ผลของอิพิแกลโลแคทิชิน-3-แกลเลต (อีจีซีจี) ต่อกำลังแรงยึดพุชเอาท์ของซีลเลอร์ชนิด เอเอชพลัสต่อเนื้อฟันส่วนรากฟัน

นางสาวสุธิดา พีร์นิธิเจริญกุล

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สาขาวิชาวิทยาเอ็นโดดอนต์ ภาควิชาทันตกรรมหัตถการ
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ปีการศึกษา 2558
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

THE EFFECT OF EPIGALLOCATECHIN-3-GALLATE (EGCG) ON THE PUSH OUT BOND STRENGTH OF AH PLUS SEALER TO ROOT DENTIN

Miss Suthida Pheenithicharoenkul

จุฬาลงกรณ์มหาวิทยาลัย
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A Thesis Submitted in Partial Fulfillment of the Requirements
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สุธิดา พีร์นิธิเจริญกุล : ผลของอิพิแกลโลแคทิชิน-3-แกลเลต (อีจีซีจี) ต่อกำลังแรงยึดพุชเอาท์ของซีลเลอร์ชนิด เอเอชพลัสต่อเนื้อฟันส่วนรากฟัน (THE EFFECT OF EPIGALLOCATECHIN-3-GALLATE (EGCG) ON THE PUSH OUT BOND STRENGTH OF AH PLUS SEALER TO ROOT DENTIN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ทญ. ดร. อัญชนา พานิชอัตรา{, หน้า.

บทนำ: การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของการใช้อิพิแกลโลแคทิชิน-3-แกลเลต (อีจีซีจี) จากชาเขียวสกัดเป็นน้ำยาล้างคลองรากฟันต่อกำลังแรงยึดพุชเอาท์ของเอเอชพลัสซีลเลอร์ต่อเนื้อฟันส่วนรากฟัน วิธีการวิจัย: นำฟันกรามน้อยถอนมนุษย์จำนวน 70 ซี่มาตัดส่วนตัวฟันและนำไปขยายคลองรากฟันด้วยไฟล์โปรเทเปอร์ยูนิเวอซัลจนถึงขนาด F3 โดยมีการล้างคลองรากฟันระหว่างเปลี่ยนเครื่องมือด้วยน้ำยาโซเดียมไฮโปคลอไรต์ ความเข้มข้นร้อยละ 2.5 สุ่มแบ่งเป็น 4 กลุ่มการทดลอง (n=16) ตามน้ำยาล้างคลองรากฟันสุดท้าย กลุ่มที่ 1 ล้างด้วยน้ำยาอีดีทีเอ ความเข้มข้นร้อยละ 17 กลุ่มที่ 2 ล้างด้วยน้ำยาอีดีทีเอ ความเข้มข้นร้อยละ 17 ตามด้วยโซเดียมไฮโปคลอไรต์ ความเข้มข้นร้อยละ 2.5 กลุ่มที่ 3 ล้างด้วยน้ำยาอีดีทีเอ ความเข้มข้นร้อยละ 17 ตามด้วยอีจีซีจี และกลุ่มที่ 4 ล้างด้วยน้ำยาอีจีซีจี คลองรากฟันอีก 6 ซี่ ล้างด้วยน้ำยาโซเดียมไฮโปคลอไรต์ ความเข้มข้นร้อยละ 2.5 เพื่อเป็นกลุ่มควบคุม ฟัน 1 ซี่จากแต่ละกลุ่มจะถูกสุ่มเลือกไปเตรียมชิ้นงานเพื่อดูด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด หลังจากนั้นนำคลองรากฟันไปอุดด้วยกัตตาเปอร์ชาและเอเอชพลัสซีลเลอร์ นำคลองรากฟันที่อุดแล้วไปตัดแนวขวางให้ได้ชิ้นงานที่ความหนา 1 มม. นำชิ้นงานไปทำการทดสอบพุชเอาท์ วิเคราะห์ค่ากำลังแรงยึดพุชเอาท์และแปลผลด้วยสถิติความแปรปรวนทางเดียว (one-way ANOVA) ร่วมกับการทดสอบทูกีย์ (Tukey post hoc test) ผลการทดลอง: กลุ่มที่ล้างคลองรากฟันสุดท้ายด้วยน้ำยาอีดีทีเอ ความเข้มข้นร้อยละ 17 ตามด้วยอีจีซีจีมีค่ากำลังแรงยึดพุชเอาท์สูงที่สุด (p<.05) กลุ่มที่ล้างคลองรากฟันด้วยอีจีซีจีมีค่ากำลังแรงยึดพุชเอาท์มากกว่ากลุ่มที่ล้างด้วยน้ำยาอีดีทีเอ (p<.05) อย่างไรก็ตามไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่ากำลังแรงยึดพุชเอาท์ระหว่างกลุ่มที่ล้างด้วยน้ำยาอีดีทีเอตามด้วยโซเดียมไฮโปคลอไรต์และกลุ่มที่ล้างด้วยน้ำยาอีดีทีเอ (p>.05) บทสรุป: การใช้อีจีซีจีเป็นน้ำยาล้างคลองรากฟันสุดท้ายเพิ่มกำลังแรงยึดของเอเอชพลัสซีลเลอร์ต่อเนื้อฟันส่วนรากฟัน

ภาควิชา ทันตกรรมหัตถการ ลายมือชื่อนิสิต ....
สาขาวิชา วิทยาเอ็นโดดอนต์ ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
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SUTHIDA PHEENITHICHAROENKUL: THE EFFECT OF EPIGALLOCATECHIN-3-GALLATE (EGCG) ON THE PUSH OUT BOND STRENGTH OF AH PLUS SEALER TO ROOT DENTIN. ADVISOR: ASST. PROF. ANCHANA PANICHUTTRA, Ph.D.{, pp.

Introduction: This study investigated the effect of epigallocatechin-3-gallate (EGCG) from green tea extract used as an endodontic irrigant on the push out bond strength of AH plus sealer to root dentin. Methods: 70 premolars with single root canals were decoronated and instrumented to size F3 (ProTaper Universal) and irrigated with 1 ml of 2.5% NaOCl between each instrument. The roots were divided into 4 groups (n=16) according to the final irrigant: group 1-17% EDTA, group 2-17% EDTA followed by 2.5% NaOCl , group 3-17% EDTA followed by EGCG, and group 4-EGCG. Six additional root canals received 5 ml of 2.5% NaOCl as the final irrigant as a control group. One root from each group (n=1) was prepared for scanning electron microscope evaluation. Then, the root canals were obturated with gutta-percha and AH plus sealer. The root canals were horizontally sectioned into one-millimeter thick slices, and the push out test was performed on root slices. The push out bond strength was analyzed using one-way analysis of variance and Tukey post hoc test. Results: Final irrigation with 17% EDTA followed by EGCG had the highest bond strength ($p<.05$). The EGCG group demonstrated significantly higher bond strength compared with the EDTA group ($p<.05$). However, there was no significant difference in bond strength between EDTA with NaOCl and EDTA groups ($p>.05$). Conclusions: The use of EGCG significantly increased the push-out bond strength between AH plus sealer and root dentin.

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Department: Operative Dentistry Student's Signature ....
Field of Study: Endodontology Advisor's Signature ....
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CHAPTER I

INTRODUCTION

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Background and Rationale

Successful endodontic therapy depends on effective cleaning and shaping of the root canal, as well as establishment of a fluid tight seal of the canal space to prevent further reinfection (1). A thorough mechanical instrumentation has been advocated to increase the success of the treatment. However, the root canal was not thoroughly cleaned after mechanical instrumentation alone. Furthermore, MI usually leaves an amorphous irregular smear layer covering the dentinal canal wall and plugging in the dentinal tubules, that may cause an obstruction of the intracanal disinfectants and sealers from penetrating the dentinal tubules (2). Various irrigating solutions have been used during root canal preparation to eradicate microorganisms that cannot be reached by mechanical instrumentation, to remove debris, necrotic pulp tissue and smear layer. Many studies suggested the EDTA combined with NaOCl irrigation to create clean root canal surface which benefited an intimate contact of root canal filling material and root dentin (3, 4).

