ผลของระยะเวลาการล้างด้วยสารละลายกรดเอทิลีนไดเอมีนเตตระอะซิติกต่อกำลังแรงยึดแบบดึงระดับจุลภาคของเรซินซีลเลอร์กับเนื้อฟันในคลองรากฟัน

นายสุทธ์ พันธุ์สว่างวงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2559

THE EFFECT OF EDTA IRRIGATION TIME ON THE MICROTENSILE BOND STRENGTH OF RESIN SEALERS AND ROOT CANAL DENTIN

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Endodontology
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สุทธ์ พันธุ์สว่างวงศ์ : ผลของระยะเวลาการล้างด้วยสารละลายกรดเอทิลีนไดเอมีนเตตระอะซิติกต่อกำลังแรงยึดแบบดึงระดับจุลภาคของเรซินซีลเลอร์กับเนื้อฟันในคลองรากฟัน (THE EFFECT OF EDTA IRRIGATION TIME ON THE MICROTENSILE BOND STRENGTH OF RESIN SEALERS AND ROOT CANAL DENTIN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ทญ. ดร. อุไรวรรณ โชคชนะชัยสกุล, 80 หน้า.

บทนำ: มีหลายการศึกษาแนะนำให้ล้างด้วยสารละลายกรดเอทิลีนไดเอมีนเตตระอะซิติก (อีดีทีเอ) และตามด้วยสารละลายโซเดียมไฮโปคลอไรท์ก่อนทำการอุดคลองรากฟันแต่อย่างไรก็ตามไม่มีการศึกษาใดที่กล่าวถึงระยะเวลาที่เหมาะสมในการล้างด้วยอีดีทีเอ ดังนั้นวิทยานิพนธ์ฉบับนี้จัดทำเพื่อรายงานผลการศึกษาและเปรียบเทียบการล้างด้วยอีดีทีเอที่ระยะเวลาต่างกันต่อกำลังแรงยึดแบบดึงระดับจุลภาคของเรซินซีลเลอร์กับเนื้อฟันในคลองรากฟัน วิธีดำเนินการวิจัย: นำฟันกรามน้อยบนรากเดี่ยวมนุษย์จำนวน 160 ซี่มาตัดส่วนตัวฟันออกและฝังลงในเรซิน ทำการเตรียมคลองรากฟันด้วยไฟล์ที่หมุนด้วยเครื่อง (Protaper Universal) ประกอบกับการล้างคลองรากฟันด้วยน้ำกลั่น นำคลองรากฟันที่ผ่านการเตรียมมาล้างด้วยสารละลายโซเดียมไฮโปคลอไรท์ 5 เปอร์เซ็นต์ ทำการแบ่งออกเป็น 5 กลุ่ม กลุ่มที่ 1 ล้างด้วยน้ำกลั่น ในขณะที่กลุ่มที่ 2 ถึง 5 ล้างด้วยอีดีทีเอ 17 เปอร์เซ็นต์เป็นระยะเวลา 1, 3, 5 และ 10 นาทีตามลำดับ จากนั้นตามด้วยล้างน้ำกลั่น นำคลองรากฟันที่ผ่านการเตรียมพื้นผิวแล้ว 2 คลองรากต่อกลุ่มมาตรวจดูพื้นผิวด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด นำรากฟันส่วนที่เหลือในแต่ละกลุ่มมาแบ่งออกเป็น 2 กลุ่มตามชนิดของซีลเลอร์ที่ใช้อุดคลองรากฟัน ได้แก่ เอเอชพลัสและเมทาซีล (n=15) หลังจากอุดคลองรากฟันทำการตัดเตรียมชิ้นงานแบบแท่งสำหรับการทดสอบกำลังแรงยึดด้วยแรงดึงระดับจุลภาค ทำการใส่แรงดึงจนกระทั่งเกิดการหลุดของชิ้นงานออกจากกัน วิเคราะห์รูปแบบความล้มเหลวของชิ้นงานและนำค่ากำลังแรงยึดที่ได้มาวิเคราะห์ทางสถิติด้วยการวิเคราะห์ความแปรปรวนแบบทางเดียวและวิธีการของทูกีย์ ผลการทดลอง: ในกลุ่มเมทาซีลกลุ่มตัวอย่างที่ถูกเตรียมพื้นผิวด้วยอีดีทีเอเป็นเวลา 10 นาที (กลุ่มที่ 5) ให้ค่ากำลังแรงยึดที่สูงกว่ากลุ่มตัวอย่างที่ไม่ใช้อีดีทีเอ (กลุ่มที่ 1) ($p<0.001$) พบความล้มเหลวแบบผสมเป็นส่วนมากในทุกกลุ่ม จากผลการตรวจดูพื้นผิวด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดพบว่ากลุ่มที่ล้างด้วยสารละลายโซเดียมไฮโปคลอไรท์พบชั้นเสมียร์ปกคลุมพื้นผิวเนื้อฟันส่วนกลุ่มที่ล้างด้วยอีดีทีเอตั้งแต่ 1 ถึง 10 นาทีไม่พบชั้นเสมียร์หลงเหลืออยู่และมีการสูญเสียแร่ธาตุของเนื้อฟันที่ระดับความลึกต่างๆกันและมีการเผยผึ่งของคอลลาเจน สรุปผลการวิจัย: ระยะเวลาในการล้างอีดีทีเอมีผลต่อกำลังแรงยึดแบบดึงระดับจุลภาคของเมทาไครเลตเรซินซีลเลอร์ (เมทาซีล) ต่อเนื้อฟันในคลองรากฟัน

ภาควิชา ทันตกรรมหัตถการ ลายมือชื่อนิสิต ....

สาขาวิชา วิทยาเอ็นโดดอนต์ ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

ปีการศึกษา 2559

5675822932 : MAJOR ENDODONTOLOGY
KEYWORDS: AH PLUS / EDTA / METASEAL / MICROTENSILE BOND STRENGTH / ROOT CANAL SEALER

SUTT PANSAWANGWONG: THE EFFECT OF EDTA IRRIGATION TIME ON THE MICROTENSILE BOND STRENGTH OF RESIN SEALERS AND ROOT CANAL DENTIN. ADVISOR: URAIWAN CHOKECHANACHAISAKUL, Ph.D., 80 pp.

Introduction: Several studies have recommended the use of EDTA as a final flush before root canal obturation, but the optimal irrigating time remains unverified. The aim of the study was to determine how the duration of EDTA irrigation affects microtensile bond strength. Materials and methods: The 160 extracted human premolars were decoronated and embedded in resin block. Root canals were prepared by using the rotary files (Protaper Universal) and distilled water irrigation, and irrigated with 5% NaOCl. In Group 1, this was followed by irrigation with distilled water, while in Groups 2–5, this was followed by irrigation with 17% EDTA for 1, 3, 5, and 10 min, followed by distilled water. Two specimens of each group were used for scanning electron microscopic (SEM) observation. The remaining specimens were divided into 2 groups—AH Plus and MetaSEAL (n = 15 each). The specimens were prepared for microtensile tests. The failure mode was identified, and the bond strength value was analyzed using one-way ANOVA and Tukey's HSD post-hoc test. Results: The 10-min EDTA-treated specimens (Group 5) showed greater microtensile bond strength than non-EDTA-treated specimens (Group 1) ($p < 0.001$) in MetaSEAL group. Mixed failure accounted for the majority of failures in all groups. In SEM, the NaOCl group showed a smear layer covering the dentin surface, but the EDTA groups showed an absence of smear layer and various depths of demineralized dentin and exposed collagen. Conclusions: The duration of EDTA irrigation affected on the microtensile bond strength of the methacrylate resin sealer and root dentin.

Department:	Operative Dentistry	Student's Signature	....
Field of Study:	Endodontology	Advisor's Signature	....
Academic Year:	2016		

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CHAPTER I
INTRODUCTION

1.1 Background and Rationale

The objective of endodontic treatment is elimination of the infection within root canal system, followed by three-dimensional hermetic filling of the entire root canal spaces (1, 2). The hermetic sealing is a primary functions of the root canal fillings which are against ingrowth of bacteria from the oral environment, the entombment of remaining microbes, and the entire obturation at microscopic level to prevent stagnant fluid from accumulating and serving as nutrients for bacteria from any source (3).

Microleakage of root canal-treated tooth is the main cause of endodontic failure (4-6). The traditional obturation technique of gutta-percha and zinc oxide eugenol based sealer has been found more leakage because it has high solubility and does not effectively seal the root canal space (7, 8). Therefore, root canal sealer is important in sealing ability to reduce the leakage. As a consequence of root canal sealer development in order to improve the sealing quality, resin sealers are well-known in endodontists. The high bond strength of root canal sealer might create low leakage (9).

The internal surface of radicular dentin is necessary to condition in order to properly bond the root canal in each type of sealers. The root canal obturation technique with resin sealer requires dentin surface treatment such as removing smear layer to improve bond strength (10, 11) commonly by final flush with EDTA and sodium hypochlorite (12, 13). However, sodium hypochlorite, a strong oxidizing agent, leaves behind an oxygen rich layer on dentin surfaces that inhibits polymerization of methacrylate resins (14) and effects decrease of bond strength (15).

Nowadays, the studies about appropriate irrigation protocol for resin sealer-root canal obturation are not available. The majority studies tended to recommend

EDTA and follow by water as a final flush (9, 11, 16). Because EDTA which is a chelating agent occur chemical elimination reaction of inorganic part within root canal dentine (12, 13). Therefore, concentration, volume, and time duration of irrigation affect removal of smear layer, dentin plug, and inorganic material on the intertubular dentin in order to occur appropriate dentin surface to bond with resin sealer. The appropriate irrigating time of EDTA has not been studied yet. Thus, the aim of this study was to verify the time duration of EDTA irrigation that affect bond strength.

1.2 Research objective

To evaluate and compare microtensile bond strength of resin sealers and root canal dentin when irrigation with EDTA in difference duration time.

1.3 Hypothesis

H_0: There would be no significant difference in microtensile bond strength of resin sealers and root canal dentin among different EDTA irrigation time.

H_1: There would be significant difference in microtensile bond strength of resin sealers and root canal dentin among different EDTA irrigation time.

1.4 Limitations of research

1. This was an *in vitro* study which might not be the evidence to be totally applied to the clinical work.
2. The study was designed to obturate the root canals with only root canal sealers without core materials. It was not same as clinical situations that core materials were used with sealers. For the reason, this study was interested in evaluation of the only one interface (between root canal dentin and the root canal sealer).

3. The only coronal third was used because the middle and apical third of the root canal were too small to prepare the specimens for the microtensile test.

1.5 Expected benefit and application

The results of the research project could lead to clinical application of irrigation protocol in the root canals filled with resin based sealer.

1.6 Research design

In vitro experimental study

1.7 Ethical consideration

The research was approved by the Ethics Review Committee of Research Involving Human Research Subjects, Chulalongkorn University because of using extracted human teeth on June 2, 2015 (No. 042/2015)

CHAPTER II
LITERATURE REVIEW

2.1 Root canal sealers in endodontics

Root canal sealers are essential to seal the space between dentinal wall and the obturating core interface, to fill voids and irregularities in the root canal and to serve as lubricants during obturation (17). The American Association of Endodontist stated that root canal sealers were used in conjunction with a biologically acceptable semi-solid or solid obturating material to establish an adequate seal of the root canal system (18).

The ideal properties of an ideal root canal sealer (19) as following

1) Exhibits tackiness when mixed to provide good adhesion between itself and the canal wall when set
2) Establishes a hermetic seal
3) Radiopaque, so that it can be seen on a radiograph
4) Very fine powder, so that it can mix easily with liquid
5) No shrinkage on setting
6) No staining to tooth structure
7) Bacteriostatic, or at least does not encourage bacterial growth
8) Exhibits a slow set
9) Insoluble in tissue fluids
10) Tissue tolerant, non-irritating to periradicular tissue
11) Soluble in a common organic solvent, if it is necessary to remove the root canal filling

The development of root canal sealers starts with the introduction of a zinc oxide eugenol based sealer in 1933. Zinc oxide eugenol sealers were then improved in the several formulas to enhance their antimicrobial properties (20-23), flow (24), and film thickness (25). In 1951, a resin based sealer was introduced to provide adhesion to root canal dentin. According to antimicrobial effects, a zinc oxide eugenol sealer containing paraformaldehyde was marketed in 1965. However, formaldehyde based sealer was not recommended by AAE because of their extremely toxic properties. Calcium hydroxide was used to fabricate root canal sealers in late 1970s because calcium hydroxide has stimulation of periapical tissue properties and antimicrobial effects (26). In 1979, glass ionomer was suggested as a root canal sealer in account of bonding ability to dentin, fluoride release, antimicrobial activity, and biocompatibility. In 1984, calcium phosphate cement, its chemical composition and crystal structure similar to tooth and bone was introduced as a root canal sealer (27). In early 2000s, silicone was developed as a root canal sealer to provide adhesion. Later, the biocompatible materials such as MTA and bioceramic were introduced as root canal sealers. The details of sealers mentioned above will be described below as follows.

