EFFECT OF DESENSITIZING TOOTHPASTE ON MICROTENSILE BOND STRENGTH BETWEEN RESIN COMPOSITE AND DENTIN

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ผลของยาสีฟันลดเสียวฟันต่อความแข็งแรงดึงระดับจุลภาคระหว่างเรซินคอมโพสิตและเนื้อฟัน

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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กชนิภา สายโสภา : ผลของยาสีฟันลดเสียวฟันต่อความแข็งแรงดึงระดับจุลภาคระหว่างเรซินคอมโพสิตและเนื้อฟัน. (EFFECT OF DESENSITIZING TOOTHPASTE ON MICROTENSILE BOND STRENGTH BETWEEN RESIN COMPOSITE AND DENTIN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ทญ. ดร. ศิริวิมล ศรีสวัสดิ์, 85 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินและเปรียบเทียบผลของยาสีฟันลดเสียวฟันสองชนิดกับยาสีฟันฟลูออไรด์ปกติ ต่อกำลังแรงยึดแบบดึงระดับจุลภาคของสารยึดติดต่อเนื้อฟัน

วัสดุและวิธีการ ฟันตัดวัวจำนวน 40 ซี่ นำมาขัดให้เรียบเพื่อให้เผยเนื้อฟันด้านริมฝีปาก แล้วแบ่งเป็น 4 กลุ่มตามยาสีฟันที่ใช้ดังนี้ 1) เซนโซดายน์ แรปิดรีลิฟ 2) คอลเกตเซนซิทิฟโปรรีลิฟ 3)คอลเกตรสยอดนิยม และ 4) แช่ในน้ำลายเทียม (กลุ่มควบคุม) แปรงฟันในกลุ่มที่ 1-3 โดยใช้ยาสีฟันตามกลุ่มที่กำหนดภายใต้การกดนำหนักที่คงที่ (200 กรัม) 250 ช่วงชักต่อนาที เป็นเวลา 2 นาที 2 ครั้งต่อวัน เป็นเวลาสามวัน จากนั้นแบ่งฟันในแต่ละกลุ่มอีกครั้งเพื่อการก่อเรซินคอมโพสิต โดยใช้สารยึดติดดังนี้ 1) ออพติบอนด์ เอกซ์ทีอาร์ หรือ 2) ออพติบอนด์ เอฟแอล หลังจากบ่มสารยึดติดก่อด้วยคอมโพสิตชนิดบ่มตัวด้วยแสง (พรีมิส) นำฟันตัวอย่างที่เสร็จแล้วมาตัดเป็นชิ้นตัวอย่างจำนวน 4 ชิ้น แต่ละชิ้นหนา 1±0.1 มิลลิเมตร นำไปทดสอบกำลังแรงยึดแบบดึงระดับจุลภาคโดยใช้เครื่องทดสอบสากล ความเร็วของหัวกด 0.5 มิลลิเมตรต่อนาที นำข้อมูลมาวิเคราะห์ความแปรปรวนสองทางและเปรียบเทียบเชิงซ้อนชนิดทูกีย์ ที่ระดับนัยสำคัญ $p<0.05$ และวิเคราะห์ลักษณะการแตกที่เกิดขึ้นของพื้นผิวเนื้อฟันที่เกิดพันธะด้วยกล้องจุลทรรศน์ชนิดสเตอริโอ

ผลการศึกษา กำลังแรงยึดลดลงอย่างมีนัยสำคัญทางสถิติจากการใช้ยาสีฟันลดเสียวฟัน ($p < 0.0001$) และชนิดของสารยึดติดส่งผลต่อกำลังแรงยึดอย่างมีนัยสำคัญ ($p < 0.0001$)

สรุป การใช้ยาสีฟันลดเสียวฟันอาจลดกำลังแรงยึดแบบดึงระดับจุลภาคของสารยึดติดต่อเนื้อฟัน

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KOCHANIPA SAISOPA: EFFECT OF DESENSITIZING TOOTHPASTE ON MICROTENSILE BOND STRENGTH BETWEEN RESIN COMPOSITE AND DENTIN. ADVISOR: ASST. PROF. DR. SIRIVIMOL SRISAWASDI, Ph.D., 85 pp.

The objective of this study was to evaluate and compare the effects of two desensitizing toothpastes and a regular fluoride toothpaste on microtensile bond strength of two adhesive agents to dentin. Materials and methods: The labial surfaces of forty bovine incisor crowns were ground flat, exposing dentin. The teeth were then randomly divided into four groups corresponding to the toothpaste used: 1) Sensodyne Rapid Relief (GlaxoSmithKline, UK), 2) Colgate Sensitive Pro-Relief (Colgate-Palmolive, Thailand), 3) Colgate Regular Flavor (Colgate-Palmolive, Thailand), and 4) immersed in artificial saliva (control). Each tooth in groups 1–3 was brushed with its respective dentifrice under constant loading (200 g) at 250 strokes/min for 2 minutes, twice daily for three days. Each group was then randomly divided for composite build-up using the following adhesive agents: 1) Optibond XTR (Kerr, USA), or 2) Optibond FL (Kerr, USA). After curing the adhesives, a light-cured resin composite (Premise, Kerr, USA) was used for a core build-up. The samples were sectioned into four 1± 0.1 mm thick specimens. The microtensile bond strength test was performed using a universal testing machine at a cross-head speed of 0.5 mm/min. The data were analyzed using two-way ANOVA and Tukey's multiple comparison tests with significance set at $p < 0.05$. Fracture analysis of the debonded dentin surface was performed using a stereomicroscope. Results: Bond strength was significantly reduced by the application of desensitizing toothpastes ($p < 0.0001$). Moreover, the type of adhesive agents had a significant effect on bond strength ($p < 0.0001$).

Conclusion: The use of desensitizing toothpaste may reduce bond strength of adhesives to dentin.

Field of Study:	Esthetic Restorative and Implant Dentistry	Student's Signature	....
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Chapter I

Introduction

Background and rationale

Dentin hypersensitivity is defined as short sharp pain arising from exposed dentin, typically in response to external stimuli, such as thermal, tactile, osmotic or chemical insults, that cannot be explained by any other form of dental defect or pathology (1). Dentin hypersensitivity can reduce the quality of life of those who suffer from it, because it can affect eating, drinking, and breathing habits. The most widely accepted explanation for dentin hypersensitivity is the hydrodynamic theory (2), which states that the movement of fluids or semi-fluid materials in the dentinal tubules transmits peripheral stimuli that activate the sensory nerves in the pulp, causing sharp short pain. Dentin hypersensitivity can be alleviated either by interfering with neural transmission or by occluding the dentin tubules (3). One of the most common ingredients used to treat dentin hypersensitivity is potassium nitrate. The potassium ions are thought to increase the nerve depolarization threshold, thereby reducing the sensation of pain (4).

Treatments which physically plug opened dentinal tubules have the potential to be more effective than potassium-based treatments (5). One treatment to occlude the tubules used high concentration fluoride gels or pastes. The high level of fluoride interacted with calcium in the saliva or on the tooth surface, and calcium fluoride precipitated within the tubules and occluded them (3). Clinical studies have been performed to evaluate the effectiveness of treating dentin hypersensitivity with various fluoride products (6-8). Although these agents reduced hypersensitivity, they were found to decrease the bond strength between composite resin and dentin (9, 10). This was due to the precipitation of microcrystals and mineral in the dentinal tubules, preventing proper resin infiltration.

Oxalate materials also have been used successfully for desensitization (11). These materials react with calcium ions on dentin and in dentinal fluid to form insoluble calcium oxalate crystals. Calcium oxalate crystals occluded open tubules in dentin (11). “Oxa-Gel” (Art-Dent, Brazil) is a product that contains monohydrogen-monopotassium oxalate. However, a previous study indicated that adhesive resins did not bond well to oxalate-treated dentin, because the dentin surface, including tubule orifices, was covered with calcium oxalate crystals (12). Thus, using a topical desensitizing agent, prior to tooth restoration using composite resin and a bonding agent, may result in a reduction in bond strength between dentin and the restorative material.

Kleinberg and colleagues developed a dentin hypersensitivity treatment consisting of 8% arginine (an amino acid found in saliva), bicarbonate, and calcium carbonate. This desensitizing formulation mimicked saliva's natural ability to plug and seal open dentin tubules (13). This formulation has been further developed as a daily-use dentin hypersensitivity dentifrice (Colgate Sensitive Pro-Relief™, Colgate-Palmolive, Thailand). In addition to 8% arginine and calcium carbonate, the dentifrice also contains 1450 ppm fluoride and claims to protect against the development of caries. Both *in vitro* and clinical studies have reported the efficacy of this dentifrice in reducing dentin hypersensitivity (14-17). High resolution scanning electron microscopy images revealed that the arginine-calcium carbonate desensitizing paste provided complete occlusion of open dentinal tubules, and freeze-fracture SEM images demonstrated that the plug reached a depth of two microns into the tubule (15).

Strontium-based dentifrices (10% strontium chloride) have been widely used in treating dentin hypersensitivity, and are believed to work by occluding dentinal tubules (18). Researchers have found that strontium acetate is more versatile than strontium chloride, can be formulated as a dentifrice base with almost no organoleptic downside, and was shown to successfully combine with sodium fluoride (18). A dentifrice containing 8% strontium acetate and 1040 ppm sodium fluoride was developed and has been extensively tested *in vitro, in situ*, and clinically (19-23). This technology is available as a daily-use dentin hypersensitivity dentifrice (Sensodyne® Rapid Relief, GlaxoSmithKline, UK). A study demonstrated that a single application of an 8% strontium acetate /1040 ppm sodium fluoride formulation occluded open dentinal tubules with a strontium-silica plug deep within the dentinal tubules, and this occlusion was resistant to dietary acids (22).

Therefore, we hypothesized that because the dentifrice containing 8% arginine and the dentifrice containing 8% strontium acetate function by occluding dentinal tubules, their use might affect the bond strength between dentin and bonding agents, as has been seen with other desensitizing toothpastes. The aim of this study was to evaluate and compare the effect of these two desensitizing toothpastes and regular fluoride toothpaste on the microtensile bond strength of two different adhesives to dentin.

Aim of this study

This *in vitro* study evaluates and compares the effect of a dentifrice containing 8% strontium acetate and 1040 ppm fluoride to 8% arginine, calcium carbonate, and 1450 ppm fluoride on the microtensile bond strengths of adhesives to treated dentin.

Research Questions

Do the dentifrice containing 8% strontium acetate and 1040 ppm fluoride and the dentifrice containing 8% arginine, calcium carbonate, and 1450 ppm fluoride have the effects on the microtensile bond strength of adhesive agents treated to dentin?

Hypothesis

Null hypothesis:

There is no difference in the microtensile bond strength of adhesive agents treated to dentin between the groups using desensitizing toothpastes compared to control group.

Alternative hypothesis:

There is difference in the microtensile bond strength of adhesive agents treated to dentin between the groups using desensitizing toothpastes compared to control group.