Sodium hypochlorite (NaOCl) has been widely used in endodontic treatment because of its broad spectrum antimicrobial activity and tissue dissolving ability. The use of ethylenediaminetetraacetic acid (EDTA) is necessary to remove inorganic part of smear layer. The alternative use of NaOCl and EDTA resulted in completely smear layer removal. Smear layer removal left the entrances to the dentinal tubules more open and exposed which benefited the penetration of intracanal disinfectant and sealer (5-10). However, these irrigants may cause the change in chemical composition, dentin permeability, wettability and collagen degradation of dentin which could potentially affect the sealing ability of the sealer to the root canal wall. A properly sealed root canal following cleaned and shaped root canal is one of the most important factors in successful endodontic treatment. The establishment of a fluid tight seal canal prevents reinfection, which is considered the aim of endodontic treatment.

In order to give a fluid-tight seal canal, the sealer should be capable of producing a bond between the core material and dentin wall. Bond strength of endodontic sealers to dentin is important as it maintains the integrity of the seal in the root canal filling during restorative procedures or masticatory function. Among various types of endodontic sealer, AH plus, an epoxy resin sealer, is the most commonly used due to its superior physical properties. AH plus sealer has been shown to have low solubility, good apical sealability, adequate biological performance. AH plus also showed greater bond strength than other sealers (11-14). Many studies suggested that high bond strength was related to the covalent bonds between epoxide rings of AH plus and the exposed amino group in the collagen

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network in root dentin (14, 15). Neelakantan et al (16) demonstrated a reduction in bond strength when NaOCl was used as a final irrigant because it has proteolytic ability that could remove collagen from dentinal surface.

Epigallocatechin-3-gallate (EGCG) is a major polyphenol found in green tea. EGCG has received considerable attention in the field of nutrition, health and medicine due to its antimutagenic, antioxidative, anticarcinogenic, anti-inflammatory, antimicrobial, antiviral, antihypertensive and hypocholestrolemic. Goo et al (17) showed that EGCG enhanced collagen stabilization and inhibited collagenase activity. EGCG's abilities to crosslink collagen, along with its antimicrobial, anti-inflammatory, antioxidative, and chelating ability, suggest that EGCG may be effectively used as an endodontic irrigant. We hypothesized that the use of EGCG combined with EDTA and NaOCl might increase the bond strength of AH plus. Therefore, this study investigated the effect of epigallocatechin-3-gallate on the push out bond strength of AH plus sealer to root dentin.

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Objective

To investigate the effect of epigallocatechin-3-gallate (EGCG) as a final irrigant on the push-out bond strength of AH plus sealer to root dentin.

Expected benefits and application

The results of the research project could lead to clinical application of the final irrigation protocol before root canal obturation that may improve the bond strength between root filling material and root canal dentin, and increase long-term endodontic success rate.

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CHAPTER II

LITERATURE REVIEW

Goal of endodontic treatment

Success in Endodontics depends on the thorough debridement of the root canal system, the elimination of pathogenic organisms and finally the complete sealing of the canal space to prevent further reinfection. Chemomechanical cleansing by mechanical instrumentation and copious irrigation is considered as the most important step aiming to disinfect the root canal. The development of rotary NiTi instrument fulfilled the mechanical aspect of root canal preparation (18) ; however, the biological aspects could not be achieved without the use of chemical irrigants. Main obstacles in removing those bacteria from the root canal are the complexity of the root canal system, bacterial intrusion into the tubules and smear layer formation after the mechanical instrumentation process. This layer contains inorganic and organic substances such as fragments of odontoblastic process, microorganisms, and necrotic materials. Smear layer itself may contain bacteria and protect the bacteria remaining in the dentinal tubules (19). The smear layer has also been shown to limit the optimum penetration of disinfecting agents (3, 20-23) and inhibited the complete adaptation of obturation materials to the prepared root canal surface (5-10).

Chemical irrigants

Because mechanical instrumentation alone is insufficient to disinfect the root canal system as well as it leaves the smear layer on the root canal wall, the use of adjunct chemical irrigants becomes indispensable during endodontic treatment. Main objective of chemical irrigants are:

- Gross debridement
- Elimination of microbes
- Dissolution of remnant pulp tissue
- Lubrication
- Smear layer removal

In order to obtain the main objectives of irrigation, ideal irrigant should have characteristics as stated (24).

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- Have a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms
- Dissolve necrotic pulp tissue remnants
- Inactivate endotoxin
- Prevent the formation of a smear layer during instrumentation or dissolve the latter once it has formed
- Be systemically nontoxic, non-caustic to periodontal tissues and have little potential to cause an anaphylactic reaction

Chemical irrigants used in endodontics are Sodium hypochlorite (NaOCl), Chlorhexidine (CHX), Ethylene diamine tetraacetic acid (EDTA), MTAD, Tetraclean, Etidronic acid (HEBP), QMiX, Citric acid, Hydrogen peroxide (H_2O_2), Iodine potassium iodide (IPI), Green tea and Triphala. Among various irrigants, Sodium hypochlorite (NaOCl) and Ethylenediaminetetraacetic acid (EDTA) are widely used in routine endodontic treatment.

Sodium hypochlorite (NaOCl)

Sodium hypochlorite (NaOCl) is the most popular irrigant used in endodontics. NaOCl is commonly used in concentration between 0.5 and 5.25%. It has approximately pH 11-12. Besides other good characteristics such as cheap, easily available and good shelf life, NaOCl possesses broad spectrum antimicrobial activity and tissue dissolving effect, which are the most desirable properties of endodontic irrigant.

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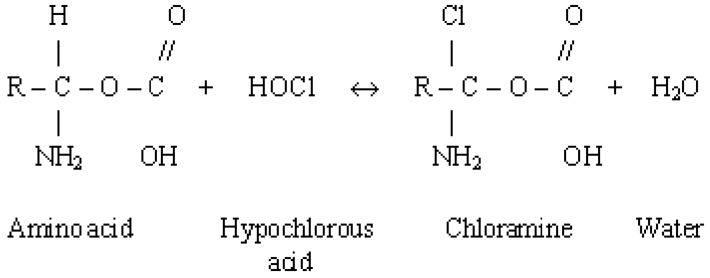
Mode of action

Antimicrobial activity: The high pH of NaOCl ($pH > 11$) interferes in the cytoplasmic inhibition, biosynthetic alterations in cellular metabolism, and phospholipid degeneration observed in lipidic peroxidation (25).

Tissue dissolving ability: Hypochlorous acid (HClO), a substance presenting in sodium hypochlorite solution, releases chlorine that combined with the protein amino group (-NH-) and forms chloramine (-NCl-) leading to amino acid degradation and hydrolysis (Fig 1).

NaOCl not only dissolves the vital and necrotic pulp remnants, it also degrades the organic, collagenous fraction of dentin.



$$R-\underset{NH_2}{\overset{H}{C}}-O-\overset{O}{\underset{OH}{C}} + HOCl \leftrightarrow R-\underset{NH_2}{\overset{Cl}{C}}-O-\overset{O}{\underset{OH}{C}} + H_2O$$

Figure 1 Chloramination reaction (25)

The higher concentration of NaOCl has greater effective in antimicrobial and tissue dissolving ability but it is also more toxic. The concentration of NaOCl therefore must be used according to “maximal antimicrobial effect with minimal toxicity” (26, 27). The antibacterial effectiveness of low concentrations of NaOCl can be enhanced by using larger volumes of irrigant and by frequent exchange of irrigant. Regular exchange and the use of large amounts of irrigant should maintain the antibacterial effectiveness of the NaOCl solution, compensating for the effects of concentration (28). Hand et al (29) and Trepagnier et al (30) recommended 2.5% NaOCl used for root canal irrigation.

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The disadvantage of NaOCl is high toxicity and unable to remove inorganic part of smear layer.

Ethylenediaminetetraacetic acid (EDTA)

EDTA, an effective chelating agent, has been used as adjuvants in endodontic therapy. It is commonly used in 17% concentration. It has approximately pH 7. It is used to enlarge root canals, remove the smear layer, and prepare the dentinal walls for better adaptation of filling materials. Several studies recommended the working times between 1 and 5 minutes for good cleaning efficacy of EDTA (31, 32). Calt and Serper (32) found peritubular and intertubular erosion after root canal was exposed to EDTA for 10 minutes.

Mode of action

Demineralization: EDTA forms a stable complex with calcium ions from dentin (Fig. 2).