2.1.1 Zinc oxide eugenol based sealers

An early zinc oxide eugenol based sealer was introduced by Rickert and Dixon in 1933. In 1958, Grossman modified the formula of the sealer that nonstained teeth (28). The powder of Grossman's formula contained zinc oxide (42%), staybelite resin (27%), bismuth subcarbonate (15%), barium sulfate (15%), and sodium borate anhydrous (1%) and the liquid contained eugenol. This formula is the prototype of various brands of the zinc oxide eugenol based sealer. The setting reaction of the zinc oxide eugenol based sealer is a chemical process combined with physical embedding of zinc oxide in a matrix of zinc eugenolate (17).

An advantage of this sealer is antimicrobial properties (21, 29-33), however, a zinc oxide eugenol based sealer displayed very low bond strength to dentin. It was suggested that it had no adhesive properties to dentin (34-36), high solubility (7) and showed inferior sealing ability in comparison to other sealers (37-39).

These sealers were marketed in a lot of brands such as Pulp Canal Sealer and Pulp Canal Sealer EWT (SybronEndo, Orange, CA, USA), Procosol (Procosol, Inc., Philadelphia, PA, USA), Roth's sealer (Roth Internation), Tubli-Seal and Tubli-Seal EWT (SybronEndo, Orange, CA, USA), and CU sealer (Faculty of Dentistry, Chulalongkorn University, Thailand) etc.

In 1965, the zinc oxide eugenol based sealer was modified by adding a paraformaldehyde according to antimicrobial and mummifying effects. However, as its severe toxicity to host tissues outweighs any antimicrobial effects, it may possess as an ingredient in endodontic materials (17, 40). There were various studies reported about the toxicity of them (41-43). Moreover, the American Association of Endodontists recommended against the use of paraformaldehyde-containing filling materials or sealers because the use of such sealer is below the standard of care for endodontic treatment (44). The example of this sealer is Endomethasone (Septodont, Paris, France).

2.1.2 Calcium hydroxide based sealer

Calcium hydroxide was first used as a root canal sealer in late 1970s (45) because it had periapical tissue healing properties and antimicrobial effects (26). For this therapeutic reason, solubility is required for release of calcium hydroxide and sustained activity. This property opposes to the purpose of a root canal sealer.

As the high solubility of calcium hydroxide based sealer (7, 25), several studies have shown that no significant difference in leakage up to 32 weeks when compared with some zinc oxide eugenol sealers and epoxy resin based sealer (46-49). On the other hand, calcium hydroxide based sealer showed a poor performance on the long term (1 year) leakage study to other sealers (50). Several studies reported that calcium hydroxide based sealers performed unsatisfactorily on dentin adhesion in both presence and absence of smear layer (34, 51-53). Some of common brand are mentioned such as Calciobiotic Root Canal Sealer (CRCS®, Coltene/Whaledent Inc., Mahwah, NJ, USA), Sealapex (SybronEndo, Orange, CA, USA), Apexit, and Apexit Plus (Ivoclar Vivadent, Schaan, Liechtenstein).

2.1.3 Glass ionomer based sealer

Glass ionomer cements were introduced as root canal sealers (54, 55), according to their advantage such as chemical bonding to dentin, fluoride release, antimicrobial activity, and biocompatibility (56). Nonetheless, studies found that glass ionomer based sealer has no antimicrobial activity with other sealers (57) or minimal antimicrobial effect (20, 31). The glass ionomer sealer had a great number of solubility (7). Moreover, the bond strength of glass ionomer sealer is low (34, 58). The example of glass ionomer sealer is Ketac Endo (3M, ESPE)

2.1.4 Silicone based sealer

Silicone (polydimethylsiloxane) has been used in dentistry for a long time especially in prosthodontics as low dimensional change and low water sorption (59). A silicone (polydimethylsiloxane) based sealer had an acceptable solubility (7, 60) and good sealing ability (59, 61), however, it had no adhesion and had low bond strength to dentin (62). The example of silicone sealer is RoekoSeal (Roeko, Langenau, Germany).

2.1.5 Resin based sealer

Resin sealers have been developed in order to provide good adhesion and good sealing ability. They were divided into 3 types according to their main compositions: polyvinyl resin, epoxy resin, and methacrylate resin (17).

Polyvinyl resin

Polyvinyl resin was introduced as a root canal sealer by Schmitt in 1951 (42). A main composition of polyvinyl resin consists of a bismuth phosphate and zinc oxide-contained powder and a liquid composed of dichlorophenol, triethanolamine, copolymers of vinyls, and propionylacetophenone (63). Solubility and leakage of this sealer is acceptable (7, 50, 64, 65), however, it had lower bond strength than contemporary resin sealer (10, 66, 67). Presently, the polyvinyl resin sealer such as Diaket (3M, ESPE) is not available.

Epoxy resin

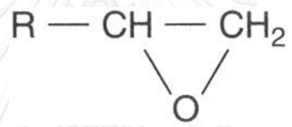


Figure 1 Chemical structure of epoxy resin

A prototype of epoxy resin sealer was first introduced as a root canal sealer in 1954 named "AH26" (Dentsply/De Tray) (68). It consists of bismuth(III) oxide, hexamethylenetetramine, silver, and titanium dioxide bisphenol-A-diglycidylether (69). It had higher bond strength than the polyvinyl resin and zinc oxide eugenol sealer (34, 66), acceptable solubility (7) and sealing ability (70). According to dentin silver-staining in the original epoxy resin sealer, the silver-free formula by absence of silver and titanium dioxide was introduced named "AH26 silver-free" (71, 72). It had higher bond strength than zinc oxide eugenol sealer (34, 58) and acceptable sealing ability (50, 65, 73). Polymerization of hexamethylenetetramine (methanamine) from both original and silver-free formula release some formaldehyde (74), which caused cytotoxic effect (69, 75, 76).

Another formula of epoxy resin is a two-paste mixing system named “AH Plus” (Dentsply DeTrey, Konstanz, Germany) which consists of an epoxide paste and an amine paste. The epoxide paste consists of diepoxide, calcium tungstate, zirconium oxide, aerosol, and pigment, while the amine paste consists of 1-adamantane amine, N,N’-dibenzyl-5-oxa-nonandiamine-1,9, TCD-Diamine, calcium tungstate zirconium oxide, aerosol, and silicone oil. These components allowed for polymerization without the unwanted formation of formaldehyde (43, 77). The monomers, diepoxides, and amine react to oligomers with epoxy- and amino- end groups, which for their part can react more with remaining monomers or other oligomers. This reaction forms high-molecular weight addition polymers to set which is called a polyaddition reaction. Its setting time is approximate 8 hours (78). This formula had lower cytotoxicity and no genotoxicity or mutagenicity when compared with the prototype included both original and silver-free formula (75, 79, 80). Nevertheless, it had similar solubility and sealing ability to the former (7, 50, 65, 81).

Methacrylate resin

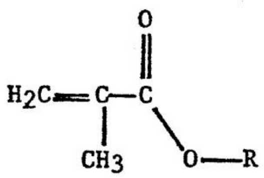


Figure 2 Chemical structure of methacrylate resin

Even though initial nonsurgical endodontic treatment using nonbonding root canal sealers have had a predictable outcome with high incidence of tooth retention (82). The development of resin-based sealers was the recognition that gutta percha does not bond to dentin or any conventional root canal sealers such as zinc oxide eugenol based sealer and epoxy resin based sealer (83). Bondable root canal sealers were developed to improve the seal, fracture resistance (84) by perfectly filled with a gap-free, solid mass (85) and promoted monoblocks of the filled root canal. The “monoblock” means a single unit, which recently can be classified into 3 types as primary, secondary, and tertiary. This classification is dependent on the numbers of interfaces between the bonding substrate and the material core (86).

A primary monoblock has only one interface that extends circumferentially between root filling material and root canal dentin. In a secondary monoblock, there are two interfaces; between cement and dentin and the between the cement and the core material. In a tertiary monoblock, there is the third interface that is a bondable coating on the surface of the core material.

The development of methacrylate resin sealers

The first generation of methacrylate resin-based sealer, “Hydron” (Hydron Technologies, Inc, Pompano Beach FL), was used in dentistry in the mid-1970s. There is poly [2-hydroxyethyl methacrylate] (poly [HEMA]) as the main ingredient that provides the sealer very hydrophilic (87-89). The use of this sealer alone for root canal obturation is an example of the primary monoblock concept. The sealer became obsolete and disused in the 1980s (84) because of its inflammation, foreign body reaction, material resorption (90), absorption of the material (91), and severe leakage (92).

The second generation of methacrylate resin-based sealer, “EndoREZ” (Ultradent Products Inc, South Jordan, UT), is a urethane dimethacrylate (UDMA) resin-based endodontic sealer. It is a dual-cured, hydrophilic and non-etching, which does not require a dentin adhesive (93, 94). EndoREZ base contains a bismuth compound as a radiopaque filler, small amounts of filler, diurethane dimethacrylate (di-UDMA), triethylene glycol dimethacrylate (TEGDMA), a peroxide initiator, and a photo initiator (not camphoquinone: CQ). Its catalyst contains a bismuth compound as the radiopaque filler, small amounts of fillers, diurethane dimethacrylate, and triethylene glycol dimethacrylate (83). This sealer can be used with resin coating gutta-percha cone or conventional gutta percha. Resin coating gutta-percha was created by reacting one of the isocyanato groups of a di-isocyanate with the hydroxyl group of a hydroxylterminated polybutadiene, as the latter is bondable to hydrophobic polyisoprene. This is followed by the grafting of a hydrophilic methacrylate functional group to the other isocyanato group of the diisocyanate, producing a gutta-percha resin coating that is bondable to a methacrylate-based resin sealer (93). The use of this system, a resin coating gutta percha with the sealer, falls into tertiary monoblock concept. Although, it created long resin tags and thin hybrid layer in radicular dentin, polymerization shrinkage of the sealer resulted in gap formation and silver leakage between gutta-percha resin-coating and the sealer (93). The second generation sealer had low bond strength when compared with other sealers (95, 96). It had high solubility (97, 98) and greater apical leakage than an epoxy resin sealer (99, 100).

The third generation of the sealers are self-etching sealers that contain a self-etching primer and a dual-cured resin composite root canal sealer (84). The self-etching primer is incorporated into smear layers that are created by instrumentation procedures along the sealer-dentin interface (101, 102). The self-etch primer contains sulfonic acid terminated functional monomer, HEMA, water, and polymerization initiator. The dentin surface is applied with an acidic primer that can penetrate through the smear layer and demineralized dentin. The primer is air-dried to remove the carrier then a dual-cure flowable resin composite sealer is applied and polymerized. “Resilon®” (Resilon research LLC, Madison, CT), a thermoplastic synthetic polymer based (polycaprolactone) root canal filling material that is based on polymers of polyester and contains bioactive glass and radiopaque fillers, was introduced to perform like gutta-percha (103). The dual-curable resin based sealer, “Epiphany®”

(Pentron Clinical Technologies, Wallingford, CT, USA) contains bisphenol-A diglycidyl dimethacrylate (BisGMA), ethoxylated Bis-GMA, urethane dimethacrylate, hydrophilic difunctional glasses, barium sulfate, silica, calcium hydroxide, bismuth oxychloride with amines, peroxide, photo initiator, stabilizers and pigment. The Resilon-Epiphany system, marketed as "RealSeal" (SybronEndo, Orange, CA),(83) is classified as a secondary monoblock type.

