FEI, Hillsboro, OR, USA).

Brushing Procedure

Teeth were randomly divided into two groups; Group A (n=24): nonbrush (control) Group B (n=26): brushed with Sensodyne Repair&Protect®. In Group B, teeth were brushed with the dentifrice slurries, which were prepared by diluting 2 g of the dentifrice in 15 ml of distilled water. A toothbrush with bristles of medium hardness was applied to the dentin surface at an inclination of about 90° under a constant loading (200 g) using a speed of 250 cycles/min for 2 minutes with a V-8 cross brushing machine (Sabri Dental Enterprise, Inc., USA). Teeth were brushed with tested toothpaste twice a day for ten days. To remove excess slurry or aqueous solution, teeth were rinsed using distilled water for 10s. During the brushing procedure, teeth were immersed in artificial saliva except for when being brushed with the brushing machine. After the brushing procedure, two teeth from brush group were confirmed for the mineral deposit using a scanning electron microscope.

Figure 3 V-8 cross brushing machine (Sabri Dental Enterprise, Inc., USA).

Bonding and restorative procedure

Both nonbrush and brush groups were then divided in to three groups (n=8 per group) for resin composite build-up using different adhesive agents as follows:

OptiBond FL$^{®}$ (Kerr, Orange, CA, USA): 37.5% phosphoric acid etching gel was applied onto prepared dentin and allowed to react for 15s, then the specimens were rinsed thoroughly with water and dried with foam pellets. OptiBond FL$^{®}$ primer was applied with a light scrubbing motion for 15s and gently air-dried for 5s until there was no visible movement of liquid. OptiBond FL$^{®}$ adhesive was then applied uniformly creating a thin coating for 15s, then light cured for 20s using a LED light curing unit (Demi Plus, Kerr Corporation, Orange, CA, USA) with 1,100 mW/cm^2 intensity.

Clearfil SE Bond® (Kuraray Medical Inc, Japan): primer was applied with rubbing motion for 20s, then dried with mild air flow for 10s. After that adhesive was applied and light cured for 20s.

Single Bond Universal® (3M ESPE, USA): adhesive was applied to the prepared tooth with rubbing motion for 20s, then gently air dried for approximately 5s to evaporate the solvent and light cured for 20s.

After bonding procedures, a silicone mold with a 14 x 8 x 4 mm^3 opening at the center was placed on the treated dentin. Resin composite (Premise, Kerr, USA) was built up incrementally to 4 mm in height, 2 mm in each layer, onto the treated dentin. Each increment was light-cured with an LED light curing unit (Demi Plus, Kerr Corporation, Orange, CA, USA) with 1,100 mW/cm^2 intensity for 40s from the top, with light tip held perpendicularly and within 1 mm superior to resin composite. Light output from the light-polymerizing unit was checked using a radiometer (Model 100 Optilux, Kerr, Orange, CA, USA) throughout the experiment.

Microtensile bond strength test

All samples were stored in water at 37°c for 24h, and then mounted onto a low-speed sectioning machine (ISOMET 1000TM, Buehler, USA), which they were subsequently sectioned in order to obtain stick-shaped microtensile specimens. Eight sticks from the middle of dentin portion were selected from each tooth. Every stick was examined using a stereomicroscope at 40X to ensure its homogeneity, without bubbles or cracks, and also to verify the exact dimension. All samples were stored in water at 37°c for 24h. Subsequently, the sectioned sticks in the same tooth were divided into 2 subgroups: 1) microtensile bond strength test and 2) thermocycling for 10,000 cycles between 5 °C and 55 °C for 30s at each temperature. All stick specimens were attached to the test apparatus using a cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Japan) and subjected to microtensile bond strength testing using a universal testing machine (EZ-S; Shimadzu Corporation, Kyoto, Japan) at a cross-head speed of 0.5 mm/min until the bond ruptured. The microtensile bond strength of each specimen was calculated as the ratio of maximum load force at fracture and cross- sectional bonding area, which was measured in each individual fractured specimen. Specimens with pretest failure were calculated as mean between 0 MPa and the lowest measured value in the specific experimental group.(61)

Figure 4 Tested microtensile specimen mounted onto a universal testing machine (EZ-S; Shimadzu Corporation, Kyoto, Japan)

Fracture Mode analysis

Fracture mode analysis of the bonded dentin surface was performed using a stereomicroscope at 40X magnification. Failure mode were classified as follow;

- Adhesive failure: fracture occurred in adhesive layer or where adhesive completely remained on top of dentin surface or resin composite (75% of failure between resin/dentin interface
- Cohesive failure in dentin: >75% of fracture or failure occurred within dentin
- Cohesive failure in restoration: >75% of fracture or failure occurred within the resin composite
- Mixed failure: failure at resin/dentin interface that included cohesive failure of the neighboring substrates

Data Collection and Analysis

All data of microtensile bond strength was analyzed statistically using a two-way ANOVA, a Tukey’s (HSD) test and a paired sample t-test, with significance set at $p < 0.05$. All statistical analyses were performed using a SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