Type of study

Randomized controlled *in vitro* study

Benefits of study

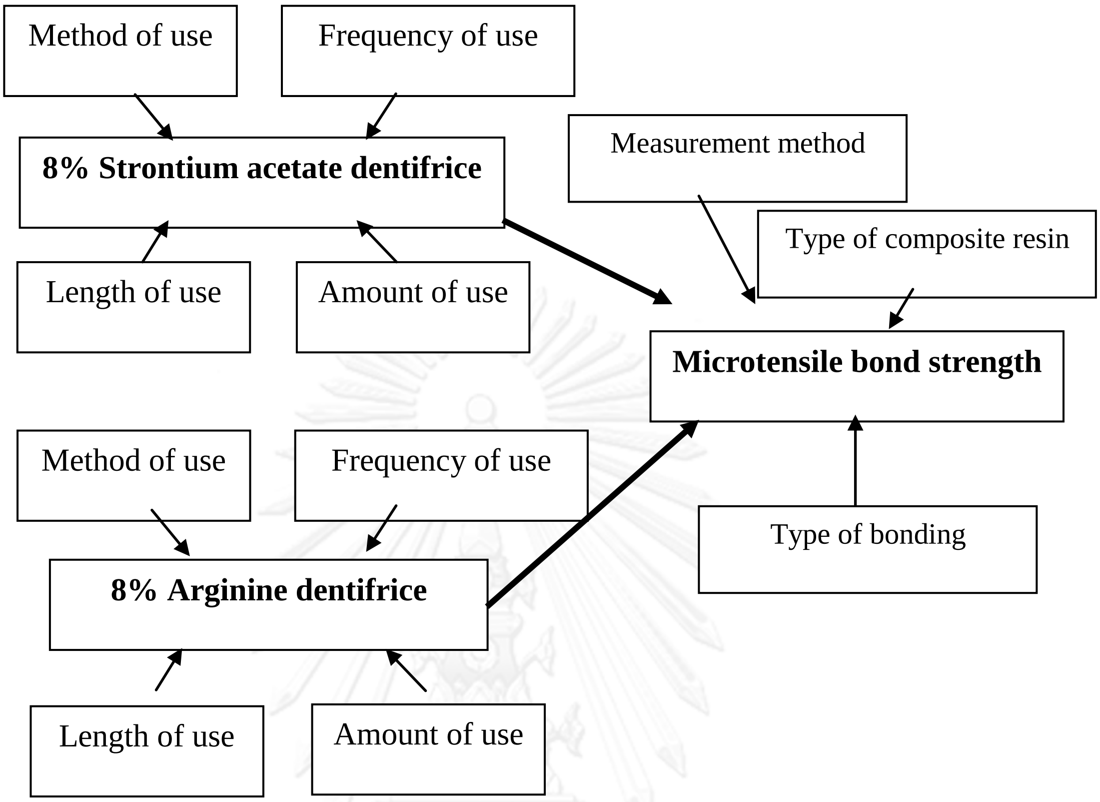
1. Information for dentists to select type of restorative materials used in the treatment of patients who use desensitizing toothpastes.

2. Knowledge for further study.

Limitation

This is an *in vitro* study, not a clinical study. Therefore, the results of this study may not be referring to the clinical result of these products.

Conceptual framework



Keywords

1. Adhesive system
2. Arginine
3. Desensitizing toothpastes
4. Microtensile bond strength
5. Strontium acetate

Chapter II

Literature Review

Mechanism of sensitivity of dentin

In the past twenty years, rapid progress was being made to elucidate the etiological factors and mechanisms underlying dentin sensitivity. Brännström and others provided experimental support behind the idea by Gysi, that stimulation evoked fluid movements in the dentinal tubules that activated intradental nerve endings (24). This process came to be known as “the hydrodynamic hypothesis of dentin sensitivity”. The evidence in support of the hydrodynamic theory 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 distorted the A-beta and A-delta fibers by mechanoreceptor action. There was also evidence that if the pressure change was large enough, the ensuring streaming potential could also trigger nerves electrically (25, 26). The consequence was the perception of the short sharp pain, typical of sensitive dentin, which usually persisted only while the stimulus was applied or shortly after removal of the stimulus. The rare cases where pain persisted as a dull ache after the intense short sharp pain, were explained by existing inflammation in the pulp (27) with recruitment of C fibers, which were stimulated chemically.

Therapeutic targets for desensitizing agents

This hydrodynamic hypothesis of dentin sensitivity suggests two general targets for desensitizing agents:

1) The intradental nerves: Agents that inactivate or reduce the responsiveness of the intradental nerves to stimuli should act as desensitizing agents.

2) The dentinal tubules: Studies examining the association between the condition of the dentin surface and sensitivity symptoms demonstrate that dentin with patent tubules is sensitive dentin. Materials that occlude the dentinal tubules should desensitize the tooth by preventing stimuli from causing movement of fluid activating the intradental nerves.

Tubule Occlusion as a management strategy for dentin hypersensitivity

Occlusive therapies for the treatment of dentinal hypersensitivity are frequently proposed because it is believed that sealing the dentinal surface diminishes the movement of fluids inside the tubule and is capable of reducing dentin hypersensitivity. The realization that smear layers could reduce dentin sensitivity symptoms by occluding the dentinal tubules lead researchers to search for desensitizing agents that could act by reducing dentin permeability.(28) There are several methods for occlusive therapies such as topical application of fluoride, oxalate materials, adhesive materials, and desensitizing toothpaste.

1. Topical application of fluoride

Topical application of fluoride by a professional has been recommended after periodontal treatment to relieve the patient's discomfort. There is also evidence that the home use of fluoridated products, as well as potassium nitrate and strontium acetate with fluoride, in the form of dentifrices and mouthwashes can benefit patients, by reducing sensitivity and dentin solubility, acting not only in reducing dentin hypersensitivity, but also in preventing caries.

Fluorides, such as sodium and stannous fluorides could reduce dentin hypersensitivity (29). The application of fluorides seemed to create a barrier by precipitation of the calcium fluoride crystals which were formed especially in the inlet of the dentinal tubules. The precipitates were slowly soluble in saliva, which may explain the transitory action of this barrier (30). The precipitate formed by substances used in the treatment of dentin hypersensitivity could disappear by the action of saliva, mechanical factors, such as brushing or chemical factors such as food, acidic beverages and the acid from dental biofilm (31).

2. Oxalate materials

Topical use of 3% potassium oxalate on exposed dentin after periodontal procedures results in a reduction of dentin hypersensitivity (32). The desensitizing action of potassium oxalate occurs by the deposition of calcium oxalate crystals on the dentin surface. Oxalate reacts with dentin calcium and promotes deposition of calcium oxalate crystals on the dentin surface and/or inside its tubules, significantly reducing hydraulic conductivity inherent to this structure, sealing the tubules more effectively than the intact smear layer. If the hydrodynamic mechanism was responsible for pain, this effect observed after the application of potassium oxalate led to the reduction of dentin hypersensitivity (33). The calcium oxalate crystals formed on the dentin surface were easily removed by daily brushing. However, when dentin was previously etched with 35% phosphoric acid, the penetration depth of oxalate buffer into the dentinal tubules was about 6-7 μm (34) and thus, pain relief could be expected for a longer period. However, there were limitations to the clinical use of potassium oxalate due to its potential toxicity (35).

3. Adhesive Materials

Resinous dentinal desensitizers, such as Gluma Desensitizer® (Heraeus Kulzer) are products which unite dentin and they can effectively seal the dentinal tubule openings. They were designed to produce an immediate long-term effect, and clinically they have been shown to fulfill these requirements. Basically, in their composition they have: hydroxyethyl methacrylate (HEMA), benzalkonium chloride, glutaraldehyde and fluoride. HEMA physically blocks the dentinal tubules and glutaraldehyde causes coagulation of plasma proteins of the tubule fluid, resulting in the reduction of dentinal permeability. HEMA can be absorbed by dentin and collagen and glutaraldehyde can form cross-links with bovine serum collagen and albumin. These results, found by Qin et al. (36), suggest that Gluma acts as a desensitizer by means of two reactions. First, the glutaraldehyde reacts with part of the serum albumin in the dentinal fluid which induces albumin precipitation, and then a second reaction of glutaraldehyde with albumin induces HEMA polymerization.

4. Desensitizing toothpaste

The use of patient-applied products containing tubule-occluding agents has the advantage of permitting repetitive application, allowing the tubule occlusion to be renewed and maintained on a frequent basis. Dentifrices are the most common vehicles for desensitizing agents. They are widely indicated, particularly because of their low cost, ease of use and home application. The act of tooth brushing applies force to the exposed dentin surface and occludes the tubules. The physical and chemical properties of the therapeutic paste must be carefully adjusted so that tubule occusion occurs in clinical use with little or no abrasive (or erosive) action exerted on the underlying dentin. There are a number of ways dentrifice constituents can cause tubule occlusion:

1) Tubule occlusion can occur through a precipitate on the dentin surface and in the tubules, as has been described for professionally applied agents. A chemical reaction between dentrifice constituents, such as calcium and phosphate salts, can form a tubule-occluding precipitate (37). Since these salts form a precipitate as soon as they react, they must be housed in separate product compartments prior to use.

2) Dentifrice constituents can reduce pain-inducing fluid flow if they can enter the tubules and set up some type of structure that resists the effect of dentin flow. The effects of various dentifrice treatments on dentin structure have been examined by SEM coupled with elemental analysis of the treated surface (38). Dentifrices containing fine particles of artificial silica and strontium salts leave a dense layer of silica particles on the dentin surface and penetrate the tubules (39). This phenomenon has been observed in both *in vitro* and *in situ* studies where this adherent material was observed to remain on the dentin surface following water washing and challenge with dietary acids (22). These results, indicating an interaction between these abrasive components and dentin, are interesting since although strontium ions penetrate and get absorbed by dentin, they do not rapidly alter dentin permeability (40). Further research is required in order to determine if this adherent layer material is capable of blocking stimulus-induced fluid shifts.

3) Dental products can contain agents that interact with the dentin surface and oral environment in such a way as to induce the formation of mineral in the tubules and on the dentin surface. This type of treatment may reduce the susceptibility of the dentin surface to wear and erosion, and alleviate sensitivity symptoms (5).

Strontium salt-based desensitizing products

Strontium can be incorporated into bone (41), and its ability to be taken up by enamel and dentin has been described (42). *In vitro* experiments showed that $SrCl_2$ treatment blocked the ability of certain dyes to stain dentin. In Pawlowska's study were indicated that strontium salts have beneficial interaction with dental hard tissue, including the ability to desensitize dentin (43).

The challenge was to formulate strontium chloride into a stable, pleasant-to-use dentifrice. Being a divalent cation, Sr^{++} binds to many anionic surfaces and molecules present in dentifrice components. This binding would hinder the release of strontium ions into the oral cavity. So to formulate an aqueous dentifrice that will release active strontium ions, many of the common abrasives such as sodium dodecyl sulphate, and gelling agents like carboxyl-cellulose, had to be replaced with less reactive components such as diatomaceous earth, a natural silicate as the abrasive (44). Since Sr^{++} reacts with fluoride to form insoluble precipitates, the original Sensodyne® toothpaste, containing 10% $SrCl_2$ hexahydrate, was introduced as a fluoride-free dentifrice for sensitive teeth. One limitation of $SrCl_2$ is its incompatibility with the anti-caries agent fluoride. This was overcome by the introduction of strontium acetate as a desensitizing agent by SmithKline Beecham in the 1980s, marketed as Macleans® Sensitive with sodium fluoride (18).

The effect on human dentin of both fluoride and strontium has been extensively studied (45); a strong desensitizing effect, in combination with an increase in the density of the treated dentin, has been demonstrated. This was measured by radiopacity and shown not to be solely a surface effect. Gedalia, et al. attributed the effect of strontium on dentin to a mineralization and/or remineralization process, similar to the mechanism of action of fluoride (45). The rate of remineralization of dentin by a calcifying solution was reported to increase in the presence of low concentrations of strontium (46), with an observed response greater than was found when fluoride was introduced at the same concentration. Ngo, et al. have investigated carious dentin exposed to strontium-containing glass ionomer restorations, and have reported that strontium can penetrate and remineralize the dentin in combination with fluoride (47). There have been several publications on an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice, confirming its ability to occlude dentin tubules.

In Vitro evidence for the occlusive effect of an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice

West et al. ran an *in vitro* study looking at occlusion afforded by a range of dentifrices, including an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice. Of the dentifrices investigated, the 8% strontium acetate dentifrice was shown to be statistically significantly better than any of the dentifrices tested at occluding patent dentin tubules, the optimum brushing time being two minutes (39).

Similarly, Parkinson and Earl evaluated eight commercial occlusion-based dentifrices, along with a non-occluding negative control dentifrice, in a hydraulic conductance model. After a single brushing application, the 8% strontium acetate, 1040 ppm sodium fluoride dentifrice demonstrated an approximately 40% reduction in dentin permeability, with an approximately 80% reduction in dentin permeability observed after three brushing applications (48).

Parkinson, et al. evaluated the utility of an acid challenge-based dentin disc occlusion model, and compared the occluding effect and acid resistance exhibited by currently marketed occlusion dentifrices, including an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice (Sensodyne® Rapid Relief, GlaxoSmithKline, UK), an

8% arginine calcium carbonate, 1450 ppm sodium monofluorophosphate dentifrice (Colgate® Sensitive Pro-Relief™ Toothpaste, Colgate-Palmolive Company, UK) and a negative control (water). The 8% strontium acetate dentifrice showed significantly better occlusion compared to the negative control on days 1 through 4, while providing statistically superior occlusion to a currently marketed 8% arginine calcium carbonate dentifrice on day 2 and day 4 (49). The ability of the 8% strontium actetate dentifrice to not only occlude, but also penetrate into the dentin tubules (to a depth of at least 15 μm) has been demonstrated in article by Earl, et al., utilizing techniques such as focused ion beam scanning electron microscopy and transmission electron microscopy (23).