There are many studies about the sealing ability of the third generation methacrylate sealers compare to conventional sealers that is still controversy (103-111). The bond strength of the third generation methacrylate sealers was lower than gutta percha/conventional nonbonding sealers (96, 112, 113).

The fourth generation methacrylate resin-based sealers function similarly to self-adhesive resin luting composites. Self-adhesive system does not require any pretreatment of the tooth surface, so it is simple to use and leaves little or no room for mistakes induced by technique sensitivity (114). 4-META (4-methacryloyloxyethyl trimellitate anhydride) is the monomer with both hydrophobic and hydrophilic groups. It is able to promote the monomer infiltration into the acid-conditioned and underlying intact dentin to create a hybrid layer after polymerization (115, 116). This monomer was used to develop self-adhesive resin sealers such as following below.

"MetaSEAL" (MetaSEAL, Parkell Inc., New York, NJ, USA) or Hybrid Root SEAL (Sun Medical, Tokyo, Japan) are a dual-cure and self-etching resin cement, which contains 4-methacryloyloxyethyl trimellitate anhydride (4-META). Both of them can be used with Resilon or gutta-percha owing to the manufacturer (106).

"RealSeal self-etch" (SE) (SybronEndo, Orange, CA) is the all-in-one step and dual-cured version of RealSeal. It incorporated the acidic resin monomer that used a polymerizable methacrylate carboxylic acid anhydride (4-META) and the self-etch primer to reduce the application step (84).

The sealing ability of the fourth generation methacrylate resin sealer was similar to the third generation methacrylate resin sealer and epoxy resin sealer (106). The bond strength of the fourth generation methacrylate resin sealers were similar or higher than the former generation of methacrylate resin sealers and the epoxy resin sealers (117-121). The obturation with methacrylate sealers is, however, high polymerization shrinkage due to high c-factor in root canals. The force of polymerization shrinkage is more than dentin bond strength and pull out resin sealer tags created voids and gaps along the sealer-dentin interface compare to the obturation with gutta percha and conventional sealer (122, 123).

2.1.6 MTA based sealer

Mineral trioxide aggregate (MTA) was introduced by Torabinejad et al. 1993 as a root end filling material (124) and it was be used to seal root perforations (125, 126). It is biocompatible (127) and also has several clinical applications (128) such as pulp capping (129), direct pulp protection after pulpotomy in permanent teeth (130), and obturation an open apex in apexification procedures (131). Moreover, MTA was recommended to use in revascularization procedures (132). MTA can produce calcium hydroxide (133-135) which is released in solution (135). The adhesion mechanism of MTA to dentin is a micromechanical bonding because MTA triggers the precipitation of carbonated apatite, promoting a controlled mineral nucleation on dentin that is the formation of an interfacial layer tag-like structures (136).

According to the advantage properties of MTA were mentioned before, MTA based root canal sealers were developed. Some of common brands such as Endo CPM Sealer (EGEO SRL, Buenos Aires, Argentina), ProRoot Endo Sealer (Dentsply Maillefer, Ballaigues, Switzerland), MTA-Obtura (Angelus, Londrina, PR, Brazil), and MTA Fillapex (Angelus Soluções Odontológicas, Londrina, PR, Brazil)

MTA based sealers showed similar sealing ability (137) or worse than conventional epoxy resin sealers (138, 139). MTA based sealers had lower (140), equal to (141), or higher bond strength than epoxy resin sealers (141).

2.1.7 Bioceramic sealer

Bioceramic-based materials were introduced in endodontics, mainly used as repair material (142, 143) and root canal sealer (144, 145). Bioceramics are a combination of calcium silicate and calcium phosphate (146). A bioceramic has a chemical bond to dentin because the releasing of calcium and hydroxyl ions from a bioceramic results in the formation of an apatite layer (147).The common brand is such as EndoSequence BC Sealer (Brasseler USA, Savannah, GA, USA; also known as iRoot SP, Innovative Bioceramix, Vancouver, Canada). It consists of calcium silicates, calcium phosphate monobasic, calcium hydroxide and zirconium oxide which includes a similar composition to white mineral trioxide aggregate (148). Its sealing ability and bond strength was comparable to an epoxy resin sealer (147, 148).

2.1.8 Calcium phosphate based sealer

Calcium phosphate based sealer was developed because its chemical composition and crystal structures is similar to tooth and bone material. The major components were tetracalcium phosphate and either dicalcium phosphate anhydrous or dicalcium phosphate dehydrate (149). The common brands are such as CAPSEAL I and CAPSEAL II (Sankin Apetite Root Canal Sealer, Sankin kogyo, Tokyo, Japan). It showed less cytotoxic than conventional root canal sealers (150, 151). Sealing ability of the calcium phosphate based sealers were similar to the epoxy resin sealer (149).

In our study, we selected AH Plus (Dentsply DeTrey, Konstanz, Germany) represented as an epoxy resin based sealer that has very good properties and a lot of success in endodontics. MetaSEAL (MetaSEAL, Parkell Inc., New York, NJ, USA) was chosen as a methacrylate resin based sealer because this sealer was able to bond to root canal dentin and ease of use. Although silicone, MTA, and bioceramic has good properties as root canal sealers, they are not available in Thailand.

2.2 Bond strength between root canal sealer and root dentin

The microleakage is a main cause of the endodontic failure (4-6, 152, 153). There are many studies about microleakage (37, 38, 111, 154), however, the study of correlation between leakage value and bond strength found conversely that mean low leakage caused high bond strength (9). Therefore, the high bond strength of root canal sealer might create low leakage. Moreover, high bond strength is able to improve the stability of root filling such as during preparation for post space (155) and prevent debonding of root canal sealer during setting reaction (123).

Root canal dentin bond strength depends on smear layer on root canal dentin surface created during mechanical instrumentation (156) and irrigation strategy in root canal. The smear layer could obstruct sealer penetration into dentinal tubule (157). The benefits from the smear layer removal were an enhancement of the sealer penetration and adaptation into dentinal tubules (158-160), increase of bond strength, and reduction of microleakage (52, 161, 162). Various studies tended to support the removal of smear layer before root canal obturation (161-166). Therefore, a widely accepted smear layer removal technique was the combination irrigation with a sodium hypochlorite (NaOCl) and a chelating agent such as ethylene diamine tetraaceric acid (EDTA). A sodium hypochlorite is essential for removal of the organic tissue elements, while an EDTA is essential for removal of the inorganic components. Several studies suggested sodium hypochlorite irrigation during mechanical instrumentation and EDTA irrigation then followed by sodium hypochlorite irrigation as final flushing before root canal obturation (12, 13, 167-170).

Hybrid layer, a resin infiltrated collagen matrix including resin tags and adhesive fillings of lateral branches of dentinal tubules, was suggested as an essential mechanism of adhesion (171). Hybrid layer between the methacrylate resin sealer and root dentin did not take place if demineralized collagen matrix had not been exposed according to the final irrigation with sodium hypochlorite (172). Moreover, the use of sodium hypochlorite as a final irrigant would inhibit the polymerization of methacrylate resins by leaving an oxygen rich layer on dentin surfaces and decreased the bond strength between methacrylate resins and root dentin (14, 15, 85, 173-177).

Moreover, irrigation strategy tended to be modified. The majority studies recommend EDTA and follow by water as a final flush instead of sodium hypochlorite (9, 11, 16, 96, 172, 178) since higher bond strength was produces EDTA treated root dentin surface decreased the wetting ability of dentinal wall as decreased surface energy, providing adhesion of hydrophobic material (179-181). High bond strength of the epoxy resin sealer to root canal dentin was hypothesized that adhesion of sealer to root dentin is associated with the formation of a covalent bond by an open epoxide ring into epoxy resin sealer to any exposed amino groups in collagen (96). Chemical bonding between the epoxy resin sealer (AH Plus) and dentinal collagen was proved by Fourier transform infrared spectroscopy method (182). However, the EDTA irrigation time of each study was varied between 2-5 minutes (9, 11, 16, 96, 172, 178).

Currently, there are no reports on an appropriate irrigation protocol for resin sealer-based root canal obturation. The appropriate irrigating time of EDTA has not been studied yet. Therefore, this study was performed to evaluate and compare bond strength of root canal dentin and resin sealer when irrigation with EDTA in difference duration time.

2.3 Bond strength test

Various methods are available for evaluating the bond strength between root canal filling materials and root canal dentin. These methods are based on the principles of shear (push-out bond strength test) (96, 113, 183) and tensile forces (microtensile bond strength test) (184, 185).

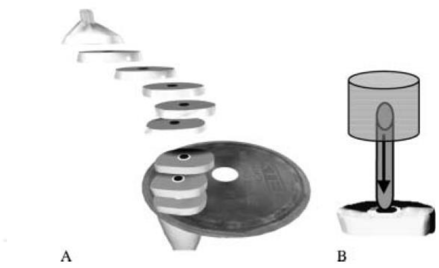


Figure 3 Push out test (186)

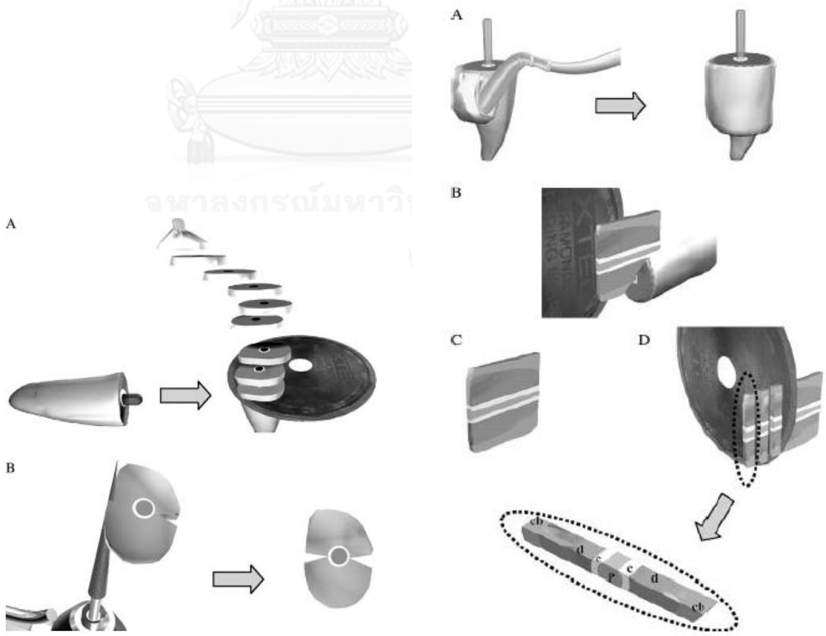


Figure 4 Microtensile test (Trimming on the left and non-trimming on the right) (186)

2.3.1 Push-out test using the punch-shear test technique which was first described by Roydhouse 1970 (187). The push out test is the force-measurement method from the shear principle that attempts to imitate three-dimensional root canal space to determine the effectiveness of bond strength between root canal filling materials and root canal dentin. The advantages of this test are less sensitive to small alterations among specimens and variations in stress distribution during load application. Moreover, it allows root canal filling materials to be evaluated even when bond strengths are low and it is easy to align samples for test (178, 188). Dislodged direction of root canal filling materials and acted force direction, however, cannot be happened in clinical situations. Several studies used push-out test and reported harmonious results (11, 113, 189-191).

2.3.2 Microtensile test was introduced by Sano et al. in 1994 (184) to reflect the true interfacial bond strength, the capability to determine bond strength in small surfaces and evaluate local variations over the bonding substrate, and obtaining multiples samples from a single tooth (192). This technique has commonly been used to test adhesion effectiveness of adhesives, resin composites, and resin cements (192-198). There are 2 methods that have been used to obtain the microtensile specimens from root dentin such as "trimming or hourglass-shaped specimen" (184, 186, 194) and "non-trimming or beam-shaped specimen" (186, 195, 196).

Trimming or hourglass shape was the first preparation technique for microtensile bond strength test to overcome the difficulties of limited area bond strength measurement (184). This technique, however, is sensitive. The important limitation of this method is a high premature failure on specimen preparation because trimming with burs might induce additional stress as reflected in the numbers of specimens that fail prior to testing especially in weaker bonds or brittle substances (186, 199). If bond strengths are relatively low (5-7 MPa), trimming specimens with a high-speed handpiece may cause premature failure of the bond due to slight eccentric movements of the bur which in caused vibrations in the specimen (192).