CHAPTER IV RESULTS

The μTBS values of all experimental groups were normally distributed ($p > 0.05$). Mean μTBS values and standard deviations of both brush and nonbrush groups in each adhesive system at 24 hours and after 10,000 cycles of thermocycling were summarized in Table 2. Two-way ANOVA revealed that μTBS values of each of adhesive system were not significantly affected by brushing with desensitizing toothpaste containing calcium sodium phosphosilicate at both 24-hour water storage ($p=0.857$) and 10,000-cycle thermocycling ($p=0.787$). On the other hand, types of adhesive had a statistically significant effect on μTBS values ($p<0.001$) as shown in Table 3 and 4. According to Tukey's (HSD) test, OptiBond FL® brush groups (FLB,FLBT) gave significant higher μTBS values than Clearfil SE Bond® brush groups (SEB,SEBT) ($p=0.036$,$p<0.001$) and Single Bond Universal® brush groups (SUB,SUBT) ($p=0.011$, $p<0.001$) at both 24-hour water storage and 10,000-cycle thermocycling. Although, SEB showed no significant difference in μTBS values to SUB group ($p=0.853$) (Table5), SEBT had a statistically significant higher μTBS values than SUBT group (p=0.038) (Table6). In addition, 10,000-cycle thermocycling did not significantly affect the μTBS values in OptiBond FL® groups (FLB, FLBT) (p=0.061) and Clearfil SE Bond® groups (SEB,SEBT) (p=0.168). In contrast, it significantly affected the μTBS values in Single Bond Universal® group (SUB,SUBT). (p=0.043) (Table 7). Failure modes were given by group in Figure 5. Adhesive failure was noticed to be a major finding in all testing groups. No pre-test failure was recorded for any other adhesives tested.

SEM image of dentin at 10000x magnification were shown in figure 6. Picture (a) showed opened dentinal tubule after immersing in a 1% citric acid. Picture (b) showed dentinal tubules occluded with deposits after brushing with desensitizing toothpaste containing calcium sodium phosphosilicate for 10,000 cycles with V-8 cross brushing machine. All arrows indicated dentinal tubules.

Table 2 μTBS values of brush and nonbrush groups in 24-hour water storage and 10,000-cycle thermocycling (means ± standard deviations (MPa) of the different experimental groups.

GROUP	24-HOUR	10,000-CYCLE THERMOCYCLING (T)
FL	$33.05\pm5.37^{A,1}$	$32.08\pm4.97^{a,1}$
FLB	$34.58\pm4.36^{A,1}$	$32.04\pm2.52^{a,1}$
SE	$28.36\pm3.20^{B,1}$	$27.69\pm4.75^{b,1}$
SEB	$30.23\pm3.43^{B,C,1}$	$28.94\pm3.96^{b,1}$
SU	$26.57\pm3.99^{D,1}$	$21.66\pm5.59^{c,2}$
SUB	$27.39\pm4.63^{C,D,1}$	$23.60\pm1.88^{c,2}$

* Similar superscripts capital letters indicate no significant differences between groups at 24-hr (left columns), similar superscript lowercase letters indicate no significant differences between groups after 10,000-cycle thermocycling (right columns), and similar superscript numbers indicate no significant differences between adhesive systems within each group (rows) according to Tukey's (HSD) test ($p > 0.05$)

Table 3 Two-way ANOVA showed the significant effects of types of adhesives systems and the effect of brushing and the interaction factor ANOVA, Analysis of variance; tested adhesives at 24-hour water storage.

Source of variation	Sum of squares	Df	Mean square	F	P
Adhesive factor	384.143	2	192.072	9.901	<0.001
Brushing factor	0.640	1	0.640	0.033	0.857
interaction	22.064	2	11.032	0.569	0.571

Table 4 Two-way ANOVA showed the significant effects of types of adhesives systems and the effect of brushing and the interaction factor ANOVA, Analysis of variance; tested adhesives at 10,000-cycle thermocycling.

Source of variation	Sum of squares	Df	Mean square	F	P
Adhesive factor	712.115	2	356.057	25.802	<0.001
Brushing factor	1.019	1	1.019	0.074	0.787
interaction	44.921	2	22.460	1.628	0.209

Table 5 Tukey's (HSD) test showed the significant μTBS values between adhesives systems at 24-hour water storage.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
FLB	SEB	5.98531	2.23411	0.036
	SUB	7.19000	2.23411	0.011
SEB	FLB	-5.98531	2.23411	0.036
	SUB	1.20469	2.23411	0.853
SUB	FLB	-719000	2.23411	0.011
	SEB	-1.20469	2.23411	0.853

Table 6 Tukey's (HSD) test showed the significant μ TBS values between adhesives systems at 10,000-cycle thermocycling.

(I) Groups	(J) Groups	Mean Difference(I-J)	Std. Error	Sig
FLBT	SEBT	5.88625	.96564	<0.001
	SUBT	8.44437	.96564	<0.001
SEBT	FLBT	-5.88625	.96564	<0.001
	SUBT	2.55813	.96564	0.038
SUBT	FLBT	-8.44437	.96564	<0.001
	SEBT	-2.55813	.96564	0.038

Table 7 Paired simple T-test showed significant effect of 100,000-cycle thermocycling in each adhesive system.

