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THE QUALITY OF ROOT FILLING IN MANDIBULAR MOLARS WITH ISTHMUSES OBTURATED BY THREE DIFFERENT TECHNIQUES

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Endodontology
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การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาเปรียบเทียบคุณภาพการอุดคลองรากฟันในฟันกรามล่างที่มีส่วนเชื่อมต่อด้วยเทคนิคการอุดคลองรากฟัน 3 เทคนิคโดยใช้เครื่องไมโครซีทีและแบบจำลองการซึมผ่านของกลูโคส โดยนำฟันกรามล่างมนุษย์จำนวน 60 ซี่ ที่ได้รับการระบุและจำแนกประเภทของส่วนเชื่อมต่อคลองรากฟันด้วยเครื่องไมโครซีทีแล้วมาขยายคลองรากฟันด้านใกล้กลางโดยใช้ไฟล์นิกเกิลไทเทเนียมชนิดหมุนด้วยเครื่อง แบ่งฟันโดยการสุ่มแบบชั้นออกเป็น 3 กลุ่มทดลอง (กลุ่มละ 20 ซี่) นำฟันที่เตรียมไว้มาอุดคลองรากฟันโดยใช้เทคนิคแลทเทอรัล คอนเดนเซชั่น แมตช์เทปเปอร์ซิงเกิลโคน และคอนตินิวอาสเวฟคอมแพคชันด้วยกัตทาเพอร์ชาและเอเอชพลัสซีลเลอร์ ฟันทุกซี่ถูกสแกนและวิเคราะห์ผ่านเครื่องไมโครซีทีในขั้นตอนก่อนและหลังการขยายคลองรากฟันและหลังการอุดคลองรากฟัน เพื่อหาปริมาณของวัสดุอุดคลองรากฟันและช่องว่างภายในคลองรากฟัน จากนั้นจึงตัดรากฟันด้านไกลกลางออก ประเมินการรั่วซึมของกลูโคส โดยใช้แบบจำลองการรั่วซึมผ่านของกลูโคสในระยะเวลา 28 วัน นำข้อมูลที่ได้มาวิเคราะห์ความแตกต่างระหว่างกลุ่มด้วยการทดสอบครัสคัล-วอลลิสที่ระดับนัยสำคัญ 0.05 ผลการศึกษาไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของปริมาณเปอร์เซนต์ของวัสดุอุดในคลองรากฟันในทุกเทคนิคและพบช่องว่างในคลองรากหลักมากขึ้นในกลุ่มทดลองที่อุดด้วยเทคนิคแลทเทอรัล คอนเดนเซชั่น การประเมินการรั่วซึมของสารไม่พบการรั่วซึมของกลูโคสในทั้ง 3 กลุ่มทดลองในระยะเวลา 28 วัน

ภาควิชา	ทันตกรรมหัตถการ	ลายมือชื่อนิสิต ....
สาขาวิชา	วิทยาเอ็นโดดอนต์	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....
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TANAWAN MANWIWATTANAKUL: THE QUALITY OF ROOT FILLING IN MANDIBULAR MOLARS WITH ISTHMUSES OBTURATED BY THREE DIFFERENT TECHNIQUES. ADVISOR: ASSOC. PROF. PIYANEE PANITVISAI, 66 pp.

This study was to compare the quality of root canal obturation in mandibular molars with isthmuses obturated by three different techniques using micro CT and glucose filtration model analysis. The isthmuses of sixty human mandibular molars were identified and classified by micro CT. The mesial root canal of each tooth was instrumented with rotary nickel-titanium files. Three groups of twenty roots were root canal obturated using lateral condensation, matched-taper single-cone and continuous wave compaction techniques with gutta-percha and AH plus sealer. All teeth were scanned before, after instrumentation and after obturation by a micro-CT scanner. Three-dimensional reconstruction of the obturated root canals was performed to analyze the volume of the filling materials and voids in the root canal system. Distal root of the specimens were removed after root canal obturation and leakage along root filling was evaluated during 28 days using a modified glucose filtration model. Statistical analysis by Kruskal-wallis at 0.05 level of confidence demonstrated that there was no statistically significant difference in the percentage of filling materials among the three obturation techniques. The presence of voids increased in the main root canal of the lateral condensation group. No leakage was found among the three experimental groups during 28 days.

Department: Operative Dentistry   Student's Signature ....

Field of Study: Endodontology   Advisor's Signature ....

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LIST OF ABBREVIATIONS

µg	microgram
µL	microliter
2D	two-dimensions
3D	three-dimensions
ABTS	Azino-bis diammonium salt
atm	Atmosphere
BLi	bucco-lingual
CEJ	cementoenamel junction
cm	centimeter
Da	Dalton
EDTA	ethylenediaminetetraacetic acid
GP	gutta-percha
h	hour
ISO	International Organization for Standardization
kPa	kilopascal
kv	terms of tube voltage
M	Molar
MD	mesio-distal
micro CT	micro computed tomography
min	minutes
mL	milliliter
mm	millimeter
mM	millimolar
MW	molecular weight
NaN_3	Sodium azide
NaOCl	sodium hypochrorite
NiTi	nickel-titanium
pH	potential of hydrogen ion

PGP	percentage of gutta-percha filled area
psi	pound per square inch
SD	standard deviation

CHAPTER I
INTRODUCTION

Background and Rationale

Obturation of the root canal systems has an important role in a long-term favorable prognosis (*1, 2*). Root canal system are complex, they compose of lateral canals, apical ramification and isthmuses. Obturating asymmetrical, irregular canals including isthmuses is a challenge. Such canals are common in most roots that contain two root canals in the same root. This includes mesial root of lower molars, first upper premolars, lower incisors and mesio-buccal root of upper molars. Currently, micro-computed tomography (micro CT) which produced three-dimensional images of external and internal tooth anatomy was used for in-depth studies of root canal anatomy. Prevalence of isthmus, evaluated by micro CT in the mesial root of mandibular first molars has been reported to range from 17%-85% (*3-5*). The canal isthmuses have clinical significance besides being a poorly accessible area, internal communication between root canal often left untouchable area from chemomechanical instrumentation. In addition, filling of these irregularities were unpredictable and might result in failure endodontics treatment (*6*).

Thermoplasticized gutta-percha has been introduced to obturate these canals in order to allow better adaptation of root filling material to the irregularities of root canal system (*7*). However, the limitations are high cost, need specific instruments and clinician's skill. Cold lateral condensation is used by clinicians throughout the world due to its simplicity and adaptability to most cases. It is often used as a standard to compare other techniques (*8-10*). However, non-filled spreader tracks are frequently present in the root filling (*11*). The method is by many practitioners considered as time-consuming and difficult to succeed.

The single cone technique consists of a single gutta-percha cone filled with sealer layer thickness that varies depending on the fit of the single cone to the walls of the canal. The technique is much faster and easier to operate. The possibility of improving and re-popularizing this technique is still discussed in the current endodontic literature. Because of the widespread use of the rotary nickel-titanium

(NiTi) file systems, manufacturers have produced gutta-percha cones that match the taper of canals prepared by these systems. Rotary instrument with constant tapers such as the ProFile and many other models were believed to better accommodate standardized greater gutta-percha cone (i.e. 0.04, 0.06, 0.08). The manufacturer claims that matched-taper, single-cone points perfectly fit canal developed to improve the quality of root canal obturations.

In in vitro studies, evaluation of the quality of obturation has commonly relied on either apical or coronal leakage. Destructive techniques such as the measure of the percentage of the cross-sectional profile of the obturated canal occupied by sealer and gutta-percha have been used. A variety of laboratory-based experimental models are used to detect and measure leakage along root filling including glucose penetration (*12*). This model allows quantitative measurements of microleakage without destroying the samples as the fluid filtration technique does. However, the glucose test may be more sensitive than the fluid filtration technique. The measurement of the fluid filtration is carried out by observing an air bubble and its movement along a capillary whilst the glucose concentration is determined by a highly sensitive enzymatic reaction measured by the spectrophotometer.