Non-trimming or beam shape version of microtensile bond test was developed to measure the bond strength of adhesive materials to root dentin (174, 175, 195, 196, 200, 201). Studies showed that the “non-trimming” version might be less traumatic to the bonding interfaces and was able to measure relatively low bond strength of materials. They suggested that the “non-trimming” technique might be more practical in to evaluate interfaces with low bond strengths (185, 186, 192).

The microtensile bond strength test has various advantages such as conservation of teeth, evaluation of regional bond strengths possible (202, 203), evaluation of bond strength to various cavity walls in restoration possible (204, 205), conductive to evaluation of the effects of polymerization shrinkage stress (206, 207), fewer cohesive failures (208), possibility to evaluate with very small areas (209), and SEM fractography can be readily performed to determine the mode of failure (210). In contrary, it has some limitations (192, 208) such as labor intensive and technically demanding, difficulty to measure in very low bond strength (<5 MPa), specimens easily dehydrate and damaged, and post-fracture specimens can be lost or damaged when removing from gripping device. There was no difference on microtensile bond strength in the range of crosshead speeds between 0.01-10.0 mm/min (211-213). Poitevin et al. 2008 demonstrated that the lower the crosshead speed is, the greater the difference between stress at maximum load and stress at breaking is. As more uniform stress-time pattern, it was suggested to use a crosshead speed at 1 mm/min (213).

In conclusion, this study tested non-trimming or beam shape of microtensile bond strength test as the reasons mentioned above.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

1. 160 Single root of human premolar teeth
2. Sickle scaler (Hu-Friedy, Chicago, IL, USA)
3. X-ray film size 2 (Carestream Dental, NY, USA)
4. Ney® Surveyor with analyzing rod (Dentsply Ceramco, York, PA, USA)
5. Clear acrylic pipe 2x2cm diameter
6. Self-cure clear resin
7. Low speed cutting machine (ISOMET™ 1000 Precision Saw, Buehler, USA)
8. 5 inches diameter Diamond wafering blade with medium grit / high concentration (0.015 inches thick) (PACE TECHNOLOGIES, Arizona, USA)
9. K-file no. 15 and 50 (Dentsply Maillefer, Ballaigues Switzerland)
10. Gates Glidden Drills no.1, 2, 3, and 4 (Dentsply Maillefer, Ballaigues Switzerland)
11. NiTi Rotary files (ProTaper Universal S1, S2, F1, F2, F3, F4, and F5, Dentsply Maillefer, Ballaigues Switzerland)
12. Torque controlled motor (X-Smart Plus, Dentsply Maillefer, Ballaigues Switzerland)
13. Paper point size L (Faculty of Dentistry, Chulalongkorn University, Thailand)
14. 25 gauge needle and syringe (Nipro (Thailand) Corporation Limited, Thailand)
15. Distilled water
16. 5% Sodium hypochlorite (Pose-Chlorite, Pose Health Care Limited, Thailand)

17. 17% Ethylenediaminetetraacetic acid (EDTA) (Faculty of Dentistry, Chulalongkorn University, Thailand)
18. 2.5% Glutaraldehyde diluted from 50% Glutaraldehyde EM grade distillation purified (Electron Microscopy Sciences, PA, USA)
19. 0.1M Phosphate buffer saline prepared from Sodium dihydrogen phosphate monohydrate and di-Sodium hydrogen phosphate dodecahydrate (Merck KGaA, Darmstadt, Germany)
20. 30%, 50%, 70%, 95%, and 100% Ethanol
21. K850 Critical Point Dryer (Quorum Technologies Limited, UK)
22. Pipette (BioPette)
23. 24-well plate
24. Ultrasonic bath (Elma®, Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany)
25. Epoxy resin based root canal sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany, batch #1507000612)

    Composition

    Epoxide paste: diepoxide, calcium tungstate, zirconium oxide, aerosol, pigment

    Amide paste: 1-ademantane amine, N,N'-dibenzyl-5-oxa-nonandiamine-1,9, TCD-Diamine, calcium tungstate, zirconium oxide, aerosol, silicone oil
26. Methacrylate resin based root canal sealer (MetaSEAL, Parkell Inc., New York, NJ, USA, batch #141001)

    Composition

    Monomethacrylates: 2-hydroxyethyl methacrylate (HEMA),

    4-methacryloyloxyethy trimellitate anhydride (4-META)

    Di(meth)acrylates
27. Glass slab

28. Cement spatula
29. LED light source; baseline output 1,100 mW/cm^2 (Demi Plus, Kerr, Orange, CA, USA)
30. Celluloid strip
31. Incubator (Contherm 160M, Contherm Scientific Ltd, New Zealand)
32. Universal testing machine (EZ-S, Shimadzu, Japan)
33. Cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin K.K., Ohtawara, Japan)
34. Stereomicroscope (SZ61, Olympus, Shibuya-ku, Tokyo, Japan)
35. Scanning Electron Microscope (Quanta 250, FEI, Oregon, USA)

3.2 Methods

1. Tooth selection

One hundred and sixty intact human premolars which were extracted for orthodontic reason with complete root formation were used in this study. Samples were cleaned with Sickle scaler and stored in distilled water at 4°C until used.

Inclusion criteria

Intact premolar with single root, one canal that was confirmed with 2 views perpendicular of conventional radiographs (X-ray film size 2, Carestream Dental, NY, USA)

1. Root length from buccal cemento-enamel junction (CEJ) to apical foramen should not shorter than 13 mm
2. Closed apex
3. No crack, fracture, caries or restoration under stereomicroscope
4. Patent root canal

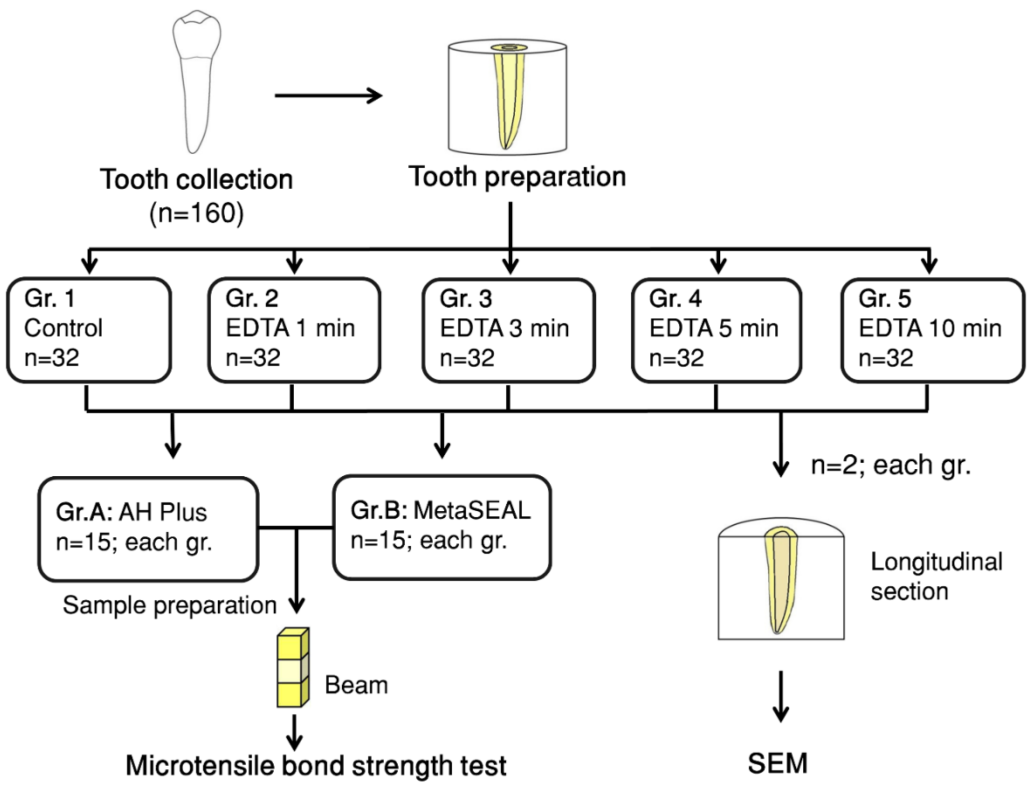


Figure 5 Schematic illustration of research methodology

2. <u>Tooth preparation</u>

One hundred and sixty human premolars were decoronated at 2 mm above CEJ by using a low speed cutting machine (Isomet^{TM} 1000 Presicion Saw, Buehler, USA) with a diamond saw (PACE TECHNOLOGIES, Arizona, USA). The working length was directly determined using K-file no. 15 (Dentsply Maillefer, Ballaigues Switzerland) inserted into the canal to the apical foramen and subtracted 1 mm. The teeth were embedded in the center clear acrylic pipes (2x2cm) with self-cure clear resin confirmed by surveyor parallel to the axis of the clear acrylic pipes.

The coronal accesses of the root canals were enlarged using Gates Glidden Drills no.1, 2, 3, and 4 then prepared the root canals by NiTi rotary files which were used for 25 canals per each file (ProTaper Universal, Dentsply Maillefer, Ballaigues

Switzerland) according to manufacturer's recommendation starting with S1 (size 18/.02) to F5 (size 50/.05). The root canals were kept patency by recapitulation with K-file no.15 and irrigation with distilled water 1 ml in needle syringe gauge 25 with slightly vertical agitation; 1 mm shorter than working length between files changing. The canal was then final flush with 1 ml of distilled water before root canal dentin surface treatment.

3. **Root canal dentin treatment**

All teeth were divided into 5 groups according to irrigation protocols as follows:

Group 1: Control group: irrigation with 10 ml of 5% sodium hypochlorite (NaOCl) for 2 minutes, followed by 10 ml of distilled water for 2 minutes (n=32)

Group 2: EDTA 1 minute: irrigation with 10ml of 5% sodium hypochlorite (NaOCl) for 2 minutes, followed by 5ml of 17% ethylenediamine tetraacetic acid (EDTA) for 1 minute, and then final flushing with10ml of distilled water for 2 minutes (n=32)

Group 3: EDTA 3 minute: irrigation with 10ml of 5% sodium hypochlorite (NaOCl) for 2 minutes, followed by 5ml of 17% ethylenediamine tetraacetic acid (EDTA) for 3 minute, and then final flushing with10ml of distilled water for 2 minutes (n=32)

Group 4: EDTA 5 minutes: irrigation with 10ml of 5% sodium hypochlorite (NaOCl) for 2 minutes, followed by 5ml of 17% ethylenediamine tetraacetic acid (EDTA) for 5 minutes, and then final flushing with10ml of distilled water for 2 minutes (n=32)

Group 5: EDTA 10 minutes: irrigation with 10 ml of 5% sodium hypochlorite (NaOCl) for 2 minutes, and followed by 5ml of 17% ethylenediamine tetraacetic acid (EDTA) for 10 minutes, and then final flushing with10ml of distilled water for 2 minutes (n=32)

4. Sample preparation

Sample preparation for microtensile bond strength test

Thirty specimens from each group were dried the canal with paper points (size L; about 7 points per canal, Faculty of Dentistry, Chulalongkorn University, Thailand). The sample of each group was divided into 2 subgroups according to the type of root canal sealer; group A: epoxy resin sealer and group B: methacrylate resin sealer; and seal the root canal as follow

A) Group A: Epoxy resin based root canal sealer group (AH Plus, Dentsply DeTrey, Konstanz, Germany, n=15), working time 4 hours at 23 °C, setting time 8 hours at 37 °C

B) Group B: Methacrylate resin based root canal sealer group (MetaSEAL, Parkell Inc., New York, NJ, USA, n=15), working time 30 minutes at 23 °C, setting time 16 hours at 37 °C

The root canal sealers were manipulated according to the manufacturer's instruction, loaded to canal by needle syringe gauge 25 and then cover the canal orifices with celluloid strip. For the methacrylate resin sealer group; they were light-cured for 20 seconds in accordance with manufacturer's instruction (LED light source, Demi Plus, Kerr, Orange, CA, USA) from the canal orifices. The filled root canals were kept in the incubator (Contherm 160M, Contherm Scientific Ltd, New Zealand) at 37°C for a period three times greater than the regular setting time of the sealers (AH Plus: 8 hours and MetaSEAL: 16 hours).