	Mean	Std. Deviation	Std. Error Mean	95% Confidence interval of difference		t	df	Sig.(2-tailed)
				Lower	Upper			
FLB-FLBT	2.53250	3.20517	1.13320	-.14709	5.21209	2.235	7	.061
SEB-SEBT	2.43344	4.47721	1.58293	-1.30960	6.17648	1.537	7	.168
SUB-SUBT	3.78688	4.33140	1.53138	.16573	7.40802	2.473	7	.043

Figure 5 Failure modes of three types of adhesive systems bonded to dentin at 24-hour water storage and after 10,000-cycle of thermocycling (T).

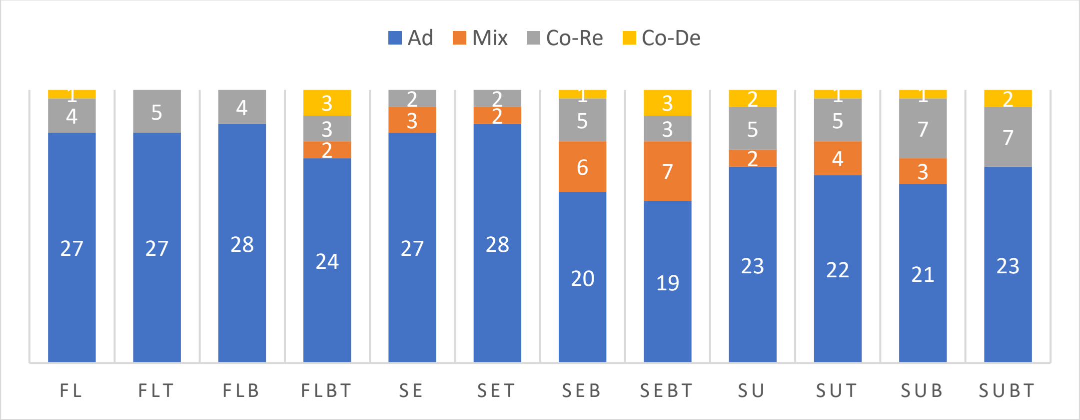
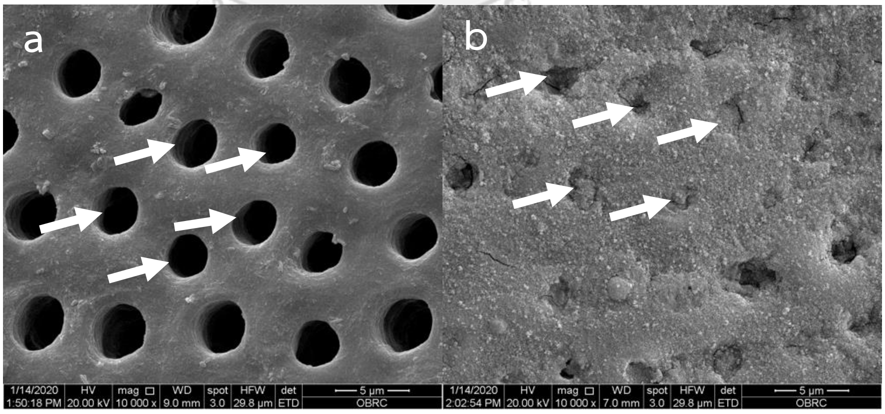


Figure 6 Representative SEM micrograph of dentinal tubule (a) after immersion in 1% citric acid revealing opening of dentinal tubules and (b) after brushing with Sensodyne Repair&Protect® 10,000 cycles with V-8 cross brushing machine (Sabri Dental Enterprise, Inc., USA) showing some deposits on dentin and in tubules. All arrows indicated dentinal tubules. (10000x magnification).



CHAPTER V DISCUSSION AND CONCLUSIONS

Discussion

This study evaluated the effect of a desensitizing toothpaste containing calcium sodium phosphosilicate on microtensile bond strength of adhesive systems treated to dentine. This desensitizing toothpaste released sodium, calcium, and phosphate ions, which consequently interacted with oral fluids and formed crystalline hydroxycarbonate apatite-like deposits, chemically and structurally similar to natural tooth mineral.(15) A previous study revealed that the mineral deposits formed by calcium sodium phosphosilicate desensitizing toothpastes were unstable(62) and not strong enough to affect the formation of the hybrid layer resulting in no interference on bond strength.(63) This was in agreement with the present study which showed that there was no significant difference in microtensile bond strength of adhesive systems treated to dentin between the groups using desensitizing toothpaste containing calcium sodium phosphosilicate compared to nonbrush group in each adhesive system. Therefore, the first null hypothesis was accepted. It could be explained that phosphoric acid from OptiBond FL® bonding system probably dissolved the calcium phosphate deposits covering the dentin leading to reopening of tubules allowing infiltration of resin monomers, favoring the micromechanical bonding.(62) However, the other two adhesive systems in this study were Clearfil SE Bond® and Single Bond Universal®, which were less acidic when compared to phosphoric acid. Since both systems did not entirely remove the smear layer from dentin and incorporated smear layer as part of hybrid, the mineral deposits accumulated on the dentin surface were unable to act as a physical barrier and compromised dentin hybridization. In agreement with the study by Aguiar et al., which showed that prolonged use of a desensitizing toothpaste containing 5 % calcium sodium phosphosilicate had no influence on bond strength of a self-etching adhesive system to dentin.(20)

As a result, OptiBond FL® brush groups gave significant higher μTBS values than Clearfil SE Bond® brush groups and Single Bond Universal® brush groups at both 24-hour water storage and 10,000-cycle thermocycling. Moreover, Clearfil SE Bond® brush groups had a statistically significant higher μTBS values than Single

Bond Universal® brush groups after 10,000-cycle thermocycling. Therefore, the second null hypothesis that there was no significant difference in μTBS between adhesive systems after treated with desensitizing toothpaste containing calcium sodium phosphosilicate was rejected.