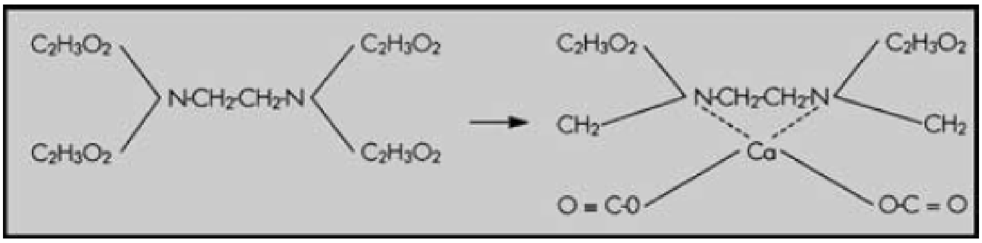

Figure 2 Chemical structure and mechanism of EDTA binding (33)

The mechanism is self-limiting owing to the change of pH during demineralization of dentin. As the reaction proceeds, acid accumulates and protonation of EDTA prevails, thus the rate of demineralization (34) is decreased as shown in following chemical reactions.

(1) $\mathrm{EDTA\ H^{3-} + Ca^{2+} = EDTACa^{2-} + H^{+}}$

(2) $\mathrm{EDTA\ H^{3-} + H^{+} = EDTAH^{2-}}$

The disadvantage of EDTA is that it does not have antimicrobial activity and tissue dissolving ability.

The use of EDTA followed by NaOCl is widely accepted as a final flush for smear layer removal before filling the root canal. Greater cleaning action and antimicrobial effect were found when these irrigants are used in combination rather than alone (4, 35, 36). A scanning electron microscopic of root canal dentin surface after the combination use of EDTA and NaOCl revealed completely removal of smear layer from the root canal wall (Fig. 3) (3, 4). The use of EDTA will dissolve inorganic components of the smear layer, decalcify peritubular and intertubular dentin and leave exposed collagen in root canal dentin. The subsequent use of NaOCl leads to the removal of organic components of the smear layer as well as collagen, leaving more open and exposed the dentinal tubules. The result mentioned above was found to enhance the penetration of the sealer into the dentinal tubule.

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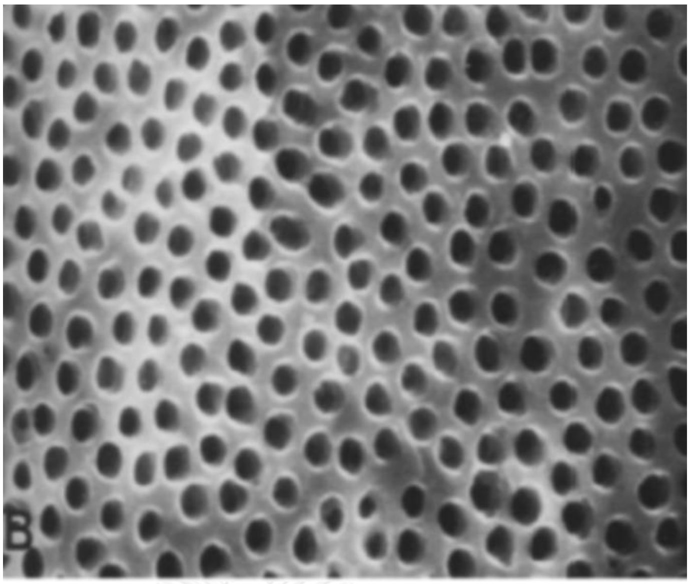

Figure 3 The smear-free dentin after final flush with 17% EDTA and 2.5% NaOCl (x700 magnification) (3)

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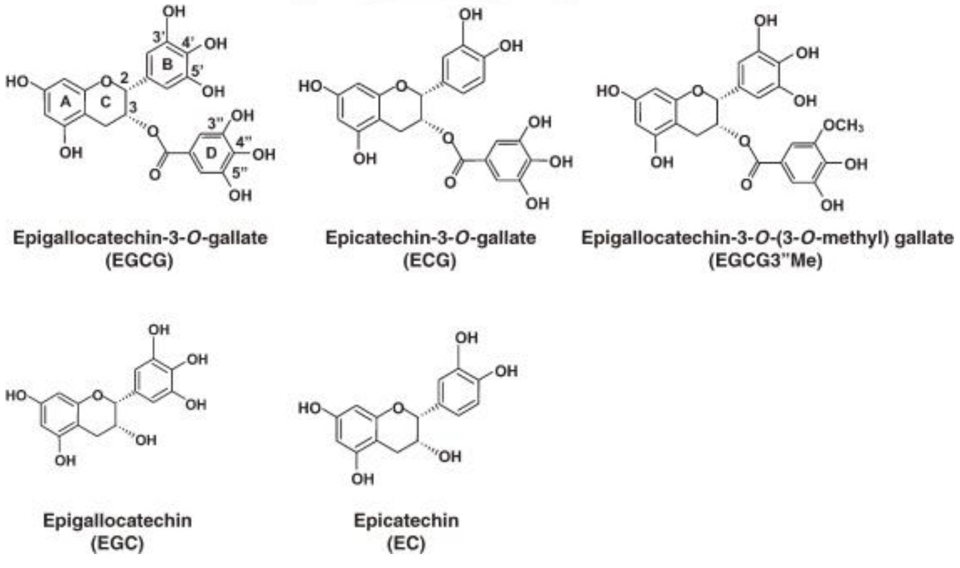
Even though irrigation should be a key role in a successful endodontic treatment, none of currently used irrigants has all of the characteristics of an ideal irrigant. The continuing search for an ideal irrigant is still in an area of interest in current researches.

Epigallocatechin-3-gallate (EGCG)

Epigallocatechin-3-gallate (EGCG) is major catechins which is a powerful polyphenol mostly found in green tea. Catechins are derived from the dried tea leaves of the plant *Camellia sinensis*. Among different types of tea, green tea is higher in catechins than black or oolong tea. During manufacturing process, black and oolong tea are subjected to fermentation that leading to polymerization of monopolyphenolic compounds such as catechins, causing a conformational change and modifying its properties. In green tea, fresh tea leaves undergo an initial heating process (steaming) which inhibits polyphenol oxidase enzyme, preserving polyphenol in monomeric form. Catechins are characterized by the dihydroxyl or trihydroxyl substitutions in the B ring and the m-5,7-dihydroxyl substitutions on the A ring (Fig. 4). The major green tea catechins are (-) epigallocatechin-3-gallate (EGCG), that represents approximately 59% of the total of catechins, (-) epigallocatechin (EGC) (19%, approximately), (-) epicatechin-3-gallate (ECG) (13.6% approximately), and (-) epicatechin (EC) (6.4% approximately). EGC is produced by hydroxylation of EC. Then ECG and EGCG are synthesized by esterification of catechins with gallic acid. The B ring is the principle site of antioxidant reactions and the antioxidant activity is further increased by the trihydroxyl in D ring (gallate) in EGCG and ECG.



Epigallocatechin-3-*O*-gallate (EGCG)

Epicatechin-3-*O*-gallate (ECG)

Epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3''Me)

Epigallocatechin (EGC)

Epicatechin (EC)

Figure 4 Chemical structures of green tea catechins (37)

It has been demonstrated that green tea constituents exhibit various biological and pharmacological properties such as anti-carcinogenic, anti-oxidative, anti-allergic, anti-virus, anti-hypertensive, anti-atherosclerosis, anti-cardiovascular disease and anti-hypercholesterolemic activities. Health benefits of green tea are derived from its antioxidative activity and reactive oxygen species scavenging ability. Among tea catechins, EGCG is the most effective form in reacting with the majority of reactive oxygen species (ROS).

Mode of action

Antioxidant activity

EGCG presents antioxidant activity by scavenging reactive oxygen species (ROS) and chelating redoxactive transitional metal ion (38). This property depends on the number of hydroxyl group presented on the flavonoid structure. If the number of hydroxyl groups is higher, the antioxidant capacity is also better.

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Anti-inflammatory activity

Various studies found that EGCG down-regulated inflammatory cytokine and chemokine expression such as TNF-α, IL-1β, IL-17 and MMP (39-41).

Collagen stabilization

Recently, EGCG was used to develop the collagen-based biomaterials for tissue repair and regeneration. EGCG-treated collagen exhibited high free-radical scavenging activity and resisted degradation by bacterial collagenase and MMP-1 (17), thus enhancing the structural integrity of collagen.