Recently, micro CT has been used for evaluation the quality of root canal obturation, to distinguish between filling material and voids, besides, it is possible to conduct volumetric measurements of root canal filling. Micro CT offers the advantages of being a rapid highly accurate and non-destructive method for in vitro evaluation of root canal filling (*13-15*). Some studies had evaluated the correlation between the outcomes of different evaluation methods (*16, 17*). However, correlation between glucose filtration and three-dimensional image analysis have never been evaluated..

Several studies compared the quality of root canal filling using matched-taper single cone techniques and lateral condensation in various root morphology such as straight canal, curved canal, round-shaped and oval-shaped canal. They concluded that no significant difference between groups (*9, 10, 18, 19*). In irregular canal anatomy such as isthmuses, little information is available for their ability to obturate and seal these complex canals. Only one study compared the percentage of gutta-percha, sealer and void and influence of isthmuses in irregular canal using matched-taper single cone techniques and lateral condensation (*20*). No data on the percentage of root filling material and void in isthmus areas have been reported. This study is to compare the quality of obturation in mandibular molars with isthmuses by lateral condensation, matched-taper single cone and thermoplasticized gutta-percha techniques using glucose filtration model and micro CT analysis..

Research Question

1. Is the quality of root filling in mandibular molar with isthmuses, different between the three obturation techniques (lateral condensation, matched-taper single cone and warm vertical condensation technique)?
2. Is there a correlation between glucose filtration measurement and percentages of canal volume filled in root canal system?

Research Objective

1. To compare the percent area occupied by root filling material and voids in root canal system of mandibular molars with isthmus obturated by lateral condensation, matched-taper single cone and warm vertical condensation technique.
2. To compare the quantity of glucose leakage of root canal of mandibular molar with isthmus obturated by lateral condensation, matched-taper single cone and warm vertical condensation technique.
3. To determine the correlation between the glucose filtration measurement and micro CT analysis.
4. Pattern of root filling penetration into isthmus areas of three obturation techniques will be described.

Hypothesis

<u>Hypothesis 1</u>

H_0: There are no differences in the areas occupied by root filling materials and voids in root canal systems of mandibular molars with isthmuses obturated by different obturation techniques.

H_1: There are differences in the areas occupied by root filling materials and voids in root canal systems of mandibular molars with isthmuses obturated by different obturation techniques.

<u>Hypothesis 2</u>

H_0: There are no differences in the glucose leakage measurement in root canal systems of mandibular molars with isthmuses obturated by different obturation techniques.

H_1: There are differences in the glucose leakage measurement in root canal systems of mandibular molasr with isthmuses obturated by different obturation techniques.

<u>Hypothesis 3</u>

H_0: There is no correlation between the glucose filtration measurement and micro CT analysis.

H_1: There is correlation between the glucose filtration measurement and micro CT analysis.

Operational Definition

Quality of root filling: There are maximal root filling material and minimal void in main root canal and isthmus area measured by micro CT image analysis and minimal glucose leakage measured by glucose filtration method.

Keyword

canal isthmuses, mandibular molars, matched-taper single cone technique, lateral condensation, continuous wave condensation, quality of root canal filling, micro CT, glucose filtration

Research Design

Experimental study

Limitation of Research

- The purpose of the study is to compare different obturation techniques in experimental models, so some procedures are not directly correlated to the clinical situation.
- Because of time limitation, this study evaluates only the leakage after root filling while sealing ability should be assessed in a longer period to ensure that apical sealing is clinically effective.

Benefits and Application

Matched-taper single cone technique which is easy to learn, save cost and create root filling area comparable to the standard technique, can be used as an alternative technique for root canal obturation in mandibular molars with isthmuses.

Ethical Consideration

The protocols used in this study were approved by the ethic committee of the Faculty of Dentistry, Chulalongkorn University.

CHAPER II
LITERATURE REVIEW

The main purpose of root canal treatment is to prepare the root canal system according to its anatomic feature, clean it, and then obturate it three-dimensionally in order to provide the maximum level of sealing ability. However, it is difficult to predictably reach this goal because of the intricate nature of root canal anatomy. The complexity of the root canal system is formed by the main canals and isthmuses communicating between them, accessory canals, apical ramifications, and anastomoses. An isthmus is generally defined as a narrow, ribbon-shaped communication between two root canals that contains pulp tissue or pulpally derived tissue (*21*). However, most literature often uses the term isthmus to mean the same as intercanal connection, transverse anastomosis or intercanal communication within root canal system. Isthmuses have been found to be present in all types of roots in which two canals are normally found, including the mesial roots of maxillary and mandibular molars, the distal root of mandibular molars, the maxillary and mandibular first and second premolars, and mandibular incisors.

Mandibular molar root system is one of the most complex morphology. The mandibular molar typically presents with two well-defined roots (*22, 23*), a mesial root characterized by a flattened mesiodistal surface and widened buccolingual surface, and a distal root mostly straight with a wide oval canal or two round canals (*24*). The morphology and buccolingual width of the mesial root allow for intercanal communications and isthmuses. The incidence of canal isthmuses in mandibular molars ranges from 6 to 83% in mesial roots of mandibular first molars which observation were performed using different method and at varying distances from the apex (*4-6, 24-26*). The incidence of isthmuses has been reported to be highest 3–6 mm from the apex (*4, 6, 25*).

Classification of Isthmuses

Hsu & Kim (*6*) classified the presence and type of canal isthmuses into 5 types that could occur on a beveled root surface. (Figure 1)

Type I is defined as two canals with no notable communication (A). Type II is defined as a hair-thin connection between the two main canals (B). Type III differs from the latter only with the presence of three canals instead of two (C). Type IV has an isthmus with extended canals into the connection (D). Type V is recognized as a true connection or wide corridor of tissue between the two main canals (E).

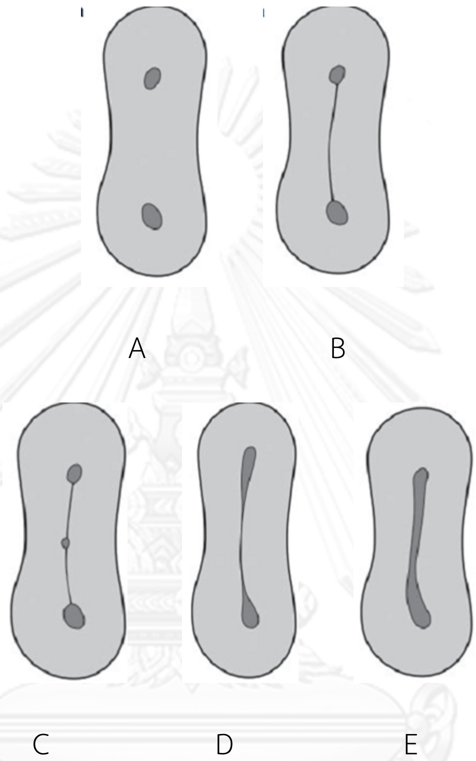


Figure 1 Types of isthmus classification determined by Hsu & Kim, 1997

However, this study was limited to microscopic assessments in a 2-dimensional plane. Recently, micro-CT has been used to investigate cross section of roots in a nondestructive manner. With the micro-CT technique, several studies found that the configuration of an isthmus might vary from the top cross section to the bottom one, and, many morphologic features of the isthmuses observed did not coincide with the classification of Hsu & Kim (*3-5*).

Fan et al. (*3*) classified isthmuses into four types on the basis of the features of reconstructed configurations in their study (Figure 2).

Type I sheet connection: narrow sheet and complete connection existing between two canals from the top to bottom of the isthmus.

Type II separate: narrow but incomplete connection existing between two canals from the top to bottom of the isthmus.

Type III mixed: incomplete isthmus existing above and/or below a complete isthmus.