Besides, desensitizing toothpaste containing calcium sodium phosphosilicate had no influence on 24-hour microtensile bond strength test results of OptiBond FL®, Clearfil SE bond® and Single Bond Universal®. Meanwhile, there was a significant decrease in microtensile bond strength of Single Bond Universal® after 10,000-cycle thermocycling in this study. Therefore, the third null hypotheses that microtensile bond strength of adhesive-dentin bond after treated with desensitizing toothpaste was not affected by thermocycling was rejected. Due to different compositions, universal bonding contained mixtures of hydrophilic and hydrophobic components within the same solution which exhibited residual solvents entrapped in the adhesive layer and might increase the permeability of the adhesive layer after polymerization leading to compromised long-term performance.(64) In addition, it was found that MDP chemically bonds to hydroxyapatite resulting in formation of

MDP-Ca salt which contribute to better bond stability.(65) Although Clearfil SE Bond® and Single Bond Universal® contain MDP as a functional monomer, they have different concentration. It has been reported that the purity of MDP and its concentration in the adhesive had crucial influence on the bonding potential.(66, 67) Previous study reported that higher purity MDP was used in Clearfil SE Bond®.(67) According to a study by Yoshida et al., it was found that Clearfil SE Bond®, containing MDP in both primer and adhesive, showed more MDP-Ca salt formation than Single Bond Universal® because of the higher concentration of MDP. As a previous study revealed that the higher the concentration of MDP, the more nano-layering intensity was found.(65) Moreover, Single Bond Universal® composed of polyalkenoic-acid copolymer which have been reported to interfere with nano-layering as it competed to react with the same calcium ion depleted from hydroxyapatite as 10-MDP.(65, 68) In addition, Nano-layering was discovered not only within the hybrid layer but also extending into adhesive layer in Clearfil SE Bond®. In Single Bond Universal®, it was found particularly near the dentinal tubule.(65)

Moreover, SEM image of dentin showed dentinal tubules occluded with deposits after brushing with desensitizing toothpaste containing calcium sodium phosphosilicate for 10,000 cycles. However, compositional analysis was not done to identify the nature of mineral occluded in tubules. Previous study revealed that EDX analysis of teeth brushed with calcium sodium phosphosilicate toothpaste showed high amounts of calcium, phosphate and small amount of silica and titanium at dentine surfaces and tubules.(8, 14) Study by Li et al. also reported that the formation of calcium phosphate as well as calcium fluoride could occur in fluoridated toothpaste. In addition, the abrasive component in toothpastes may help to form smear layers, varied widely in composition, and composed mainly of toothpaste abrasives, on dentin after brushing.(69)

In this present investigation, microtensile bond strength test was used because small-sized bonding area could reduce probability of sample internal defects and provided a more homogenous distribution of stress during loading, therefore, fewer cohesive failures in substrates occured.(54, 55) As seen in this study most failure modes were observed at the adhesive interface which indicated that the value measured when specimen cracked represented a more reliable microtensile strength in nature.(70, 71) Moreover, thermocycling was performed at 10,000 cycles in this study to simulate approximately 1 year of clinically oral function.(59) Study by Ozcan et al. found that this method was appropriate in inducing degradation of the composite resin restorations compared to other aging methods. Therefore, it represented a more challenging condition for the material tested.(60) Furthermore, no pretest failure was found.

Limitations

1. This study was an in vitro study. Therefore, the results of this study may not be totally inferred to the clinical situation.

2. This study focused on a particular brand of each adhesive system, therefore, the results of this study may not be inferred to other products.

Conclusion

Desensitizing toothpaste containing calcium sodium phosphosilicate had no effect on OptiBond FL® Clearfil SE Bond® and Single Bond Universal® in both immediate microtensile bond strength and bond strength after aging by 10,000-cycle thermocycling. In addition, 10,000-cycle thermocycling significantly reduced microtensile bond strength of Single Bond Universal® adhesive after treated with desensitizing toothpaste containing calcium sodium phosphosilicate.

Clinical implication

Restorative treatment using etch and rinse, self-etch, and universal adhesive systems may be used for longer-lasting desensitization as the lesion becomes more extensive and has to be restored in a patient with tooth hypersensitivity who has been using long-term desensitizing toothpaste containing calcium sodium phosphosilicate.

Declaration of Conflicting interest

The authors declare that there is no conflict of interest.

