การเปรียบเทียบปริมาณของน้ำยาล้างคลองรากฟันที่เกินออกนอกปลายรากเมื่อทำการล้างครั้งสุดท้ายด้วยระบบเอ็นโดแอคติเวเตอร์และการล้างด้วยวิธีปกติในฟันที่มีขนาดคลองรากฟันส่วนปลายที่แตกต่างกัน

นางสาว ณัตตยา รุ่งเจริญพร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาเอ็นโดดอนต์ ภาควิชาทันตกรรมหัตถการ
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

COMPARISON IN AMOUNT OF EXTRUDED IRRIGANT DURING FINAL FLUSH USING CONVENTIONAL METHOD AND ENDOACTIVATOR® IN ROOT CANAL SYSTEM WITH DIFFERENT APICAL PREPARATION SIZES

MISS NATTAYA RUNGCHAROENPORN

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Endodontology
Department of Operative Dentistry
Faculty of Dentistry Chulalongkorn University
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Thesis Title	Comparison in amount of extruded irrigant during final flush using conventional method and EndoActivator® in root canal system with different apical preparation sizes
By	Miss Nattaya Rungcharoenporn
Field of Study	Endodontology
Thesis Advisor	Somsinee Pimkhaokam, Ph.D.

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ณัตตยา รุ่งเจริญพร : การเปรียบเทียบปริมาณของน้ำยาล้างคลองรากฟันที่เกินออกนอกปลายรากเมื่อทำการล้างครั้งสุดท้ายด้วยระบบเอ็นโดแอคติเวเตอร์และการล้างด้วยวิธีปกติในฟันที่มีขนาดคลองรากฟันส่วนปลายที่แตกต่างกัน. (COMPARISON IN AMOUNT OF EXTRUDED IRRIGANT DURING FINAL FLUSH USING CONVENTIONAL METHOD AND ENDOACTIVATOR® IN ROOT CANAL SYSTEM WITH DIFFERENT APICAL PREPARATION SIZES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อ.ทญ.ดร.สมสินี พิมพ์ขาวขำ, 44 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบปริมาณของน้ำยาล้างคลองรากฟันที่เกินออกนอกปลายรากเมื่อทำการล้างคลองรากฟันครั้งสุดท้ายด้วยระบบเอ็นโดแอคติเวเตอร์และการล้างด้วยวิธีปกติในฟันที่มีขนาดคลองรากฟันส่วนปลายที่แตกต่างกัน โดยใช้ฟันหน้าแท้ปลายรากปิดที่ถูกถอนจำนวน 100 ซี่ นำมาแบ่งเป็น 3 กลุ่ม (30 ซี่/กลุ่ม) และขยายให้ได้ขนาดคลองรากฟันส่วนปลายที่แตกต่างกันตาม คือ ขนาด #35, #50 และ #80 ฟันแต่ละซี่หลังจากขยายคลองรากแล้วจะถูกนำมายึดในอะกาโรสเจลโมเดล และล้างทั้งวิธีปกติและเอ็นโดแอคติเวเตอร์ การเกินของน้ำยาล้างคลองรากฟันจะถูกเก็บใน 2 ส่วน คือ การเปลี่ยนสีของเจล และความแตกต่างของน้ำหนักของโมเดล จากนั้นวิเคราะห์ความแตกต่างของปริมาณของน้ำยาล้างคลองรากฟันที่เกินด้วยสถิติการทดสอบของครัสคัล-วอลลิสที่ระดับนัยสำคัญ 0.05 จากการศึกษาพบการเปลี่ยนสีของเจลในกลุ่มควบคุมบวก และโมเดลที่การเพิ่มขึ้นของน้ำหนัก โดยการเปลี่ยนสีที่พบเปลี่ยนจากไม่มีสีเป็นสีน้ำเงิน แต่ไม่พบการเปลี่ยนสีในโมเดล ในกลุ่มควบคุมลบและฟันที่มีขนาดคลองรากฟันส่วนปลายเบอร์ 35 อย่างไรก็ตามพบว่าปริมาณน้ำยาคลองรากฟันเกินออกนอกปลายรากเพิ่มขึ้นเมื่อมีการเพิ่มขนาดคลองรากฟันส่วนปลาย นอกจากนี้พบความแตกต่างอย่างมีนัยสำคัญของปริมาณน้ำยาล้างคลองรากฟันที่เกินออกนอกปลายรากระหว่างฟันที่มีขนาดคลองรากฟันส่วนปลายเบอร์ 50 และ80 ในการล้างด้วยวิธีปกติและเอ็นโดแอคติเวเตอร์ แต่ไม่พบความแตกต่างระหว่างฟันที่มีขนาดคลองรากฟันส่วนปลายเบอร์ 50 ในการล้างด้วยวิธีปกติและฟันที่มีขนาดคลองรากฟันส่วนปลายเบอร์ 80 ในการล้างด้วยวิธีเอ็นโดแอคติเวเตอร์ จากการศึกษานี้สามารถสรุปได้ว่าการเกินออกนอกปลายรากของน้ำยาล้างคลองรากฟันขึ้นกับวิธีในการล้างและขนาดของคลองรากฟันส่วนปลาย โดยเมื่อเพิ่มขนาดของคลองรากฟันส่วนปลายการเกินของน้ำยาล้างคลองรากฟันเพิ่มขึ้นและมีการเกินของน้ำยาล้างคลองรากฟันเพียงเล็กน้อยในการล้างด้วยวิธีเอ็นโดแอคติเวเตอร์ แม้ว่าปริมาณน้ำยาคลองรากฟันเกินออกนอกปลายรากเพิ่มขึ้น เมื่อมีการเพิ่มขนาดคลองรากฟันส่วนปลายในการล้างด้วยวิธีเอ็นโดแอคติเวเตอร์ แต่ยังพบว่าการเกินของน้ำยาน้อยกว่า เมื่อเทียบกับการล้างด้วยวิธีปกติ

ภาควิชา.....ทันตกรรมหัตถการ..... ลายมือชื่อนิสิต.....
สาขาวิชา.....วิทยาเอ็นโดดอนต์.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
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KEYWORDS : ENDOACTIVATOR® / EXTRUSION / APICAL PREPARATION SIZE / SODIUM HYPOCHLORITE

NATTAYA RUNGCHAROENPORN: COMPARISON IN AMOUNT OF EXTRUDED IRRIGANT DURING FINAL FLUSH USING CONVENTIONAL METHOD AND ENDOACTIVATOR® IN ROOT CANAL SYSTEM WITH DIFFERENT APICAL PREPARATION SIZES. ADVISOR: SOMSINEE PIMKHAOKHAM, Ph.D., 44 pp.

The purpose of this study was to investigate the amount of sodium hypochlorite extrusion from root canal when using the EndoActivator® with different apical preparation sizes compared to the conventional irrigation method. 100 extracted human maxillary anterior teeth with mature apices were instrumented and divided into 3 groups (30 teeth/group) based on the 3 different apical sizes: #35, #50, #80. Each tooth was fixed in agarose gel and irrigated with both needle (NG) and EndoActivator© (EA) techniques. The data of apical extrusion is collected in 2 parts, which are color changed detection and weight difference of model. Differences in apical extrusion volumes were analyzed by Kruskal-Wallis test ($p < 0.05$). In positive control group and models with increasing weights, the color change of agarose gel was observed from colorless to blue color. On the other hand, there was no color change in negative control group and in teeth with apical preparation of size #35 that corresponded to the result of no weight change. When root canal was prepared to apical size #35, weight change was not observed in both NG and EA groups. However, the extrusion volume was increasing when apical preparation size increased regardless of irrigation technique used. There were significant difference in extrusion volume among root canals with apical size #50 and 80 in NG and EA groups However, there was no significant difference between root canal with apical size #50 in NG group and apical size #80 in EA group ($p>0.05$). In conclusion, the apical extrusion depended on apical preparation size and irrigating systems. When increased in apical preparation size, amount of NaOCl extrusion was greater. There was few amount of apical extrusion when using EndoActivator®. Although, apical extrusion volume in EndoActivator® using was greater when increased in apical preparation size, the extrusion was less in EndoActivator® system when compared with needle and syringe irrigation.