Mechanism of action for collagen stabilization

1. **Macroscopic stabilization**

EGCG is capable of intermolecular crosslinking between collagen through the formation of hydrogen or esteric bonds. Seven hydroxyl groups of EGCG easily react with carboxyl group present in collagen molecule, resulting in the formation of esteric bond (17). In addition, the study demonstrated the involvement of hydrogen bonding and hydrophobic interactions as the major forces involved in the stabilization of collagen (42).

2. **Microscopic stabilization**

EGCG restricts the conformational space available to the collagen backbone.

Furthermore, EGCG inhibits the collagenolytic activity of collagenase by forming hydrogen bond and hydrophobic interaction with collagenase leading to significant changes in its conformation and activity inhibition.

There are few studies of EGCG in dentistry which all of them showed positive benefits of EGCG.

Horiba et al was the first to use Japanese green tea as intracanal medication due to its antibacterial and bactericidal activity against various strains of bacteria (43). Catechins showed a bactericidal effect against black-pigmented, gram-negative anaerobic rods which are associated with periodontitis (44). This study found that periodontal status was improved after the combined use of mechanical treatment

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and the application of green tea catechins (44). In vivo study confirmed that EGCG inhibited alveolar bone resorption via the inhibition of MMP-9 expression in osteoblast and osteoclast formation (45). Recent studies found that EGCG had preservative potential for use as storage medium that may promote favorable outcome of tooth replantation. The studies found the highest PDL cell viability in the EGCG storage, regardless of its improper pH and osmolallity of EGCG (46-48).

Obturation

This smear-free layer will be sealed afterwards with root canal filling by mean of gutta percha and sealer for the final objective of endodontic treatment. Although the chemomechanical preparation is the most important stage in endodontic treatment, the three dimensional obturation of the root canal system must not be neglected to achieve final goal of endodontic treatment. There are two main purposes of the obturation.

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1. Sealing any remaining bacteria or any irritants after appropriate shaping and cleaning of the canals.
2. Elimination of all avenues of leakage from the oral cavity or the periradicular tissues into the root canal system.

Gutta percha and sealer have long been used as the standard root canal filling. Gutta percha itself does not bond to the canal wall. Therefore, the sealing ability of sealer is essential to obtain fluid-tight seal canal. Sealer not only enhances an impervious seal, it also serves as filler for canal irregularities and minor discrepancies between the root canal wall and core filling material. Sealer serves as one of the important factors in successful endodontics.

Types of root canal sealer and brand available

1. Zinc oxide eugenol based sealer (eg. Roth sealer, Kerr PCS, Procoseal, Endomethasone)
2. Resin based sealer (eg. AH Plus, AH26, Topseal, 2-seal)
3. Calcium hydroxide sealer (eg. Sealapex, Apexit, CRCS, Sealer 26)
4. Glass ionomer sealer (eg. Ketac endo)
5. Calcium silicate sealer (eg. Endosequence, iroot SP, iroot BP, Bioaggregate)
6. Silicone based sealer (eg. RoekoSeal, Gutta flow)
7. MTA sealer (eg. Endo-CPM-Sealer, MRA Obtura, ProRoot Endo Sealer, MTA fillapex)
8. Calcium phosphate based sealer (eg. CPS1, CPS2)
9. Methacrylate based sealer (eg. EndoREZ, Realseal, Realseal SE, Epiphany, Metaseal SE)

AH plus

AH plus, an epoxy resin sealer, is the most popular used resin sealer in endodontics. It consists of two pastes as follows:

Paste A: Bisphenol-A epoxy resin, Bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, Silica, Iron oxide pigments

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Paste B: Dibenzyldiamine, Aminoadamantane, Tricyclodecane-diamine, Calcium tungstate, zircronium oxide, silica, silicone oil

AH plus has a good performance on physical properties compared to other sealers. Besides its excellent physical properties such as longer setting time, low solubility, higher flow rate and less volumetric polymerization shrinkage, studies showed that epoxy resin-based sealers produced higher bond strength to dentin than zinc oxide, glass ionomer, calcium hydroxide and even methacrylate resin-based sealers (49-52). The high bond strength of AH plus is owing to the covalent bond between epoxy ring of AH plus and amino group of exposed collagen in dentin (14, 53).

The most desirable property of the sealer is to have a good sealing ability. In addition, root canal sealer should create a bonded interface between core filling material and root dentin. An interface serves as two important purposes. (1) In static situation, it produces a better adaptation of the root filling to the dentinal walls. Adaptation of root canal filling to dentinal walls could prevent the percolation of fluid or microbial between the filling and the wall causing reinfection. (2) In dynamic situation, it resists dislocation of the root filling during subsequent procedures such as post space preparation.

The chemical irrigants used during chemomechanical preparation could cause the change in physical and chemical composition, dentin permeability, wettability and collagen degradation of dentin. It also influences the bond between sealer and

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root dentin surface. The final flush with NaOCl after EDTA which has been routinely used before obturation results in collagen degradation in root dentin, decreasing the bond strength of AH plus. The decrease in push out bond strength of AH plus was found after using NaOCl as a final flush (12, 16, 54).

Push out test

The push-out test is a method used to measure bond strength. It has been widely used to measure the bonding of post in root canals. Interfacial shear bond strength is determined by measuring the compressive force required to dislodge the post segment using a slightly smaller diameter punch. Later, the push-out test has been used to evaluate the bond strength of root filling materials in root canals.

To give a detail of a test, the root that had been shaped and filled was sectioned into thin slices. The root slice was then subjected to a compressive load using a stainless steel cylindrical plunger. The force was applied in apico-coronal direction until bond failure occurred, which was manifested by extrusion of the filling material and a sudden drop along the load deflection.

Although the push out test in gutta percha will deform plastically under test condition, the study showed that this test was appropriated for ranking the bond strength of root canal filling material including gutta percha. Pane et al recommended the use of 90% canal diameter punch size, thin slice sample (1 or 2 mm) to reduce sliding friction, and the deviation of vertical alignment sample should be less than 10° for the accuracy of the test.

The force needed to dislodge the filling material (N) was transformed into tension (MPa) by dividing the force by the adhesive area of the filling material (mm^2)

Push out bond strength (MPa) = $\frac{maximum\ load\ (N)}{adhesion\ area\ of\ root\ filling\ (\ mm^2)}$

The adhesion (bonding) surface area of each section was calculated as: ($\pi r_1 + \pi r_2$) × L, where L = $\sqrt{(r1 - r2)^2 + \ h^2}$; where π is the constant 3.14, r1 and r2 are the larger and smaller radii, respectively, and h is the thickness of the section in mm.

Bond strength of AH plus

The bond strength can be enhanced by two means: First is to develop the sealer properties. Lastly, is to prepare the dentin surface that suitable for the optimal seal of the sealer.

The recent researches attended to find methods to improve the bond of AH plus and root dentin which are

- Using 980 nm diode laser to irradiate root canal dentin (55)
- Drying canal method with low vacuum by using a Luer adapter for 5 seconds, followed by 1 paper point for 1 second instead of paper point to leave the canals slightly moist before filling (56)
- Using 70% isopropyl alcohol instead of paper point to the dry canal before obturation (57)

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- Smear layer removal using using 17% EDTA followed by 5.25% NaOCl before obturation (58)
- The obturation technique: Lateral condensation (59, 60)
- Using ultrasonic activation and manual dynamic activation (61)

Whereas Andrade-Junior (65) found decrease in bond strength after using a bonding agent.

Also, there were studies in the effect of various irrigants to bond strength of AH plus. Many of the studies reported the negative effect of NaOCl which is commonly used as a final flush after 17% EDTA to the bond strength of AH plus (12, 15, 16, 54).

Nunes et al (12) reported that push out bond strength decreased after final flushed with 1% NaOCl when compared to 1% NaOCl followed by 17% EDTA in spite of no statistical significance.

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Vilanova et al (54) showed that the push out bond strength of AH plus decreased in the group that final flushed with 1% NaOCl. Groups irrigated with 1% NaOCl and 17% EDTA, 17% EDTA, 24% EDTA (gel) and 2% Chlorhexidine (gel) showed significantly higher bond strength.

Neelakantan et al (15, 16) and Rocha et al (62) also found the lowest bond strength after final flushed with 2.5% NaOCl. While final flush with decalcifying agent such as 17% EDTA, 7% maleic acid or etidronic acid increased the bond strength of

AH plus. The result from the study suggested a final flush with a decalcifying agent before obturation could enhance bond strength of AH plus.