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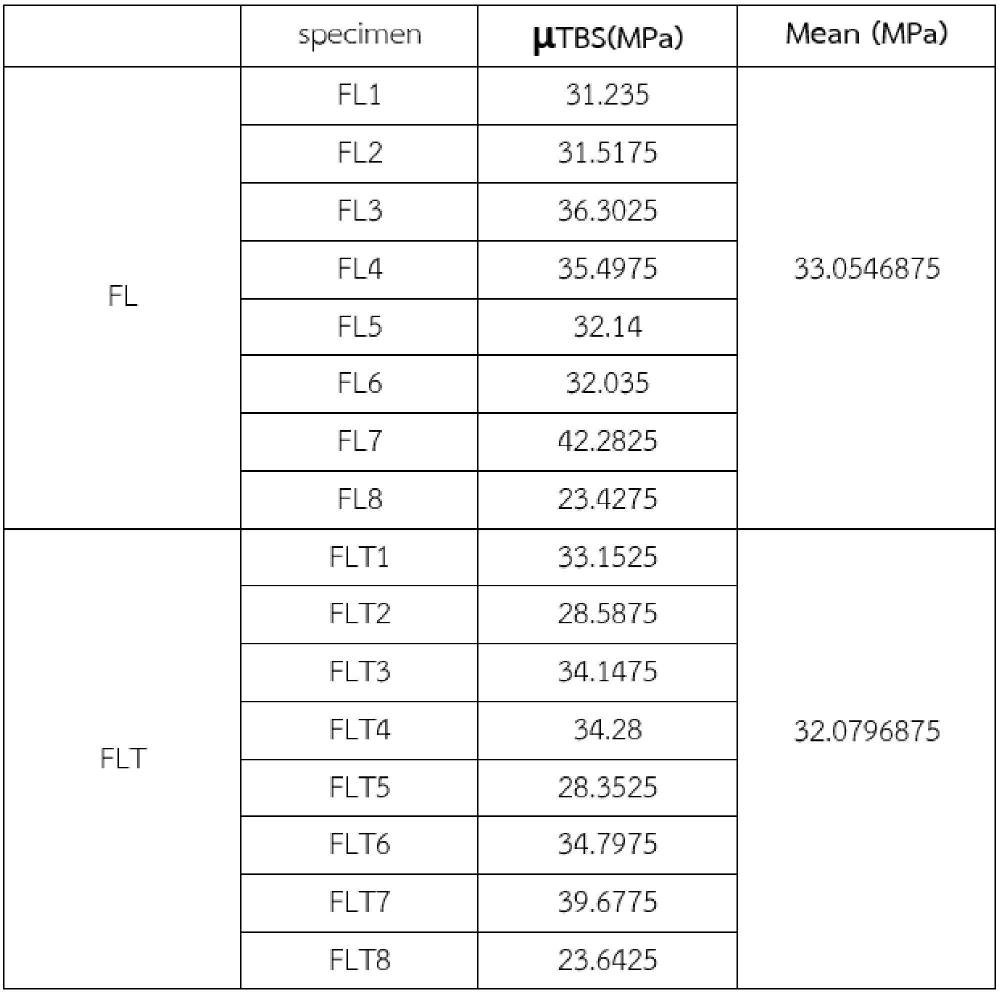
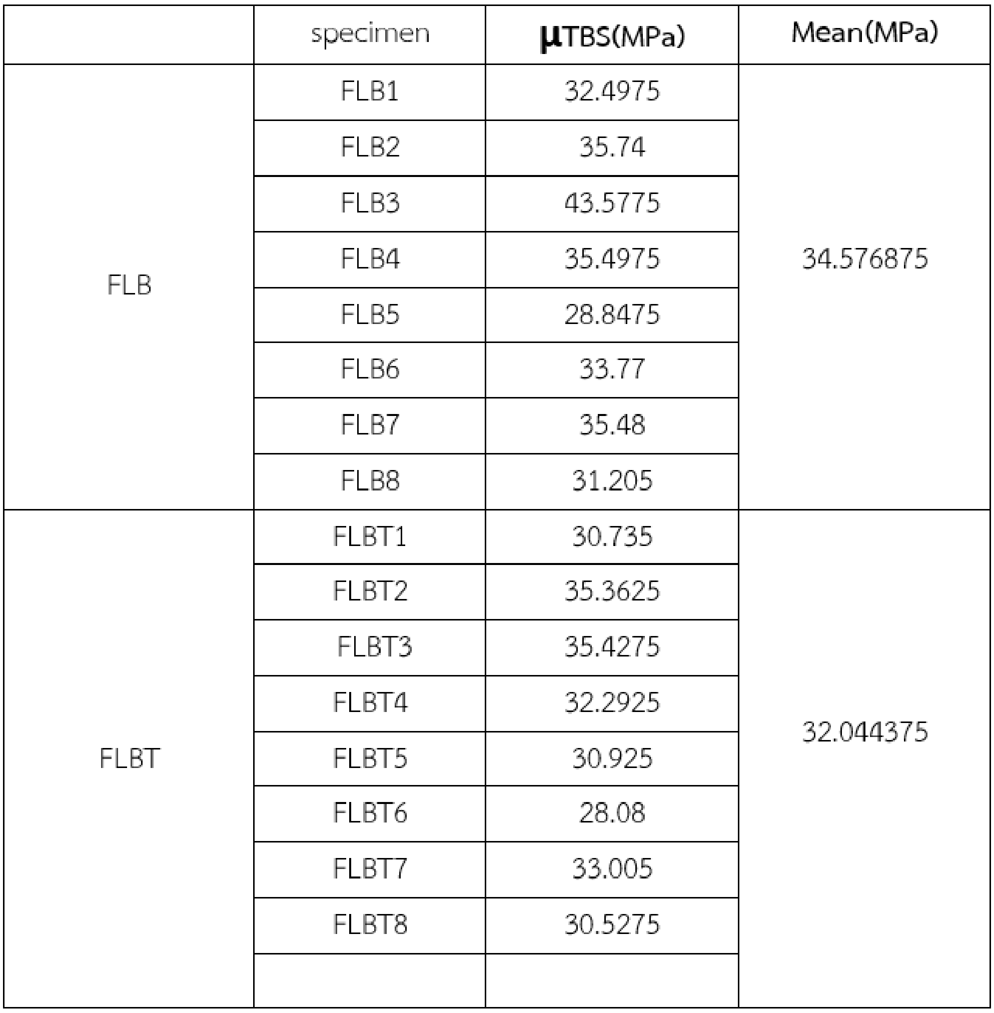
Appendices