Department :..... Operative Dentistry..... Student's Signature.....
Field of Study :.....Endodontology..... Advisor's Signature.....
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LIST OF ABBREVIATIONS

µl	microlitre
CFD	computational fluid dynamic
CHX	chlorhexidine digluconate
CPM	cycles per minute
EA	EndoActivator® irrigating technique
EDTA	ethylenediaminetetraacetic acid
Hz	Hertz
ISO	International organization for standardization
kPa	kilopascals
min	minute
ml	millilitre
mm	millimetre
NaOCl	sodium hypochlorite
NG	needle irrigation technique
pH	potential of hydrogen ion
SD	standard deviation
sec	second
WL	working length

CHAPTER I

INTRODUCTION

Background and rational

It has been established that successful endodontic treatment depends on the efficacy of root canal debridement by means of instrumentation, copious irrigation, and intracanal medication (1). The ideal irrigating solutions should possess many significant properties. The irrigant should demonstrate powerful washing action but should not weaken the tooth structure (2). The solution should not be cytotoxic and not irritate/damage all vital periapical tissue but should facilitate the dentin removal during root canal preparation by providing lubricating properties. The ideal solution should be able to dissolve both inorganic and organic tissue as well as to penetrate into the root canal periphery. Additionally, the ideal irrigating solution should have antimicrobial and antifungal activity.

Many irrigating solutions have been used in root canal treatment, for example, sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), chlorhexidine digluconate (CHX) and various combinations of these compounds. However, none of these available irrigating solutions can be regarded as the most optimum. Currently, the most widely accepted irrigating solution is NaOCl due to its antibacterial and lubricating properties, and its ability to dissolve pulp remnants (3, 4). However, NaOCl has unpleasant taste, is toxic and cannot completely remove the smear layer.

Generally, during root canal preparation and irrigation the excess irrigating solution will extrude beyond the apical foramen (5-8). From the survey of diplomats of the American Board of Endodontics, 42 percentages of diplomates of the American Board of Endodontic had experienced at least one NaOCl accident (9). This accidental extrusion of sodium hypochlorite through the root canal foramen into the periapical tissues can lead to the extensive soft tissue or nerve damage as well as the airway compromise (10). The NaOCl extrusion depends on many factors including needle insertion depth, needle tip size and design, delivery technique, flow rate of irrigating solution, size of apical foramen, and canal tapering.

In vitro studies showed significant amounts of debris began to extrude from apex when root canal was prepared to the size #50 while *in vivo* study extruded irrigants into periapical tissue was observed in necrotic pulp tissue case when instrumented to

file size #45 (11). Thus, it appears that increasing the apical preparation size results in a higher risk of apical extrusion. However studies using Computational Fluid Dynamic model showed an increase in root canal taper and apical preparation sizes improved irrigant replacement and wall shear stress, thus reducing the risk for irrigant extrusion (12, 13).

Many irrigating systems have been developed to improve root canal cleanliness. These systems can be divided in two broad categories, manual agitation techniques and machine-assisted agitation devices (14). Automated system that is designed for agitation in root canal include sonic, ultrasonic, and pressure alternation devices. Many *in vitro* and *in vivo* studies attempted to evaluate the debridement efficacy of automated system. Most of these devices appeared to increase root canal debridement efficacy (15).

The EndoActivator® system (Dentsply Maillefer, Ballaigues, Switzerland), a sonic irrigation device, consists of a portable hand piece and the disposable flexible polymer tips with 3 different sizes. The standard clinical protocol of using the EndoActivator® is to agitate an intracanal solution of full strength NaOCl for 60 seconds. This process should be repeated for every intracanal irrigation until the irrigating fluid in the pulp chamber is observed to be clean (16). Manufacturer claimed that the EndoActivator® can provide a safer, better, and faster method to disinfect root canal during irrigation. Two studies comparing the amount of irrigant extruded from the root canal when using an EndoActivator®or other delivery systems indicated that only small amounts of irrigating solution extruded from the canal when irrigated using an EndoActivator® (17, 18).

The purpose of this study was to assess the amount of sodium hypochlorite apically extruded from teeth instrumented to different apical preparation sizes during irrigation using an EndoActivator® compared to conventional irrigation technique.

Research question

Is the amount of sodium hypochlorite extrusion when using EndoActivator® in root canals with various apical preparation sizes different from that when using conventional irrigation technique?

Research objective

The objective of the research was to assess the amount of sodium hypochlorite apically extruded from teeth instrumented to different apical preparation sizes during irrigation using an EndoActivator® compared to conventional irrigation technique.

Hypothesis

- Hypothesis 1

- H_0 : There would be no difference in the amount of sodium hypochlorite extruded from the root canal using the EndoActivator® compared to conventional needle and syringe irrigation technique.
- H_A : The amount of sodium hypochlorite extrusion from the root canal using the EndoActivator® different from conventional needle and syringe irrigation technique.

- Hypothesis 2

- H_0 : There would be no difference in the amount of sodium hypochlorite extruded from the root canal with various apical preparation sizes.
- H_A : The amount of sodium hypochlorite extrusion from teeth root canal is directly proportional to the apical preparation sizes.

- Hypothesis 3

- H_0 : There would be no difference in the amount of sodium hypochlorite extruded from the root canal using the EndoActivator® at various apical preparation sizes compared to conventional needle and syringe irrigation technique
- H_A: The amount of sodium hypochlorite extrusion from the root canal using the EndoActivator® at various apical preparation sizes different from conventional needle and syringe irrigation technique.

Field of Research

The research was to assess the amount of sodium hypochlorite apically extruded from root canals of anterior extracted teeth instrumented to different apical preparation sizes during irrigation using an EndoActivator® compared to conventional needle and syringe irrigation technique.

Keywords

EndoActivator®, Extrusion, Apical preparation size, Sodium hypochlorite

Research design

In vitro laboratory experimental study

Limitations of research

1. This study was an *in vitro* study that may not be directly applied to virtual clinical practice to the effect of patient's periapical tissue.
2. The measurement used in this study was weighing the summation mass of agarose gel and various amount of extruded sodium hypochlorite. The weighing was not sensitive to detect when the extruded volume was less than 10 μl. However, the measurement by color changing of pH indicator was sensitive to extruded volume more than 1 μl. The present study used both measurement methods.

Obstacle and strategies to solve the problems

1. There are natural variations of the sample root canals, both in size and shape. However, the strategic problem solving will be done by controlling the canal preparation using the same instrumentation system and by randomly sampling.
2. The color change of thymolphthalein blue, a pH indicator, is not stable. The extruded sodium hypochlorite will change thymolphthalein blue from colorless to blue. The blue color will be stable for only 3 minutes. This problem will be solved by instantly measuring the

changing color of every test after irrigating sodium hypochlorite into the root canal, suction the overflow irrigant with high-power suction and canal drying with paper point.

3. The pH transition range of thymolphthalein blue can be altered by high temperature. So adding of thymolphthalein blue to the teeth holding agarose gel has to be done at gel temperature below 40 °C.

Expected benefit & Application

The results of this study on the amount of sodium hypochlorite extrusion from teeth root canal when using different irrigation methods at various apical preparation sizes will be beneficial in clinical application. The extrusion toxicity of sodium hypochlorite that was normally used to clean teeth root canal was evaluated. Also the effect of various degrees of apical preparation sizes was also analyzed. Therefore, these findings will be applied to both clinical dental work and future dental research.

Ethical consideration

The protocols used in our study were approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University.

CHAPTER II
LITERATURE REVIEW

The goal of endodontic treatment is to eliminate the microorganisms from the root canal system and to prevent the patient from microbial infection. The chemomechanical cleansing by instrumentation and copious irrigation are the most widely recommended to disinfect the entire root canal system. Many cleansing irrigating solutions have been potentially used. The major aims of using the solution are washing root canal, reducing instrument friction during irrigation, facilitating dentin removal, dissolving inorganic tissue and organic matter, penetrating into the canal periphery and killing bacteria and yeasts. The irrigating solutions should not irritate or damage the vital periapical tissue, not be cytotoxic and not weaken tooth structure (2). At present, none of the available irrigating solutions can meet all of these criteria. However, sodium hypochlorite is the most promising irrigating solution.

Sodium hypochlorite (NaOCl)

Sodium hypochlorite (NaOCl) is the most popular irrigating solution, which was recommended to use in endodontics as the main irrigating solution. NaOCl is commonly used in concentration between 0.5% and 6%. It is a potent antimicrobial agent, effectively dissolves necrotic and vital organic tissue, cheap, easily available, and good shelf life. It has approximately pH 11-12. The high pH of NaOCl interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alteration in cellular metabolism and phospholipid degradation observed in lipid peroxidation (19).