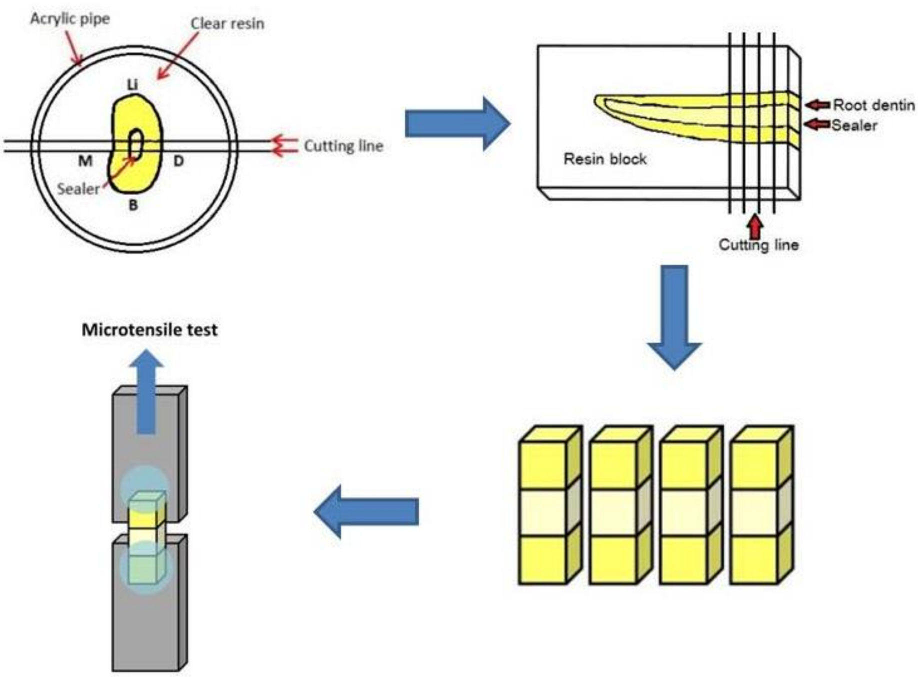


Figure 6 Schematic illustration of sample preparation method for microtensile bond strength test

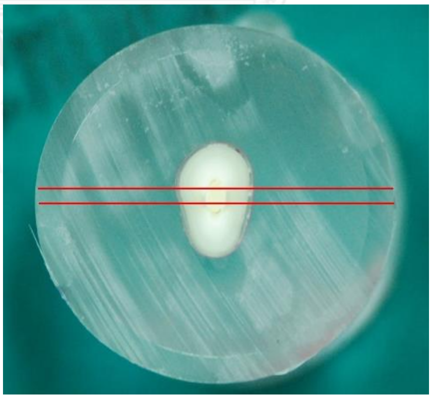


Figure 7 Decoronated filled root canal with imaginary cutting line

The specimens were decoronated to create 13 mm root length and cut into beam-shaped samples from the coronal one-third of the root canal, using the slow-speed diamond saw. The 0.6 × 0.6-mm-thick beams (Figure 9) were cut at the widest part of the canal that consisted of 2 interfaces (Figure 6 and 7). Four samples were cut from each specimen. Prematurely failure of prepared samples were excluded form the test. The median bond strength of these samples was recorded as the microtensile bond strength of that root canal. In case even numbers of samples were prepared from each specimen, the bond strength value which was similar to the average bond strength of each specimen was recorded as the microtensile bond strength of that root canal.

Figure 8 Slow-speed diamond saw; Isomet^{TM} 1000 Presicion Saw, Buehler, USA

Figure 9 Microtensile bond strength test specimen (0.6 x 0.6 mm—thick beam)

Sample preparation for SEM observation

Two of specimens from all irrigation groups were prepared for SEM observation. They were cut perpendicularly to the root axis in a controlled root region (13 mm from the root apex) to observe the dentin surface; then, they were cut longitudinally through the center of the bucco-lingual width of the canal, to expose their internal portion by using the slow-speed diamond saw.

The specimens were cleaned with distilled water in the ultrasonic bath (Elma®, Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany) for 5 minute. They were immersed in fixative solution containing 2.5% glutaraldehyde for 24 hours rinsed with 1 ml of phosphate buffer saline 3 times for 5 minutes each and stored at 4°C before processing. The specimens were dehydrated by immersion in 50%, 70%, 95%, and 100% ethanol for 15 minutes each respectively and completely dried in K850 Critical Point Dryer (Quorum Technologies Limited, UK). The internal and lateral surfaces of the root canal, representing the cross-sectional and longitudinal views of dentinal tubules, were viewed by a scanning electron microscope (SEM; Quanta 250, FEI, Oregon, USA) after being sputter-coated with gold.

5. **SEM observation**

The root canal surfaces of prepared specimens were observed using the SEM (Figure 10) at magnifications of 10000x and 25000x. They were examined in both cross sectional and longitudinal views and photographed into TIFF images.

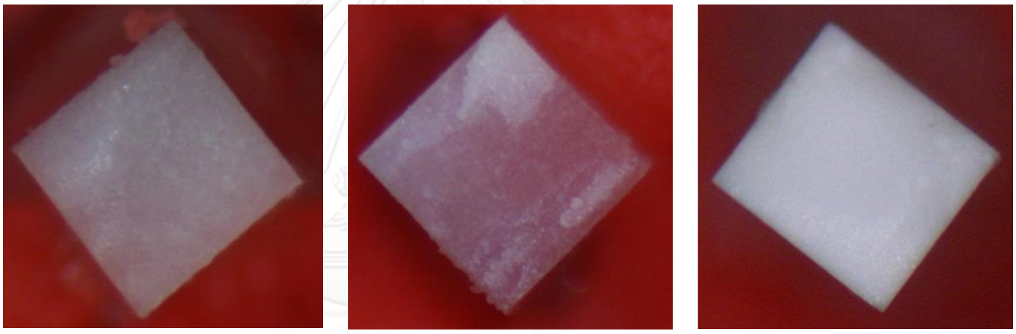
Figure 10 Scanning electron microscope (SEM, Quanta 250, FEI Company, USA)

6. **Microtensile bond strength testing**

One of the two interfaces of each beam was randomly selected for microtensile bond strength testing. One of the root dentin side and the interface between the opposite root dentin and sealer of each beam were glued onto a testing device in universal testing machine (EZ-S, Shimadzu, Japan) using a cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin K.K., Ohtawara, Japan) (Figure 11) and were subjected to a tensile force at a crosshead speed of 1 mm min^{-1}. After fracture, each beam was measured the cross-sectional area calculated into mm^2 under 45x magnification with stereomicroscopic (SZ61, Olympus, Japan) (Figure 12) and determined the failure modes. The maximum tensile force that fractures the specimen was recorded and it was divided by the bonded cross-sectional surface area and calculated into the bond strength (MPa). The failure mode was classified as one of the following: adhesive failure, cohesive failure in sealer, cohesive failure in dentin, and mixed failure. Adhesive failure means that the specimen fracture within the interface between the sealer and the dentin and none of remaining sealer on fracture surface specimen. Cohesive failure in sealer means that the specimen fracture within sealer and remaining sealer covers all of fracture surface specimen. Cohesive in dentin means that the specimen fracture within dentin and none of remaining sealer on fracture surface specimen. Mixed failure means that both of sealer and exposed dentin surface remain on the surface of fracture surface specimen.

Figure 11 Attached specimen on the universal testing machine (EZ-S, Shimadzu, Japan)

Figure 12 Stereomicroscope (SZ61, Olympus, Japan)



(A) (B) (C)

Figure 13 Failure mode: (A) Adhesive failure, (B) Mixed failure, and (C) Cohesive in sealer

3.3 Statistical analysis

Bond strength values of each type of sealer were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test (α = 0.05). All statistical analyses were performed using SPSS software version 22 (SPSS Inc., Chicago, IL, USA).

CHAPTER IV

RESULTS

4.1 Microtensile bond strength test

Table 1 Microtensile bond strength (MPa, mean ± standard deviation) of 2 resin sealers after 5 irrigation protocols (n=15)

Group	NaOCl DW	NaOCl EDTA 1 min DW	NaOCl EDTA 3 min DW	NaOCl EDTA 5 min DW	NaOCl EDTA 10 min DW
AH Plus	10.45±2.97	12.62±3.17	11.38±2.98	12.23±4.71	12.62±5.05
MetaSEAL	14.90±5.41^{A}	20.53±8.10^{A,B}	20.24±7.37^{A,B}	20.91±5.45^{A,B}	26.15±5.93^{B}

Bond strength is given in MPa; measurements are given as mean ± standard deviation The same superscript capital letters indicate the absence of significant differences in microtensile bond strength for each row ($p > 0.05$).

DW, distilled water

In AH Plus group, 40 out of 300 samples (13.33%) and 53 out of 300 samples (17.67%) in MetaSEAL group were failed prematurely during the cutting phase.

The means and standard deviations of microtensile bond strength are given in Table 1. For AH Plus, treatment of the root canal dentin surface with NaOCl, 1-, 3-, 5, or 10-min EDTA, followed by distilled water (groups 2-5) did not show higher bond strength than non-EDTA group (group 1). For MetaSEAL, 1-, 3-, and 5-min EDTA irrigation group (group 2-4) showed not significantly higher than treatments without EDTA (group 1) ($p = 0.139$, $p = 0.179$, and $p = 0.099$, respectively), whereas 10-min EDTA irrigation group (group 5) promoted significantly higher bond strength than treatments without EDTA ($p < 0.001$).

4.2 Failure mode

The failure mode is presented in Figure 14. The predominant failure mode throughout groups was mixed failure, no cohesive failure within the dentin occurred. A markedly higher number of cohesive failures in the sealer were found in the 10-min EDTA groups of MetaSEAL.

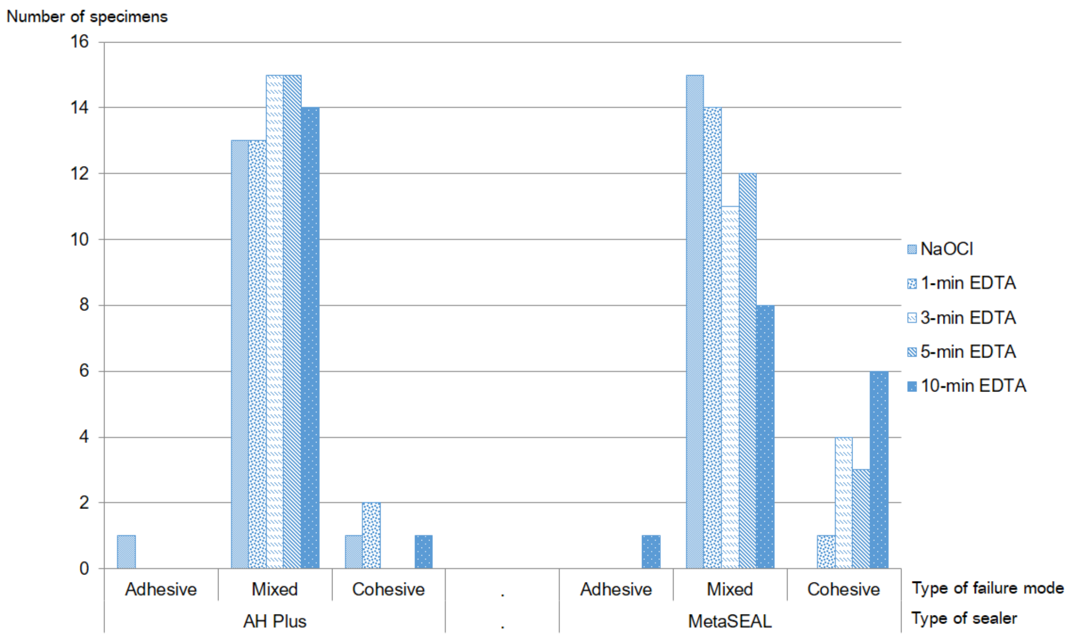
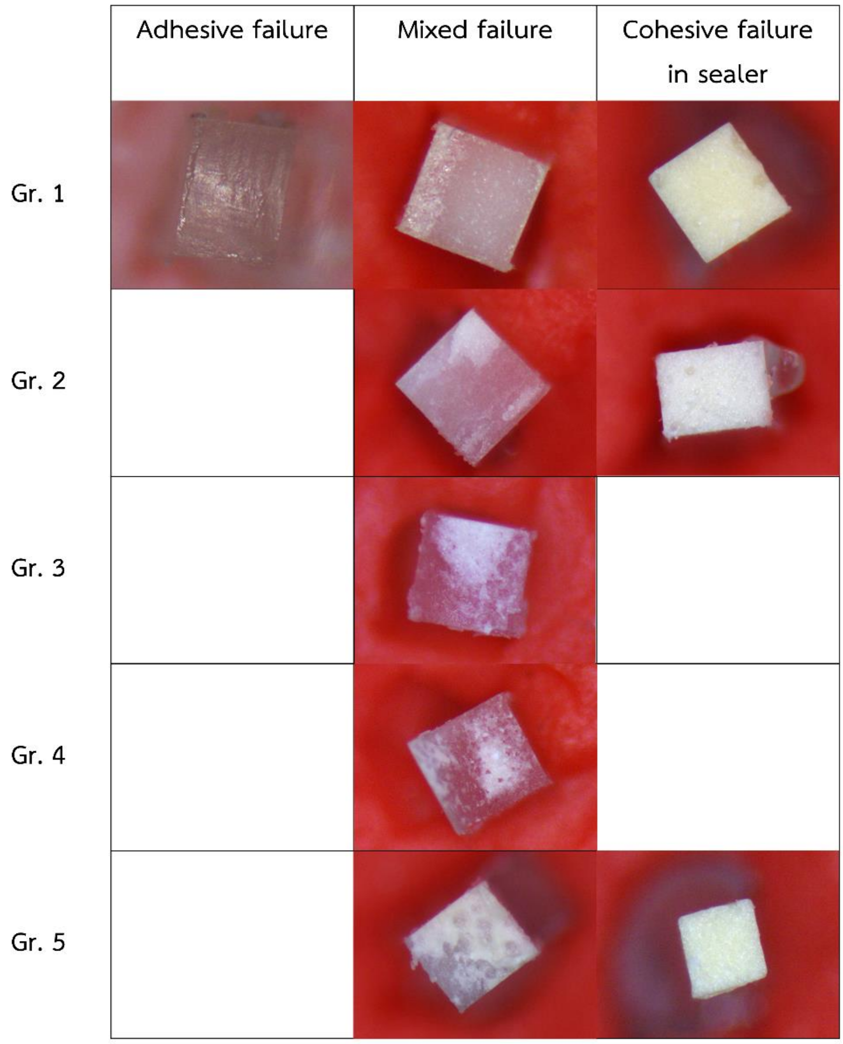