Appendix A : μTBS values of nonbrush groups of OptiBond FL® in 24-hour water storage and 10,000-cycle thermocycling



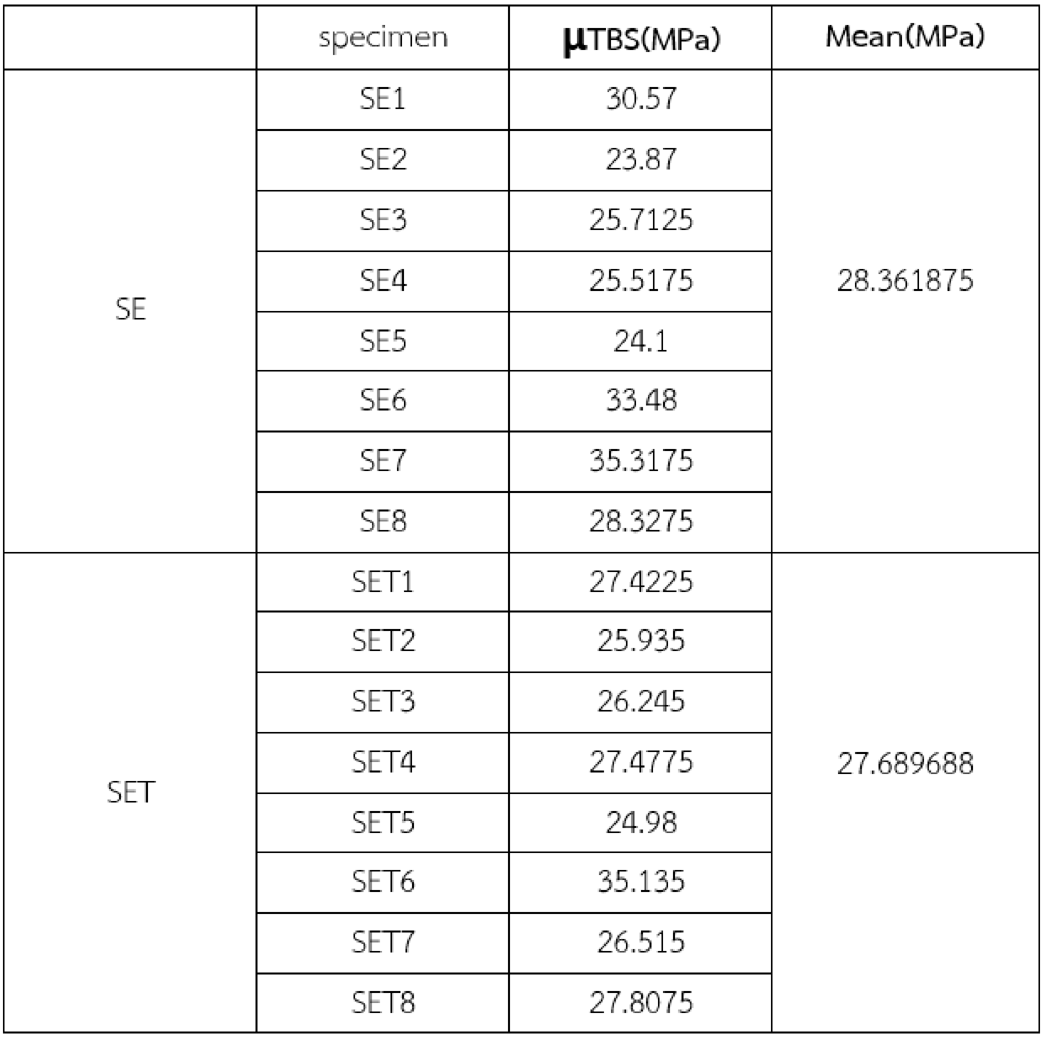
	specimen	μTBS(MPa)	Mean (MPa)
FL	FL1	31.235	33.0546875
	FL2	31.5175	
	FL3	36.3025	
	FL4	35.4975	
	FL5	32.14	
	FL6	32.035	
	FL7	42.2825	
	FL8	23.4275	
FLT	FLT1	33.1525	32.0796875
	FLT2	28.5875	
	FLT3	34.1475	
	FLT4	34.28	
	FLT5	28.3525	
	FLT6	34.7975	
	FLT7	39.6775	
	FLT8	23.6425	