Because of the antimicrobial and tissue-dissolving properties, NaOCl is highly toxic therefore inducing tissue irritation. The previous study of NaOCl toxicity in biological models showed that it caused hemolysis and loss of membrane cellular proteins. The high concentration of NaOCl led to moderate to severe irritation of rabbit eyes (20).

It was reported that NaOCl was much more toxic to gingival fibroblasts than other endodontics agents, for example iodine, potassium iodide and calcium hydroxide (21). The disadvantages of NaOCl are not only its cytotoxicity but also its unpleasant

taste and its ability to completely remove smear layer. Nevertheless, NaOCl should be used with appropriate management to minimize risk and potential complication.

Factors involved in apical extrusion

Two important goals must be balanced and maintained during irrigation. They are safety and effectiveness. Sufficient volumes of NaOCl and other irrigating solutions in apical part of root canal while keeping minimal apical pressure are the important goals of irrigation (2). Many model *in vitro* studies and *in vivo* studies were designed to detect factors that involved in apical extrusion. These factors are:

1. **Needle depth**. Computational fluid dynamics (CFD) model was currently developed based on SST k-ω turbulence model in order to simulate various conditions and perform parametric investigations in root canal environment (22). They validated this model by using camcorder in *in vitro* model. They also found that this CFD model had the potential to serve as a platform for the study of root canal irrigation.

   Shen et al. used CFD to determine influence of needle design to irrigation flow patterns. They found that apical pressure was highest in the beveled needle and lowest in the side-vented closed-end needle (23). They also found that reducing distance of needle from apex in root canal led to high apical pressure.

   This study corresponded to another study which found that positioning of the needle closer to the working length improved the irrigating solution replacement in the apical part of the root canal (indicated by a high axial z-component of velocity) but this led to increase mean pressure at the apical foramen (24). This indicated an increased risk of irrigating solution extrusion toward the periapical tissue. They also found that the side-vented needle achieved irrigation replacement to the working length only at the 1-mm position, whereas the open-ended flat needle was able to achieve complete replacement even when positioned at 2 mm short of the working length.

2. **Needle size**. Although the smaller needles allowed delivery of the irrigating solution close to the apex, this also led to increase apical extrusion (2). Root canals are typically prepared to sizes 0.5-2 mm in diameter at the coronal third

of root canal and taper down to less than 0.3 mm in mature roots. Therefore, a 27- gauge needle having 0.21 mm in diameter will penetrate to the apical third of the prepared canal (15).

The effects of needle sizes on irrigating solution flow rate, intra-barrel pressure, irrigation duration and volume of irrigating solution were evaluated by Boutsioukis C et al (25). Duration of delivery and flow rates significantly decreased as the needle diameter increased, whilst pressure increased up to 400–550 kPa. They also concluded that smaller diameter needles required increased effort to deliver the irrigating solution and resulted in higher intra-barrel pressure. This phenomenon could lead to increase risk of apical extrusion.

3. **Needle tip designs**. There are many available commercial irrigation needle tips. They are categorized as flat open-ended, beveled open-ended, side-cut open-ended, close-ended and single side-opening, and close-ended and multiple side-openings (15).

Shen et al. investigated effect of needle tip designs on the irrigation flow inside a prepared root canal using a CFD model. They found that apical pressure was highest when using the beveled needle and lowest when using the side-vented close-ended needle (26). Boutsioukis C et al. also used CFD model to evaluate effect of needle tip designs on irrigation flow. They found that the flow pattern of the open-ended needles was different from the close-ended needles, resulting in more irrigating solution replacement in front of the open-ended needles but also higher apical pressure (27).

4. **Flow rate**. CFD model was used to investigate fluid flow rate in root canal. Boutsioukis C et al. found that flow rates in needle type irrigation were 0.02-0.79 ml/sec. However, irrigating solution replacement occurred only when flow rates in the range of 0.53– 0.79 ml/sec were used (28). Nevertheless, higher initial velocities will increase possibilities of irrigating solution or air extrusion toward the periapical tissue (29).

5. **Delivery systems**. There were two studies that evaluated the safety of various intracanal irrigation systems by measuring the apical extrusion of irrigating solution (17, 18). In the first study, the irrigation systems used in the study were

EndoVac® Micro and Macro Cannulae, EndoActivator ®, manual irrigation with Max-I-Probe® needle, Ultrasonic Needle Irrigation, and Rinsendo®.

This study showed that the EndoVac® did not extrude irrigating solution after deep intracanal delivery from the chamber and simultaneous evacuation of irrigating solution to full working length. EndoActivator ® had a minimal, although statistically insignificant, amount of irrigating solution extruded out of the apical foramen. Manual, Ultrasonic, and Rinsendo® groups had significantly greater amount of extrusion compared with EndoVac® and EndoActivator ®(17).

In second study, the irrigation systems used to evaluate were EndoActivator ®, EndoVac®, RispiSonic/MicroMega 1500, passive ultrasonic irrigation, and syringe irrigation with a slot-tipped needle. The result of this study corresponded with the previous study that only few amount of extrusion was observed in EndoActivator ® group. The extent of extrusion in passive ultrasonic irrigation and syringe irrigation with a slot-tipped needle was greater than RispiSonic/MicroMega 1500, EndoActivator ®, and EndoVac®(18).

6. **Apical preparation size**. From the study of Vande Visse and Brilliant in 1975, they found that when root canalwas prepared to the size of file #50 significant amounts of debris began to extrude from the apex (11). *In vivo* study, irrigating agents could penetrate in the apical half of the root canal when instrumented to no. 35 file and extruded into periapical tissues when instrumented to no. 45 file in necrotic pulp tissue cases (30). However, apical extrusion was not detected in vital pulp tissue cases. They suggested that vital pulp and periapical tissues might provide a natural barrier to prevent extrusion.

The effect of apical preparation sizes on apical extrusion is still inconclusive. Two studies determined the apical extrusion of extracted tooth with different apical preparation sizes by using weighting of the extruded debris and irrigating solution collection method. They found that there was no statistically significant relationship between foramen sizes and amount of extruded irrigating solutions (6, 31). However, Boutsioukis C et al. used CFD model to evaluate effect of apical preparation size on irrigation flow. They found that larger apical preparation sizes produced less apical pressure (12). They also suggested that the relatively low apical pressure for large preparation sizes could be translated

to an important decrease in the risk for apical extrusion and could be considered an indication to prepare root canals to sizes larger than 25.

The study that evaluated extent of extrusion in different apical preparation sizes and irrigation systems (18). It found that the frequency of apical extrusion was dependent on the apical preparation sizes and type of irrigating systems. The extent of extrusion increased from 36% in apical preparation size 35/.06 to 60% in apical preparation size 50/.06. There was not significant different in apical preparation size 50/.06 with various irrigating systems. However, the significant difference was found in different irrigating system with apical preparation sixe 35/.06.

7. **Canal tapering.** Boutsioukis C et al. used CFD model to evaluate the effect of root canal tapering on irrigating solution flow inside the prepared root canal. They found that apical pressure gradually decreased when increased the canal tapering (13). However, this phenomenon could also find when increased apical preparation size. Therefore, they concluded that in order to decrease apical pressure and reduce risk of apical extrusion, minimally tapered root canal with a large apical preparation size or tapered root canals with a smaller apical preparation size should be prepared in instrumentation procedures.

EndoActivator®

The principle of EndoActivator® system (Dentsply Maillefer, Ballaigues, Switzerland) is using the sonic energy to irrigate root canal systems. The instrument comprises of 2 components, a portable handpiece and a series of activator tips. It is recommended to use following the root canal preparation by agitating NaOCl for 60 seconds. The usage of this device should be repeated for every changing of the new intracanal solution or repeated until the fluid in pulp chamber is observed to be cleaned. When vibrating the tip, in combination with moving the tip up and down for 2-3 mm, this sonic device will produce the powerful hydrodynamic force which can increase the washing potency of the irrigating solution (16).