In Situ evidence for the occlusive effect of an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice

Claydon, et al. conducted two randomized, crossover, blinded *in situ* clinical studies evaluating the efficacy of different occlusion activities, including an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice, on patent dentin tubules. Healthy participants wore intraoral appliances retaining dentin samples over four days, with the dentin samples brushed twice daily with a test dentifrice. Assessment utilized surface topological analysis with a replica-based methodology under scanning electron microscopy. Results demonstrated that 8% strontium acetate dentifrice occluded dentin tubules significantly better than negative controls (20).

The inclusion of an acid challenge into an *in situ* model by Banfield and Addy was able to show that 8% strontium acetate, 1040 ppm sodium fluoride dentifrice provided good levels of occlusion, an outcome little changed by the introduction of a dietary acid challenge (orange juice) into the model. The 8% strontium acetate, 1040 ppm sodium fluoride dentifrice demonstrated acid resistant occlusion (22).

Clinical evidence for the relief of dentin hypersensitivity provided by an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice

Mason, et al. reported the results of a clinical study investigating immediate relief from sensitivity as a “dab-on” application followed by twice-daily brushing for three days, compared to a control (1450 ppm fluoride dentifrice). The 8% strontium acetate dentifrice demonstrated significant reductions in sensitivity across all measures following an initial dab-on application. Between-treatment analyses showed the 8% strontium acetate dentifrice to be significantly better at relieving subjects’ sensitivity across all measures compared to the control. Subjects then used the products as a replacement to their regular dentifrice, brushing twice daily for three days. Significant reductions in dentin hypersensitivity compared to baseline were observed at the end of the clinical study for both treatment groups for all measures. Between-treatment analyses showed the 8% strontium acetate dentifrice to be significantly better at relieving subjects' sensitivity across all measures compared to the control (50).

Hughes, et al. report the results of a clinical study investigating the longtitudinal relief from twice-daily brushing for eight weeks with an 8% strontium acetate, 1040 ppm sodium fluoride dentifrice compared to a marketed occlusion-based dentifrice containing 8% arginine calcium carbonate, and 1450 ppm sodium monofluorophosphate. Both subject groups exhibited significant cumulative reductions in dentin hypersensitivity compared to baseline for each of the post-baseline visits. No significant difference was observed between treatments for any of the time points (day 14, 28, 56) and measures, except for tactile sensitivity at Day 56, in which the 8% strontium acetate dentifrice was statistically superior to the marketed 8% arginine calcium carbonate, 1450 ppm sodium monofluorophosphate control dentifrice (51).

Arginine-based desensitizing products

The research by Kleinberg and coworkers, have resulted in the development and validation of a new in-office treatment for dentin hypersensitivity. This treatment, a desensitizing prophyraxis paste (ProClude®, Ortek Therapeutics, USA) based upon 8.0% arginine, an amino acid naturally present in saliva, bicarbonate, a pH buffer, and calcium carbonate, a source of calcium, has been marketed in the United States for the management of dentin hypersensitivity during professionally administered prophylaxis procedures (13). Clinical studies have shown that this treatment is effective in providing instant sensitivity relief, when burnished onto sensitived teeth following professional dental prophylaxis, and that sensitivity relief lasts for at least 28 days following a single in-office treatment (52).

In contrast to other products which occlude dentin tubules, this technology is unique in that two of its key components, arginine and calcium, are found naturally in saliva, and the arginine and calcium carbonate work together to accelerate the natural mechanisms of occlusion by depositing a dentin-like mineral, containing calcium and phosphate, within the dentin tubules and in a protective layer on dentin surface (15). In 2009, the technology has been re-launched as an in office-desensitizing polishing paste under the brand name Colgate® Sensitive Pro-Relief™

(Colgate-Palmolive Company,USA), and additional clinical studies were conducted. The innovative technology has been suggested later by the manufacturer as a daily-use dentin hypersensitivity dentifrice. In addition to 8.0% arginine and calcium carbonate, the dentifrice contains 1450 ppm fluoride, as sodium monofluorophosphate (MFP), for cavity protection (53).

***In Vitro* evidence for the occlusive effect of an 8% arginine and calcium carbonate dentifrice**

Petrou, et al. used confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) to assess tubule occlusion and used energy dispersive x-ray (EDX) and electron spectroscopy for chemical analysis (ESCA) to identify the composition of the dentin plug. Hydraulic conductance has been used to assess the effectiveness of the arginine-calcium carbonate technology in arresting dentin fluid movement, to evaluate the effects of pulpal pressure on the robustness of the occlusion, and to confirm the resistance of the occlusion to an acid challenge (15).

Visualization of the dentin surface by CLSM, SEM, and AFM has clearly shown that the combination of arginine plus calcium carbonate is highly effective in occluding open dentin tubules. The combination of arginine, calcium, and alkaline pH appear to be key components in determining that effective occlusion occurs. This observation supports the hypothesis that arginine facilitates the adherence of calcium carbonate to the surface. The presence of arginine alone, in the absence of alkalinity, is insufficient to create conditions that favor deposition of an arginine-calcium agglomerate on the dentin surface or within the dentin tubules. The hydraulic conductance study demonstrate that the occlusion provided by the arginine-calcium carbonate technology results in highly significant reductions in dentinal fluid flow, and that the tubule plug is resistant to normal pulpal pressure and acid challenge (15).

Clinical evidence for the relief of dentin hypersensitivity provided by an 8% arginine, calcium, 1450 ppm fluoride dentifrice

Two eight-week dentin hypersensitivity clinical studies were conducted comparing arginine-based dentrifice to a commercial desensitizing toothpaste containing 2% potassium ion as the active ingredient (17, 54). Both studies demonstrated that the dentrifice containing 8% arginine, calcium carbonate, and 1450 ppm fluoride provides superior sensitivity relief compared to a dentrifice containing 2% potassium ion, as 3.75% potassium chloride, and 1450 ppm fluoride, as a sodium fluoride. In an eight-week study by Ayad, et al., the 8% arginine toothpaste provided statistically significant reductions in dentin hypersensitivity in response to tactile and air blast measures compared to the commercial desensitizing toothpaste at two, four, and eight weeks (54). In another eight-week study by Docimo, et al., the 8% arginine toothpaste also provided statistically significant reductions in dentin hypersensitivity in response to tactile and air blast measures compared to the commercial desensitizing toothpaste at two, four, and eight weeks (17).

Ayad, et al. reported the results of a study conducted among 120 subjects in Canada. The first phase of the study consisted of a single topical application in which subjects applied a pea-size amount of their assigned toothpaste directly onto the hypersensitive surface of each of the two baseline-designated hypersensitive teeth, and massaged each surface for one minute. The second phase of the study consisted of twice-daily at-home brushing with the assigned toothpaste for three days. Relative to the desensitizing and the fluoride toothpaste control groups, the 8% arginine toothpaste group exhibited statistically significant reductions in dentin hypersensitivity on both tactile and air blast measures immediately after direct application. Relative to the desensitizing and the fluoride toothpaste control groups, the 8% arginine toothpaste group also exhibited statistically significant reduction in sensitivity, after completion of the brushing phase of the study (16).

Dentin adhesion

Bonding to dentin presents a much greater challenge than enamel. Several factors account for this difference between enamel and dentin bonding. Although enamel is highly mineralized tissue composed of more than 90% (by volume) hydroxyapatite, dentin contains a substantial proportion of water and organic material, primarily type I collagen. Dentin also contains a dense network of tubules that connect the pulp with dentinoenamel junction. A cuff of hypermineralized dentin called peritubular dentin lines the tubules. The less mineralized intertubular dentin contains collagen banding. The intertubular dentin is penetrated by submicron channels, which allow the passage of tubular liquid and fibers between neighboring tubules, forming intertubular anastomeses.

Dentin is an intrinsically hydrated tissue, penetrated by a maze of 1- to 2.5 micron-diameter fluid-filled dentin tubules. Movement of fluid from the pulp to the DEJ is a result of a slight but constant pulpal pressure (55). Dentinal tubules enclose cellular extensions from the odontoblasts and are in direct communication with the pulp. Inside the tubule lumen, other fibrous organic structures are present, such as lamina limitants, which substantially decreases the functional radius of the tubule. The relative area occupied by dentin tubules decreases with increasing distance from the pulp. The tubules occupy an area of only 1% of the total surface near the DEJ, whereas they occupy 22% of the surface close to pulp (56).

Adhesion can be affected by the remaining dentin thickness after tooth preparation. Bond strengths are generally less in deep dentin than in superficial dentin (57). Nonetheless, some dentin adhesives, including some that contain the 4-META monomer, do not seem to be affected by dentin depth (58).

Whenever tooth structure is prepared with a bur or other instrument, residual organic and inorganic components form a "smear layer" of debris on the surface (59). The smear layer fills the orifices of dentin tubules, forming "smear plugs", and decreases dentin permeability by 86% (60). The composition of the smear layer is basically hydroxyapatite and altered denatured collagen. This altered collagen can acquire a gelatinized consistency because of the friction and heat created by the preparation procedure (61). Submicron porosity of the smear layer still allows for diffusion of dentinal fluid. The removal of the smear layer and smear plugs with acidic solutions results in an increase of the fluid flow onto the exposed dentin surface. This fluid can interfere with adhesion because hydrophobic resins do not adhere to hydrophilic substrates even if resin tags are formed in the dentin tubules (62).

Several additional factors affect dentin permeability. Factors such as the radius and length of the tubules, the viscosity of dentin fluid, the pressure gradient, the molecular size of the substances dissolved in the tubular fluid, and the rate of removal of substances by the blood vessels in the pulp affect permeability (63). All of these variables make dentin a dynamic substrate and consequently a difficult substrate for bonding (64).

1. Etch-and-rinse adhesive systems

Although the smear layer acts as a “diffusion barrier” that reduces the permeability of dentin, it also can be considered an obstacle that must be removed to permit resin bonding to the underlying dentin substrate. Removal of the smear layer via acid etching led to significant improvements in the *in vitro* bond strengths of resins to dentin (65). Because the clinical technique involves simultaneous application of an acid to enamel and dentin, this method is commonly known as the total-etch technique. Also called the etch-and-rinse technique, it was the most common stategy for dentin bonding the 1990s and remains popular today.

Etch-and-rinse adhesive systems can be either three- or two-step materials depending on whether primer and bonding are separated or combined in a single bottle. The adhesion strategy involves at least two steps and, in its most conventional form, three steps with successive application of the conditioner (acid etchant), followed by the primer (adhesion promoting agent), and eventually, application of the bonding agent (adhesive resin). The simplified two-step version combines the second (priming) and third (bonding) steps, but still follows a separated etch and rinse phase (66-68).

1.1 Three-step etch and rinse adhesives

Application of acid to dentin results in partial or total removal of the smear layer and demineralization of the underlying dentin. Acids demineralize intertubular and peritubular dentin, open the dentin tubules, and expose a dense filigree of collagen fibers, increasing the microporosity of the intertubular dentin (69).

The total-etch concept originated in Japan, with phosphoric acid etching of dentin before the application of a phosphate ester type of bonding agent (70). Despite the obvious penetration of this early adhesive into the dentinal tubules, etching did not result in a significant improvement in bond strengths, possibly as a result of the hydrophobic nature of the phosphonated resin (71). The three essential components of three-step adhesives are 1) a phosphoric acid etching gel that rinsed off 2) a primer containing reactive hydrophilic monomers in ethanol, acetone, or water, and 3) an unfilled or filled resin bonding agent. The last mentioned contains hydrophobic monomers, such as Bis-GMA, frequently combined with hydrophilic molecules, such as HEMA.