Type IV cannular connection: narrow cannular between two canals

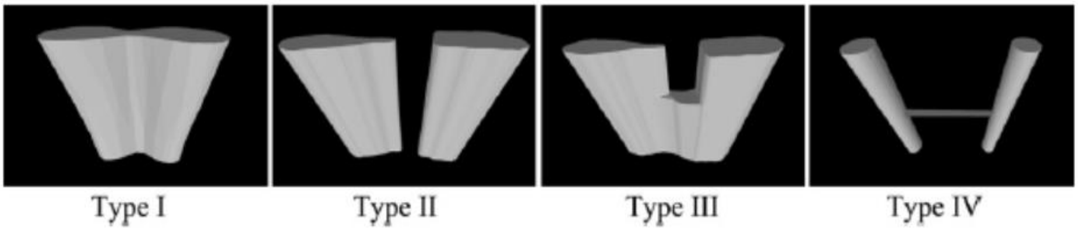


Figure 2 Classification of canals with isthmus determined by Fan et al, 2010

In addition, Gu et al. (*4*) found that the incidence of isthmuses in the mesial root of mandibular molars significantly decreased with age.

The management of the isthmus area was considered a potential impact on the long-term prognosis of root canal treatment because the small size of this area often precludes present-day mechanical instrumentation. Dentin debris and smear layer were formed during instrumentation and soft-tissue remnants, both remaining in the isthmus area and canal anastomoses, prevent root filling material to fully occupy this space (*27*). The cleaning phase can only be accomplished with caustic tissue solvents such as sodium hypochlorite (NaOCl). Insufficient exposure to chemicals or failure to obturate the space may leave residual irritants, voids for leakage, or both. Thus, when conventional root canal therapy appears to have been optimally performed and pathosis persists, uncleaned, unfilled accessory canals must be considered as a potential etiology for failure to heal (*6, 28*). Therefore, root canal preparation and obturation are important factor for the success of endodontic treatment in irregular canals.

Root Canal Preparation

Root canal preparation is accomplished using a combination of mechanical instrumentation and chemical irrigation. Root canals are prepared to eliminate micro-organisms, to remove pulp tissue and debris and to shape the canal system allowing placement of a three-dimensional root filling, all this without inflicting iatrogenic damage to the root canal or to the periradicular tissues. This goal is easier to achieve today, because of the introduction and use of rotary NiTi file system.

Table 1 Variety of instrument designs and brands

Instrument	Year	Cross-section	Taper	Tip
Light Speed	1992	U file	02	Pilot
ProFile	1993	U file	02-06	Pilot
Quantec	1996	Modified K-file	02-12	Various
GT rotary	1998	U file	04/06-12	Pilot
Hero 642	1999	Modified H-file	02-06	Modified active
RaCE	1999	Modified K-file	02-10	Pilot
Flex Master	2000	Modified K-file	02-06	Modified active
ProTaper	2001	Modified K-file	Multiple/reverse	Modified active
K3	2001	Modified K-file	02-10	Pilot
Endostar	2001	Modified K-file	02-10	Pilot
NiTi-Tee	2002	Modified S-file	02-12	Pilot
MFile	2003	Modified K-file	02-06	Pilot

Most NiTi rotary systems have constant proportion of diameter increments with ISO–sized tip (Table 1). Among these, ProFile has been the most widely researched NiTi rotary instrument in endodontics over the last 10 years, establishing it as the gold standard against which others are measured. ProFile instrument was sold as the series 29 in 0.04 taper, while instrument with .06 taper were added later by Dentsply Maillefer (Ballaigues, Switzerland). This set is claimed to be better accommodate standardized gutta-percha cones, which are predominantly manufactured in the market.

On cleaning ability of rotary instruments, several studies discovered uninstrumented areas with remaining debris in all areas of the canals irrespective of the preparation techniques with the worst results for the apical third (*29-32*). Besides, root canal preparation produces hard tissue debris or smear layer on instrumented surface, the accumulation of this debris into canal fins, isthmuses, and ramifications certainly has a negative impact on the sealability of root canals (*33*).

Some areas of irregular root canals and the area facing the isthmus in ribbon-shaped canal cannot be adequately prepared by current rotary files. All current rotary NiTi files tend to prepare the root canal into a form that has a round crosssection. Substantial untouched areas may be left on the side facing the isthmus areas or other irregularities within the root canal. Therefore, irrigant agitation is a necessary adjunct to mechanical instrumentation as a method of chemical debridement to remove debris and bacteria from the canal system.

There has been increased interest in recent years in the effect of irrigation on the isthmus area. Successful debridement of posterior teeth is hampered by the difficulty in flushing debris effectively from the isthmus of roots containing multiple canals. Although there is no hard tissue debris associated with these noninstrumented regions, dissolution of soft tissue remnants and eradication of microbes associated with infected pulpal tissues rely on the efficacy of irrigants to access these hard-to-reach regions. No single solution or technique has been found to achieve complete canal debridement, however the use of ultrasonic as an adjunct to clean and shape has shown increased canal cleanliness. An addition of 1-minute ultrasonically activated NaOCl, following hand/rotary root canal cleaning and shaping, cleaned canals and isthmuses significantly greater than hand/rotary instrumentation alone (*34-39*).

Besides proper cleaning and shaping of the root canal, the complete and hermatic obturation of the root canal system is another major objective in root canal treatment. Several techniques have been developed to improve the seal of the prepared root canal.

Obturation Technique

1. Lateral condensation technique

Currently, the most accepted technique is the cold lateral condensation of gutta-percha in combination with root canal sealer. Lateral condensation technique traditionally uses a master .02 taper standard gutta-percha cone that has a diameter consistent with the final file used in the canal at the working length followed by the addition of accessory cones. To increase the volume of gutta-percha with lateral condensation, a spreader is repeatedly forced in an apical direction, to create space for accessory cones (*40*). In clinical practice, however, there is no clear evidence whether ISO or unstandardized cones are preferred. But laboratory investigations suggest that ISO master cones allowed deeper spreader penetration and a larger number of accessory cones to be inserted (*41, 42*). Lateral condensation is regarded as safe, cost-effective and user-friendly.

A disadvantage to lateral condensation is that the process does not produce a homogenous mass (*43*). The accessory and master cones are laminated and remain separate. It is expected that the space between each of the cones is filled with sealer. Besides, this technique may not fill canal irregularities (*7, 9*) and considered as time-consuming.

2. Single cone technique

The single-cone obturation technique was introduced with the development of ISO standardization for endodontic instruments and filling points. The single cone technique consists of a single gutta-percha cone filled with sealer layer thicknesses that vary depending on the fit of the single cone to the walls of the canal. The single cone method has been advocated for narrow canal and the cone fit the canal loosely (*40*). However, the space between the single cone and the walls of the canal may be wide. A thorough coating of sealer is therefore absolutely necessary before placement of the single cone. The seal depends on whether a sufficient volume of sealer is placed into the canal. Single-cone obturations have not been well regarded because of the use of large amounts of sealer. Porosities in large volumes of sealer, setting contraction and dissolution of the sealer are the main disadvantages of this technique. However, unless coronal or apical leakage occurs, resulting in the gradual dissolution of the sealer cement, there may be little difference between filling the canal space with sealer cement versus gutta-percha.

3. Matched-taper gutta-percha cone

Because of the widespread use of the rotary NiTi systems, obturation cones are now produced to match the taper and size of canals prepared with rotary instruments in order to provide three dimensional obturation of the root canal over its entire length. This obturation can be achieved without requiring accessory cones or spending time on lateral condensation. Matched-taper gutta-percha cones were classified as standard taper, which are gutta-percha cones with increased taper and ISO-sized tips such as 0.04, 0.06, 0.08 (Diadent: Diadent Group International Inc., Vancouver, BC, Canada; Lexicon: Dentsply Tulsa Dental, Tulsa, OK; Maxima: Henry Schein, Melville, NY) and matched-taper for specific design rotary file system which manufacturers claim that they can replicate tapered canals effectively (ProTaper: Dentsply Maillefer, Ballaigues, Switzerland, K3: Sybronendo, Sybron Dental Specialties Inc). Thus, the use of a NiTi file-matched-taper cone system may promotes the single-cone cementation technique and makes the single-cone technique popular again.