Figure 14 The percentage of failure modes of 2 resin sealers after 5 irrigation protocols

(A)


Adhesive failure
Mixed failure
Cohesive failure in sealer
Gr. 1
Gr. 2
Gr. 3
Gr. 4
Gr. 5

(B)

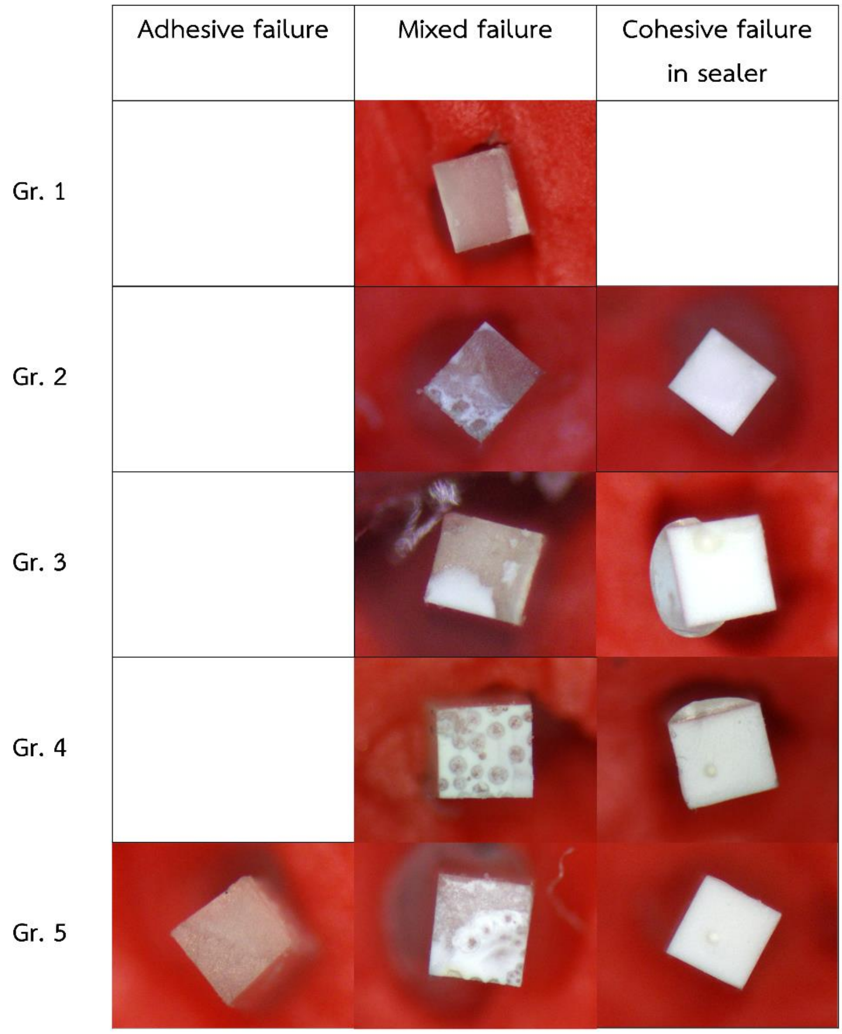


Figure 15 Failure modes of (A) AH Plus groups, (B) MetaSEAL groups

4.3 SEM observation

Group 1 (Sodium hypopchlorite; Figure 16A and 16B) showed an amorphous smear layer covering the dentin surface, and no dentinal tubules were seen. Longitudinal sections of dentinal tubules (Figure 16C) demonstrated short collagen fibrils in the intertubular dentin, but rarely in the peritubular dentin (Figure 16C; arrows).

Group 2 (1-min EDTA, Figure 16D and 16E) showed no smear layer, and generally patent dentinal tubules, demineralized dentin surface in some areas, and generally exposed integral collagen fibrils. In longitudinal sections (Figure 16F), collagen fibrils on the intertubular dentin were more visible than in Group 1, and collagen fibrils were exposed on most of the peritubular dentin.

Group 3 (3-min EDTA; Figure 16G and 16H) showed the absence of a smear layer, entirely patent dentinal tubules, generalized demineralization of the dentin surface (which was deeper than that seen in Group 2), and a vast integral collagen fibril network. In longitudinal sections (Figure 16I), the collagen fibril appearance on the intertubular dentin and peritubular dentin were similar to that in Group 2, but a collagen fibril network was present in the demineralized dentin on the wall of the root canal (left side).

Group 4 (5-min EDTA; Figure 16J and 16K) showed a similar surface to that in group 3, but the demineralized dentin area and exposed integral collagen fibril network were larger than those in Group 3. In longitudinal sections (Figure 16L), more collagen fibrils were present on the intertubular dentin and peritubular dentin and along dentinal tubules than in Group 3.

Group 5 (10-min EDTA; Figure 16M and 16N) appeared similar to Group 4; however, dentin demineralization was deeper and dense collagen bands were present. In longitudinal sections (Figure 16O), dense collagen bands were seen, and other areas were similar to Group 4.

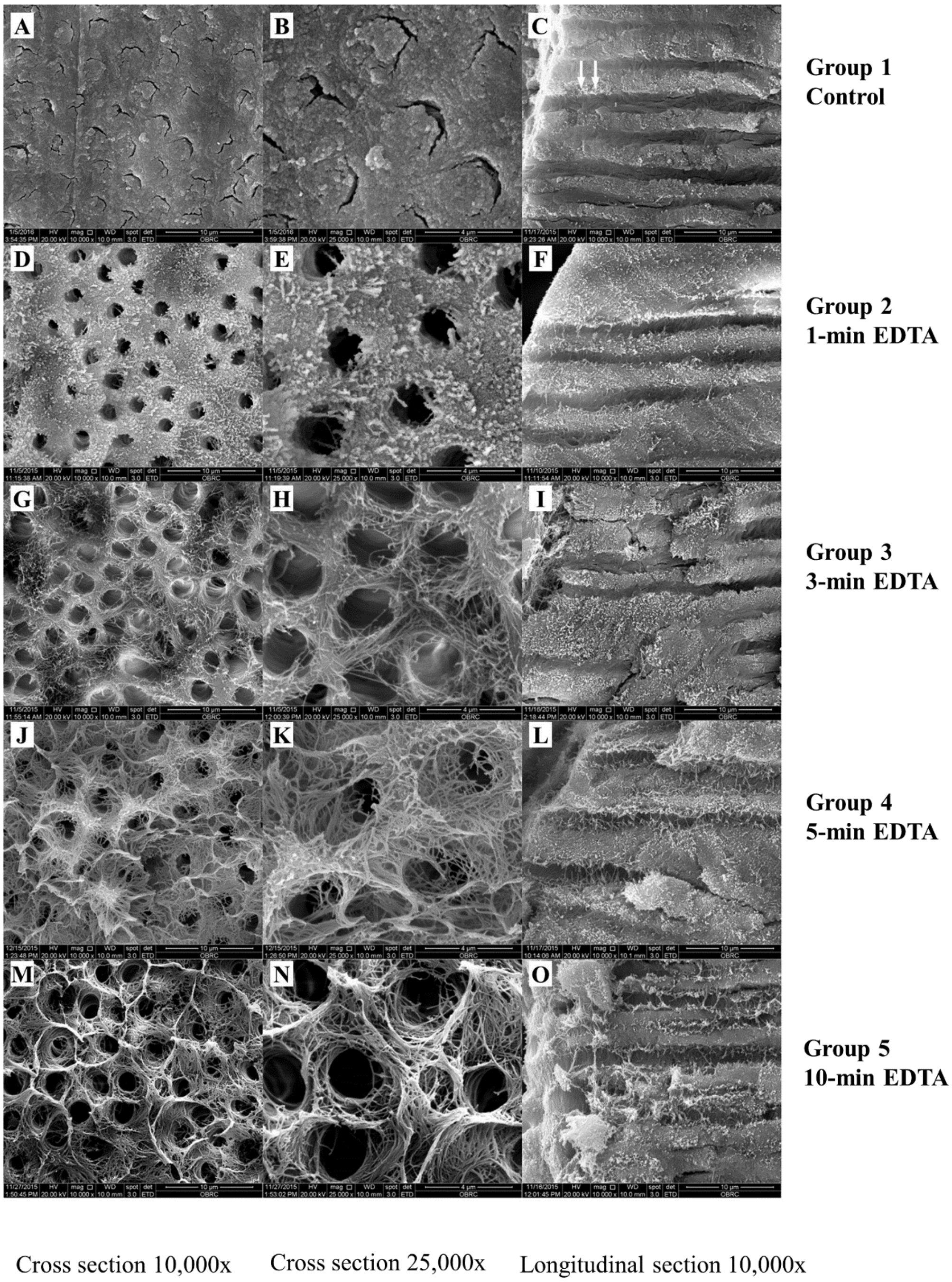


Figure 16 Representative scanning electron microscope micrograph of radicular dentin specimens.

CHAPTER V
DISCUSSION

Root canal obturation is aimed at comprehensive three-dimensional filling of the root canal, to prevent reinfection into the root canal system (2, 3). The bondability of the root canal sealer to root dentin is thought to improve sealing ability and stability of the root-filling materials. Several irrigation protocols were suggested in order to modify dentin surface to promote bond strength of root canal sealers.

Dentin is a porous biologic compound made up of apatite crystal filler in a collagen matrix that is formed developmentally by odontoblasts (214). The dentin consists of 70% of inorganic material, 20% of organic material, and 10% water on a weight basis (215). The inorganic material is mainly composed of calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ and type 1 collagen is the most common protein in organic matrix material (216).

Dentin is a heterogenous composite material which contains tubules lined by a highly mineralized peritubular dentin that may be termed "intratubular dentin". The peritubular dentin is composed mainly of crystals of carbonated apatite with a small amount of collagen (217). It is embedded within a partially mineralized intertubular dentin that mainly composed of a matrix of type 1 collagen reinforced by apatite (218). The collagen fibrils are oriented approximately at right angle to dentinal tubules (219).