The acid-etching step not only alters the mineral content of the dentin substrate, but also changes its surface free energy (72). The latter is an undesirable effect, because for good interfacial contact, any adhesive must have a low surface tension, and the substrate must have a high surface free energy (61). Substrates are characterized as having low or high surface energy. Among dental materials, hydroxyapatite and glass-ionomer cement filler particles are high-energy substrates, whereas collagen and composite have low energy surfaces (73). Consequently, dentin consists of two distinct substrates, one of high surface energy (hydroxyapatite) and one of low surface energy (collagen). After etching with acidic agents, the dense web of exposed collagen is a low surface energy substrate. There is a correlation between the ability of an adhesive to spread on the dentin surface and the concentration of calcium on that same surface (74). The primer in a three-step system is designed to increase the critical surface tension of dentin, and direct correlation between surface energy of dentin and shear bond strengths has been shown. HEMA (2-hydroxymethyl methacrylate) is a very popular monomer which is in widespread use (75). It is much employed either in three- and two-step etch-and-rinse systems and one reason for this preference is related to its hydrophilicity that makes it an excellent adhesion promoter enhancing bond strength.

1.2 Two-step etch and rinse adhesives

In the two-step systems the hydrophilic and hydrophobic monomers are combined with solvents in the same bottle. A separation etching step still is required. When a commercially available proves to be satisfactory under *in vitro* conditions but takes several time-consuming steps to be applied *in vivo*, the clinician usually prefers a material that would be easier to apply. Manufacturers have been attempting to reduce the number of steps needed and the corresponding application time, making more user-friendly adhesive systems. Since two-step etch and rinse adhesives contain higher percentages of hydrophilic monomers co compared to three-step adhesive (76), they exhibit greater permeability after polymerization, thus facilitating the presence of water-filled areas within hybrid layer (77). Recently, it can be noted the trend towards decreasing the amount of strong hydrophilic monomers, such as HEMA, and replacing this portion by UDMA or TEGDMA (78).

2. Self-etch adhesive systems

2.1 Self-etching primer systems

The self-etch approach is an alternative based on the use of non-rinse acidic monomers that simultaneously condition and prime tooth tissues. In contrast to conventional etchants, self-etching primer systems are not rinsed off. The bonding mechanism of self-etching primer systems is based on the simultaneous etching and priming of enamel and dentin, forming a continuum in the substrate and incorporating smear plugs into the resin tags (79). In addition to simplifying the bonding technique, the elimination of rinsing and drying steps reduces the possibility of overwetting or overdying, either of which can affect adhesion adversely. (80) Also, water is always a component of self-etching primer systems because it is needed for the acidic monomers to ionize and trigger demineralization of hard dental tissues; this make self-etching primer systems less susceptible to variations in the degree of substrate moisture.

Self-etching primer systems are less technique-sensitive than total-etch adhesives. Additionally, self-etching primer systems are less likely to result in a discrepancy between the depth of demineralization and the depth of resin infiltration (81) because self-etching primer systems demineralize and infiltrate dentin simultaneously. Self-etching primer systems do not remove the smear layer from dentin completely, which is the main reason that they might result in less postoperative sensitivity than total-etch adhesives (82).

2.2 Self-etching adhesives (All-in-one)

Continuing the trend toward simplification, no-rinse, self-etching materials that incorporate the classic steps of etching, priming, and bonding into one solution have becoming popular. In contrast to conventional adhesive systems that contain an intermediate light-cured, low-viscosity bonding resin to join the primed dentin/enamel substrate to the composite restorative material, all-in-one adhesives contain uncured ionic monomers that contact the composite restorative material directly (83). Additionally, all-in-one adhesives tend to behave as semipermeable membranes, resulting in a hydrolytic degradation of the resin-dentin interface (77). Because all-in-one adhesives must be acidic enough to be able to demineralize enamel and penetrate dentin smear layers, the hydrophilicity of their resin monomers, usually organophosphates and carboxylates, also is high. Some of these resin monomers might be too hydrophilic, which makes them liable to water degradation (84).

Microtensile bond strength

Microtensile bond testing was originally designed to permit evaluation of bond strengths between adhesive materials and small regions of dental tissue (eg, occlusal vs middle vs cervical third of enamel (85), normal vs adjacent caries-affected dentin (86), occiusal vs gingival walls of Class V wedge-shaped lesions). (87) This test offered versatility that could not be achieved using conventional shear and tensile methods. “Micro” tensile bond strength (μTBS) lends itself to additional research designs that the “macro” tests do not, such as, the elimination of tooth dependency through balanced designs (88), and has shown reduced test variance (89). Microtensile bond strengths tend to be much higher than that of macro TBS values because the defect concentration in the small cross-sectional interfacial areas is lower (89). The measured bond strength and the failure mode or debond pathway produced is dependent, among other things, upon: flaws existing within or between materials, specimen size and geometry, material properties of each component of the bonded assembly, and method of load application. Adhesive resin–tooth bond strength testing involves two separate substrates and complicated interphases or zones of interdiffusion between these components, all possessing different material properties. Therefore, even if a perfectly uniform tensile load could be applied across a resin-based-composite–dentin adhesively bonded joint, a non-uniform stress distribution will occur in the adhesive joint. Smaller test specimens are ‘stronger’ than larger ones due to the lower probability of having a critical sized defect present and aligned in a crack opening orientation relative to the applied load. The measured “bond strength” at failure will be dependent not only upon the fracture strength but the presence of flaws. This volume dependency of strength tells us that a smaller test specimen will be less likely to have a larger flaw that leads to its failure.

There are several advantages of microtensile bond strength testing which include (90, 91) conservation of teeth, possible evaluation of regional bond strengths (92), remaining dentin thickness effects (93), intra- and inter-tooth variability, bond strength to various cavity walls in restoration (94), bond strength to intra-radicular dentin, conducive to evaluation of the effects of rein composite polymerization shrinkage stress (95), more uniform loading may be possible due to less bending offset, relative to conventional tensile testing, due to alternative gripping method, fewer cohesive failures in substrates, bond strengths are higher than those measures from conventional tensile and shear bond strength tests due to the decreased number of defects in the substrate or at the bond interface, additional research designs can be performed to account for tooth dependency, such as multiple surfaces within a cavity, various substrates within a tooth, durability testing by aqueous storage (88), accelerated environmental aging is feasible by aqueous storage due to short diffusional distances (96), possible to evaluate very small surface areas when necessary (86), can minimize shear effect by tensile testing a relatively flatter region of tooth when not preparing surface (97), SEM fractography can be readily performed to determine the mode of failure (98), clinically retrieved restorations can be evaluated (99), and conducive to comprehensive examination of research question, such as mechanical, morphologic and chemical studies on same sample (100).

On the other hand, there are several limitations of μTBS testing which include (90, 91) labor intensive, technically demanding, difficult to measure very low bond strength (<5MPa), specimens easily dehydrate and damaged, post-fracture specimens can be lost or damaged when removing from active gripping devices that use glue, difficult to fabricate with consistent geometry, surface finish and damage history without aid of special equipment, and lack of consensus exists for conduct of test, reporting of pre-test failures and fractures outside of the designated.

To measure μTBS, the specimens were trimmed to an hourglass profile to produce uniform stressing of the smallest cross-sectional area. To measure the modulus of elasticity in tension, the need of a known gauge-length required that the specimens be trimmed to the outline of an " I " beam (101), In the microtensile bond test, the occlusal surface of the tooth was ground flat. The entire surface was bonded, and a large resin composite build up was created (90). Indeed, there is theoretically no need to produce a flat surface using polishing devices. The surface to be bonded can be fractured, polished, or bur cut. Microtensile testing can be done on teeth prepared exactly as they are restored clinically, because the bonded surface area is determined after bonding, not before bonding, by trimming. Due to the strong effect of cross-sectional area on resin-dentin bond strength (102), it is important to make certain that there is no statistically significant difference in bonded cross-sectional areas between experimental groups, if differences exist, one must adjust for the covariant of bonded area using the Least-Squares Means test, which adjusts for such differences prior to comparing bond strengths. Pashley, et al. suggested to trim specimens to 0.8 to 1.0 mm (89). In the microtensile bond test, a large resin-bonded molar yielded 5 to 8 slabs when vertically sectioned, depending upon the thickness of the blade and the desired thickness of the slabs. Thus, each tooth yielded 5 to 8 specimens for bond testing, instead of a single specimen using conventional testing. Pashley et al. suggested pooling the specimens of several teeth for calculation (91).

Chapter III

Methodology

Sample description

Dentin samples in this study were prepared from permanent bovine incisors with normal shape and size. Deciduous bovine incisors with craze or crack, and bovine incisors with abnormal shape or excessive dental wear were excluded from this study.

Sample size

The number of specimens for each group was calculated from the result of the pilot study (table 5) with the formula:

$$\text{Sample size per group (n)} = \frac{\sigma^2 (Z_{\alpha/2}+Z_{\beta})^2}{(\mu_1 - \mu_2)^2}$$

$$\alpha = 0.05$$

$$\beta = 0.10$$

$$Z_{\alpha/2} = Z_{0.05/2} = 1.96$$

$$Z_{\beta} = Z_{0.10} = 1.28$$

The maximum sample size per group was 349.92. However, due to the reason of time and budget, we chose to use 20 specimens per group (5 teeth per group).

Materials

1. Bovine incisors
2. Thymol solution (Merck, Germany)
3. Self-curing resin (Suksapan, Thailand)
4. Silicone (Suksapan, Thailand)
5. 320-600-1200 grit silicon carbide paper (TOA, Thailand)
6. Artificial saliva (Faculty of Dentistry, Chulalongkorn University, Thailand)
7. Sensodyne® Rapid Relief toothpaste (GlaxoSmithKline, UK)
8. Colgate® Sensitive Pro-Relief™ toothpaste (Colgate-Palmolive, UK)
9. Colgate® Regular Flavor toothpaste (Colgate-Palmplive, Thailand)
10. Distilled water (Faculty of Dentistry, Chulalongkorn University, Thailand)
11. Composite resin (Premise™, Kerr, USA)
12. Self-etch bonding agent (Optibond® XTR, Kerr, USA)
13. 3-step etch and rinse bonding agent (Optibond® FL, Kerr, USA)
14. Cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Japan)

15. Disposable microbrush (Kerr, USA)

16. 2.5% glutaraldehyde in cacodylate buffer solution (Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand)

17. Ethanol (Emsure®, Merck, Germany)

18. Hexamethyldisilazane (Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand)

Equipments

1. Polishing machine (NANO 2000 Grinder-polisher, Pace Technologies, USA)

2. Stereomicroscope (ML 9300, Meiji Techno Co. Ltd., Japan)

3. Light curing unit (EliparTriLight Curing Light; 3M ESPE, USA)

4. Low speed cutting machine (ISOMET 1000™, Buehler, USA)

5. Digital vernier caliper (Mitutoyo Co., Japan)

6. Universal testing machine (EZ-S, Shimadzu, Japan)

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

8. Scanning electron microscope (JSM-5410LV, JEOL, Japan)

Dentin sample preparation

Forty extracted bovine incisors were collected, cleaned and stored in 0.01% thymol solution for 1 week, and then stored in distilled water at 4°C for a maximum of 1 month after extraction. Roots were removed at 1 mm apical to the cemento-enamel junction (Fig.1A). Pulpal tissue was carefully removed with pliers. Teeth were subsequently embedded into a self-curing resin (Fig.1B). Labial surface was ground flat using a polishing machine (NANO 2000 Grinder-polisher, Pace Technologies, USA) with a series of 320,600, and 1200 grit silicon carbide paper, under running water until the enamel was completely removed (Fig.2). Each tooth was carefully inspected to ensure that it was free of enamel using a stereomicroscope (ML 9300; Meiji Techno Co. Ltd., Japan) at 40X.