4. <u>Warm vertical condensation technique</u>

Warm vertical condensation is another technique promoted by Herbert Schilder (*43*). His approach was described as '3-dimensional', indicating an intention to fill all ramifications of the pulp space, rather than just the primary root canal. In order to employ the warm gutta-percha technique, the root canal must be shaped for a continuously tapering funnel and keeping the apical foramen as small as possible. The technique involves fitting a master cone short of the correct working length with resistance to displacement. Following the adaptation of the master cone, it is removed and sealer applied. Heat is applied through the instrument which has a narrow enough tip to reach within 5 mm of root-end. A plugger is inserted into the canal and the gutta-percha is condensed, forcing the plasticized material apically. The process is repeated until the apical portion has been filled. The coronal canal space is backfilled using small pieces of gutta-percha. The sectional method consists of placing section of gutta-percha, apply heat, and compacting the mass with a plugger. Alternative backfill methods include filling with injection-molded gutta-percha, delivered from a gun in increments of 3–4 mm and compacted by hand to counteract cooling contraction (*44*). Advantages of the warm vertical compaction include filling of canal irregularities and accessory canals. Disadvantages are less length control, the potential for extrusion of material into the periradicular tissue and requiring of specific instruments.

The Quality of Root Filling Assessment

There are various methods to evaluate the quality of root canal obturation. Clinically, the quality of root filling is evaluated using radiograph which are normally taken in the bucco-lingual direction. In in vitro studies, bidirectional (bucco-lingual and mesio-distal) radiographs are used to evaluate the quality of root filling (*16, 17*).

The mesio-distal radiograph reveals more inadequacies in the root filling than the bucco-lingual radiograph. Therefore, a good quality determined by two-dimensional clinical bucco-lingual radiographs does not guarantee true good quality (*45, 46*).

1. <u>Percentage of canals filled in cross-sections</u>

The standard root filling is a combination of sealer cement with a central core material, which until now has been almost exclusively gutta-percha. Root canal sealer plays an important role in sealing the root canal, fill the space between gutta-percha and the root canal wall. In many studies the percentage of gutta-percha filled area (PGP) has been measured. The assessment of the quality of a root filling is determined by cross-sectional analysis of the space occupied by gutta-percha, sealer and void (*9, 16, 20, 47, 48*). The remaining sealer and amount of gutta-percha core allows for comparisons between studies and obturation techniques.

2. <u>Micro CT analysis</u>

High resolution micro-computed tomography (micro CT) is a new technology with several applications in different fields of dentistry. Early studies using conventional CT technology for the examination of teeth were limited by resolution. Low resolution of conventional CT was insufficient for adequate reconstruction of small objects such as teeth or root canals. With the development of CT technology, micro CT has been established in recent years. This technology was used as a research tool in various applications. In the field of endodontic research, micro CT has been used for the evaluation of root canal anatomy, assessment of root canal morphology after instrumentation and root canal obturation (*3-5, 13, 49*). Micro CT offers the advantages of a rapid, high accurate and non-destructive way of evalution. Moreover, with the help of a 3D-reconstruction, information about distribution of the component of a root canal filling can be obtained. Specimens can be both quantitatively and qualitatively examined. Volumes are calculated by dedicated software, whilst it is possible to localize specific details with visual image analysis. This technology is capable of distinguishing filling materials, voids and tooth structures with high accuracy and spatial resolution (*13, 15*).

3. Sealing ability assessment

The development and maintenance of a hermatic seal is considered to be a measure prerequisite for success in root canal treatment. Therefore the evaluation of the quality of root canal filling using leakage model tests is still relevant. Generally sealing quality is estimated by measuring microleakage that allows the tracer agent to penetrate the filled canal. A variety of laboratory based experimental models are used to detect and measure the leakage along root filling.

a) Dyes penetration method

The methodology using tooth immersion in dyes reported for the first time by Grossman in 1939. Dye penetration is perhaps the most widely used method, mainly because it is easy to perform. The phenomenon of capillarity is the utmost importance in this passive method used mainly for assessing apical leakage, as the tooth apex is submerged in the dye that penetrates through any space between the canal wall and filling material (*50*). Then, the teeth are sectioned longitudinally, transversely, or cleared and the linear penetration of the dye is recorded. There are various types of dyes such as methylene blue, India ink, silver nitrate, etc. Particle molecule size, pH and chemical reactivity of dyes are expected to affect the degree of penetration (*51*).

One of the major considerations in dye penetration studies is air entrapped in voids along the root canal filling which may hinder fluid movement. A comparison of techniques for assessment of dye leakage emphasized the importance of the use of reduced air pressure in dye penetration. Passive dye penetration resulted in incomplete void filling, regardless of void size, whereas reduced air pressure technique resulted in complete void filling (*52*). Tooth positioning had a significant effect on linear dye penetration under reduced pressure. The need to standardize factors that may influence penetration was emphasized when assessing the methodology of leakage studies.

b) Fluid filtration or transportation method

The fluid filtration method, in which the sealing capacity is measured by means of air bubble movement inside a capillary tube, was developed by Pashley 's group in 1987 and modified by Wu et al. (*53*) for use in root canals. It consists of a filled canal that has its coronal portion connected to a tube filled with water under atmospheric pressure, and its apex to a 20 µL glass capillary tube 170 mm long and of uniform caliber filled with water. Finally, a pressure of 0.1 atm is applied through the coronal part, which forces the water through the empty spaces along the root canal. The results are generally expressed in µL / min (*54*).

The fluid filtration method presents many advantages in comparison with dye penetration method. As the samples are not destroyed, therefore it allows the sealing to be assessed after a long period of time. Furthermore, the results are recorded automatically, thus providing quantitative measurements and avoiding operator errors. The results are precise, as small volume can be recorded and it would be more sensitive than dye penetration in detecting empty spaces along the canal. System sensitivity can be adjusted by altering the pressure used or the diameter of the micropipette (*55*). However, the materials and methods used in this technique are not standardized. As the pressure used may range from 10 to 20 psi, and the measuring time from 1 min to 3 h, this would alter the result obtained. Since lower filtration values have been found associated with longer recording time, and the values recorded were higher when high pressure was used in comparison with low pressure. It was suggested that 20 psi pressure would appear to be far too high because it corresponds to 1406 cm H_2O pressure. Therefore, to be as close as possible to physiological pressure, 15 cm by H_2O would appear to be sufficient when highly sensitive equipment is used. The pressures should include in the results and should be expressed as μL/min cm H_2O instead of μL/min (*54*). As various parameters could affect the test results, diameter of the capillary that contains the bubble, bubble length, measuring time and pressures applied, must be mentioned in the materials and methods section (*54*).

A new computerized fluid filtration meter based on light refraction at starting and ending positions of air bubble movement inside micropipette was developed (*56*). It has some advantages over the conventional ones with the computer control and digital air pressure arrangement. Additionally, the movement of air bubbles can be observed by laser diodes which are computer controlled rather than visual findings.

c) Dye extraction Method

This method developed by Zakariasen (*57*). In the dye extraction or dissolution method, the teeth are dissolved in acids that release all the dye from the interface and the optical density of the solution is measured by a spectrophotometer. This technique is based on the assumption that the amount of tracer uptake is related to the width of the gap appearing between the root filling and the root canal wall. This technique should provide reliable results in endodontic experiments because it measures all of dye taken up in the root. It is fast and can be carried out with equipment available at most universities (*50*).