According to the manufacturer, EndoActivator® system has three levels of frequencies. The level 1 is 33 Hz with 2000 cycles per minute (CPM), level 2 is 100 Hz with 6000 CPM, and level 3 is 166 Hz with 10,000 CPM. However, when using the high-speed camera to determine the removal of dentin debris from the root canal by the

EndoActivator® it was reported that the frequency level was not corresponded to the manufacturer's levels (32). They found level 1 was 160±5 Hz, level 2 was 175±5 Hz, and level 3 was 190±5 Hz. This reported showed that frequencies in all mode greater than manufacturer sale brochure. The sonic tip showed only one node (at attachment point) and one anti-node (at the free end) during oscillation. Nevertheless, this study showed a lot of wall contact of the sonic tip during activation, and no cavitation was observed

The activation tips of the EndoActivator® system are made of smooth polymer based material that is claimed to be strong and flexible, does not break easily and does not directly cut the dentin. From A. Al-Jadaa et al. 2009, they compare the effects of different ultrasonic tips and the EndoActivator® activation tip on transportation of the simulated main canal. They found that ultrasonically activated nickel-titanium tips and the EndoActivator® activation tip did not cause canal transportation (33).

The EndoActivator® tips have three sizes. Their color-coded activator tips correspond to file nomenclature sizes 20/.02, 25/.04, and 35/.04. The tips are 22 mm long with depth gauge rings positioned at 18, 19, and 20 mm. Tip selection is based on the size of fully prepared canal. The tip should fits loosely and to within 2 mm of working length in order to promote irrigating dynamic. A possible disadvantage of the activator tips used in the EndoActivator® system is that they are radiolucent. Although these tips are designed to be disposable and do not break easily during use, it would be difficult to identify them if part of a tip separates inside a root canal (14).

There are many studies that attempted to evaluate the effectiveness of the EndoActivator® system in irrigation. The EndoActivator® system showed that it increased debris removal in straight and less curve root canal systems (32, 34). However, this device has been shown that it did not enhance debris removal in curved root canal (35).

The ability of the EndoActivator® system in smear layer removal was also assessed. It was found that the system could promote smear layer removal in curve root canal (35, 36) but could not promote smear layer removal in straight root canal compared to the conventional Max-I-Probe® irrigation with NaOCl and EDTA (37).

The effectiveness of the EndoActivator® system in killing the bacteria and removing the biofilm are still inconclusive. Two studies found that this device promoted bacterial removal in the root canal system when compared with conventional needle irrigation (38, 39). However, there were another three studies reported that there was no

significant difference in bacteria and biofilm removal between the EndoActivator® system and the conventional needle irrigation (23, 40, 41).

The effect of currently used irrigation and activation systems on the penetration of sodium hypochlorite into simulated lateral canals and on the working length in a closed system was evaluated (42). It was shown that the penetrating efficacy of EndoActivator® system into the lateral canals and working length was less than that of the ultrasonic activation. But this study did not compare the efficacy with the conventional needle irrigation. Paragliola R et al. examined the penetrating efficacy of an agitated endodontic irrigating solution into the dentinal tubules of different root canal and found that EndoActivator® was less effective than the ultrasonic irrigation but more effective than the conventional irrigation with manual activation (43).

Safety of the EndoActivator® system was also analyzed using various intracanal irrigation systems by measuring the apical extrusion of irrigating solution (17, 18). They reported that the EndoActivator® system had a minimal amount of extruded irrigating solution out of the apex when compared with other delivery systems. However, the performance steps should be sequentially ordered, delivering irrigating solution into the pulp chamber, placing the tip into the canal, and initiating the sonic energy.

The extent of apical extrusion in EndoActivator® was greater when increased apical preparation size from 35/.06 to 50/.06. However, There were only few amount of apical extrusion when used EndoActivator®(18).

Apical extrusion assessment methods

The extrusion of irrigating solution beyond apical foramen can generally occur during root canal preparation and irrigation (5-8). Generally, measurement of NaOCl extrusion calculates from total volumes of NaOCl extrusion but the exact amount of NaOCl is difficult to measure. Nevertheless, there are many methods developed to test apical extrusion. They are:

1. **Weighting of the extruded debris and irrigating solution collection**. This method was first reported by Martin H and Cunningham WT in 1982 (44) and was modified by many later studies. First, tooth was rigidly fixed in pre-weighted glass/plastic vial and then this model was irrigated by irrigating

protocol. Finally, the debris and irrigating solution that extruded from the apical foramen were collected and weighted again (8).

This method was later modified by equalizing the air pressure in and outside the bottle using needle inserted into through the rubber stopper (17). Pressure suction to evacuate the coronal excess irrigating solution was performed. This modified method was used to evaluate the safety of various intracanal irrigation systems by measuring the apical extrusion of irrigating solution. It was used by many later studies because of its simplicity. However, it cannot simulate the vital pulp and periapical tissue, which may restrict apical solution extrusion.

2. **Spectrometric analysis of the extruded debris**. Brown et al. developed this method in 1995 to assess the volumes of NaOCl that extruded apically during root canal preparation in different irrigating techniques (5). They positioned the tooth in the opening of a small glass specimen bottle containing one millilitre of distilled water. The bottle was supported in place by vinyl polysiloxane impression material. A 27- gauge needle was placed through the impression material in order to equalize the pressure between inside and outside. The bottle collected the apically extruded debris and irrigating solution during irrigation. The volumes of extruded solution were measured by calculating the concentration of sodium present in the extruded debris. This measurement was done with atomic emission spectrophotometer.

   However, some sodium could also be detected when distilled water was used as control. This may be the cellular sodium from fixed pulp tissue and/or tooth structure. Therefore, the disadvantage of this method is misinterpretation due to the cellular sodium. The extruded volumes of this method are always greater than expected. This method also cannot simulate the vital pulps or periapical tissues.

3. **Dye extrusion measurement**. This method measures fluid extrusion that is caused by laser pulses using a matrix design. The tooth is horizontally mounted on a glass slide with the height of the apical foramen at 2 to 3 mm above plain paper sheet 10 cm long and 5 cm wide. The paper functions as a recorder of the extruded dye that can be photographed by a digital camera mounted at a fixed distance under constant lighting conditions.

Finally, the amount of extruded dye (relative to image pixels) is determined with the aid of a 10-mm grid (45).

This method is easy and simple to perform. This method can be modified to create an environment external to the tooth apex using air pressures to simulate the resistance of an intact periodontium and its associated tissue fluids. However, anterograde movement of the dye cannot be avoided, rendering the inaccuracy of measurement the dye extrusion.

4. **Discoloration area of gel**. This method was firstly reported by Y. Fukumoto et al in 2006. This technique was used to evaluate the effectiveness of a new root canal irrigating technique, intracanal aspiration in removing the smear layer, and to assess the extrusion of irrigating solution *ex vivo*. The root was secured in a plastic case containing normal saline agar colored with 1% acid red to evaluate the extrusion of NaOCl. When NaOCl extruded out of the apical foramen into the gel, the agar changed color from pink to colorless. After irrigation, the color alteration of agar was recorded by an image scanner. The magnitude of the discolored area was calculated using Photoshop© software (46).

   This technique was used to compare apical extrusion of NaOCl delivered with either a 27-gauge slot tipped endodontic irrigation needle or the EndoVac® (47). The tooth was fixed and secured to a modified flat-sided clear plastic container embedded with a 0.2% agarose gel containing 1 mL 0.1% m-Cresol purple. M-Cresol purple has a pH sensitive color change from yellow at a pH of 7.4 to purple at a pH of 9. A color change to purple indicated the extrusion of NaOCl (pH=11.4) into the gel. Digital photograph was taken on light box after 20 minutes of initial irrigation. Adobe Photoshop 7© was used to determine the area of the color change expressed in pixels.

   Fixing tooth into the gel created a closed system that simulates the *in vivo* condition. This method is very sensitive because it can detect even small amount of NaOCl extrusion at the apical foramen. However, the areas of discoloration cannot exactly represent the amount of extrusion volume because the area depends on many factors such as gel pore sizes, irrigating solution penetration, and amount of solution penetration. Nevertheless, this method cannot be used with unstable pH indicator because discoloration will rapidly fade to its original color.