Hashem et al (63) found the adverse effect of MTAD and MTAD with Chlorhexidine used as a final irrigant to the bond strength of AH plus.

It has also been shown that the irrigation protocols influenced the bond strength of AH plus (64). The bond strength was higher when phosphoric acid was used followed NaOCl or used Chlorhexidine with EDTA.

Whereas Saleh et al and Stelzer et al (11, 65) found no significant difference in bond strength of AH plus regardless of irrigants used.

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Table 1 Studies in the effect of various irrigants in the bond strength of AH plus

Study	Irrigants used	Test	Result
Saleh 2002 (11)	37% phosphoric acid, 25% citric acid, 17% EDTA	Tensile bond strength	Decalcifying agents reduced bond strength.
Nunes 2008 (12)	Distilled water, 1%NaOCl, 1%NaOCl+17%EDTA	Push out	AH plus presented greater adhesion to dentin than Epiphany regardless of the treatment of root canal wall.
Hashem 2009 (64)	EDTA, EDTA+CHX, MTAD, MTAD+CHX	Push out	Higher bond strength was found when using EDTA as the final irrigant. Whereas the bond strength was adversely affected by MTAD and CHX.

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Neelakantan 2011 (15)	Water, 3% NaOCl, 17% EDTA,7% maleic acid, 2%CHX, 3%NaOCl+17%EDTA +3%NaOCl, 3%NaOCl+3%NaOCl +17%EDTA, 3%NaOCl+7%MA+3%NaOCl, 3%NaOCl+3%NaOCl+7%MA	Push out Leakage test (fluid transport)	Final irrigation with decalcifying agent (EDTA or maleic acid) resulted in superior sealing ability and bond strength.
Vilanova 2012 (54)	1% NaOCl, 1% NaOCl+17%EDTA, 17% EDTA, 24%EDTA(gel), 2%CHX(gel)	Push out	Lowest bond strength was found in NaOCl group.
Rocha 2012 (63)	Normal saline, NaOCl+EDTA, CHX+EDTA	Push out	2.5% NaOCl decreased bond strength of AH plus.

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Neelakantan 2012 (16)	Mixture of 5% NaOCl and 18% Etidronic acid(HEBP), EDTA, NaOCl	Push out	Continuous chelation irrigation protocol optimized the bond strength.
Prado 2013 (65)	NaOCl+EDTA, NaOCl+Phosphoric acid(PA), NaOCl+EDTA+CHX, NaOCl+PA+CHX, CHX+EDTA, CHX+PA, CHX+EDTA+CHX, CHX+PA+CHX	Push out	Higher bond strength was found in NaOCl+PA and CHX+EDTA group.
Stelzer 2014 (66)	3% NaOCl, 17% EDTA, 2% CHX	Push out	No difference in the bond strength was found regardless of the irrigants used.

Although the chemical bond between epoxide ring of AH plus and exposed collagen network in root dentin has recently been proved (53), there is no clear instruction for the proper irrigant used for final irrigation before filling the root canal. NaOCl followed EDTA is still used as a final flush even if a decrease in bond strength was found after the final irrigation protocol. The use of EGCG, a collagen crosslinking agent, therefore may improve the bond strength of AH plus to dentinal wall.

This study aimed to evaluate the effect of EGCG as a final irrigant on the push out bond strength of AH plus sealer.

CHAPTER III

RESEARCH METHODOLOGY

Target Population

Human root canal

Sample

Human premolar with intact single root canal

Independent Variable

Different final irrigation protocols

1. 17% EDTA 5 min
2. 17% EDTA 5 min + 2.5% NaOCl 1 min
3. 17% EDTA 5 min + EGCG 5 min
4. EGCG 10 min

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Dependent Variables

Push out bond strength (MPa)

Control Variable

Needle gauge, rate of irrigation, canal preparation size

Confounding Factors

Root canal irregularities of each tooth, error from laboratory technique

Hypothesis

Ho: There is no difference in push out bond strength of AH plus sealer to root dentin among four final irrigation protocols

Ha: There is a difference in push out bond strength of AH plus sealer to root dentin among four final irrigation protocols

Materials

1. Chemicals

- 2.5% Sodium hypochlorite (NaOCl)
- 17% Ethylenediaminetetraacetic acid (EDTA)
- EGCG capsule (Giffarine, Thailand)
- EGCG standard (Sigma-Aldrich)
- Distilled water
- Glutaraldehyde
- PBS buffer

2. Slow speed, water-cooled diamond saw (Isomet 1000, Buehler, USA)
3. Universal testing machine (Shimadzu, ez-s, Japan)
4. HPLC (Shimadzu)
5. Stereomicroscope
6. Scanning electron microscope
7. K-file #10 (Dentsply Maillefer, Ballaigues, Switzerland)

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8. Plastic syringes 1, 5, 10 ml
9. 30-gauge needles
10. Timer
11. ProTaper Universal rotary file (Dentsply Maillefer)
12. AH plus (Dentsply)
13. Gutta-percha main cone

Methods

1. Quantitative analysis of EGCG capsule concentration by high-performance liquid chromatography

Instrumentation and chromatographic conditions

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HPLC system (Shimadzu) consisted of a LC-10AD delivery system with a membrane degasser, SPD-10A detector. The analytical column was a Symmetry-C18 reversed-phase, 150 mm x 3.9 mm i.d., packed with 5 µm C18 modified silica. A 20 mm x 3.9 mm i.d. guard column packed with 5 µm diameter Symmetry-C18 was utilized.

The chromatographic separation was performed under an isocratic condition. The mobile phase consisted of 0.1% acetic acid and methanol (70:30, v/v). The mobile phase was filtered through Nylon66 membrane filters (47 mm, 0.45 µm) and degassed by an ultrasonic bath. A flow rate of 1.0 ml/min was maintained and a UV detection was set at 230 nm. An aliquot of sample solution (10 µl) was injected onto the analytical column with an auto HPLC injector.

Preparation of standard solutions

EGCG was prepared as aqueous solution with concentration 1, 0.5 and 0.25 mg/ml

Preparation of sample solutions

Samples were prepared by mixing the powder in the EGCG capsule (Giffarine) with 35 ml distilled water to 1 mg/ml concentration and filtered through a filter paper (Whatman No.1).

2. Push out test and failure mode

Sample preparation

Seventy human premolars were selected from teeth extracted for orthodontic purposes and stored in 0.1% thymol solution. The study protocol was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University. Any teeth with resorption, calcification, cracks, open apex, or apical curvature were excluded.

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The teeth were decoronated using a slow-speed, water-cooled diamond saw (Isomet 1000, Buehler, USA) to a length of 15 mm. A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to determine canal patency and length and 1 mm was subtracted to establish the working length. The root canals were shaped with the ProTaper Universal NiTi rotary system (Dentsply Maillefer) to a master apical file #30, 0.09 taper, and irrigated with 1 ml of 2.5% NaOCl (Faculty of Dentistry Chulalongkorn University) between files. Irrigation was performed using a 5 ml disposable plastic syringe with a 30-gauge needle passively placed up to 2 ml from the working length without binding.

EGCG was prepared by mixing the powder in the EGCG capsule (Giffarine) with 35 ml distilled water, resulting in a 1 mg/ml concentration, which was confirmed by high performance liquid chromatography.

The roots were divided into four groups (n=16) according to the final irrigant: group 1-5 ml of 17% EDTA 5 min, group 2-5 ml of 17% EDTA 5 min followed by 5 ml of 2.5% NaOCl 1 min, group 3-5 ml of 17% EDTA 5 min followed by 5 ml of EGCG 5 min, and group 4-5 ml of EGCG 10 min. Six additional root canals received 5 ml of 2.5% NaOCl for 1 min as a control group.

Push-out test

The root canals were dried with paper points and obturated with gutta-percha cones and AH plus per the manufacturer's instructions using a lateral condensation technique. The specimens were stored at 37°C in a humidified atmosphere for 48 hours and embedded in clear self-cured resin in a plastic PVC mold. The resin was polymerized overnight and each root was horizontally sectioned into 1 mm slices. Two slices were collected from the middle third of each root (The coronal third slices were excluded because it was unable to obtain circular shape). The root slice was mounted on a universal testing machine (Shimadzu, ez-s, Japan) with the smaller diameter of the slice positioned up, and a crosshead speed of 1 mm/min was applied perpendicular to the root canal filling material (Fig. 5). The round metallic load applicator was 0.5 mm in diameter and only contacted the root filling. The force at failure was recorded in Newtons when interfacial failure or dislodgement was indicated by a sudden drop in the load-displacement curve. The push out bond strength was measured in MPa by dividing the force by the bonding area, according to the following formula (66):

$$\text{Push out bond strength (MPa)} = \frac{maximum\ load\ (N)}{adhesion\ area\ of\ root\ filling\ (\ mm^2)}$$

The adhesion (bonding) surface area of each section was calculated as: $(\pi r_1 + \pi r_2) \times L$, where $L = \sqrt{(r1 - r2)^2 + \ h^2}$; where r1 and r2 were the larger and smaller radii, respectively, and h was the thickness of the section in mm.