There was no correlation between dye penetration and the fluid filtration which determine micro leakage. However, the dye extraction method and the fluid filtration were statistically correlated. The lack of correlation between the dye penetration and the dye extraction techniques cannot be related to a lack of physical coherence, because the same roots were dipped into the same dye and were subsequently compared. The dye penetration relies on randomly cutting the root into two pieces, without knowing if the section goes through the deepest dye penetration and the small size of the root limits this possibility. On the other hand, dye extraction technique involves recovering all of the dye penetration, thereby avoiding the limitations of sectioning the root. The fluid filtration technique gives results similar to the dye extraction technique because it takes into account all of the porosities of the interfaces between the filling material and the root. In addition, both techniques are based on quantitative measurements of the passage of a liquid within these interfaces (*50*).

d) Bacteria and toxin infiltration method

The use of bacteria to assess leakage (mainly coronal) is considered to be of greater clinical and biological relevance than the dye penetration method (*58*). Many different strains of bacteria have been used to assess marginal leakage and this has led to contradictory results, because methods depend on the type of bacteria used. Moreover, if the sealer has antimicrobial activity, it is unfeasible to employ the bacteria method (*59*). The system generally comprises two chambers and enables the apical and coronal extremities of each specimen to be completely separated. The turbidity of the broth in apical chamber is the first indication of contamination by microorganisms (*60*). For example, Torabinejad et al. found that 50% of filled root canals were completely contaminated by both *S.epidermidis* and *P.vulgalis* within 42 days (*61*). On the other hand, Wu et al. found that after 50 days of exposure to *P.aeruginosa*, only 7% of the filled canals allowed passage of this bacterium (*62*). The difference between the two studies may arise from difference in bacterial species or from difficulties in maintaining aseptic conditions throughout all steps of the procedure. These bacterial studies have been qualitative rather than quantitative. If only one bacterium passes through the obturated root canal, it may multiply in the enriched broth and cause turbidity (*63*).

The differences in behavior between bacteria and endotoxins must be related to their chemical activities. Endotoxins are lipopolysaccharides of the external membrane of gram-negative bacteria and consist of a lipid portion, Lipid A, and a polysaccharide portion, which is the external part of the membrane. The possibility of the bacteria exerting enzymatic action on the gutta-percha, sealer and dentin, and creating a passage through the seal has not yet been demonstrated (*60*). It has been reported that endotoxin proceeded bacterial penetration of canal system (*64*)

e) Glucose filtration method

A method for analysis of endodontic microleakage based on the filtration rate of glucose along the root canal filling was introduced by Xu et al (*12*). The model consists of a tube containing concentrated glucose solution was connected to the coronal aspect of the tooth, and whilst the apical region is in water. Glucose that accumulates in apical chamber is measured. In an assay reaction, glucose is oxidized by the enzyme glucose oxidase using molecular oxygen to gluconic acid and hydrogen peroxide as products in the reaction (1). The first reaction is coupled with the reaction of peroxidase enzyme and chromogenic substrate. The concentration of the hydrogen peroxide product is reduced by chromogenic substrate (Dye $_{(red)}$) to produce oxidized form (Dye $_{(ox)}$). Dye $_{(ox)}$ is proportional to the glucose concentration and is determined by absorbance in visible region.

$$\text{D-glucose} + O_2 + 2H^+ \xrightarrow{\textbf{Glucose oxidase}} \text{D-glucono-}\delta\text{-lactone} + H_2O_2 \qquad (1)$$

$$H_2O_2 + \text{Dye}_{(red)} + 2H^+ \xrightarrow{\textbf{Peroxidase}} 2H_2O_2 + \text{Dye}_{(ox)} \qquad (2)$$

Therefore, the glucose concentration is referred to the amount of leakage and is quantified using UV-visible spectrophotometry. Glucose was selected as the tracer because of its small molecular size (MW=180 Da). The glucose filtration method is expected to be more sensitive than the measurement of the fluid transport because the measurement of fluid transportation is observed by movement of an air bubble along a capillary whilst the glucose concentration assayed by glucose oxidase is a highly sensitive enzymatic reaction.

However, the fluid filtration model and glucose penetration model were similar. Fluid filtration and glucose filtration occur only through voids that are completely opened, while cul-de-sac type voids prevent fluid transport or glucose penetration. It has been shown that neither water nor glucose solution penetrate through root dentin. Thus, evidence of fluid transport or glucose penetration demonstrates the existence of at least 1 continuous void along the root filling. Among the available leakage evaluation methodologies, glucose filtration was preferred as the method of assessment for allowing measurement of endodontic leakage of a high number of specimens continuously over time independent from the observer and standardized technique. Besides, glucose penetration was reported to be more sensitive than fluid filtration (*65*).

To evaluate the quality of root filling, some studied have combined radiographs, fluid filtration method (*17*) whereas other studies used radiograph and dye penetration (*66*), or radiographs, dye penetration and PGP (*45*) or radiographs, fluid filtration method and PGP (*16*). The correlation between the different tests was evaluated. For example, Beyer-Olsen et al. (*66*) found a small correlation between radiograph and dye penetration in some groups. In the Wu et al. study, the correlation between radiographs and fluid filtration method was highly significant (*17*). Kersten et al. showed limited correlation between radiographs, dye penetration and PGP (*45*). van der Sluis et al.(*16*) found that there was no correlation between the fluid filtration method and PGP or fluid filtration method and radiograph, however, the correlation between the radiographic score of the mesio-distal radiograph and the PGP at 4 and 6 mm was significant. Besides, Souza et al. (*67*) evaluated the fluid filtration method and glucose filtration method, a positive correlation was found. However, none of these methodologies are evaluated with correlation between glucose filtration methods and the PGP or micro CT imaging.

Related Work and Similar Studies

Previously studies involving quality of root canal filling on teeth obturated using a single-cone method have shown different results. Single cone methods of obturation have been shown to be inferior in providing an apical seal in numerous in vitro studies (*68-70*). On the other hand, Zmener et al. (*71*) prepared the root canals using a rotary system and obturated with single-cone and lateral condensation techniques. They reported that with the use of a methacrylate-based sealer, the difference between single-cone and lateral condensation obturation was not significant. However, gutta-percha main cones in these studies are 0.02 taper cones which may not provide three-dimensional obturation of the root canal.

Recently, matched-taper gutta-percha cones were developed and the single-cone technique has become popular again. Inan et al. (*18*) compared the sealing ability of root canal prepared in round-shape, straight root canal with Protaper rotary NiTi instruments and obturated with 0.02 tapered gutta-percha master cone using lateral condensation and matched-taper gutta-percha cone using single-cone technique and found no difference between the groups. Besides, Karapınar-Kazandağ et al. evaluated microleakage by glucose filtration analysis. Various root filling systems including resilon + Epiphany, EndoRez, Activ GP and conventional AH Plus +gutta-percha technique were tested using the lateral condensation and single cone technique in round-shaped, straight canals. They concluded that no statistically significant difference was observed between the glucose leakage values of all the groups (*72*). Moreover, Wu et al. (*73*) studied the leakage of single-cone fillings using a silicone-based sealer for 1 year and concluded that single cone fillings prevented fluid transport for 1 year. However, this study did not compare with other filling techniques. Recently, Somma et al. (*15*) studied the quality of root canal filling by two thermoplaticized gutta-percha techniques (Thermafil and system B) and matched-taper single cone technique using micro CT analysis and concluded that all techniques produced comparable results in term of percentage of filling and voids distribution.

In curved canal, Wu et al. (*17*) evaluated the quality of root filling in small and curved canals with 0.02 tapered gutta-percha cone comparing lateral condensation technique and single cone technique. Evaluation was done by radiographs and fluid filtration method and was concluded that similar quality of root fillings were obtained in small and curved root canals filled using either a single cone or laterally compacted gutta-percha and epoxy resin– based sealers. However, gutta-percha main cones in this study were not matched-taper single cone which may not provide three-dimensional obturation of the root canal. Gordon et al. (*8*) compared the area occupied by gutta-percha, sealer, or void in standardized 0.06 taper prepared simulated curved canals and in mesio-buccal canals of extracted maxillary first molars filled with a single 0.06 gutta-percha points and sealer or lateral condensation of multiple 0.02 gutta-percha points and sealer. They found that 0.06 taper single cone technique was comparable with lateral condensation in the amount of gutta-percha occupying a prepared 0.06 tapered canal, however, this study filled only one canal of MB root in maxillary molar which had a high incidence of communicating isthmus and patent second mesiobuccal canals, so, the obturation quality would have been considerably better had both the MB 1 and MB 2 canals been prepared and filled. Horsted-Bindslev et al. (*10*) compared the quality of root canal filling performed with the single cone and lateral compaction technique using 0.02 taper in lateral condensation technique and 0.04 taper in single-cone technique using radiographs and concluded that there was no significant impacted of the filling technique on the quality, however, no data on degree of curvature of root canal or root shape which may give variability of the internal anatomy and crossectional area that making it difficult to compare quality of filling. On the contrary, Schäfer et al. (*74*) compared different obturation techniques in severely curved canals in terms of the percentage of gutta-percha filled area and voids, using 0.04 matched-taper single cone, cold lateral compaction with 0.04/0.02 gutta-percha master cone and concluded that at all level, lateral condensation produced significantly higher PGP and lower sealer filled areas than matched-taper single cone. However, no data on crossectional root shape, which may give variability of the crosssectional area and making it difficult to compare the quality of filling.