CHAPTER III
MATERIALS AND METHODS

Materials

1. Chemicals
   - Sodium hypochlorite (NaOCl) was obtained from Chlorinated soda solution 2.5% (Faculty of Dentistry Chulalongkorn University)
   - Normal saline
   - 17% Ethylenediaminetetraacetic acid (EDTA)
   - Agarose gel
   - TBE bufffer
   - Thymolphthalein blue (Merck, KGaA, Darmstadt, Germany)
   - 50% ethanol
   - 10% formalin solution

2. Instruments, glassware and plastic ware
   - EndoActivator® (Dentsply Tulsa Dental Socialties, Tulsa, OK
   - Pipette tips (Bioline, London, UK) Pipette tip 20, 200, 1000 µl
   - Pipette 10, 25 µl
   - Thermometer
   - Polystyrene tissue culture dishes with 6 cm in diameter (Corning Costar Corporation, Cambridge, USA)
   - X-ray film size 2
   - Round and safe-tip diamond burs
   - Gates Glidden drills size 2-4
   - ProTaper rotary instruments (Dentsply Tulsa Dental Socialties, Tulsa, OK)
   - K-files ISO size 15-80 (Dentsply Tulsa Dental Socialties, Tulsa, OK)
   - Gutta percha main cone ISO size 35, 50, and 80
   - Periphery wax
   - Nail varnish
   - Plastic syringes 5 ml
   - Monoject 27-gauge needles (Nipro Corp., Osaka, Japan).
   - Precisa XT2200C weighing machine (Precisa Instruments AG, Dietikon, Switzerland)

Methods

1. Teeth preparation

One hundred single canal extracted human maxillary anterior teeth with mature apices were collected from patients whose permission was received with the signed consent forms. Every collected teeth should have the straight root canal to reduce effect of canal curvature. After extraction, the teeth were cleaned and stored in 10% formalin solution until used. Radiographic images of the buccal/lingual and mesial/distal aspects of each tooth were taken to explore the root canal curvature. The degree of curvature was assessed according to the method of Schneider (48). Exclusion criteria were teeth with root canal curvatures more than 20 degrees, teeth with calcified root canals, teeth with root canals allowing introduction of an instrument exceeding ISO size 25 to the apical foramen, teeth with root caries, and teeth with root resorption.

Each tooth was accessed with high speed diamond round bur #2 and safe-tip diamond bur with water spray. A size 15 K-file was placed in each canal until it was visible at the apical foramen. The working length (WL) was established at 1mm short of this length. Each coronal aspect of the root canal was flared by using Gate Glidden drills size 2-4. The remaining canal was shaped by using ProTaper rotary instruments and the apical instrument at WL was completed with F3 file (size 30/.09). Between instrumentation each root canal was irrigated with 5 ml of 2.5% NaOCl solution using a 5 ml syringe and a monoject 27-gauge needle. Apical patency was done with a size 10 K-file between each instrument.

All teeth were randomly divided into 3 groups (30 teeth/group) based on the 3 different apical sizes: #35, #50 and #80. The root canals of teeth of these 3 groups were instrumented to the corresponding sizes using K-file. During apical preparation, the root canals were irrigated with 5 ml of 2.5% NaOCl solution using a 5 ml syringe and a monoject 27-gauge needle. After apical preparation, each tooth was dried with paper point and air-dried at room temperature for one hour. The root surface of each tooth was double coated with nail varnish except their apical foramens.

2. Preparation of teeth holding models

The models used in this study were modified from those of a previous study (47). Each tooth was inserted into a hole cut through the lid of a polystyrene tissue culture dish (Costar Corp., Cambridge, MA, USA) and was rigidly secured with border wax when the root apex was 0.5 mm from the bottom of the culture dish.

Five ml of 0.2% agarose gel containing 0.1 ml of 0.2% thymolphthalein blue (Merck Darmstadt, Germany) was then poured into the culture dish, and the lid with the tooth affixed was placed on top (Fig 1). In order to prevent gel leaking into the root canal, a master apical file was inserted to the working length during model preparation.

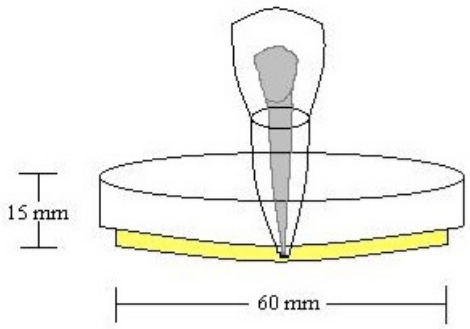

Figure 1 Picture of teeth holding model

3. Irrigating solution extrusion testing

Each gel holding tooth in a culture dish was weighed using Precisa XT2200C weighing machine (Precisa Instruments AG, Dietikon, Switzerland). The weighing machine was standardized every time before use. Each model was rinsed with 2 techniques. The irrigating techniques were needle with 5 ml syringe (NG) and an EndoActivator® with 35/04 tip (EA). After each technique, each root canal was dried with paper point and weighed with the same weighing machine. Then each tooth was removed from gel, cleaned, dried, and re-fixed in a new gel model. The weight of new model was measured before the next irrigating technique.

In NG technique, the root canal of each tooth was constantly rinsed with 3 ml of 2.5% NaOCl solution using a 5 ml syringe and monoject 27-gauge needle in up and down motion. A monoject 27-gauge needle attached to a 5 ml syringe was placed loosely into the root canal 2 mm short of the working length. The rubber stop was placed on irrigation needle at 2 mm short of working length between irrigation. The rate of irrigation was approximately 0.5 ml/min. The overflow of irrigant was suctioned with a high-speed evacuator.

In the EA technique, the root canal was flushed with 2.5% NaOCl using a monoject 27-gauge needle and 5 ml syringe. The needle was placed loosely into the root canal 2 mm short of working length. The root canal was flushed with NaOCl until the irrigant filled the root canal up to the access cavity and overflow was observed. The EndoActivator® with a 35/.04 size tip was then placed in the root canal 2 mm short of the working length and was used with up and down motion for 1 minute during vibration on mode 3 setting (166 Hz).

Ten teeth were divided to positive and negative control groups (n=5). The root canals of both groups were instrumented at the working length to a master apical file size #80. The root surface of the positive control group was double coated with nail varnish except at the apical foramen. The master apical file was placed to the working length while the tooth was fixed in a gel model. After the gel set, 3 ml of NaOCl was delivered into the root canal with a monoject 27-gauge needle and a 5 ml syringe at the working length for 1 minute.

For the negative control group we coated the entire tooth with nail varnish, including the apical foramen. This group was also prepare to apical preparation size #80 and fixed in the gel model. They was irrigated with 3 ml of NaOCl at the working length for 1 minute by using a monoject 27-gauge needle and a 5 ml syringe.

4. **Apical extrusion measurement**

The data of apical extrusion was collected in 2 parts, which were color changed detection and weights of model. The weighing was not able to detect when the extruded volume was less than 10 μl. However, color change of pH indicator was visible when extruded volume was more than 1 μl. Firstly, when each irrigating technique was finished, color change was observed right after the root canal was dried with paper point. Extruded irrigant was visually

detected when the pH indicator changed from colorless to blue. The data was recorded as color change or no color change.

Secondly, after each irrigation techniques, the models were weighed again with the same weighing machine. The weights of the models before and after each irrigation technique were compared. The weight difference was divided by the density of NaOCl to generate the volume of extruded irrigant. The density of 2.5% NaOCl is 1.06 g/ml (49).

5. Statistical analysis

All experiments were measured in three times. Data were expressed as the means of each sample $\pm$ standard deviation (SD).

For statistical analysis, the Kruskal-Wallis test was performed by using the SPSS software version 17 to test the group differences in relative irrigating techniques and apical preparation sizes followed by Mann-Whiney rank sum test for pairwise comparisons at the 95% confidence interval.

6. Budgets

• Materials	50,000	BHT
• EndoActivator®	30,000	BHT
• Others	5,000	BHT
Total	85,000	BHT

CHAPTER IV
Results

Color changed detection of agarose gel

There was no color change observed in the negative control group or in teeth apically prepared to size #35 after irrigating with NG and EA irrigating techniques, corresponding to the result of no weight change. In contrast, in the positive control group and models with increased weight subsequent to irrigation, the color of agarose gel changed from colorless to blue.

Extrusion volume of NaOCl in agarose gel model

The extrusion volume of 2.5% NaOCl was shown in Table 1. We found that when the root canals were prepared to apical size #35, no weight change was observed in both NG and EA irrigating techniques. Therefore, the calculated extrusion volume of NaOCl was zero.

In both EA and NG groups, extrusion volumes of teeth with apical size #80 was significantly greater than that of teeth with apical size #50.