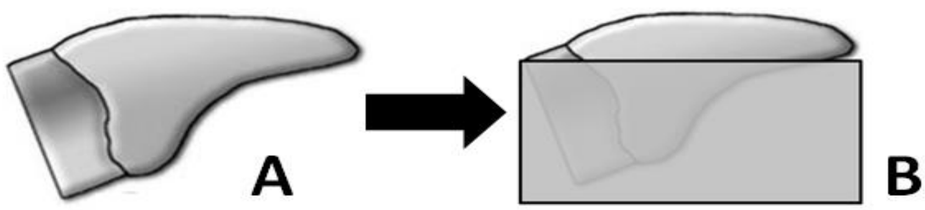


Fig.1 Root of bovine tooth was removed and embedded into a self-curing resin

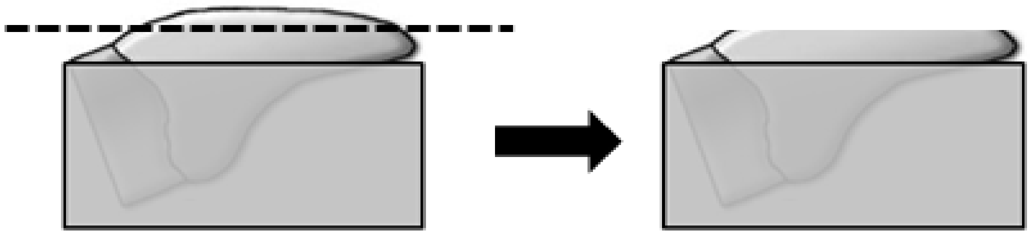


Fig.2 The labial surface was ground flat until the enamel was completely removed

Experimental design

Teeth were then randomly divided into four groups (Fig. 5)

Group 1: Brushing with Sensodyne® Rapid Relief

Group 2: Brushing with Colgate® Sensitive Pro-Relief™

Group 3: Brushing with 1,000 ppm fluoride toothpaste (Colgate® Regular Flavor)

Group 4: (Control) Immersed in artificial saliva

Compositions of desensitizing toothpastes used in this study were summarized in Table 1. Each tooth from Groups 1-3 was brushed with the dentifrice slurries, which were prepared by diluting 2 g of the dentifrice in 6 ml of distilled water. (103) 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) for 250 strokes/min for 2 minutes (103) with V-8 cross brushing machine (Sabri Dental Enterprise, Inc., USA). Teeth were brushed with tested toothpastes twice a day for three days. To remove excess slurry or aqueous solution, teeth were rinsed using distilled water for 10 s. During the three day brushing procedure, teeth were immersed in artificial saliva except for when being brushed by the brushing machine.

Table 1 Major composition of tested toothpastes

Treatments	**Manufacturer**	**Active ingredients**
Sensodyne® Rapid Relief	GlaxoSmithKline Ltd., UK	Strontium acetate, Sodium fluoride, silica
Colgate® Sensitive Pro-Relief™	Colgate-Palmolive, Thailand	Arginine bicarbonate, Sodium silicate, Sodium monofluorophosphate
Colgate® Regular Flavour	Colgate-Palmolive, Thailand	Sodium silicate, Sodium monofluorophosphate

After the above mentioned procedures, each group was then divided for composite build-up (Fig. 5) using adhesive agents as followed:

1) Self-etch bonding agent (Optibond® XTR, Kerr, USA): Applied OptiBond XTR Primer to the dentin surface using a disposable applicator brush and scrubbed the surface using a brushing motion for 20 seconds. Then, applied OptiBond XTR adhesive to the dentin surface using light brushing motion for 15 seconds, gently air dried for 5 seconds, and light cured with a visible light-polymerization unit (EliparTriLight Curing Light; 3M ESPE, USA) for 10 seconds.

2) 3-step etch and rinse bonding agent (Optibond® FL, Kerr, USA): Placed Kerr Gel Etchant with 37.5% phosphoric acid on dentin for 15 seconds and rinsed with water until etchant has been completely removed (approximately 15 seconds). Then, gently air dried for 5 seconds. Applied OptiBond FL Prime (Bottle #1) over dentin surfaces with a light agitating motion for 15 seconds, gently air dried for approximately 5 seconds. After applied OptiBond FL Adhesive (Bottle #2) over dentin uniformly creating a thin coating, aired thin for 5 seconds, light cured with a visible light-polymerization unit (EliparTriLight Curing Light; 3M ESPE, USA) for 20 seconds.

After placing and curing the adhesive according to the manufacturer's instruction, a silicone mold with a 14 x 8 mm^2 opening at the center was placed on the treated dentin. (Fig. 3) A light-cured composite (Premise™, Kerr, USA) was built up to approximately 4 mm in height by incremental placement onto the treated dentin surface.(Fig. 4) Each 2 mm increment was polymerized for 40 seconds using a visible light-polymerization unit (EliparTriLight Curing Light; 3M ESPE, USA).

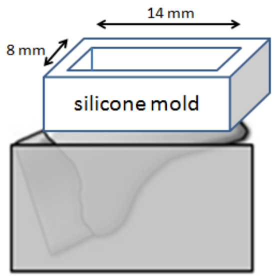


Fig. 3 Silicone mold placed on preparation dentin surface

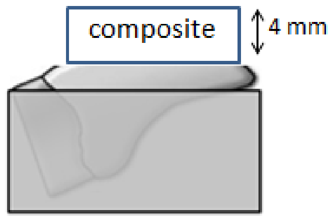


Fig. 4 Composite resin was built up to approximately 4 mm in height

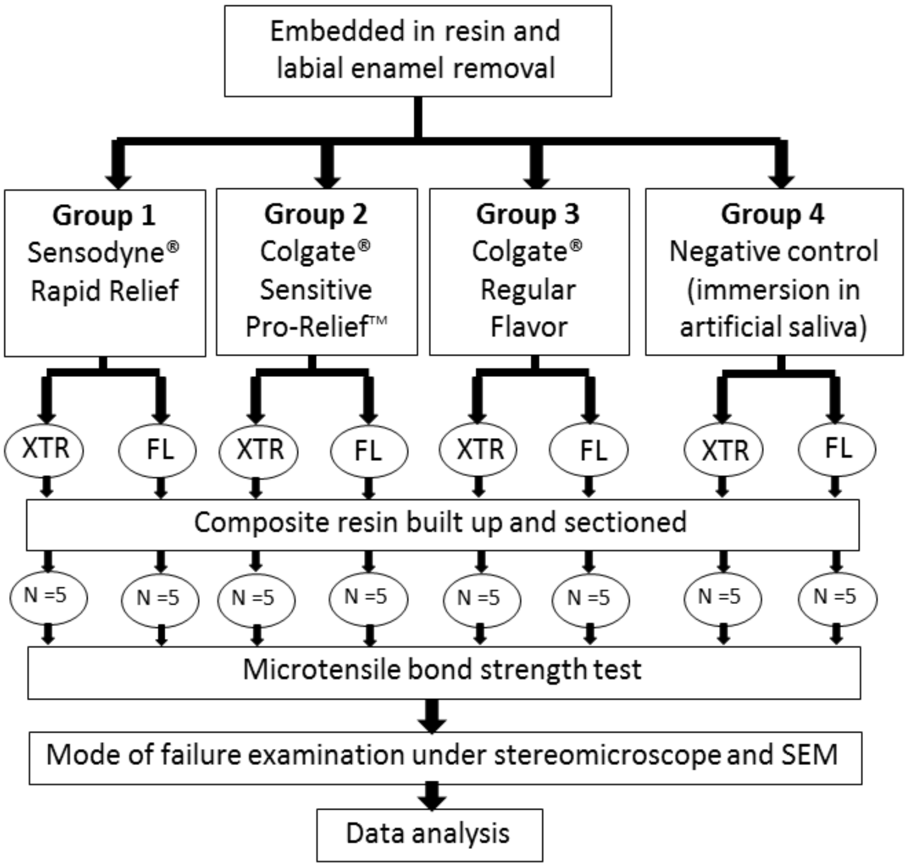


Fig.5 Diagram of study design

Microtensile bond strength measurement

The completed tooth sample was stored in distilled water at 37°c for 24 h, then mounted onto a low speed cutting machine (ISOMET 1000™, Buehler, USA), and was subsequently sectioned both mesial-distally and inciso-cervically in order to obtain stick-shaped microtensile specimens from each tooth. Only four sticks in the middle were used for further test. (Fig. 6 and 7) The square cross section of each stick was approximately 1.0 mm^2 (1±0.1 mm x 1±0.1 mm). (Fig. 8) Each stick was carefully examined using a stereomicroscope at 40X to ensure its homogeneity without bubbles or cracks. The dimension of each specimen was measured using a digital vernier caliper (Mitutoyo Co., Japan).

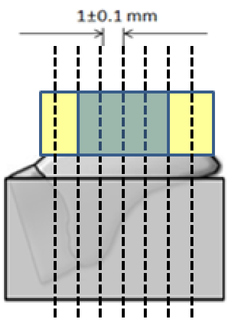


Fig.6 Cutting of the stick from side view

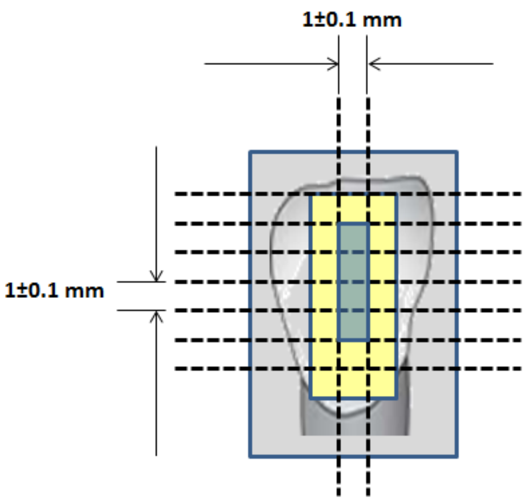


Fig.7 Cutting of the stick from top view

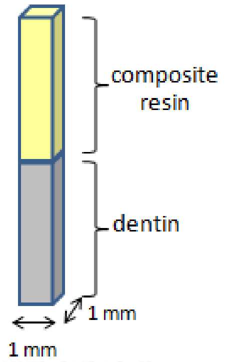


Fig.8 Stick specimen

All stick specimens were attached to the test apparatus using a cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Japan) (Fig. 9) and stressed to failure in tension using a universal testing machine (EZ-S, Shimadzu, Japan) at a cross-head speed of 0.5 mm/min. The microtensile bond strength of each specimen was calculated as the ratio of the maximum load force at the fracture and the cross-sectional bonding area which was measured in each individual fractured specimen.

Fig. 9 The specimen was fixed to the apparatus

Fracture analysis

Fracture analysis of the bonded dentin surface was performed using a stereomicroscope at 40X magnification. Failures were classified as **adhesive** (>75% of failure between tooth and restorative material), **cohesive** (>75% of the failure was within the restorative material or dentin) or a **mixture** of two. Specimens with pre-test failure were excluded from the study.

Randomly selected samples with adhesive fractures from each group were processed for scanning electron microscopy (JSM-5410LV, JEOL, Japan) using standard SEM specimen processing techniques; i.e. fixed in a 2.5% glutaraldehyde cacodylate buffer solution, dehydrated in graded ethanols, chemically dried using hexamethyldisilazane, and gold-sputter coated. The diagram of study design is shown in figure 5.

Data analyses

Microtesile bond strength

The microtensile bond strength data were statistically analyzed using two-way ANOVA and Tukey's post-hoc tests, with significance set at $p < 0.05$. All statistical analyses were performed using SPSS v17.0 for Windows (SPSS Inc., USA).

Chapter IV

Results

Table 2 summarizes the mean microtensile bond strength values and standard deviations of the test and control groups. Two-way ANOVA indicated that the type of toothpaste ($p < 0.0001$), the type of adhesive agent ($p < 0.0001$) and their interaction ($p < 0.05$) had a significant effect on microtensile bond strength. The microtensile bond strengths in Colgate® Regular Flavor (group 3) and the control groups (group 4) were significantly higher than in Sensodyne® Rapid Relief (group 1) and Colgate® Sensitive Pro-Relief™ group (group 2) ($p < 0.05$). However, there was no significant difference in bond strength between groups 1 and 2 ($p = 0.760$) and groups 3 and 4 ($p = 0.104$). There were significant differences in bond strength between adhesive agents in group 1 ($p < 0.05$) and 2 ($p < 0.0001$), but no significant differences were found in group 3 ($p = 0.859$) and 4 ($p = 0.879$). Premature failures occurred in group 1 + Optibond® XTR (n = 2) and group 2 + Optibond® XTR (n =1). The distribution of failure modes is presented in Table 3. The bond failure type in each group was predominantly adhesive (83% or higher), with the remainder exhibiting cohesive failures, and no mixed failures (Fig 12).