Appendix B : μTBS values of brush groups of OptiBond FL® in 24-hour water storage and 10,000-cycle thermocycling



	specimen	μTBS(MPa)	Mean(MPa)
FLB	FLB1	32.4975	34.576875
	FLB2	35.74	
	FLB3	43.5775	
	FLB4	35.4975	
	FLB5	28.8475	
	FLB6	33.77	
	FLB7	35.48	
	FLB8	31.205	
FLBT	FLBT1	30.735	32.044375
	FLBT2	35.3625	
	FLBT3	35.4275	
	FLBT4	32.2925	
	FLBT5	30.925	
	FLBT6	28.08	
	FLBT7	33.005	
	FLBT8	30.5275	

Appendix C: μTBS values of nonbrush groups of Clearfil SE Bond® in 24-hour water storage and 10,000-cycle thermocycling



	specimen	**μTBS(MPa)**	**Mean(MPa)**
SE	SE1	30.57	28.361875
	SE2	23.87	
	SE3	25.7125	
	SE4	25.5175	
	SE5	24.1	
	SE6	33.48	
	SE7	35.3175	
	SE8	28.3275	
SET	SET1	27.4225	27.689688
	SET2	25.935	
	SET3	26.245	
	SET4	27.4775	
	SET5	24.98	
	SET6	35.135	
	SET7	26.515	
	SET8	27.8075	

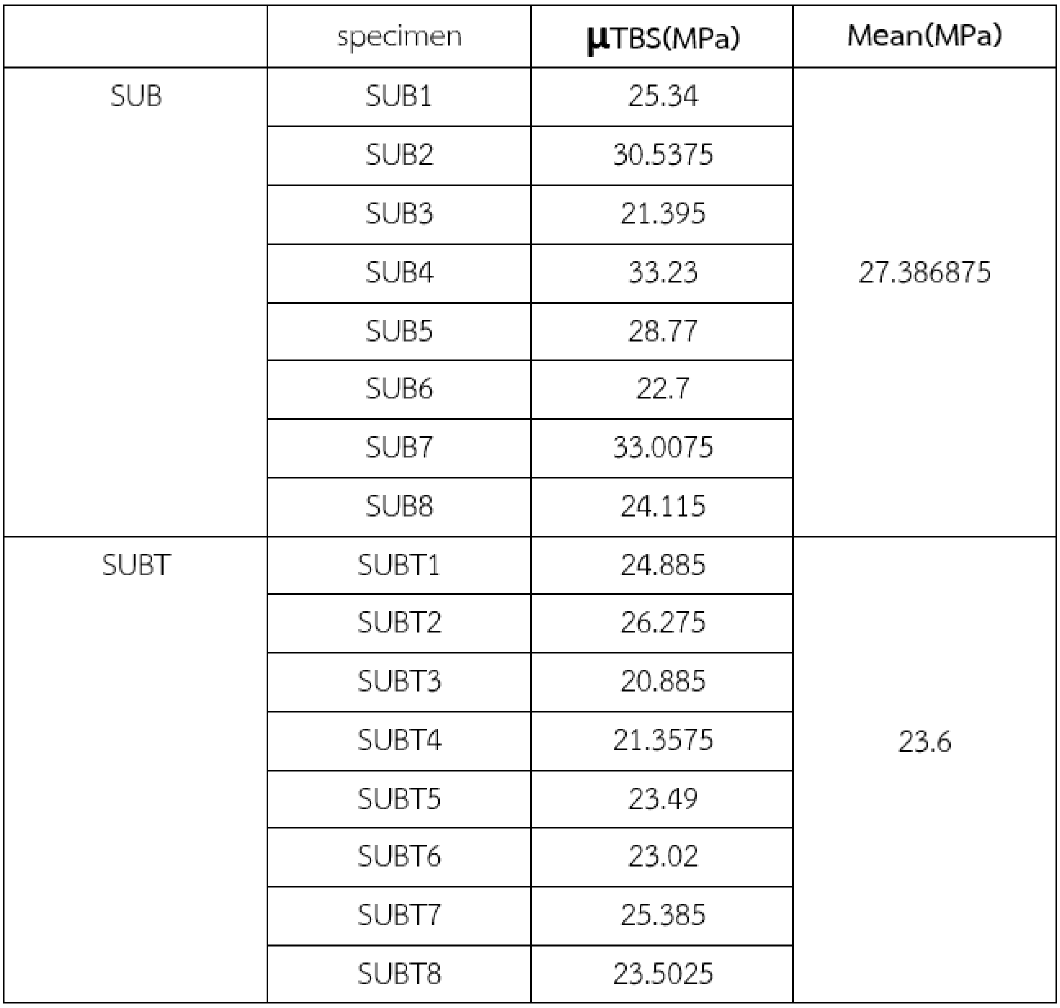
Appendix D: μTBS values of brush groups of Clearfil SE Bond® in 24-hour water storage and 10,000-cycle thermocycling

	specimen	μTBS(MPa)	Mean(MPa)
SEB	SEB1	27.7325	30.2340625
	SEB2	30.765	
	SEB3	35.0775	
	SEB4	28.995	
	SEB5	35.55	
	SEB6	29.77	
	SEB7	26.215	
	SEB8	27.7675	
SEBT	SEBT1	22.6775	28.936875
	SEBT2	24.92	
	SEBT3	28.9325	
	SEBT4	33.26	
	SEBT5	34.3125	
	SEBT6	29.525	
	SEBT7	30.76	
	SEBT8	27.1075	