We did find, however, that apical extrusion occurred when apical preparation size was increased regardless of the irrigation technique used (Table 1). In teeth prepared to a size #50, the NG group had a mean extrusion of 0.1903 ml, which was significantly higher than that for the EA group at 0.0107 ml, an approximately 20-fold difference. Preparing teeth to a size #80 resulted in a mean extrusion of 0.8381ml in NG group; this was significantly different than that of the EA group, where a mean of 0.0877 ml was found, an approximately 10 fold difference. The mean extrusion in either group was significantly higher in teeth size #80 compared to the corresponding group in the teeth size #50. However, there was no significant difference in extrusion volume between teeth prepared to apical size #50 in the NG group or to apical size #80 in the EA group ($p>0.05$).

Table 1 Extrusion volume (ml, means ± standard deviation) in 2.5% NaOCl during final flush using conventional method and EndoActivator® in root canal system with different apical preparation sizes (*n*=90)

Apical preparation size (#)	Irrigating technique	Extrusion volume (ml)
35	NG	0.00 ± 0.00^{A}
	EA	0.00 ± 0.00^{A}
50	NG	0.1903 ± 0.8693^{B}
	EA	0.0107 ± 0.01502^{C}
80	NG	0.8381 ± 0.17378^{D}
	EA	0.0877 ± 0.06786^{B}

Mean values that share the same superscript letter were not significantly different at p=0.05 (Kruskal-Wallis test and the Mann-Whiney rank sum test for pairwise comparisons)

CHAPTER V
Discussion

It is accepted that elimination of bacteria, removal of tissue remnants and debridement in root canal treatment cannot be accomplished solely by mechanical instrumentation (50). Irrigation is always performed as complement to mechanical preparation (51). The outcome of irrigation depends on the ability of the irrigant to penetrate through the root canal system which is influenced by the innate anatomy of the root canal (4). The use of appropriate irrigants with antimicrobial properties is essential for the bacterial elimination. The irrigant flushing also provides lubrication of the canal dentinal walls, dissolution, and removal of contaminated debris.

Sodium hypochlorite (NaOCl), at concentrations ranging from 0.5 to 5.25%, is routinely used to clean and disinfect the root canal system (2). Although it is generally believed safety, the cytotoxicity of NaOCl cannot be ignored if it is accidentally extruded into the periradicular space. It can dissolve both vital and necrotic pulp remnants indistinguishably leading to tissue ulcerative and necrosis (10).

In the present study, the amount of apically extruded NaOCl was assessed by the weight change in tooth models pre and post irrigation, and the color change of the pH indicator in the model gel. The volume of the extruded irrigant was calculated by dividing the density value of NaOCl (1.06 g/ml) by the weight change. Using teeth embedded in agarose gel created a closed system that simulated the in vivo anatomy.

The models that were used in this study combine two methods to detect extrusion. The advantages of gel discoloration detection and weighing of the extruded irrigating solution combination methods were earlier detection of NaOCl extrusion and represented the amount of extrusion volume under periapical tissue simulated condition. The collection and weighing of the extruded debris and irrigating solution have been used in many previous studies (6, 17, 44). This method was used by many later studies because of its simplicity. However, teeth in these studies were fixed in empty bottles, therefore it could not simulate the vital pulp and periapical tissue anatomy, which may restrict apical solution extrusion.

Agarose gel was used in the model of this study because it could create a closed system and could simulate the periapical tissue while the pH indicator could detect the small amount of extruded irrigant. Thymolpthalein blue mixed in agarose gel

is a pH indicator with a transition range at approximately pH 9-10.5. When the pH is below this range, it is colorless, but when the pH is higher, it becomes blue. NaOCl has a pH value of 11 (2). Therefore when it is extruded through the apical foramen, the pH indicator will change from colorless to blue.

In the previous study, areas of the gel discoloration were calculated and correlated to the amount of extrusion volume (18, 46, 47). This correlation was doubtful because penetration of NaOCl into the gel depended also on various factors such as gel pore sizes and gel concentration. Besides, the penetration pattern was, most of the time, asymmetrical in shape. Consequently, area of gel discoloration could not represent the amount of extrusion volume. The measurement used in this study were both weighing the summation mass of the model with various amount of extruded sodium hypochlorite and discoloration of the gel. The weighing method could represent the amount of extruded NaOCl volume. The observed color change in the gel model confirmed that the measured weight change of the tooth model was the result of NaOCl extrusion from the apical foramen.

The result of this study revealed that both irrigating techniques and apical preparation size played a role in apical extrusion of 2.5% NaOCl. There were no weight changes in both NG group and EA group with apical size #35. The highest extrusion volume of NaOCl was in NG group with apical size #80. In teeth with apical size of #50 and #80, the extrusion volume increased with the larger apical sizes. and was less in EA groups when compared with NG groups. All the models with unchanged weights also showed no change in the color of the agarose gel. Consequently, the amount of sodium hypochlorite extrusion from the root canal using the EndoActivator® at various apical preparation sizes was different from conventional needle and syringe irrigation technique.

In negative control group, we could not detect color changed in agarose gel and the weights of the models did not increased. In positive control group, we could detect color changed and the weights of the model also increased. However, none of the model was detected color changed and the weights of the model did not increased. This finding may imply that the extrusion volumes of NaOCl in the model that increased weights were more than 10 μl. The previous study showed that 10 μl of NaOCl with concentration more than 0.1% were found to be cytotoxic to human PDL cell. Increased NaOCl concentration not only caused cell cytotoxicity but also reduced mitochondrial activity and protein synthesis (52). In clinical situation, we used at concentrations

ranging from 0.5 to 5.25%, this higher concentration caused more periapical tissue damage.

From the study of Vande Visse and Brilliant, they found that when root canal was prepared to the size of file #50 significant amounts of debris began to extrude from the apex (11). This finding corresponded to our study that NaOCl was initially observed to extrude through the apical foramen when prepared to size of fle #50.

However, in Mitchell et al. study found that extrusion of NaOCl occurred in teeth with apical size 35, 6% tapering when using syringe with 27- gauge slot-tipped needle and EndoActivator® irrigating systems (18). This could be explained by different in tapering of root canal preparation. Boutsioukis C et al. used computational fluid dynamic (CFD) model to evaluate the effect of root canal tapering on irrigating solution flow inside the prepared root canal. They found that apical pressure gradually decreased when increased the canal tapering (13). In this study, root canals were instrumented by using Protaper® rotary files with 9%. tapering. Because of greater tapering, this might explain no apical extrusion observed in teeth with apical size #35 when compared with Mitchell et al. study.

From the result of this study, the apical extrusion increased when the apical preparation size was greater. This finding also corresponded with previous study (18). They found that increasing apical preparation size resulted in greater extent of extrusion. Although, large apical preparation could eradicate more debris and bacteria in root canal, the greater apical preparation also led to more apical extrusion. Therefore, in order to reduce risk of apical extrusion, apical preparation should be done as minimum as possible.

The principle of EndoActivator® system (Dentsply Maillefer, Ballaigues, Switzerland), is using the sonic energy to irrigate root canal systems. The instrument comprises of 2 components, a portable handpiece and series of activator tips. It is recommended to use following the root canal preparation by agitating NaOCl for 60 seconds. The using of this device should be repeated for every changing of the new intracanal solution or repeated until the fluid in pulp chamber is observed to be clean (16). This sonic device will produce the powerful hydrodynamic force that can increase the washing potency of the irrigating solution.

However, the effectiveness of the EndoActivator® system in cleanliness of the root canal system is still inconclusive. Many studies found that this device promoted debris, smear layer and bacteria removal in the root canal system when compared with conventional needle irrigation (34, 38, 39). However, there were also many studies reported that there was no significant difference in debris, smear layer and bacteria and biofilm removal between the EndoActivator® system and the conventional needle irrigation (23, 35, 37, 40, 41).

Safety of the EndoActivator® system was also analyzed using various intracanal irrigation systems by measuring the apical extrusion of irrigating solution (17, 18). It was reported that the EndoActivator® system had a minimal amount of extruded irrigating solution out of the apex when compared with other delivery systems. However, the performance steps should be sequentially ordered, delivering irrigating solution into the pulp chamber, placing the tip into the canal, and initiating the sonic energy. Even in the large apical preparation size 50 (9% tapering) group of this study, apical extrusion of EndoActivator® group was less than needle and syringe irrigating system. This finding was also corresponded with two previous studies that was mention above (17, 18).