Figures 10 and 11 show representative SEM images of the debonded dentin specimens. It was possible to observe partial obstruction of dentinal tubules in specimens of group 1 + Optibond® XTR (Fig. 10a) and group 2 + Optibond® XTR (Fig. 10b).

Table 2 Mean ± standard deviation microtensile bond strength values (MPa) between dentin and resin composite and pre-testing failures (n)

Adhesive agent	**Group 1 Sensodyne® Rapid Relief**	**Group 2 Colgate® Sensitive Pro-Relief™**	**Group 3 Colgate® Regular Flavor**	**Group 4 Negative Control**
Optibond® XTR	27.9501 ± 4.9057^{Aa} (2)	28.3655 ± 5.4310^{Aa} (1)	41.8800 ± 3.4454^{Ba} (0)	43.8628 ± 2.7549^{Ba} (0)
Optibond® FL	32.7260 ± 3.2008^{Ab} (0)	34.1199 ± 3.7559^{Ab} (0)	43.5586 ± 3.7308^{Ba} (0)	45.4874 ± 2.5832^{Ba} (0)

Means ± standard deviation followed by the same superscript capital letters in the row or lower case letters in the column indicates no statistical difference (*p > 0.05*)

Table 3 The fracture modes of the tested groups

Group	Adhesive agents	Adhesive failure	Cohesive failure	Mixed failure
Sensodyne® Rapid Relief	Optibond® XTR (n = 18)	15 (83.33%)	3 (16.67%)	0
	Optibond® FL (n = 20)	20 (100%)	0	0
Colgate® Sensitive Pro-Relief™	Optibond® XTR (n = 19)	16 (84.21%)	3 (15.79%)	0
	Optibond® FL (n = 20)	17 (85%)	3 (15%)	0
Colgate® Regular Flavor	Optibond® XTR (n = 20)	17 (85%)	3 (15%)	0
	Optibond® FL (n = 20)	20 (100%)	0	0
Negative control	Optibond® XTR (n = 20)	17 (85%)	3 (15%)	0
	Optibond® FL (n = 20)	18 (90%)	2 (10%)	0

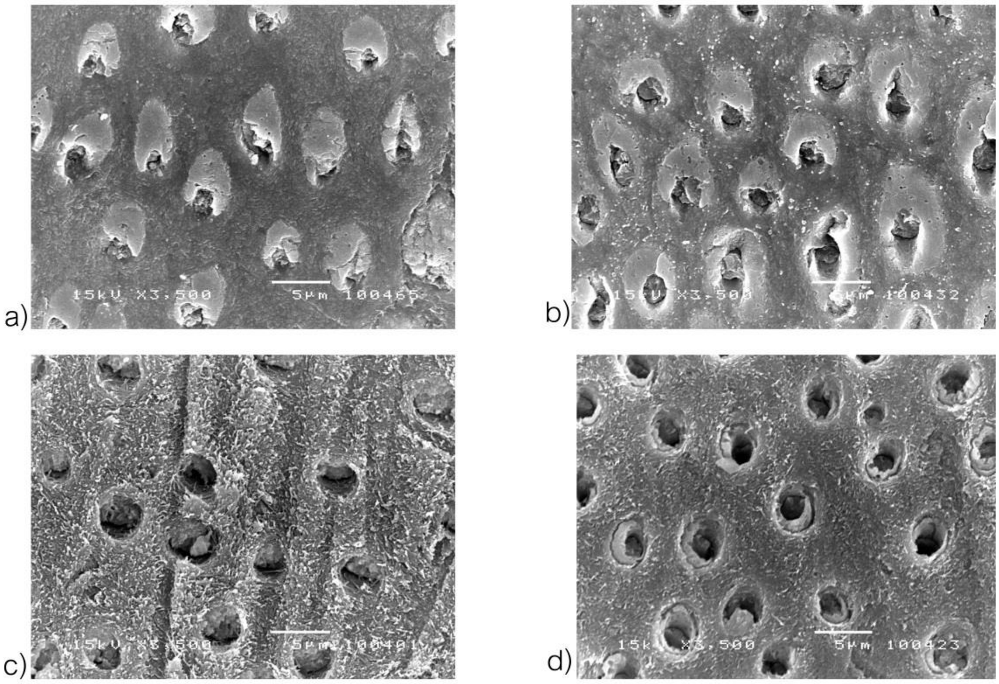


Fig. 10 Scanning electron micrographs of debonded specimens treated with Optibond® XTR in each group (x 3,500): a) **group1** - Sensodyne® Rapid Relief, b) **group 2** - Colgate® Sensitive Pro-Relief™, c) **group 3** - Colgate® Regular Flavor, and d) **group 4** – Negative control

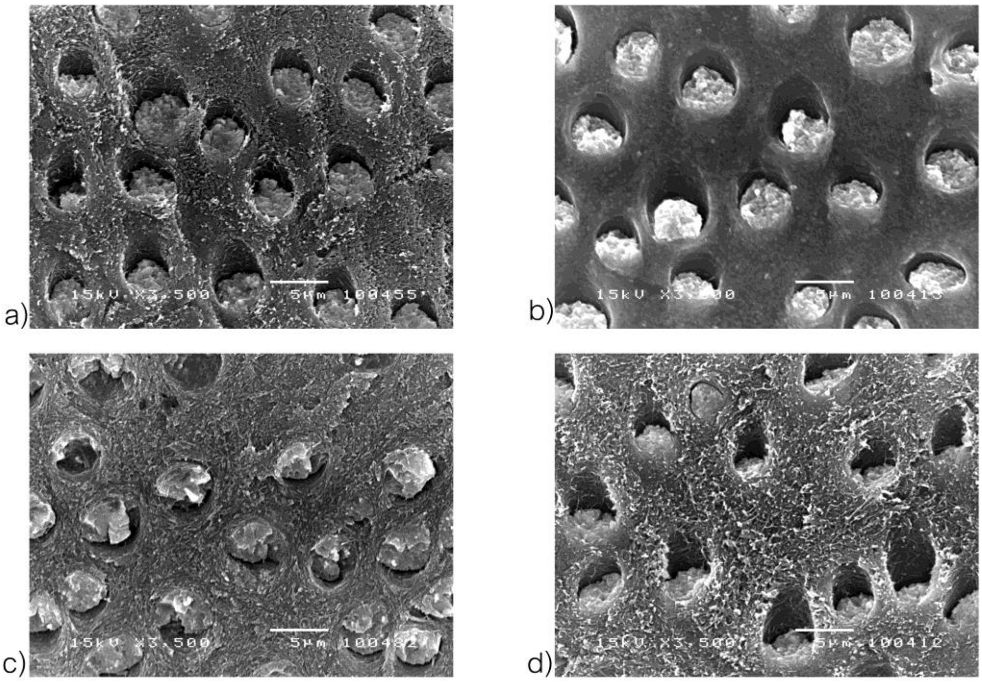


Fig. 11 Scanning electron micrographs of debonded specimens treated with Optibond® FL in each group (x 3,500): a) **group1** - Sensodyne® Rapid Relief, b) **group 2** - Colgate® Sensitive Pro-Relief™, c) **group 3** - Colgate® Regular Flavor, and d) **group 4** – Negative control

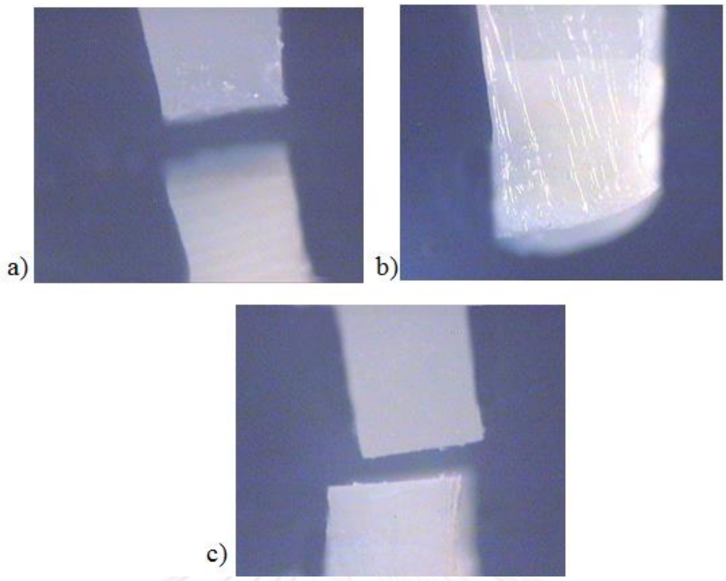


Fig. 12 The strereomicroscopic view of a) adhesive failure, b) cohesive failure in dentin, and c) cohesive failure in composite resin (magnification: 40X).

Chapter V

Discussion

The hydrodynamic theory has been widely accepted as the principal mechanism for dentin hypersensitivity. Based on this theory, a substance occluding the dentinal tubules can cause a decrease in dentinal fluid flow, thereby reducing the clinical symptoms of dentin hypersensitivity (5). Previous studies have demonstrated that using an 8% arginine or an 8% strontium acetate toothpaste resulted in significant tubule occlusion compared with the negative control (15, 49, 104-106). However, the use of these dentifrices may alter the microtensile bond strengths of adhesives to dentin.

The results of the present study revealed that both 8% arginine and 8% strontium toothpastes significantly reduced the microtensile bond strengths of adhesives to dentin. This may be because these two desensitizing toothpastes occluded dentinal tubules, and made dentin more resistant to acid challenge (15, 49, 104, 105). *In vitro* and *in situ* studies have demonstrated that following acid challenge (grapefruit juice (49, 105), Coca-Cola® (15), and citric acid(107)), dentin samples treated with 8% arginine or 8% strontium toothpaste had significantly more occluded dentin tubules than the negative control (15, 49, 104, 105). These desensitizing toothpastes formed occluding layers that were resistant to acid challenge (15, 49, 104, 105). Therefore, these layers may be resistant to acid etching used in bonding procedures, and may chemically and physically prevent complete penetration of the bonding agents. We found that the desensitizing paste groups bonded with Optibond® XTR (pH = 1.6-2.4), which was less acidic than Optibond® FL (pH = 1.8), demonstrated a significantly lower mean microtensile bond strength than the Optibond® FL groups. An etchant with lower acidity may result in less tubular penetration of the bonding agent, resulting in lower microtensile bond strength.

A previous study indicated that Colgate Sensitive Pro-Relief™ desensitizing paste did not have a significant effect on the shear bond strength of the composites bonded to enamel (108). Similarly, a previous study by Canares, *et al*. reported that 8% arginine desensitizing toothpaste had no effect on the bond strength of composites bonded to dentin (109). The differences in findings between these *in vitro* studies and the present study may be due to differences in study design. The formers only applied the desensitizing toothpaste once and did not immerse the samples in artificial saliva to simulate the oral environment, therefore, the environment and application methods of these studies may not be sufficient for the precipitation process to occur, generating results dissimilar to those of the present study.

SEM analysis of our samples demonstrated partial obstruction of the dentinal tubules in group 1 using Optibond® XTR and group 2 using Optibond® XTR. The groups with blocked tubules also had significantly lower bond strength compared to the other groups suggesting that tubule occlusion was responsible, at least in part, for decreasing bond strength. In addition, the SEM analysis from our pilot study (Fig. 13) after brushing dentin specimens with the same desensitizing toothpaste also demonstrated similar phenomenon. However, further compositional analysis may be needed to determine exactly what was obstructing the dentinal tubules of these specimens. Petrou, *et al.* treated dentin specimens with an 8% arginine desensitizing paste, which occluded the dentinal tubules, and analyzed them by electron spectroscopy for chemical analysis (15), finding that calcium, oxygen, and phosphorus levels were significantly increased. Carbonate compound was also detected on the treated dentin surface. They concluded that the treated surfaces had been remineralized, and that calcium carbonate was simultaneously deposited on the dentin surface (15). Earl, *et al.* analyzed dentin specimens treated with an 8%

strontium acetate dentifrice using energy dispersive x-ray spectroscopy (EDX). EDX analysis indicated the presence of strontium within the dentin tubules (23).