In irregular canal, Ozawa et al. (*9*) compared the effectiveness of obturation techniques for oval-shaped canal using matched-taper single cone, lateral condensation and thermoplastic obturation and concluded that percentage occupied by sealer cement in the thermoplastic obturation group was significantly lower than the single cone group or the lateral condensation group and there were no significant differences between the single cone obturation group and the lateral condensation group in each level. Besides, Marciano et al. (*20*) studied to compare the PGP, sealer and void and the influence of isthmuses in mesial root canals of mandibular molar filled with matched-taper single cone, lateral condensation and System B/Thermafil. They concluded that single cone and lateral condensation techniques had a higher incidence of voids, no relationship was found between the presence of isthmuses and the sealer distribution in all the evaluated techniques. The presence of isthmuses increased the presence of voids only in the lateral condensation group or the presence of isthmuses may influence the quality of root filling. However, this study did not separate the analysis between main canals and isthmus areas which the quality of obturation depended considerably on the extent of sealer cement or gutta-percha into unprepared recesses of canal and obturation technique may influence the quality of filling in this areas.

CHAPTER III

METHODOLOGY

Population and Sample

1. Target population: Human teeth with irregular root canal with isthmuses
2. Study population: Mesial roots of extracted mandibular molars
3. Sample: Mesial roots of human mandibular teeth were collected from hospitals in different parts of Thailand. The teeth were extracted from patients older than 20 years old, complete root formation with separate mesial canals and two distinct major foramens, no crack or fracture, no root caries and no root resorption. The curvature of the roots varied between 10-25 degrees as determined by Schneider (*75*). The length of the selected teeth was between 19 and 21 mm. The root canals showed isthmuses under micro CT scanning.

Materials

1. **Chemicals**
   - 0.1 % thymol solution 1000 mL
   - 2.5 % sodium hypochlorite solution 3000 mL
   - 17% ethylenediaminetetraacetic acid solution 1000 mL
   - AH plus sealer (Dentsply Maillefer, Ballaigues, Switzerland)
   - Silicone sealant (3M™ St. Paul, Minnesota, United States)
   - Cavit™ (3M™ St. Paul, Minnesota, United States)
   - 1 M glucose solution in 0.1% benzoic acid solution
   - D-(+)-glucose (S.M Chemical Supplies CO., LTD, Bangkok, Thailand)
   - 0.2% Sodium azide solution in 50 mM phosphate buffer
   - Ampicillin (T.P Drug Laboratories (1969) Co., Ltd., Bangkok, Thailand)
   - 2, 2’-Azino-bis (3-ethyl benzothiazine-sulfonic acid) diammonium salt (ABTS) (S.M Chemical Supplies CO., LTD, Bangkok, Thailand)
   - Glucose oxidase (S.M Chemical Supplies CO., LTD, Bangkok, Thailand)
   - Peroxidase (S.M Chemical Supplies CO., LTD, Bangkok, Thailand)

2. **Instruments**

- K file NO.10, 15 (Dentsply Maillefer, Ballaigues, Switzerland)
- ProFile® rotary instrument (Dentsply Maillefer, Ballaigues, Switzerland)
- ProTaper® rotary instrument (Dentsply Maillefer, Ballaigues, Switzerland)
- Irrigating needle 30 gauge (Max-I-Probe Dentsply Maillefer, Ballaigues, Switzerland)
- Syringe irrigate (size 5 mL)
- Paper point
- ISO size 35 gutta-percha (Dentsply Maillefer, Ballaigues, Switzerland)
- 0.04 taper gutta-percha size 35 (Dentsply Maillefer, Ballaigues, Switzerland)
- Accessory cones gutta-percha size FF (Dentsply Maillefer, Ballaigues, Switzerland)
- Spreader size D11TS, NiTi spreader (Hu-Friedy, Pearson Dental Supply Company, Chicago, IL.USA)
- Endodontic plugger
- Glass slab and metal spatula
- Long glass tube 20 mm.
- Rubber cap
- Incubator 37° (Memmert, United instrument Co., Ltd. Thailand)
- Ultrasonic (P5 newtron XS, Acteon Thailand)
- System B (SybronEndo, Orange, CA.USA)
- Obtura II (Obtura Spartan ,Fenton, MO. USA)
- Test tube
- Pipette tip (size 100 μL, 1 mL)
- Cuvette
- Spectrophotometer (Biomat, Becthai Bangkok Equipment & Chemical CO.,Ltd, Bangkok Thailand)
- Peristaltic pump (Pharmacia Fine Chemicals, Stockholm Sweden)
- Low speed saw (ISOMET®, Buehler, IL. USA)
- Micro CT (μCT 35 scanners; Scanco, Bruttisellen, Switzerland)

Methods

1. Selection and preparation of teeth

Seventy extracted human mandibular first and second molars were selected for the study. The teeth were stored in 0.1% thymol solution. The teeth were immersed in 2.5% sodium hypochlorite for 5 min to remove organic tissue from the root surface. Any remaining tissue was mechanically removed using a curette with intention not to damage the root surface. The teeth were radiographed and examined in the BLi projection and in the MD projection to evaluate the root canal morphology and curvature. The curvature of the roots varied between 10-25 degrees as determined by Schneider (*75*). The teeth were scanned by a micro CT system from the apex to crown. A µCT 35 high resolution micro-CT scanner (Scanco, Bruttisellen, Switzerland) was used to scan the specimens at energy of 70 kVP, 800 ms integration time, an isometric voxel size of 20 µm. The cross-sections of the root canals with or without an isthmus were identified first, and then the root canals with isthmuses were reconstructed. Subsequently, the 3-D morphology of the canals with isthmuses was investigated using SCANCO µCT software (SCANCO medical AG). Four types of isthmuses were classified on the basis of the features of reconstructed configurations as determined by Fan et al. (*3*). Each type of isthmus teeth was randomly divided by stratified randomization into 3 groups in different obturation techniques: lateral condensation technique, matched-taper single-cone technique and continuous wave compaction technique. Each group consists of 20 samples, and 10 specimens were used as positive and negative randomly.

The crowns of the selected teeth were sectioned with low speed saw to gain 15 mm. long roots. All teeth were stored in gauzed moistened with sterile saline.

2. Instrumentation and preparation of teeth

Mesial canals of the seventy selected teeth were prepared. Apical patencies were determined with a size 10 K file. The working length was established 1 mm. short of the apical foramen. A glide path was confirmed with a size 15 K file. Root canal instrumentation was completed by using a crown-down, continuous taper technique with nickel-titanium rotary files: ProTaper S1, S2, F1 and Profile 25/.04, 30/.06, 30/.04, 35/.06 and 35/.04. Each rotary file was used for 10 root canals. All canals were instrumented to size 35/.04. The purpose of preparation regimen is to create a uniform size of canal. 3 mL of 2.5% sodium hypochlorite solution was used for irrigation between each file size. After the master file, the canals were irrigated for 1 min with 2.5% sodium hypochlorite solution using passive ultrasonic irrigation. The power setting of the ultrasonic unit was set to maximum. The ultrasonic file was placed to the pre-measured depth and, upon activation, moved passively up-and-down to ensure that it did not bind within the root canal. The energized ultrasonic file was used continuously for 1 min in each canal while sodium hypochlorite in tank of ultrasonic unit was delivered continuously through the ultrasonic file (*35*). High speed suction was maintained at all times on the distal cusp of the experimental tooth.