Using EndoActivator® in large apical sizes or immature tooth with opened apex should be done with caution in order to reduce extrusion of NaOCl. As shown in this study that there was no significant difference of NaOCl extrusion volume in teeth with apical size #80 of EA group and size #50 of NG group. Apical extrusion of irrigant is likely to occur in larger apical sizes regardless of irrigation techniques used.

In the needle and syringe groups, the open-ended needle was inserted 2 mm shorter than the working length and constantly delivered using an up and down motion. This technique led the irrigant replacement up to the working length resulting in lower apical pressure in large apical preparation sizes (12). However, enlarging the foramen results in a marked increase in surface area (by a power of two) and circumference. This could increase the potential for apical extrusion. In order to reduce effect of large apical preparation size, using side-vented needle could produce less apical pressure (27).

EndoActivator® was commonly used as final rinse before medication and obturation procedures. Syringe with needle irrigation was also the major method that was used through out the chemomechanical phase. In the clinical situation both

techniques need to be combined. This study limited investigation of NaOCl extrusion in a final rinse and not during complete instrumentation process.

During final rinse, there was a replenishment of the irrigant when using needle and syringe, however with EndoActivator®, there is no replenishment of the irrigant. This might be one of the reasons that caused less irrigant extrusion when using EndoActivator®.

Ultrasonic irrigation significantly reduced number of *Enterococcus faecalis* in root canal when agitated with NaOCl for 1 minute. This protocol could equally reduce the number of bacteria when compared with calcium hydroxide medication for 1 week. (53). When compared effectiveness of ultrasonic agitation, EndoActivator® in removing bacteria, the previous study showed that the ultrasonic and EndoActivator® had similar bacteria removal ability. Both of them were significantly more effective than syringe and needle (38). Due to ability of EndoActivator and Ultrasonic irrigation in removing bacteria, these protocols were suggested as the adjuctive irrigation during final flush irrigation.

This study was also an *in vitro* study, which results could not directly applied to clinical situation because it might be effected by periapical tissues (30). The periapical tissues had many structures including periodontal ligament, bones, blood vessel and nerve supplied which might restrict to apical extrusion of irrigating solution.

From previous *in vivo* study, irrigation in necrotic teeth could lead to apical extrusion when prepared the root canal to size #45. However, the irrigant reached the apex sooner during irrigating than it did in vital teeth. The teeth with necrotic pulp had a radiolucent area at the apex, thus there was no vital periapical tissues that might provide a natural barrier. This natural barrier may have an apical resistance during irrigation (11). The agarose gel that was used in this study could provide the apical barrier. However, it could not imitate all the characteristic of periapical tissues.

Up until now, the exact amount of NaOCl extrusion that can damage periapical tissue is still unknown. However, reduction in apical extrusion as much as possible can reduce potential problems caused by NaOCl toxicity. Further studies should be carried out in order to investigate factors that involved in apical extrusion such as tapering of the root canal, needle depth, flow rates of irrigation, irrigating systems and immature tooth model.

In conclusion, the null hypothesis was rejected. The apical extrusion depended on apical preparation sizes and irrigating systems when their apical preparation sizes were larger than size #35. Regardless of irrigation technique, NaOCl apical extrusion was not observed in teeth prepared to an apical size #35. However, increased amounts of NaOCl extruded from the apex when the apical canal portion was prepared up to file size #50 and this extrusion increased in teeth prepared to apical size #80. The use of the EndoActivator® resulted in less apical extrusion than with needle and syringe irrigation.

Further studies should be done to find other factors that involved in apical extrusion. Increased amount of NaOCl extrusion was found when increasing apical preparation size. Nevertheless, this model should be applied to evaluate the apical extrusion in immature tooth samples. However, this model could be improved to evaluate the effectiveness of root canal debridement and relate the outcome to the apical extrusion.

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APPENDIX

Preliminary report

Pilot study was carried as written in material and method of this study. From the pilot study, we could not detect the color change of agarose gels in negative control group. This result was corresponded with the result from weighing, that the weights of models did not increase after irrigating with NG and EA irrigating techniques. On the other hand, agarose gels changed from colorless to blue color in positive control group and the weight of models also increased after irrigation.

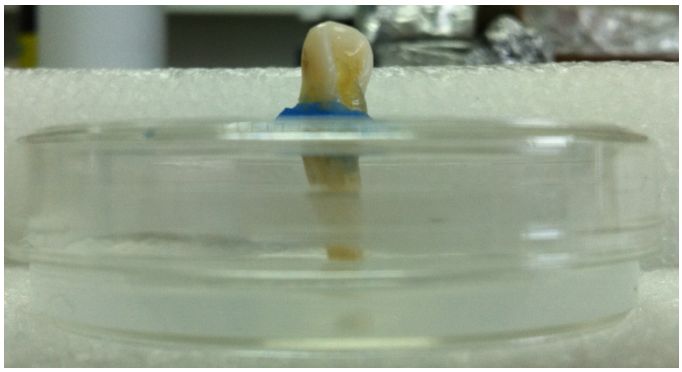

Figure 2 No color change of agarose gels in negative control group

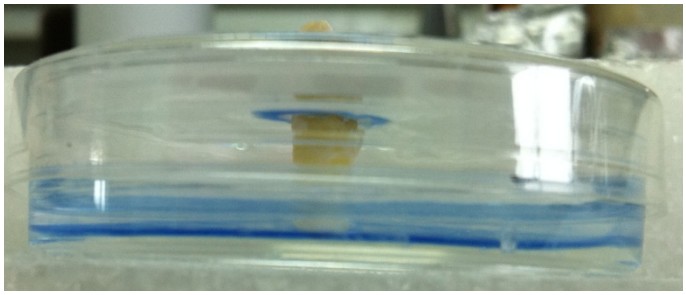

Figure 3 Agarose gels changed from colorless to blue color in positive control group

Table 2 Weight of agarose gel model in apical preparation size #35

No.	Conv. Be	Conv. Af	EA. Be	EA. Af	Conv. Diff	EA. Diff
1	13.78	13.78	17.17	17.17	0	0
2	16.09	16.09	13.77	13.77	0	0
3	15.39	15.39	14.95	14.95	0	0
4	13.32	13.32	14.42	14.42	0	0
5	13.13	13.13	16.41	16.41	0	0
6	17.07	17.07	14.03	14.03	0	0
7	16.28	16.28	15.34	15.34	0	0
8	20.24	20.24	13.01	13.01	0	0
9	18.5	18.5	13.82	13.82	0	0
10	16.43	16.43	14.53	14.53	0	0
11	17.06	17.06	13.43	13.43	0	0
12	14.28	14.28	13.01	13.01	0	0
13	14.46	14.46	12.19	12.19	0	0
14	16.22	16.22	14.53	14.53	0	0
15	13.76	13.76	13.98	13.98	0	0
16	14.3	14.3	16.82	16.82	0	0
17	15.46	15.46	15.94	15.94	0	0
18	13.54	13.54	13.82	13.82	0	0
19	14.84	14.84	15.84	15.84	0	0
20	12.14	12.14	11.51	11.51	0	0
21	15.43	15.43	16.47	16.47	0	0
22	15.32	15.32	12.86	12.86	0	0
23	11.93	11.93	13.8	13.8	0	0
24	16.46	16.46	13.13	13.13	0	0
25	14.8	14.8	15.71	15.71	0	0
26	11.92	11.92	10.69	10.69	0	0
27	12.95	12.95	11.71	11.71	0	0
28	13.55	13.55	14.2	14.2	0	0
29	11.85	11.85	11.93	11.93	0	0
30	11.49	11.49	13.81	13.81	0	0