Figure 5 The push out test on a root slice

Failure mode

After the push out test, the tooth slice was examined under stereomicroscope to determine the mode of failure. Each sample was evaluated at 20x magnification and classified into following categories (57).

1-adhesive failure at the dentin/sealer interface (no sealer visible on dentin walls)

2-cohesive failure within the sealer (dentin walls totally covered with sealer)

3-mixed failure (both adhesive and cohesive failures could be observed)

3. SEM investigation

SEM investigation of root canal dentin surface

After having been received final irrigation protocols, one root canal from each group was taken for SEM investigation. The groove was cut on the buccal and lingual surface. The roots were then split with a hammer and chisel. The root halves were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 and prepared with an ethanol dehydration protocol. Each specimen was mounted on aluminium stubs, coated with gold/palladium and examined under SEM.

SEM investigation of resin-dentin interface

The apical third of the root canals slice was air dried, mounted in stubs and sputter coated and imaged for view sealer/dentin interface.

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Statistical analysis

The effect of final irrigation protocols on push-out bond strength was analyzed by one-way analysis of variance. Post hoc comparisons were performed using Tukey multiple comparisons. The significance level was set at $p<0.05$.

CHAPTER IV

RESEARCH RESULTS

1. Quantitative analysis of EGCG concentration by high-performance liquid chromatography

The retention time of the EGCG powder (Giffarine) on the HPLC column was 2.222 min (Fig. 6), which was comparable to that of the EGCG standard (Sigma-Aldrich). The 1 mg/ml concentration of the EGCG solution was determined by comparison with an EGCG standard calibration curve.

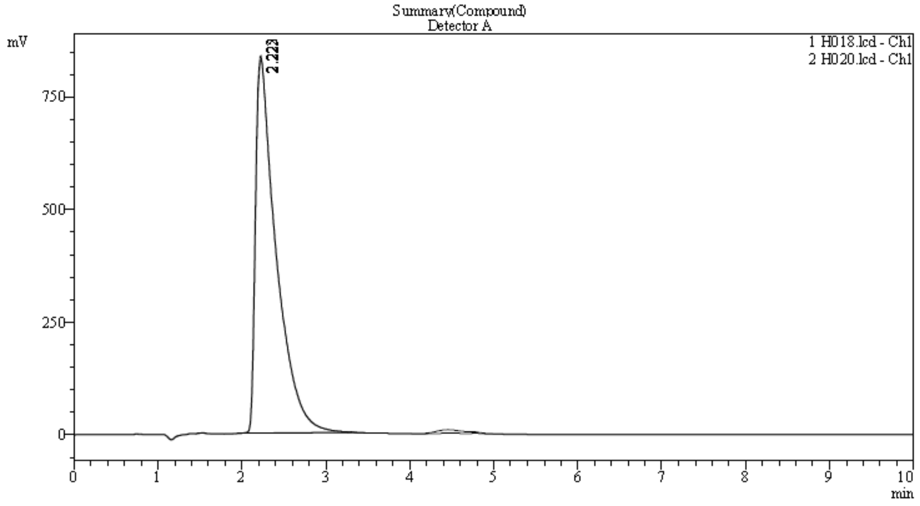

Figure 6 HPLC chromatograms of EGCG capsule sample and standard EGCG

2. Push out test and failure mode

Table 2 shows the mean and standard deviation of the bond strength and failure patterns of each group. The push out bond strength of the final irrigation with

EDTA and EGCG group was significantly higher than that of the other groups ($p<.05$). The EGCG group demonstrated significantly higher bond strength compared with the EDTA group ($p<0.05$). There was no significant difference in the mean bond strength between the EDTA and EDTA+NaOCl groups ($p>0.05$). Final irrigation with NaOCl (control group) resulted in the lowest bond strength (0.37±0.11 MPa).

Table 2 Bond strength values and failure patterns of the experimental groups

Groups	Bond strength (MPa) Mean±SD	Failure pattern (n)		
		Adhesive	Cohesive	Mixed
EDTA	1.53 ± 0.31^{c}	0	12	18
EDTA+NaOCl	1.31 ± 0.27^{c}	0	9	21
EDTA+EGCG	3.59 ± 0.38^{b}	0	18	12
EGCG	2.16 ± 0.42^{a}	0	11	19

Different capital letters represent significant differences between the groups ($p<0.05$)

Examination of the failure mode revealed that mixed and cohesive failures predominated in all groups. Adhesive failure was only found in the control group (five out of ten).

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3. SEM investigation

Figure 7 shows the SEM images of the root canal dentin surface following the different final irrigants. An exposed intact porous collagen network can be seen in all groups except for the control group. In the control group, the dentin surface was covered with a thick smear layer (Fig. 8).

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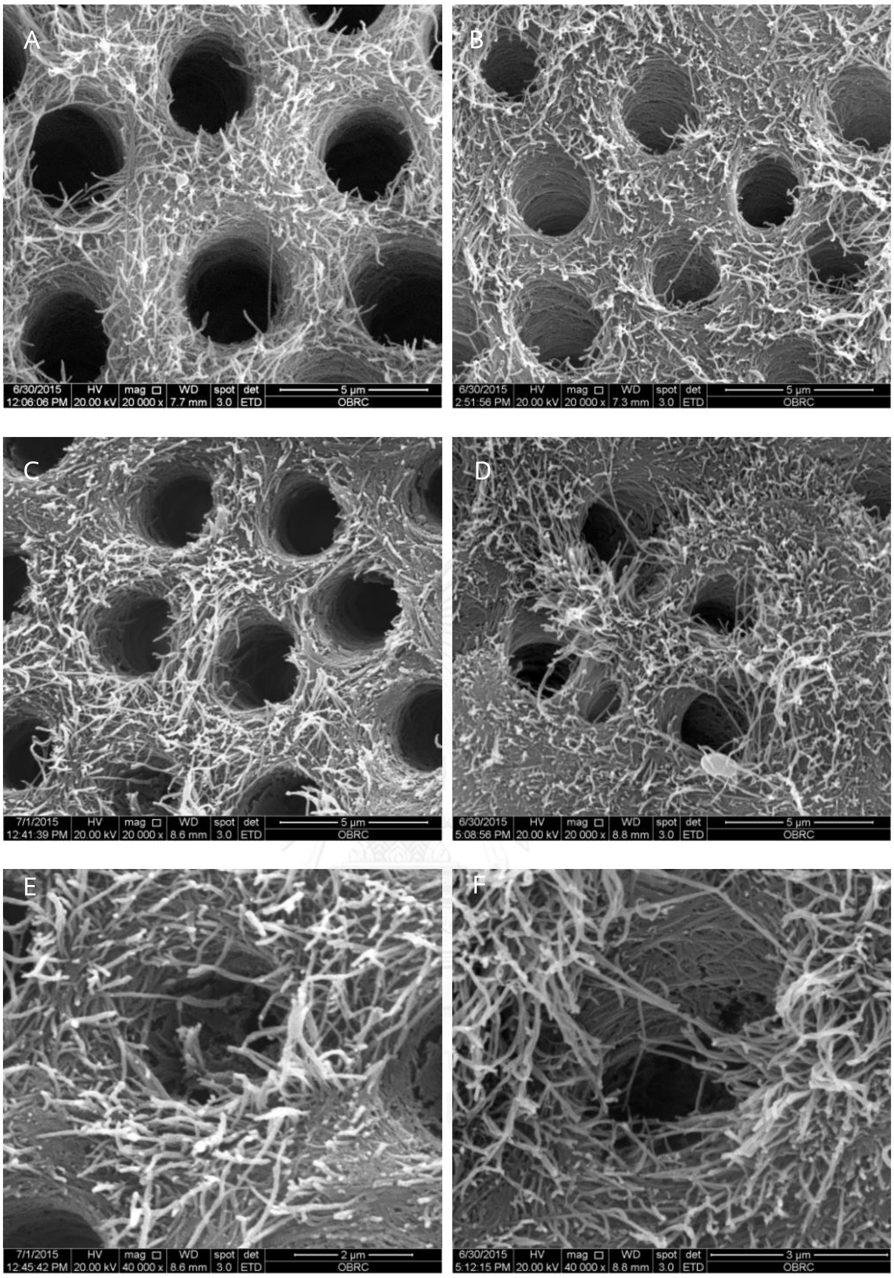