The smear layer was removed with 1 mL 17% ethylenediaminetetraacetic acid (EDTA) for 1 min, followed by a final flush of 3 mL of 2.5% NaOCl solution (*76*). After irrigation, each canal was dried with paper points. Smear layer removal was ensuring under SEM. Finally, all specimens were stored in sterile saline moistened gauze. After root canal preparation, the specimens were scanned using a micro CT scanner and analyzed using 3-D reconstruction software (Figure 3). To standardize the samples, volume analysis were done only from apical foramen to floor of pulp chamber.

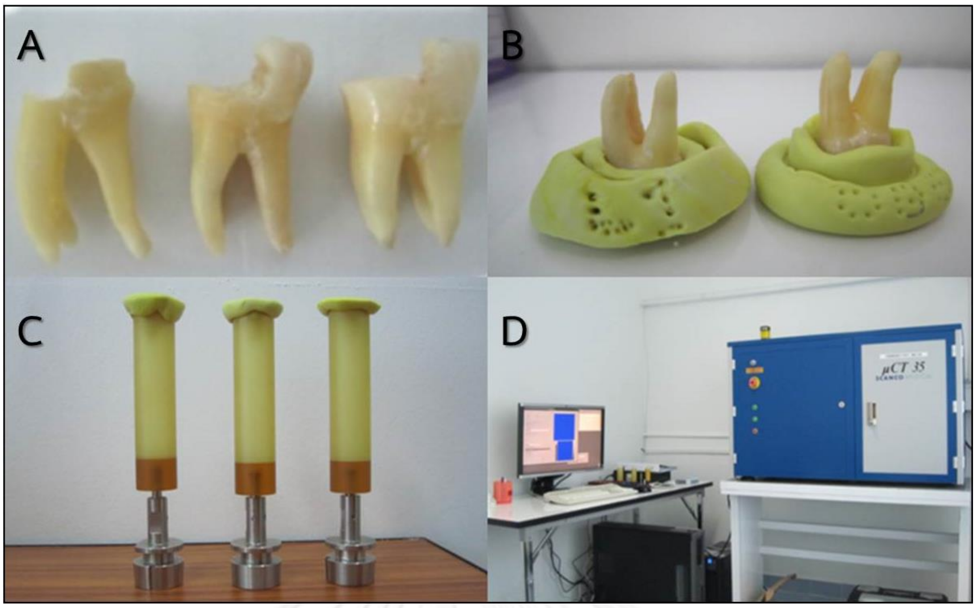


Figure 3 Preparation of tooth and micro CT scanning. (A) The teeth were sectioned to gain 15 mm long root. (B) Prepared teeth were put in silicone jig to fix position. (C) The specimen in sample holder for micro CT scanning (D) Micro CT

3. Root canal obturation

After the preparation procedure, five roots were obturated with gutta-percha cone (size 35/.02 tapered standardized cone) without sealer to serve as positive control. Five roots were completely coated with silicone sealant covering the root canal orifice, serving as the negative control. The remaining sixty teeth were randomly assigned between the three experimental groups. AH plus was used as a sealer and mixed according to the manufacturer’s instructions. The ratio of a paste A: paste B ratio was 1:1 at room temperature and introduced into the prepared canals with a size 25 lentulo spiral in a slow speed handpiece until the sealer could be seen at the apical foramen. Finally, the root canals were obturated with the following techniques:

Group 1: matched-taper single-cone technique, gutta-percha cone (size 35/.04 tapered gutta-percha cone) was coated with sealer and inserted into the canal to the working length.

Group 2: the cold lateral condensation, the master cone (size 35/.02 tapered standardized gutta-percha cone) was coated with sealer and inserted into the canal. The root canals were filled with the lateral condensation technique using a nickel titanium spreader inserted 1 mm short of the apical foramen and FF sized gutta-percha accessory cones were used to fill the entire length of the root canal.

Group 3: the continuous wave compaction technique, the cone (size 35/.04 tapered gutta-percha cone) was placed in the canal. The system B (SybronEndo, Orange, CA. USA) was used to remove the coronal portion of the gutta-percha cone and softens the remaining material in the canal. A System B plugger was inserted into the canal and the gutta-percha condensed, forcing the plasticized cone apically. Radiographs were taken in a bucco-lingual direction to evaluate the apical portion. The process was repeated until the apical portion filled up completely. The coronal canal space was back-filled using Obtura II thermoplastic injection (Obtura Spartan, Fenton, MO. USA).

After obturation, all the root canal orifices were sealed with temporary filling material (Cavit ™ 3M ESPE Dental Products United States). All specimens were stored in gauzes moistened with sterile saline at 37° C, 100% humidity for 1 week to allow complete set of the materials. After filling the canal, radiograph was taken in a bucco-lingual direction for each specimen. The quality of the filling was evaluated by an endodontist (blinded observer). The evaluating criteria was according to the general guideline for good clinical practice, gutta-percha was well adapted to the canal wall and show only a few, minor air bubbles (less than 0.25 mm in diameter) (*45*)

4. Micro CT analysis

After root filling, all specimens were rescanned using micro CT, then, 3D reconstructed model was established. Superimposition of root canal and root filling was performed. Different colors were used to designate state of the root canal using micro CT software (Figure 4).

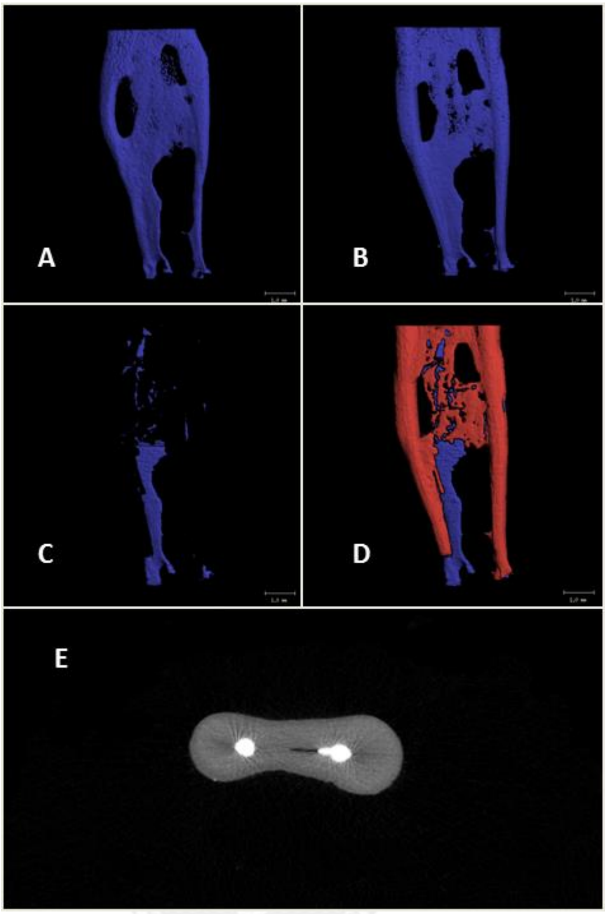


Figure 4 3D reconstructed model of a sample root canal system representative for type III isthmuses. (A) Before instrumentation reconstruction. (B) After instrumentation reconstruction. (C) After filling, subtraction reconstruction representative for the distribution of voids and non-filling area. (D) Superimposed reconstruction after filling, root filling material is indicated in orange, non-filling area and voids is indicated in gray. (E) A cross-section of middle thirds of root canal representative the inner structure of the filled roots

The micro CT was used to quantify the root filling volume in root canal system (mm^3) and the volume of the void distribution in root canal system (mm^3)

5. Glucose filtration method

After the micro CT analysis, each root was evaluated using a modified glucose penetration study design that represents a modification of the previously reported protocol by Xu et al. (*12*). Each root was connected to a 20 cm long glass tube with silicone sealant. All the root canal specimens were coated with silicones sealant, except for the 3 mm of the apical part in order to allow glucose penetration via apical region. The assembly were then placed in a test tube with a rubber cap and sealed with silicone sealant (Figure 5). A solution of 50 mM sodium phosphate buffer pH 7.0 containing 0.2% sodium azide (NaN_3) and 100 µg/mL ampicillin was dispensed into the test tube. After that, the 3 mm apical portion of root canal specimens was immersed in the solution. NaN_3 and ampicillin were used to inhibit the growth of microorganisms. The tracer use in this study was 1 M glucose containing of 0.1% benzoic acid.