Table 3 Weight of agarose gel model in apical preparation size #50

No.	Conv. Be	Conv. Af	EA. Be	EA. Af	Conv. Diff	EA. Diff
1	12.21	12.42	15.98	15.98	0.21	0
2	13.45	13.64	12.53	12.54	0.19	0.01
3	12.53	12.87	15.92	15.94	0.34	0.02
4	14.98	15.14	13.06	13.06	0.16	0
5	15.67	15.78	13.55	13.56	0.11	0.01
6	13.43	13.53	15.52	15.53	0.1	0.01
7	12.92	13.03	12.36	12.37	0.11	0.01
8	13.64	13.88	12.48	12.48	0.24	0
9	13.49	13.62	13.07	13.08	0.13	0.01
10	14.72	14.87	15.84	15.84	0.15	0
11	15.83	16.13	15.35	15.35	0.3	0
12	14.77	14.9	16.11	16.13	0.13	0.02
13	12.89	13.05	15.02	15.02	0.16	0
14	16.46	16.59	13.36	13.36	0.13	0
15	14.23	14.33	14.84	14.87	0.1	0.03
16	14.04	14.33	12.01	12.01	0.29	0
17	15.11	15.26	14.26	14.27	0.15	0.01
18	14.5	14.63	15.37	15.37	0.13	0
19	14.23	14.33	16.36	16.36	0.1	0
20	13.29	13.68	15.86	15.9	0.39	0.04
21	15.19	15.37	13.33	13.34	0.18	0.01
22	14.37	14.52	14.74	14.75	0.15	0.01
23	16.5	16.65	12.67	12.7	0.15	0.03
24	15.76	15.94	15.24	15.26	0.18	0.02
25	14.18	14.49	14.56	14.59	0.31	0.03
26	13	13.22	12.23	12.23	0.22	0
27	15.4	15.76	13.17	13.17	0.36	0
28	12.55	12.91	11.35	11.42	0.36	0.07
29	11.88	12.04	12.42	12.42	0.16	0
30	12.03	12.39	13.14	13.14	0.36	0

Table 4 Weight of agarose gel model in apical preparation size #80

No.	Conv. Be	Conv. Af	EA. Be	EA. Af	Conv. Diff	EA. Diff
1	15.32	16.46	13.75	13.79	1.14	0.04
2	15.97	17.06	14.64	14.65	1.09	0.01
3	15.7	16.81	16.08	16.18	1.11	0.1
4	12.56	13.57	15.15	15.25	1.01	0.1
5	13.49	14.59	14.19	14.24	1.1	0.05
6	15.02	15.76	15.63	15.75	0.74	0.12
7	16.77	17.66	13.31	13.41	0.89	0.1
8	15.3	16.51	13.27	13.35	1.21	0.08
9	14.68	15.41	13.04	13.12	0.73	0.08
10	13.87	14.97	13.19	13.26	1.1	0.07
11	12.84	13.48	13.29	13.33	0.64	0.04
12	13.45	14.48	11.91	11.96	1.03	0.05
13	13.41	14.18	13.63	14.02	0.77	0.39
14	13	14.05	12.54	12.76	1.05	0.22
15	12.59	13.59	12.69	12.79	1	0.1
16	13.16	14.05	16.21	16.38	0.89	0.17
17	13.64	14.65	14.14	14.26	1.01	0.12
18	14.19	15.16	16.24	16.33	0.97	0.09
19	15.77	16.73	15.23	15.29	0.96	0.06
20	14.9	15.78	14.41	14.43	0.88	0.02
21	13.91	14.62	15.34	15.43	0.71	0.09
22	15.46	16.06	14.41	14.47	0.6	0.06
23	14.46	15.37	16.16	16.24	0.91	0.08
24	16.25	16.97	14.95	15.07	0.72	0.12
25	15.62	16.53	16.46	16.57	0.91	0.11
26	10.67	11.19	12	12.09	0.52	0.09
27	13.98	14.65	12.71	12.84	0.67	0.13
28	10.78	11.61	11.46	11.48	0.83	0.02
29	15.81	16.41	13.17	13.21	0.6	0.04
30	14.01	14.87	11.37	11.41	0.86	0.04

Statistic analysis

Table 5 Normal distribution test by using One-sample Kolmogorov-Smirnov Test

One-Sample Kolmogorov-Smirnov Test

Method	MAF			ExtVol
Conventional	MAF35	N		30
		Normal Parameters[a,,b]	Mean	.0000
			Std. Deviation	.00000[c]
	MAF50	N		30
		Normal Parameters[a,,b]	Mean	.1903
			Std. Deviation	.08693
		Most Extreme Differences	Absolute	.208
			Positive	.208
			Negative	-.135
		Kolmogorov-Smirnov Z		1.138
		Asymp. Sig. (2-tailed)		.150
	MAF80	N		30
		Normal Parameters[a,,b]	Mean	.8381
			Std. Deviation	.17378
		Most Extreme Differences	Absolute	.094
			Positive	.090
			Negative	-.094
		Kolmogorov-Smirnov Z		.517
		Asymp. Sig. (2-tailed)		.952
EndoActivator	MAF35	N		30
		Normal Parameters[a,,b]	Mean	.0000
			Std. Deviation	.00000[c]
	MAF50	N		30
		Normal Parameters[a,,b]	Mean	.0107
			Std. Deviation	.01502
		Most Extreme Differences	Absolute	.267
			Positive	.267

		Negative	-.238
	Kolmogorov-Smirnov Z		1.461
	Asymp. Sig. (2-tailed)		.028
MAF80	N		30
	Normal Parameters[a,,b]	Mean	.0877
		Std. Deviation	.06786
	Most Extreme Differences	Absolute	.220
		Positive	.220
		Negative	-.131
	Kolmogorov-Smirnov Z		1.207
	Asymp. Sig. (2-tailed)		.109

a. Test distribution is Normal.

b. Calculated from data.

c. The distribution has no variance for this variable. One-Sample Kolmogorov-Smirnov Test cannot be performed.

The result showed that data was not normal distribution ($p < 0.05$) in both NG and EA irrigating techniques with apical preparation size #35 and EA irrigating technique with apical preparation size #50. Nevertheless, Kruskal Wallis test was used to analyze in irrigating techniques and apical preparation sizes.

Table 6 Mean rank from Kruskal Wallis test

Ranks

	Group	N	Mean Rank
ExtVol	conv35	30	37.50
	conv50	30	132.23
	conv80	30	165.50
	EA35	30	37.50
	EA50	30	62.68
	EA80	30	107.58
	Total	180	

c. The distribution has no variance for this variable. One-Sample Kolmogorov-Smirnov Test cannot be performed.

Table 7 Kruskal Wallis test

Test Statistics[a,b]

	ExtVol
Chi-Square	166.895
df	5
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Group

Table 8 Mann-Whiney rank sum test for pairwise comparisons

Group comparison	$Z_{\alpha}/k(k-1)$	N	ni	nj	$\overline{R}_i$	$\overline{R}_j$	$\overline{R}_i - \overline{R}_j$		critical value
0 and 1	2.394	180	30	30	37.50	132.23	94.73	>	32.2080
0 and 2	2.394	180	30	30	37.50	165.50	128.00	>	32.2080
0 and 3	2.394	180	30	30	37.50	37.50	0.00	<	32.2080
0 and 4	2.394	180	30	30	37.50	62.68	25.18	<	32.2080
0 and 5	2.394	180	30	30	37.50	107.58	70.08	>	32.2080
1 and 2	2.394	180	30	30	132.23	165.50	33.27	>	32.2080
1 and 3	2.394	180	30	30	132.23	37.50	94.73	>	32.2080
1 and 4	2.394	180	30	30	132.23	62.68	69.55	>	32.2080
1 and 5	2.394	180	30	30	132.23	107.58	24.65	<	32.2080
2 and 3	2.394	180	30	30	165.50	37.50	128.00	>	32.2080
2 and 4	2.394	180	30	30	165.50	62.68	102.82	>	32.2080
2 and 5	2.394	180	30	30	165.50	107.58	57.92	>	32.2080
3 and 4	2.394	180	30	30	37.50	62.68	25.18	<	32.2080
3 and 5	2.394	180	30	30	37.50	107.58	70.08	>	32.2080
4 and 5	2.394	180	30	30	62.68	107.58	44.90	>	32.2080

0 = NG and apical preparation size 35

1 = NG and apical preparation size 50

2 = NG and apical preparation size 80

3 = EA and apical preparation size 35

4 = EA and apical preparation size 50

5 = EA and apical preparation size 80

BIOGRAPHY

Nattaya Rungcharoenporn was born on 20th May 1984 in Bangkok. She graduated with D.D.S. (Doctor of Dental surgery) from the Faculty of Dentistry, Thammasat University in 2008, and had worked as a lecturer at Faculty of Dentistry, Naresuan University for 2 years. She studied in a Master degree program in Endodontology at Graduate School, Chulalongkorn University in 2011. At the present, she has worked as a lecturer at Faculty of Dentistry, Naresuan University.