Figure 7 Demineralized uncollapsed collagen network is present (A) after final flush with 17% EDTA followed by 2.5% NaOCl (x20,000) and (B) after final flush with 17% EDTA (x20,000), (C) after final flush with 17% EDTA followed by 1 mg/ml EGCG for 5 min (x20,000), and (D) after final flush with 1mg/ml EGCG 10 min. (x20,000) (E, F) Higher magnification of C and D, respectively (x40,000)

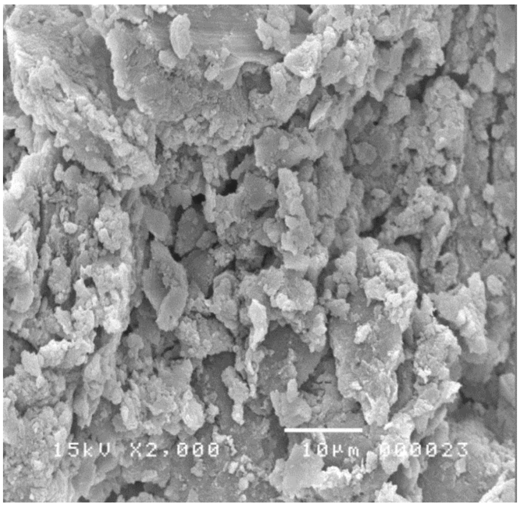

Figure 8 Dentin surface covering with a thick smear layer along the whole canal in NaOCl (control) group (x2000)

Figure 9 shows the SEM image of the resin - dentin interface of each group. A gap between interfaces was obviously seen with magnification x20, 000 in the control group (Fig. 9B). The experimental groups showed an intact interface. The sealer can penetrate into the dentinal tubule.

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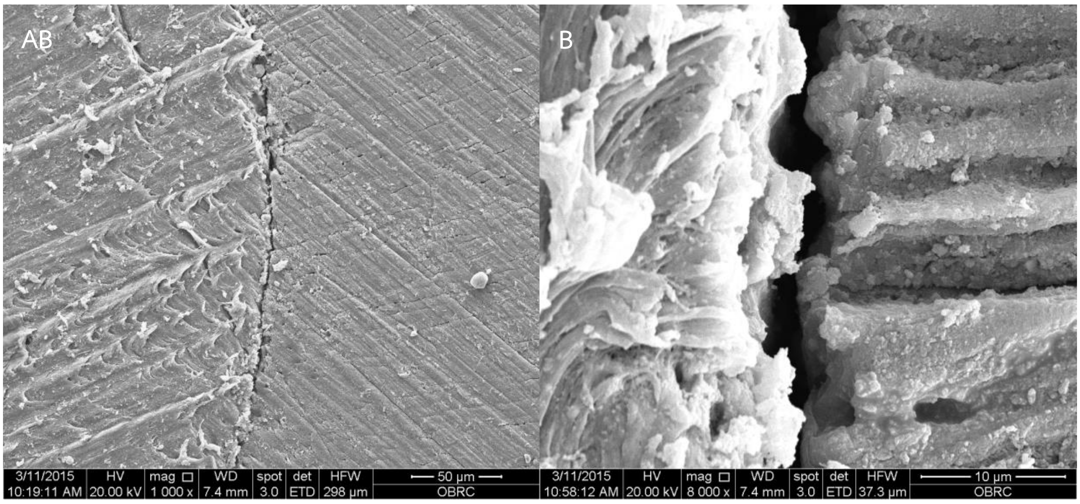

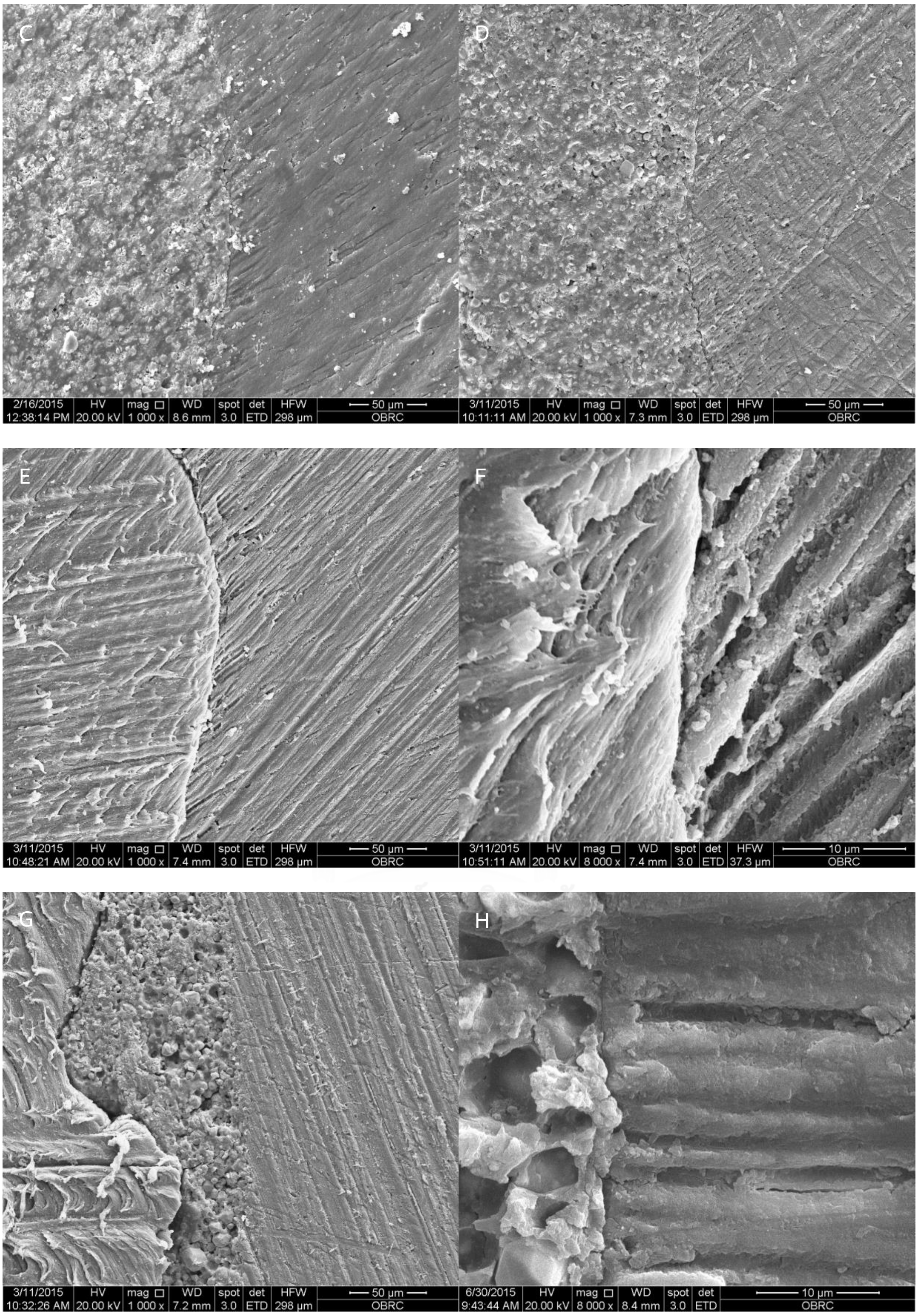
C
2/16/2015 12:38:14 PM | HV 20.00 kV | mag □ 1 000 x | WD 8.6 mm | spot 3.0 | det ETD | HFW 298 µm | 50 µm | OBRC
D
3/11/2015 10:11:11 AM | HV 20.00 kV | mag □ 1 000 x | WD 7.3 mm | spot 3.0 | det ETD | HFW 298 µm | 50 µm | OBRC
E
3/11/2015 10:48:21 AM | HV 20.00 kV | mag □ 1 000 x | WD 7.4 mm | spot 3.0 | det ETD | HFW 298 µm | 50 µm | OBRC
F
3/11/2015 10:51:11 AM | HV 20.00 kV | mag □ 8 000 x | WD 7.4 mm | spot 3.0 | det ETD | HFW 37.3 µm | 10 µm | OBRC
G
3/11/2015 10:32:26 AM | HV 20.00 kV | mag □ 1 000 x | WD 7.2 mm | spot 3.0 | det ETD | HFW 298 µm | 50 µm | OBRC
H
6/30/2015 9:43:44 AM | HV 20.00 kV | mag □ 8 000 x | WD 8.4 mm | spot 3.0 | det ETD | 10 µm | OBRC