Although reduction of resin tag or hybrid layer in dentinal tubules may affect the microtensile bond strength, previous studies indicated that tubular occlusion not always affect dentin bond strength (110, 111). The penetration of resin tags into the dentinal tubules is believed to contribute in only a minor way to overall dentin bond strength (112). In addition, the finding that bond strength value drop when deeper dentin is prepared and intertubular dentin occupies less of the total bonding sites confirms the major involvement of intertubular dentin in the eventual bond stability (113). Moreover, a recent in vitro study suggested no influence of resin tags on bond strength, as microtensile bond strengths decreased with or without the presence of resin tags after thermocycling (114). Therefore, the cause of lower bond strength in the desensitizing toothpaste groups might not only come from the dentinal tubule occlusion.

Both Sensodyne® Rapid Relief and Colgate® Sensitive Pro-Relief™ were able to plug dentinal tubules through an interaction between their respective active ingredients, abrasive agents, and the dentin itself (49, 54). However, there were some differences between these two desensitizing pastes. Arginine was absorbed onto the surface of calcium carbonate forming a positively charged alkaline agglomerate (15). This alkaline agglomerate had a high affinity to dentin, and relied on the deposition of calcium and phosphate from saliva to deposit onto dentin and to occlude the dentin tubules. The presence of saliva was, therefore, essential for the mechanism of action of arginine (15). However, strontium-based dentifrices function based on a different mechanism of action. Strontium is an alkaline earth metal, which has a strong inherent absorptive capacity to calcified tissues, especially those with a high organic content such as dentin (115). This may be because strontium permeated into dentin and adsorbed into or onto organic connective tissues, including odontoblast processes, as was claimed in an earlier study using the metallic compound strontium chloride (116). Another study showed that strontium penetrated dentinal tubules, and was thought to occlude the tubules by substituting for calcium in hydroxyapatite (105).

In the present study, Optibond® FL was chosen to represent etch and rinse adhesives, and Optibond® XTR, self-etch adhesives. Optibond® FL has had long-term clinical track (68, 117), and has been considered to be the gold standard for adhesives (118). Optibond® XTR is a simplified version of adhesive, a mild two-step self-etch system, utilizing a functional monomer similar to that of Optibond® FL, glycerol phosphate dimethacrylate, which is a phosphate monomer that has been used for bonding to dentin for over 50 years (119). However, it has only recently been introduced; therefore, there have been few studies on its dentin bonding strength. In the studies that have been published, Optibond® XTR did not demonstrate a lower bond strength compared with Optibond® FL (118, 119). These findings are consistent with the results of the present study, where no significant difference was found between specimens bonded with Optibond® XTR and Optibond® FL in the regular fluoride toothpaste group or the negative control group. Nevertheless, these two adhesives performed differently in the two desensitizing toothpaste groups.

The presence of smear layer during dentin preparation by using a series of silicon carbide paper can also be a co-factor on bonding strength (120). Etch and rinse system and self-etch system have different interaction with smear layer. Etch and rinse adhesives are characterized by an initial etching step, followed by a compulsory rinsing procedure which is responsible for the complete removal of smear layer and smear plugs (120). Differently from etch and rinse system, self-etch adhesives do not require a separate etching step, as they contain acidic monomers that simultaneously etch and prime the dental substrate. Due to such acidic characteristics, self-etch adhesives are able to dissolve the smear layer and demineralize the underlying dentin (81). The finding that the self-etch adhesives did not totally remove the smear layer or open all the tubules may be important for the bond strength. If the adhesive's capacity to dissolve the smear layer is limited, the bond strengths to the dentin with a thick smear layer may be reduced (121). However, the self-etch systems were gradually modified in the last few years and one important change was the increase in their aggressiveness (122). According to the manufacturer, the pH of Optibond® XTR primer is 2.4 until it is dispensed. Acetone rapidly evaporates from the material, increasing the concentration of glycerol phosphate dimethacrylate and thereby reducing the pH to 1.6 (118). Thus, using Optibond® XTR may have less effect by smear layer than former mild self-etch adhesives.

The present study employed microtensile bond strength test to minimize the occurrence of dentin cohesive failure, which has been reported to occur in up to 80% of the specimens in conventional shear and tensile tests (90, 123). A characteristic feature common to all variations of the microtensile bond strength test method is the use of a relatively small cross-sectional surface area of 1 mm^2 or less. A smaller bonding area reduces the probability of sample internal defects and provides a more homogeneous distribution of stress during loading, thus minimizing the chance of dentin cohesive failure. The small size of the dentin/resin composite slabs allows for testing multiple specimens derived from the same tooth, which makes it necessary to treat the respective bond strength values as repeated measurements in the statistical analysis (90).

The pre-test failure specimens were excluded from the data analysis to avoid the high scatter of the bond strength data (124). The pre-test failure can be an additional source for the scatter in the microtensile test results. Spontaneous interfacial debonded specimens are not treated in the same statistical manner by all research papers. Many of papers report the number of pre-test failures but not include them in the statistical, whereas others include these as zero values in the statistical analysis (124).

Our *in vitro* study was performed using extracted teeth without simulating dentinal fluid pressure, so it is difficult to compare the results with the clinical situation. When dentin is clinically exposed to the oral cavity, dentinal fluid may move from pulp to exposed dentin surface because of the pulpal interstitial fluid pressure. Studies have reported that dentinal fluid flow affected the ingress of adhesive resins into the dentinal tubules (125, 126). Therefore, the results of the present study should be confirmed by a clinical study.

Conclusion

The present study demonstrated that:

1) The microtensile bond strength of adhesive to dentin specimens treated with 8% arginine or 8% strontium acetate desensitizing toothpaste were significantly lower than specimens treated with a regular fluoride toothpaste and specimens in the negative control groups.
2) The type of toothpaste and the type of adhesive agent had a significant effect on microtensile bond strength.

There were significant differences in bond strength between the type of adhesive agent in specimens treated with 8% arginine and 8% strontium acetate desensitizing toothpaste.

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APPENDIX

Pilot study

Part I

Method

1. Forty bovine incisors were collected, cleaned and stored in 0.01% thymol solution.

2. Roots were removed 1 mm apical to the cemental-enamel junction and were embedded into a self-curing resin.

3. The labial surface was ground flat using a polishing machine.

4. The specimens were then randomly divided in 20 groups (2 teeth per group):

Group 1: Brushed with Sensodyne® Rapid Relief for 1 day and bonded with Optibond® FL.

Group 2: Brushed with Sensodyne® Rapid Relief for 3 days and bonded with Optibond® FL.

Group 3: Brushed with Sensodyne® Rapid Relief for 7 days and bonded with Optibond® FL.

Group 4: Brushed with Sensodyne® Rapid Relief for 1 day and bonded with Optibond® XTR.

Group 5: Brushed with Sensodyne® Rapid Relief for 3 days and bonded with Optibond® XTR.

Group 6: Brushed with Sensodyne® Rapid Relief for 7 days and bonded with Optibond® XTR.

Group 7: Brushed with Colgate® Sensitive Pro-Relief™ for 1 day and bonded with Optibond® FL.

Group 8: Brushed with Colgate® Sensitive Pro-Relief™ for 3 days and bonded with Optibond® FL.

Group 9: Brushed with Colgate® Sensitive Pro-Relief™ for 7 days and bonded with Optibond® FL.

Group 10: Brushed with Colgate® Sensitive Pro-Relief™ for 1 day and bonded with Optibond® XTR.

Group 11: Brushed with Colgate® Sensitive Pro-Relief™ for 3 days and bonded with Optibond® XTR.

Group 12: Brushed with Colgate® Sensitive Pro-Relief™ for 7 days and bonded with Optibond® XTR.

Group 13: Brushed with Colgate® Regular Flavor for 1 day and bonded with Optibond® FL.

Group 14: Brushed with Colgate® Regular Flavor for 3 days and bonded with Optibond® FL.

Group 15: Brushed with Colgate® Regular Flavor for 7 days and bonded with Optibond® FL.

Group 16: Brushed with Colgate® Regular Flavor for 1 day and bonded with Optibond® XTR.

Group 17: Brushed with Colgate® Regular Flavor for 3 days and bonded with Optibond® XTR.

Group 18: Brushed with Colgate® Regular Flavor for 7 days and bonded with Optibond® XTR.

Group 19: Immersed in artificial saliva for 1 day and bonded with Optibond® FL.

Group 20: Immersed in artificial saliva for 1 day and bonded with Optibond® XTR.

5. light-cured composite was built up onto the treated dentin surface.

6. The completed specimens were stored in distilled water at 37°c for 24 h then cut into 4 slabs per tooth (8 slabs per group) by a low speed cutting machine.

7. In each slab, a gentle curve with the narrowest portion at the resin-dentin interface was prepared using a diamond finishing bur.

8. Microtensile bond strength were measured using a universal testing machine.

Results

Table 4 Pilot study result

Group (N=8)	Toothpaste	Duration	Bonding agent	Mean (MPa)	Standard deviation (MPa)
1	Sensodyne® Rapid Relief	1	Optibond® FL	37.5407	3.2750
2	Sensodyne® Rapid Relief	3	Optibond® FL	33.5234	2.3475
3	Sensodyne® Rapid Relief	7	Optibond® FL	32.5634	4.0123
4	Sensodyne® Rapid Relief	1	Optibond® XTR	35.0043	3.2044
5	Sensodyne® Rapid Relief	3	Optibond® XTR	31.7050	4.1002
6	Sensodyne® Rapid Relief	7	Optibond® XTR	31.2905	2.5690
7	Colgate® Sensitive Pro-Relief™	1	Optibond® FL	37.4221	3.1580
8	Colgate® Sensitive Pro-Relief™	3	Optibond® FL	32.7702	3.8500
9	Colgate® Sensitive Pro-Relief™	7	Optibond® FL	32.0679	2.2476
10	Colgate® Sensitive Pro-Relief™	1	Optibond® XTR	35.7743	2.9754
11	Colgate® Sensitive Pro-Relief™	3	Optibond® XTR	31.5705	3.3321

Group (N=8)	Toothpaste	Duration	Bonding agent	Mean (MPa)	Standard deviation (MPa)
12	Colgate® Sensitive Pro-Relief™	7	Optibond® XTR	30.9220	3.1970
13	Colgate® Regular Flavor	1	Optibond® FL	38.0054	4.0004
14	Colgate® Regular Flavor	3	Optibond® FL	36.2215	3.9778
15	Colgate® Regular Flavor	7	Optibond® FL	35.8965	2.0103
16	Colgate® Regular Flavor	1	Optibond® XTR	34.1324	3.0800
18	Colgate® Regular Flavor	7	Optibond® XTR	31.0056	4.1984
19	None	1	Optibond® FL	38.2995	3.0150
20	None	1	Optibond® XTR	35.2146	3.4320

Data Analysis

After analyzing the pilot study's results using 1-way ANOVA, we found that there were statistically significant differences between 1 and 3 days period in every toothpaste ($p<0.05$). In contrast with comparing the 3 and 7 days groups, there were no statistically significant differences ($p>0.05$).

Consequently, we choose 3 days-duration for this study.

Table 5 Results from 3 days-duration pilot study

Bonding type	Mean microtensile bond strength (SD)			
	Artificial saliva	Sensodyne® Rapid Relief (3 days)	Colgate® Sensitive Pro-Relief™ (3 days)	Colgate® Regular Flavor (3 days)
Total-etch	38.2995(3.0150)	33.5234(2.3475)	37.4221(3.8500)	38.0054(3.9778)
Self-etch	35.2146(3.4320)	31.7050(4.1002)	31.5705(3.3321)	31.0657(2.0005)

Sample size calculation form test group comparison

1. Artificial saliva total-etch and Sensodyne® Rapid Relief total-etch = 0.21

2. Artificial saliva total-etch and Colgate® Sensitive Pro-Relief™ total-etch = 10.4976

3. Artificial saliva total-etch and Colgate® Regular Flavor total-etch = 108.17

4. Sensodyne® Rapid Relief total-etch and Colgate® Sensitive Pro-Relief™ total-etch = 1.56

5. Sensodyne® Rapid Relief total-etch and Colgate® Regular Flavor total-etch= 1.39

6. Colgate® Sensitive Pro-Relief™ total-etch and Colgate® Regular Flavor total-etch =

0.49

7. Artificial saliva self-etch and Sensodyne® Rapid Relief self-etch = 13.89

8. Artificial saliva self-etch and Colgate® Sensitive Pro-Relief™ self-etch = 0.01

9. Artificial saliva self-etch and Colgate® Regular Flavor self-etch = 1.25

10. Sensodyne® Rapid Relief self-etch and Colgate® Sensitive Pro-Relief™ self-etch = 349.92

11. Sensodyne® Rapid Relief self-etch and Colgate® Regular Flavor self-etch = 113.913

12. Colgate® Sensitive Pro-Relief™ self-etch and Colgate® Regular Flavor self-etch= 74.32

Part II

Method

1. Eight bovine incisors were collected, cleaned and stored in 0.01% thymol solution.

2. Roots were removed 1 mm apical to the cemental-enamel junction and were embedded into a self-curing resin.

3. The labial surface was ground flat using a polishing machine.

4. The specimens were then randomly divided in 4 groups (2 teeth per group):

Group 1: Brushed with Sensodyne® Rapid Relief for 3 days.