Collagen is an extracellular structural protein. It is the representative of the major composite of all connective tissues. It is a triple-helical structure that is formed by three polypeptide chains and bound by hydrogen bonds and hydrophobic interactions. Collagen contains hydroxyproline and hydroxylysine amino acids. The three main amino acid components are glycine, proline, and hydroxyproline (220). Exposed dentinal collagen which is occurred form root canal irrigation procedure is the essential factor for adhesion between resin sealer and root dentin such as hybrid layer formation (116, 221).

Irrigation process is the important disinfection procedure in endodontic. The principal irrigating solutions have consisted of sodium hypochlorite (NaOCl) and ethylene diamine tetraaceric acid (EDTA) (222). EDTA was widely suggested to use as a final irrigation combined with a sodium hypochlorite (NaOCl) in order to modify root dentin surface before root canal obturation (9, 11, 16). Combination use of both irrigants is able to patent the dentinal tubule by smear layer removal and demineralize the root dentin surface (13, 167, 169, 223). The sequence of irrigation affected the dentin surface that irrigation with NaOCl and followed by EDTA caused demineralized dentin surface with exposed collagen fibrils but irrigation with EDTA and followed by NaOCl showed absence of exposed collagen fibril (223).

There are various types of root canal sealer in endodontics such as zinc oxide eugenol based sealers, calcium hydroxide based sealer, glass ionomer based sealer, silicone based sealer, resin based sealer, MTA based sealer, bioceramic sealer, and calcium phosphate based sealer. Presently, resin based sealers are widely used in endodontics such as an epoxy resin sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany) and a methscrylate resin sealer (MetaSEAL, Parkell Inc., New York, NJ, USA). The adhesion of epoxy resin sealer (AH Plus) to dentin was found that it adhered by mechanical lock from sealer penetration in dentinal tubules (10, 160) and chemically bonds to dentin (182). It has been theorized that chemical bond of the epoxy resin sealer to dentin is the formation of a covalent bond between the amino groups of the dentin collagen and epoxide rings of AH Plus (96). For methacrylate resin sealer (MetaSEAL), it is the self-adhesive sealer which contains 4-META (4-methacryloyloxyethyl trimellitate anhydride) as the key factor for self-adhesion. The 4-META is the acidic monomer with both hydrophobic and hydrophilic groups which is able to promote the infiltration of monomer into demineralized surface and dentinal collagen fiber mesh and underlying intact dentin to create a hybrid layer which is an essential mechanism of adhesion after polymerization (116, 221). However, MetaSEAL was incapable to etch beyond the smear layer that was created by root canal preparation process into the underlying intact radicular dentin (224). Measurement of sealing ability of resin sealer was capable to evaluate bond strength value as well owing to the correlation of sealing ability and bond strength (9).

From the reasons mentioned in above paragraph, irrigation procedure affected bond strength of both epoxy and methacrylate resin sealers. Absence of smear layer and presence of exposed dentinal collagen were important factors to enhance bond strength of the resin sealers. Scanning electron microscopy observation showed that irrigation root canal with NaOCl and followed by EDTA and distilled water was capable to remove smear layer and create exposed collagen. Therefore, irrigation with NaOCl and followed by EDTA and distilled water should enhance bond strength of the resin sealers.

From the result, treatment the root canal dentin surface with NaOCl, EDTA (1- to 10-min), followed by distilled water did not significantly increase the microtensile bond strength of the epoxy resin sealer (AH Plus) compared with the control group. The result did not well correlate with previous findings (9, 11) which reported that a high bond strength of resin sealers was associated with final irrigation using a decalcifying agent such as EDTA. From SEM observations, a 1-min EDTA irrigation resulted in demineralization of dentin in some areas and short exposed collagen fibrils, while longer EDTA irrigation (3–10 min) tended to result in deeper demineralization and longer exposed collagen fibrils in a duration-dependent manner. All EDTA irrigation groups showed patent dentinal tubules, absence of smear layer, the integrity of collagen fibrils and no denatured collagen fibrils were observed. From this finding, it seems that penetration of the sealer into dentinal tubules (155) and the quality and amount of collagen fibrils may less affect the bond strength of the epoxy resin sealer. For the methacrylate resin sealer (MetaSEAL), the higher bond-strength value in the EDTA groups correlated with previous findings (11, 16, 225). A longer duration of EDTA irrigation tended to promote a higher strength of resin sealer-dentin bonding. From SEM examination described above, irrigation with EDTA causes chelation of calcium from the exposed dentinal collagen, which is important for adhesion of the methacrylate resin sealer. However, the sealer was incapable of etching through the smear layer (224). Based on this finding, it seems that removal of the smear layer and the integrity and quantity of collagen fibrils affect the bond strength of the methacrylate resin sealer.

The failure modes of all irrigation protocols of epoxy resin sealer (AH Plus) were predominant mixed mode (93.33%) which the crack line mainly occurred both in sealer-dentin interface; reflect bond between dentin and sealer, and crack in sealer itself. The result showed the EDTA irrigation time did not effect the bond of epoxy resin sealer (AH Plus) whenever increased duration of irrigation. In MetaSEAL group, the failure modes of all irrigation protocols were mainly mixed mode (80%). These also showed the methacrylate resin sealer could bond to dentin. A longer duration of EDTA irrigation in methacrylate resin sealer (MetaSEAL) group tended to result in more cohesive failure than no EDTA irrigation duration which related to the bond strength result. This result meant bond strength between dentin and methacrylate resin sealer higher than bond strength within the resin or there were errors in sealer while loading. Cohesive failure could occur due to errors in alignment of the specimen along the long axis of the testing device, from microcracks during cutting of the specimens (226). The methacrylate resin sealer showed higher bond strength than the epoxy resin sealer because the chemical bonding theory of the epoxy resin sealer (96, 182) might have little effect on bond strength while the hybridization theory of the methacrylate resin (116, 221) sealer perform strong bond to dentin.

Several bond-strength testing methods have been used previously, for example, push-out test (225, 227), shear test (10, 228), and microtensile test (195, 200). Push-out test has been used to evaluate the bond strength between the root canal filling material and the post. The push-out test measures bond strength by dislocation resistance which comprises friction force and bond strength (227, 229). The shear test measures adhesion force parallel to the interface between the material and tested surface like the push-out test and it is simply reproducible model (58). In our study, the microtensile test, which is commonly used to test adhesion effectiveness of bonding agents was selected because it reflects the interfacial bond strength in small area, and minimize friction force (192).

The study is limited in that only the coronal third of the root was used, because the middle and apical third of the root canal were too small for preparing specimens for microtensile testing. To evaluate bond strength at only one interface (between root dentin and sealer), it was necessary to fill the root canal only with sealers without core materials.

In summary, the duration of EDTA irrigation affects the microtensile strength of the bond between the methacrylate resin sealer (MetaSEAL) and root canal dentin. Final irrigation with 5% NaOCl, 17% EDTA and distilled water increased the bond strength of resin sealers. Ten minutes of EDTA irrigation could enhance adhesion of the methacrylate resin sealer to the root canal dentin. However, as various factors enhance bond strength, further studies are warranted.

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APPENDIX

Data of bond strength and failure mode of AH Plus

AH Plus			
Protocol	Number	Bond strength (MPa)	Failure mode
NaOCl + DW	1	9.09	Mixed failure
	2	6.89	Mixed failure
	3	13.11	Mixed failure
	4	13.33	Cohesive failure in sealer
	5	11.73	Mixed failure
	6	5.57	Mixed failure
	7	12.32	Adhesive failure
	8	9.02	Mixed failure
	9	14.67	Mixed failure
	10	8.94	Mixed failure
	11	10.28	Mixed failure
	12	8.99	Mixed failure
	13	15.78	Mixed failure
	14	7.04	Mixed failure
	15	10.02	Mixed failure
NaOCl + 1-min EDTA + DW	1	18.09	Mixed failure
	2	11.40	Mixed failure
	3	9.05	Mixed failure
	4	7.92	Mixed failure
	5	10.19	Mixed failure
	6	13.25	Cohesive failure in sealer
	7	12.50	Mixed failure
	8	19.88	Cohesive failure in sealer
	9	12.91	Mixed failure
	10	13.15	Mixed failure
	11	10.66	Mixed failure
	12	9.82	Mixed failure
	13	14.13	Mixed failure
	14	13.79	Mixed failure

	15	12.60	Mixed failure
NaOCl + 3-min EDTA + DW	1	6.29	Mixed failure
	2	13.59	Mixed failure
	3	15.01	Mixed failure
	4	13.07	Mixed failure
	5	12.02	Mixed failure
	6	8.75	Mixed failure
	7	13.49	Mixed failure
	8	8.80	Mixed failure
	9	8.14	Mixed failure
	10	6.37	Mixed failure
	11	14.59	Mixed failure
	12	14.54	Mixed failure
	13	11.36	Mixed failure
	14	13.23	Mixed failure
	15	11.45	Mixed failure
NaOCl + 5-min EDTA + DW	1	7.97	Mixed failure
	2	7.11	Mixed failure
	3	12.76	Mixed failure
	4	13.14	Mixed failure
	5	13.93	Mixed failure
	6	4.32	Mixed failure
	7	22.68	Mixed failure
	8	11.62	Mixed failure
	9	13.78	Mixed failure
	10	10.90	Mixed failure
	11	9.81	Mixed failure
	12	17.54	Mixed failure
	13	16.38	Mixed failure
	14	14.65	Mixed failure
	15	6.91	Mixed failure
NaOCl + 10-min EDTA + DW	1	6.97	Mixed failure
	2	6.55	Mixed failure
	3	10.73	Cohesive failure
	4	12.74	Mixed failure

	5	12.44	Mixed failure
	6	11.27	Mixed failure
	7	8.09	Mixed failure
	8	13.78	Mixed failure
	9	13.54	Mixed failure
	10	26.10	Mixed failure
	11	12.52	Mixed failure
	12	14.43	Mixed failure
	13	9.86	Mixed failure
	14	20.36	Mixed failure
	15	9.88	Mixed failure

Data of bond strength and failure mode of MetaSEAL

MetaSEAL			
Protocol	Number	Bond strength (MPa)	Failure mode
NaOCl + DW	1	24.76	Mixed failure
	2	12.18	Mixed failure
	3	13.38	Mixed failure
	4	17.51	Mixed failure
	5	4.10	Mixed failure
	6	18.27	Mixed failure
	7	14.27	Mixed failure
	8	16.34	Mixed failure
	9	11.66	Mixed failure
	10	5.54	Mixed failure
	11	12.34	Mixed failure
	12	17.49	Mixed failure
	13	15.60	Mixed failure
	14	19.52	Mixed failure
	15	20.54	Mixed failure
NaOCl + 1-min EDTA + DW	1	13.58	Mixed failure
	2	15.47	Mixed failure
	3	15.53	Mixed failure
	4	13.99	Mixed failure
	5	17.96	Mixed failure
	6	32.99	Mixed failure
	7	29.24	Mixed failure
	8	20.06	Mixed failure
	9	9.23	Mixed failure
	10	12.70	Mixed failure
	11	13.24	Mixed failure
	12	24.57	Mixed failure
	13	32.32	Mixed failure
	14	26.01	Cohesive failure in sealer

	15	31.11	Mixed failure
NaOCl + 3-min EDTA + DW	1	35.56	Mixed failure
	2	18.83	Mixed failure
	3	18.41	Cohesive failure in sealer
	4	23.90	Mixed failure
	5	8.56	Mixed failure
	6	15.22	Cohesive failure in sealer
	7	15.51	Mixed failure
	8	16.72	Mixed failure
	9	22.36	Mixed failure
	10	27.77	Cohesive failure in sealer
	11	20.40	Mixed failure
	12	12.65	Mixed failure
	13	21.83	Mixed failure
	14	32.29	Cohesive failure in sealer
	15	13.56	Mixed failure
NaOCl + 5-min EDTA + DW	1	24.27	Mixed failure
	2	16.33	Mixed failure
	3	32.57	Cohesive failure in sealer
	4	12.21	Mixed failure
	5	27.15	Mixed failure
	6	21.48	Mixed failure
	7	16.54	Mixed failure
	8	22.35	Mixed failure
	9	15.89	Mixed failure
	10	17.29	Cohesive failure in sealer
	11	14.42	Mixed failure
	12	22.10	Cohesive failure in sealer
	13	25.26	Mixed failure
	14	24.07	Mixed failure
	15	21.75	Mixed failure
NaOCl + 10-min EDTA + DW	1	23.97	Mixed failure
	2	27.78	Adhesive failure
	3	20.49	Cohesive failure in sealer
	4	21.66	Mixed failure