Appendix E: μTBS values of nonbrush groups of Single Bond Universal® in 24-hour water storage and 10,000-cycle thermocycling

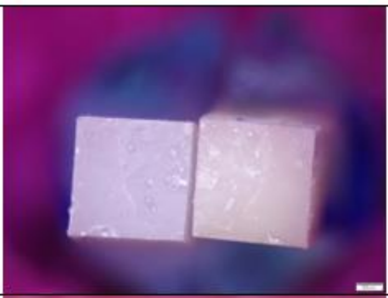
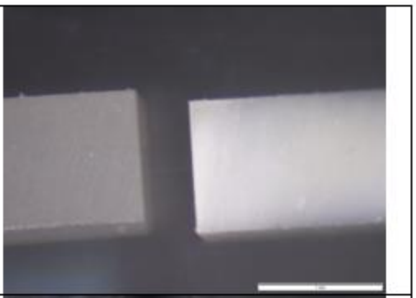
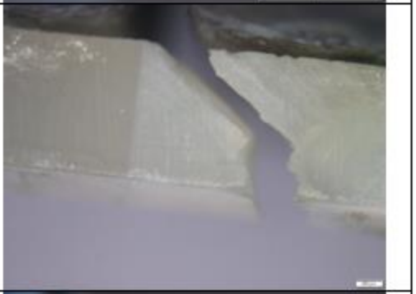
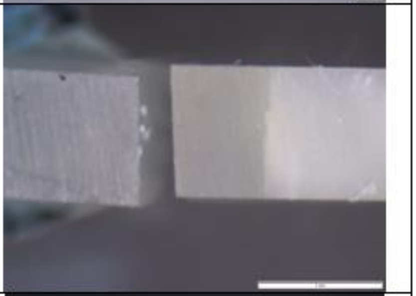
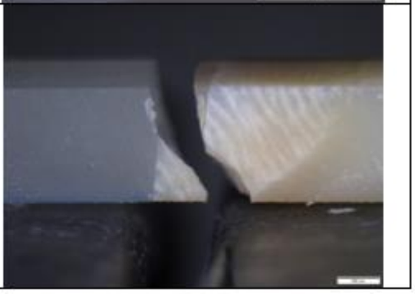
	specimen	μTBS(MPa)	Mean(MPa)
SU	SU1	31.17	26.57375
	SU2	32.4525	
	SU3	29.5325	
	SU4	23.6225	
	SU5	26.3925	
	SU6	22.68	
	SU7	22.2625	
	SU8	24.4775	
SUT	SUT1	23.7075	21.6603125
	SUT2	28.33	
	SUT3	30.9325	
	SUT4	18.155	
	SUT5	15.0475	
	SUT6	18.8525	
	SUT7	17.2175	
	SUT8	21.04	

Appendix F: μTBS values of brush groups of Single Bond Universal® in 24-hour water storage and 10,000-cycle thermocycling



	specimen	**μTBS(MPa)**	**Mean(MPa)**
SUB	SUB1	25.34	27.386875
	SUB2	30.5375	
	SUB3	21.395	
	SUB4	33.23	
	SUB5	28.77	
	SUB6	22.7	
	SUB7	33.0075	
	SUB8	24.115	
SUBT	SUBT1	24.885	23.6
	SUBT2	26.275	
	SUBT3	20.885	
	SUBT4	21.3575	
	SUBT5	23.49	
	SUBT6	23.02	
	SUBT7	25.385	
	SUBT8	23.5025	

Appendix G: failure mode

Adhesive failure		
Cohesive failure in dentin		
Cohesive failure in restoration		
Mixed failure		

Appendix H: raw data from pilot study

specimen	**μTBS(MPa)**	**Mean(MPa)**
Nonbrush1	27.7	35.89
Nonbrush2	31.92	
Nonbrush3	38.17	
Nonbrush4	45.76	
Brush1	45.05	32.19
Brush2	37.39	
Brush3	19.97	
Brush4	26.36	

$$S_1^2 = \frac{\sum(x-\bar{x})^2}{n-1}$$

$$S_1^2 = \frac{67.08 + 15.76 + 5.20 + 97.42}{3-1}$$

$$S_1^2 = 61.82$$

$$S_2^2 = \frac{\sum(\mathrm{x}-\bar{\mathrm{x}})^2}{n-1}$$

$$S_2^2 = \frac{165.38 + 27.04 + 149.33 + 33.99}{3-1}$$

$$S_2^2 = 125.25$$

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