Figure 9 SEM image of the sealer-dentin interface in (A) NaOCl (control) group (x1000), (B) a higher magnification of A (x8000), (C) EDTA group (x1000), (D) EDTA+EGCG group (x1000), (E) EDTA+EGCG group (x1000), (F) a higher magnification of E (x8000), (G) EGCG group (x1000) and (H) a higher magnification of G (x8000)

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CHAPTER V

DISCUSSION

Inability to establish fluid-tight seal root canal after chemomechanical preparation may lead to leakage, reinfection and treatment failure. From a clinical point of view, it is likely that leakage test is suitable to investigate sealing ability of root canal filling. However, the results were contradictory and varied with the methods (67). Therefore, the bond strength test has gained more attention. Although there has been no correlation between the bond strength and clinical success, it would appear that root canal filling with higher bond strength may produce better adaptation of root canal filling to dentinal wall and consequently resist it from dislocation during following procedures such as post space preparation (68, 69).

Root canal sealer is important to create an impervious seal during obturation procedure. There has been a continuous development of resin sealer in order to improve the sealing ability and potentially create monoblock. AH plus, a conventional epoxy resin root canal sealer, seem to have greater properties (70, 71) and higher bond strength than other sealers (49-52, 55). An epoxy resin sealer is still a commonly used sealer and may consider as gold standard for new root canal sealer testing. The covalent bond forming between epoxide ring and exposed collagen network in root dentin has first been theorized (14) with no scientific proof but has recently been confirmed using a fourier transform infrared spectroscopy analysis (53).

The coronal third slices of the specimen were excluded in this study because it was unable to obtain circular shape canal. In addition, coronal third slices of all groups presented only cohesive failure since the oval-shaped canal was irrelevant to circular-shape load applicator.

The chemical irrigants used during chemomechanical preparation cause the change in chemical and structural composition of dentin which consequently affect the seal of root canal sealer to dentinal wall. In this present study, the push out bond strength of AH plus was shown to be affected by the final irrigation protocols. The result of the bond strength to root canal dentin can be ordered as follows: NaOCl(control)<EDTA+NaOCl<EDTA<EGCG<EDTA+EGCG.

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EGCG, a group of polyphenol, is well known for its powerful antioxidative activity. In addition, EGCG is capable of intermolecular crosslinking between collagen. Seven hydroxyl groups of EGCG react with carboxyl group in collagen molecule, forming esteric bond (17). Moreover, Madhan et al (42) demonstrated the involvement of hydrogen bonding and hydrophobic interactions as the major forces involving in the stabilization of collagen. EGCG has been treated to collagen-based biomaterial in order to improve their mechanical strength and biodegradation (17). We suggested that collagen stabilization by EGCG resulted in the highest push out bond strength in EDTA and EGCG group. In addition, EGCG group showed greater bond strength over EDTA because EGCG also has chelating ability and able to stabilize the collagen. The exposed Collagen after using EDTA in fact undergoes collapse according to the formation of interpeptide hydrogen bonding (72). EGCG not only bonds to collagen causing collagen stabilization and preventing from access of collagenase to the active site, EGCG itself also potentially inhibits the collagenolytic activity by collagenase (17).

This study found no statistical difference between final flush with EDTA and EDTA followed by NaOCl in contrast to the findings of Neelakantan et al (15, 16). NaOCl caused collagen degradation leading to the decrease in the bond strength of an epoxy resin sealer as epoxy ring of an epoxy resin sealer formed covalent bond with exposed amino group in the dentin collagen network (14). However, in this present study, the SEM image revealed that the exposed porous collagen network remained intact even after final irrigation with 2.5% NaOCl for 1 minute following 17% EDTA. This may explain contradictory result of endodontic literature that whether the final flush with NaOCl effects the bond strength of an epoxy resin sealer (12, 15, 16, 54, 64, 65). The use of NaOCl final flush in higher concentration or longer time period may result in collagen degradation decreasing the bond strength of an epoxy resin to root dentin (15) providing the integrity of sealer-dentin interface from the biodegradation over time.

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Analysis of bond failure after the push out test revealed that the most common types of failure mode were mixed and cohesive failure in accordance with Prado et al (64). This indicates high bond strength of an epoxy resin sealer to root dentin. The adhesive failure was found only in control group because the dense smear layer along the whole canal inhibited the penetrating and bonding of an epoxy resin sealer (2).

SEM image of the intact porous collagen network in root canal dentin could be seen in all groups except for the control group which was covered with dense smear layer along the root canal dentin. The use of 2.5% NaOCl 1 following 17% EDTA did not result in collagen degradation. There was no difference in SEM image of collagen in root canal dentin between experimental groups. However, only the quantity not the quality of the collagen in root canal dentin could be seen in SEM image.

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Growing attention in resin-based sealer to obtain the ultimately sealed canal, the interest should be considered in the irrigants used before root canal obturation. The irrigants can cause change in chemical composition, dentin wettability and collagen degradation which potentially affect the sealing ability of the resin-typed sealer. The exposed collagen network should be preserved when using an epoxy resin sealer. The final flush with EGCG results in collagen stabilization which increases the strength of covalent bonding to epoxide ring of an epoxy resin sealer. Further study is needed to determine proper concentration and time used of EGCG and evaluate the biodegradation resistance of root dentin over time.

Conclusion

Within the limitations of this study, a final flush with 1mg/ml EGCG 5 min after 17% EDTA 5 min resulted in the highest push-out bond strength of AH plus sealer to root dentin. The result was confirmed by the SEM image of an intact resin-dentin interface. Further study is needed to determine the proper concentration and time for using EGCG and to evaluate the resistance to biodegradation of the root dentin over time.

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APPENDIX

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Table 3 Kolmogorov-Smirnov test for normal distribution

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Group	Statistic	df	Sig.	Statistic	df	Sig.
Strength	EDTA	.110	30	.200[*]	.942	30	.100
	EDTA+NaOCl	.125	30	.200[*]	.943	30	.110
	EDTA+EGCG	.153	30	.070	.936	30	.071
	EGCG	.116	30	.200[*]	.948	30	.149

a. Lilliefors Significance Correction

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

The result showed that the data of all groups was normal distribution ($p>0.05$). One-way analysis of variance was used to analyse the effect of final irrigation protocols on push-out bond strength

Table 4 Levene's test for homogeneity of variences

Test of Homogeneity of Variances

Strength

Levene Statistic	df1	df2	Sig.
1.895	3	116	.134

The groups variances were equal.

Table 5 ANOVA test

ANOVA

Strength

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	94.776	3	31.592	254.872	.000
Within Groups	14.378	116	.124		
Total	109.154	119			

Table 6 Tukey's test for multiple comparisons

Multiple Comparisons

Strength

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
EDTA	EDTA+NaOCl	.22767	.09090	.064	-.0093	.4646
	EDTA+EGCG	-2.05400*	.09090	.000	-2.2910	-1.8170
	EGCG	-.62767*	.09090	.000	-.8646	-.3907
EDTA+NaOCl	EDTA	-.22767	.09090	.064	-.4646	.0093
	EDTA+EGCG	-2.28167*	.09090	.000	-2.5186	-2.0447
	EGCG	-.85533*	.09090	.000	-1.0923	-.6184
EDTA+EGCG	EDTA	2.05400*	.09090	.000	1.8170	2.2910
	EDTA+NaOCl	2.28167*	.09090	.000	2.0447	2.5186
	EGCG	1.42633*	.09090	.000	1.1894	1.6633
EGCG	EDTA	.62767*	.09090	.000	.3907	.8646
	EDTA+NaOCl	.85533*	.09090	.000	.6184	1.0923
	EDTA+EGCG	-1.42633*	.09090	.000	-1.6633	-1.1894

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

VITA

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