	5	13.81	Mixed failure
	6	26.28	Mixed failure
	7	33.01	Cohesive failure in sealer
	8	27.95	Cohesive failure in sealer
	9	32.83	Mixed failure
	10	37.03	Mixed failure
	11	31.28	Cohesive failure in sealer
	12	27.60	Cohesive failure in sealer
	13	24.25	Mixed failure
	14	21.01	Cohesive failure in sealer
	15	23.31	Mixed failure

Statistical analysis

AH Plus

Descriptive statistical analysis of bond strength of each irrigation protocol of AH Plus

Case Processing Summary

	Irrigant	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
Bond strength	Na DW	15	100.0%	0	0.0%	15	100.0%
	Na ED1 DW	15	100.0%	0	0.0%	15	100.0%
	Na ED3 DW	15	100.0%	0	0.0%	15	100.0%
	Na ED5 DW	15	100.0%	0	0.0%	15	100.0%
	Na ED10 DW	15	100.0%	0	0.0%	15	100.0%

Descriptives

	Irrigant			Statistic	Std. Error
Bond strength	Na DW	Mean		10.4520	.76643
		95% Confidence Interval for Mean	Lower Bound	8.8082	
			Upper Bound	12.0958	
		5% Trimmed Mean		10.4272	
		Median		10.0200	
		Variance		8.811	
		Std. Deviation		2.96839	
		Minimum		5.57	
		Maximum		15.78	
		Range		10.21	
		Interquartile Range		4.17	
		Skewness		.202	.580
		Kurtosis		-.743	1.121

Na ED1 DW	Mean		12.6227	.81930
	95% Confidence Interval for Mean	Lower Bound	10.8654	
		Upper Bound	14.3799	
	5% Trimmed Mean		12.4807	
	Median		12.6000	
	Variance		10.069	
	Std. Deviation		3.17315	
	Minimum		7.92	
	Maximum		19.88	
	Range		11.96	
	Interquartile Range		3.60	
	Skewness		.918	.580
	Kurtosis		1.044	1.121
Na ED3 DW	Mean		11.3800	.77021
	95% Confidence Interval for Mean	Lower Bound	9.7281	
		Upper Bound	13.0319	
	5% Trimmed Mean		11.4611	
	Median		12.0200	
	Variance		8.898	
	Std. Deviation		2.98299	
	Minimum		6.29	
	Maximum		15.01	
	Range		8.72	
	Interquartile Range		4.84	
	Skewness		-.568	.580
	Kurtosis		-1.067	1.121
Na ED5 DW	Mean		12.2333	1.21520
	95% Confidence Interval for Mean	Lower Bound	9.6270	
		Upper Bound	14.8397	
	5% Trimmed Mean		12.0926	
	Median		12.7600	
	Variance		22.151	
	Std. Deviation		4.70644	
	Minimum		4.32	
	Maximum		22.68	
	Range		18.36	

	Interquartile Range		6.68	
	Skewness		.403	.580
	Kurtosis		.448	1.121
Na ED10 DW	Mean		12.6173	1.30375
	95% Confidence Interval for Mean	Lower Bound	9.8211	
		Upper Bound	15.4136	
	5% Trimmed Mean		12.2054	
	Median		12.4400	
	Variance		25.496	
	Std. Deviation		5.04941	
	Minimum		6.55	
	Maximum		26.10	
	Range		19.55	
	Interquartile Range		3.92	
	Skewness		1.521	.580
	Kurtosis		2.921	1.121

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Irrigant	Statistic	df	Sig.	Statistic	df	Sig.
Bond strength	Na DW	.143	15	.200*	.967	15	.816
	Na ED1 DW	.184	15	.183	.926	15	.235
	Na ED3 DW	.181	15	.199	.899	15	.093
	Na ED5 DW	.104	15	.200*	.978	15	.952
	Na ED10 DW	.226	15	.037	.862	15	.026

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

a. Lilliefors Significance Correction

One-way analysis of variance of bond strength of each irrigation protocol of AH Plus

Test of Homogeneity of Variances

Bond strength

Levene Statistic	df1	df2	Sig.
.907	4	70	.465

ANOVA

Bond strength

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	52.612	4	13.153	.872	.485
Within Groups	1055.958	70	15.085		
Total	1108.569	74			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Bond strength

Tukey HSD

(I) Irrigant	(J) Irrigant	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Na DW	Na ED1 DW	-2.17067	1.41822	.546	-6.1419	1.8006
	Na ED3 DW	-.92800	1.41822	.965	-4.8992	3.0432
	Na ED5 DW	-1.78133	1.41822	.719	-5.7526	2.1899
	Na ED10 DW	-2.16533	1.41822	.549	-6.1366	1.8059
Na ED1 DW	Na DW	2.17067	1.41822	.546	-1.8006	6.1419
	Na ED3 DW	1.24267	1.41822	.905	-2.7286	5.2139
	Na ED5 DW	.38933	1.41822	.999	-3.5819	4.3606
	Na ED10 DW	.00533	1.41822	1.000	-3.9659	3.9766
Na ED3 DW	Na DW	.92800	1.41822	.965	-3.0432	4.8992
	Na ED1 DW	-1.24267	1.41822	.905	-5.2139	2.7286
	Na ED5 DW	-.85333	1.41822	.974	-4.8246	3.1179
	Na ED10 DW	-1.23733	1.41822	.906	-5.2086	2.7339
Na ED5 DW	Na DW	1.78133	1.41822	.719	-2.1899	5.7526
	Na ED1 DW	-.38933	1.41822	.999	-4.3606	3.5819
	Na ED3 DW	.85333	1.41822	.974	-3.1179	4.8246
	Na ED10 DW	-.38400	1.41822	.999	-4.3552	3.5872
Na ED10 DW	Na DW	2.16533	1.41822	.549	-1.8059	6.1366
	Na ED1 DW	-.00533	1.41822	1.000	-3.9766	3.9659
	Na ED3 DW	1.23733	1.41822	.906	-2.7339	5.2086
	Na ED5 DW	.38400	1.41822	.999	-3.5872	4.3552

MetaSEAL

Descriptive statistical analysis of bond strength of each irrigation protocol of MetaSEAL

Case Processing Summary

	Irrigant	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
Bond strength	Na DW	15	100.0%	0	0.0%	15	100.0%
	Na ED1 DW	15	100.0%	0	0.0%	15	100.0%
	Na ED3 DW	15	100.0%	0	0.0%	15	100.0%
	Na ED5 DW	15	100.0%	0	0.0%	15	100.0%
	Na ED10 DW	15	100.0%	0	0.0%	15	100.0%

Descriptives

	Irrigant			Statistic	Std. Error
Bond strength	Na DW	Mean		14.9000	1.39595
		95% Confidence Interval for Mean	Lower Bound	11.9060	
			Upper Bound	17.8940	
		5% Trimmed Mean		14.9522	
		Median		15.6000	
		Variance		29.230	
		Std. Deviation		5.40648	
		Minimum		4.10	
		Maximum		24.76	
		Range		20.66	
		Interquartile Range		6.09	
		Skewness		-.439	.580
		Kurtosis		.429	1.121
	Na ED1 DW	Mean		20.5333	2.09200
		95% Confidence Interval for Mean	Lower Bound	16.0464	
			Upper Bound	25.0202	
		5% Trimmed Mean		20.4693	

	Median		17.9600	
	Variance		65.647	
	Std. Deviation		8.10227	
	Minimum		9.23	
	Maximum		32.99	
	Range		23.76	
	Interquartile Range		15.66	
	Skewness		.378	.580
	Kurtosis		-1.443	1.121
Na ED3 DW	Mean		20.2380	1.90363
	95% Confidence Interval for Mean	Lower Bound	16.1551	
		Upper Bound	24.3209	
	5% Trimmed Mean		20.0356	
	Median		18.8300	
	Variance		54.357	
	Std. Deviation		7.37274	
	Minimum		8.56	
	Maximum		35.56	
	Range		27.00	
	Interquartile Range		8.68	
	Skewness		.663	.580
	Kurtosis		.130	1.121
Na ED5 DW	Mean		20.9120	1.40712
	95% Confidence Interval for Mean	Lower Bound	17.8940	
		Upper Bound	23.9300	
	5% Trimmed Mean		20.7478	
	Median		21.7500	
	Variance		29.700	
	Std. Deviation		5.44977	
	Minimum		12.21	
	Maximum		32.57	
	Range		20.36	
	Interquartile Range		7.94	
	Skewness		.355	.580
	Kurtosis		-.069	1.121
Na ED10 DW	Mean		26.1507	1.53019

	95% Confidence Interval for Mean	Lower Bound	22.8687	
		Upper Bound	29.4326	
	5% Trimmed Mean		26.2319	
	Median		26.2800	
	Variance		35.122	
	Std. Deviation		5.92638	
	Minimum		13.81	
	Maximum		37.03	
	Range		23.22	
	Interquartile Range		9.62	
	Skewness		-.107	.580
	Kurtosis		.182	1.121

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Irrigant	Statistic	df	Sig.	Statistic	df	Sig.
Bond strength	Na DW	.141	15	.200*	.964	15	.753
	Na ED1 DW	.198	15	.116	.899	15	.092
	Na ED3 DW	.120	15	.200*	.961	15	.702
	Na ED5 DW	.147	15	.200*	.963	15	.753
	Na ED10 DW	.114	15	.200*	.981	15	.975

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

a. Lilliefors Significance Correction

One-way analysis of variance of bond strength of each irrigation protocol of MetaSEAL

Test of Homogeneity of Variances

Bond strength

Levene Statistic	df1	df2	Sig.
1.716	4	70	.156

ANOVA

Bond strength

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	952.779	4	238.195	5.564	.001
Within Groups	2996.786	70	42.811		
Total	3949.565	74			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Bond strength

Tukey HSD

(I) Irrigant	(J) Irrigant	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Na DW	Na ED1 DW	-5.63333	2.38918	.139	-12.3234	1.0567
	Na ED3 DW	-5.33800	2.38918	.179	-12.0281	1.3521
	Na ED5 DW	-6.01200	2.38918	.099	-12.7021	.6781
	Na ED10 DW	-11.25067*	2.38918	.000	-17.9407	-4.5606
Na ED1 DW	Na DW	5.63333	2.38918	.139	-1.0567	12.3234
	Na ED3 DW	.29533	2.38918	1.000	-6.3947	6.9854
	Na ED5 DW	-.37867	2.38918	1.000	-7.0687	6.3114
	Na ED10 DW	-5.61733	2.38918	.141	-12.3074	1.0727
Na ED3 DW	Na DW	5.33800	2.38918	.179	-1.3521	12.0281
	Na ED1 DW	-.29533	2.38918	1.000	-6.9854	6.3947
	Na ED5 DW	-.67400	2.38918	.999	-7.3641	6.0161
	Na ED10 DW	-5.91267	2.38918	.108	-12.6027	.7774
Na ED5 DW	Na DW	6.01200	2.38918	.099	-.6781	12.7021
	Na ED1 DW	.37867	2.38918	1.000	-6.3114	7.0687
	Na ED3 DW	.67400	2.38918	.999	-6.0161	7.3641
	Na ED10 DW	-5.23867	2.38918	.195	-11.9287	1.4514
Na ED10 DW	Na DW	11.25067*	2.38918	.000	4.5606	17.9407
	Na ED1 DW	5.61733	2.38918	.141	-1.0727	12.3074
	Na ED3 DW	5.91267	2.38918	.108	-.7774	12.6027
	Na ED5 DW	5.23867	2.38918	.195	-1.4514	11.9287

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

VITA

Sutt Pansawangwong was born on 5th February 1988 in Bangkok. He graduated with D.D.S. (Doctor of Dental Surgery) from the Faculty of Dentistry, Chulalongkorn University in 2011, and had worked as a dentist at the special clinic of Faculty of Dentistry, Mahidol University, Bangkok for 1 year. At the present, he has studied in a Master degree program in Endodontology at Faculty of Dentistry, Chulalongkorn University.