Group 2: Brushed with Colgate® Sensitive Pro-Relief™ for 3 days.

Group 3: Brushed with Colgate® Regular Flavor for 3 days.

Group 4: Immersed in artificial saliva for 3 days (negative control).

5. The specimens were sectioned longtitudinal and were processed for scanning electron microscopy using standard SEM specimen processing techniques.

Figure 13 demonstrated the results of this pilot study.

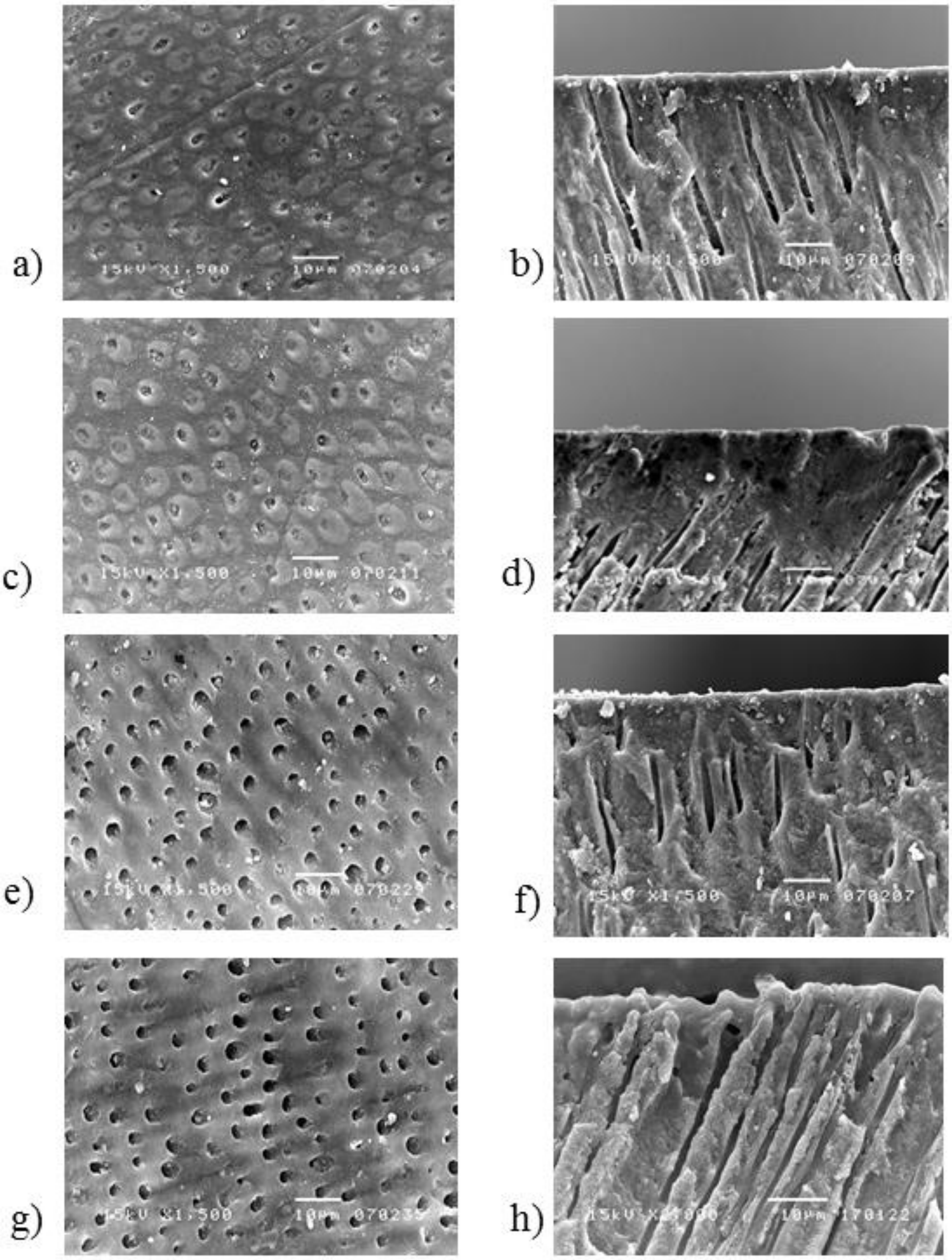

Fig. 13 Scanning electron micrographs of dentin specimens 3-day treated with Sensodyne® Rapid Relief (a and b), Colgate® Sensitive Pro-Relief™ (c and d), Colgate® Regular Flavor (e and f), and Negative control (g and h)

Raw data

Table 6 Microtensile bond strength values (MPa) and the fracture modes of specimens in group Sensodyne® Rapid Relief and Optibond® XTR

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	27.3402	Adhesive
2	22.0812	Adhesive
3	23.0931	Adhesive
4	30.7494	Adhesive
5	26.9463	Adhesive
6	33.0840	Cohesive in resin composite
7	Pre-test failure	Adhesive
8	32.0484	Adhesive
9	20.7384	Adhesive
10	34.0931	Cohesive in resin composite
11	27.6648	Adhesive
12	36.0294	Adhesive
13	24.2384	Adhesive
14	22.9899	Adhesive
15	35.0010	Adhesive
16	29.9744	Adhesive
17	21.0920	Cohesive in dentin
18	Pre-test failure	Adhesive
19	29.4502	Adhesive
20	26.4877	Adhesive
Mean ± SD	**27.9501 ± 4.9057**	

Table 7 Microtensile bond strength values (MPa) and the fracture modes of specimens in group Sensodyne® Rapid Relief and Optibond® FL

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	37.5961	Adhesive
2	34.0127	Adhesive
3	32.1094	Adhesive
4	36.1775	Adhesive
5	31.6509	Adhesive
6	30.7677	Adhesive
7	33.8275	Adhesive
8	29.9914	Adhesive
9	28.8498	Adhesive
10	36.0021	Adhesive
11	33.9760	Adhesive
12	26.0312	Adhesive
13	34.1773	Adhesive
14	32.2074	Adhesive
15	32.5639	Adhesive
16	37.6483	Adhesive
17	35.5538	Adhesive
18	28.2734	Adhesive
19	28.9100	Adhesive
20	34.1937	Adhesive
Mean ± SD	**32.7260 ± 3.200**	

Table 8 Microtensile bond strength values (MPa) and the fracture modes of specimens in group Colgate® Sensitive Pro-Relief™ and Optibond® XTR

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	28.3495	Adhesive
2	38.2203	Cohesive in resin composite
3	36.9477	Adhesive
4	32.1928	Adhesive
5	33.3048	Adhesive
6	21.4058	Adhesive
7	18.2034	Cohesive in dentin
8	28.2203	Adhesive
9	Pre-test failure	Adhesive
10	23.0442	Adhesive
11	19.2947	Adhesive
12	26.0384	Adhesive
13	25.0384	Adhesive
14	29.3048	Cohesive in resin composite
15	28.8894	Adhesive
16	27.4975	Adhesive
17	33.0452	Adhesive
18	32.0475	Adhesive
19	30.4048	Adhesive
20	27.4947	Adhesive
Mean ± SD	**28.3655 ± 5.4309**	

Table 9 Microtensile bond strength values (MPa) and the fracture modes of specimens in group Colgate® Sensitive Pro-Relief™ and Optibond® FL

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	34.6987	Adhesive
2	39.4526	Cohesive in resin composite
3	38.1002	Adhesive
4	28.0192	Adhesive
5	29.0291	Adhesive
6	37.0845	Adhesive
7	40.1178	Adhesive
8	33.0927	Adhesive
9	34.6695	Adhesive
10	37.5847	Adhesive
11	34.0967	Adhesive
12	32.0057	Adhesive
13	27.4386	Cohesive in dentin
14	30.8563	Adhesive
15	33.5856	Adhesive
16	37.7096	Adhesive
17	37.0287	Adhesive
18	35.4854	Cohesive in resin composite
19	30.3047	Adhesive
20	32.0375	Adhesive
Mean ± SD	**34.1199 ± 3.756**	

Table 10 Microtensile bond strength values (MPa) and the fracture modes of specimens in group Colgate® Regular Flavor and Optibond® XTR

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	45.1039	Adhesive
2	44.0495	Adhesive
3	40.9753	Adhesive
4	48.2098	Adhesive
5	47.3058	Adhesive
6	45.3086	Adhesive
7	43.0050	Adhesive
8	37.4480	Adhesive
9	39.0595	Adhesive
10	46.2433	Adhesive
11	38.6485	Adhesive
12	48.0833	Adhesive
13	36.0495	Adhesive
14	42.7769	Adhesive
15	45.6495	Adhesive
16	40.9896	Adhesive
17	47.6183	Adhesive
18	42.2293	Adhesive
19	44.4095	Adhesive
20	48.0085	Adhesive
Mean ± SD	**43.5586 ± 3.7308**	

Table 11 Microtensile bond strength values (MPa) and the fracture modes of specimens in group Colgate® Regular Flavor and Optibond® FL

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	41.6539	Adhesive
2	46.7369	Adhesive
3	37.0384	Cohesive in resin composite
4	36.0484	Adhesive
5	42.0394	Adhesive
6	41.3837	Adhesive
7	45.5632	Adhesive
8	39.0485	Adhesive
9	43.0595	Adhesive
10	38.6574	Adhesive
11	48.8464	Adhesive
12	40.0091	Adhesive
13	41.2834	Cohesive in resin composite
14	39.0495	Adhesive
15	42.4752	Adhesive
16	44.9583	Adhesive
17	42.0484	Cohesive in resin composite
18	47.2533	Adhesive
19	38.4625	Adhesive
20	41.9844	Adhesive
Mean ± SD	**41.8800 ± 3.4454**	

Table 12 Microtensile bond strength values (MPa) and the fracture modes of specimens in group negative control and Optibond® XTR

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	48.2350	Adhesive
2	48.1094	Cohesive in resin composite
3	46.0182	Adhesive
4	50.1023	Adhesive
5	47.1079	Cohesive in resin composite
6	42.4020	Adhesive
7	41.0157	Adhesive
8	43.7029	Adhesive
9	44.8203	Adhesive
10	41.5102	Adhesive
11	43.0032	Cohesive in dentin
12	43.6692	Adhesive
13	42.4849	Adhesive
14	41.7203	Adhesive
15	43.7331	Adhesive
16	40.7118	Adhesive
17	39.9187	Adhesive
18	42.2104	Adhesive
19	43.0172	Adhesive
20	43.7640	Adhesive
Mean ± SD	**43.8628 ± 2.7549**	

Table 13 Microtensile bond strength values (MPa) and the fracture modes of specimens in group negative control and Optibond® FL

Specimen No.	Microtensile bond strength values (MPa)	Fracture modes
1	45.3765	Adhesive
2	47.0862	Adhesive
3	45.9604	Adhesive
4	44.5200	Adhesive
5	47.3302	Adhesive
6	41.0297	Adhesive
7	42.1520	Adhesive
8	41.7536	Adhesive
9	43.0162	Adhesive
10	41.6283	Adhesive
11	48.9213	Cohesive in resin composite
12	49.1183	Adhesive
13	47.1002	Adhesive
14	48.1023	Adhesive
15	47.8263	Adhesive
16	48.8821	Cohesive in resin composite
17	45.2100	Adhesive
18	44.1923	Adhesive
19	45.7600	Cohesive
20	44.7821	Adhesive
Mean ± SD	**45.4874 ± 2.583**	

VITA

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