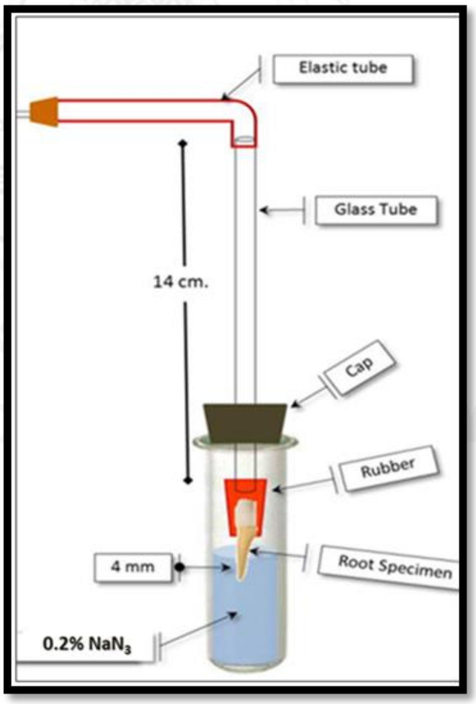


Figure 5 Glucose filtration model

The solution of 1 M glucose was filled in a glass tube using peristaltic pump to avoid air bubbles inside the glass tube of a tooth sample. The glucose solution was flowed into the glass tube in the slow rate of 0.7 mL/min through long silicone tube until the level of solution reached the length of 14 cm higher than the root canal specimen which created a hydrostatic pressure of 1.5 kPa or 15 cm H_2O (Figure 6).

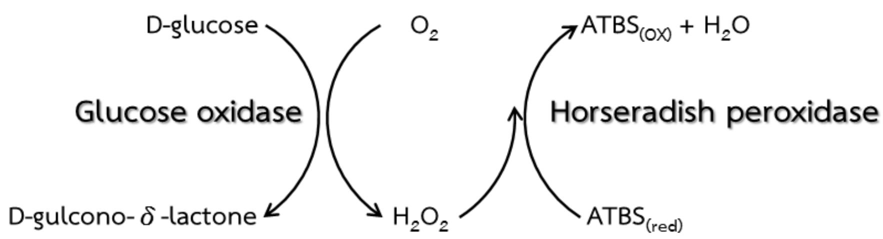
Figure 6 Preparation of glucose filtration test

All specimens were stored in the incubator at 37°C for 28 days. For glucose assay, a solution of 15 µL was taken from the test tube using a micropipette at the time of 1, 4, 7, 10, 13, 16, 19, 22, 25, 28 days. After sampling solution, a solution of 15 µL of 50 mM sodium phosphate buffer solution containing 0.2% NaN_3 solution and 100 µg/mL ampicillin was added into the test tube to maintain a constant volume of 2 mL. The glucose concentration in samples was analyzed three times per one sample using couple assay of glucose/peroxidase system as described. The results of glucose concentration (mM) were referred and calculated to rate the leakage in all groups.

The determination of glucose concentration were done using couple assay of glucose oxidase/peroxidase system as following

-50 mM sodium phosphate buffer pH 7.0	785	µL
-ABTS (chomogenic substarte for peroxidase) (10 mM)	200	µL
-Glucose oxidase (30 mg/mL)	5	µL
-Horseradish peroxidase (0.20 mg/mL)	5	µL
-Sample	5	µL
Total volume	1	mL

The final concentrations of each reagent are 5 mM ABTS, 150 µg/mL glucose oxidase and 200 µg/mL horseradish peroxidase. The reactions was left for 5 minutes to complete oxidation of glucose as described in the following reaction



An increase in oxidized dye concentration was monitored at 420 nm. The glucose concentration in couple reaction was calculated using extinction coefficient of oxidized ABTS (ε_{420} = 4.2 x 10^3 $M^{-1}cm^{-1}$). The glucose concentrations (M) in samples was corrected by dilution factor of 200 as the following equation

$$[glu\cos e] = \frac{\Delta A_{420}}{4.2 \times 10^3} \times 200$$

Statistical Analysis

The result data was statistically test for normal distribution with one sample Kolmogorov-Smirnov test. As a result of the absence of normal distribution, statistical analysis was performed using the non-parametric Kruskal-Wallis at $p<0.05$ between the 3 groups by using SPSS software version 21 (IBM® SPSS® statistics North, Armonk, NY, U.S.A.).

CHAPTER IV
RESULTS

Micro CT Analysis

Each reconstructed isthmus model of the specimen was assigned to 1 of the following 4 categories with descriptions of specific morphology (Figure 7).

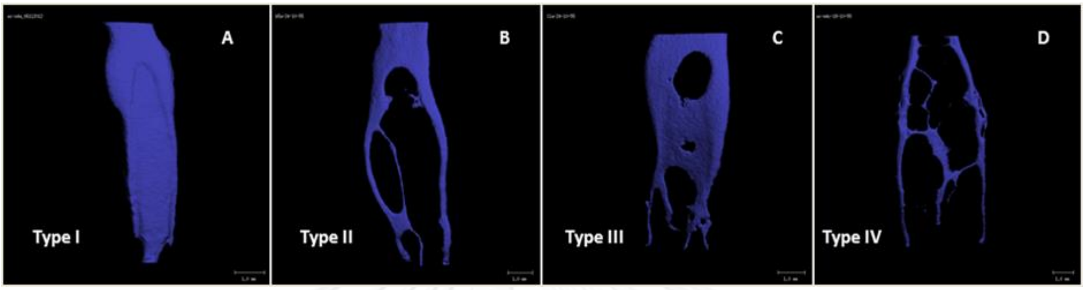


Figure 7 Reconstruction of the root canal system with isthmuses 4 categories. Type I: sheet connection, Type II: separate, Type III: mixed, Type IV: cannular connection

The teeth were randomly divided into 3 groups for different obturation techniques (Table 2). After root canal preparation, no significant differences were found amongst the experimental groups for the volume of root canal system ($p<0.05$). Mean of the root canal system volume of each group was presented in Table 2.

Table 2 Distribution of teeth for different obturation technique and mean of root canal volume

group	Type 1	Type 2	Type 3	Type 4	Total	Mean of root canal volume (mm^3)
Continuous wave	1	1	11	7	20	12.27 ± 3.12^a
Lateral condensation	1	2	10	7	20	10.60 ± 3.03^a
Single cone	1	2	10	7	20	12.03 ± 2.44^a

Value represents mean and standard deviation. Different letter in each column indicates statistically significant differences ($p<0.05$)

As the data is not normal distributed, median and range were used to present the percentage of filling material volume (% Filling) and voids (% void) in Table 3. There was no significant difference of amongst the three obturation techniques. In different isthmus types, type I and type II could not compare due to too small sample size. In type III and IV, were no significant difference amongst the three obturation techniques ($p<0.05$).

Table 3 The percentage of filling material and void in root canal system

group	n	% Filling	% Void
Continuous wave	20	99.11 (93.81-99.79)[a]	0.11 (0.02-0.50)[a]
Lateral compaction	20	98.37 (97.46-99.15)[a]	0.14 (0.06-0.50)[a]
Single cone	20	98.78 (95.09-99.54)[a]	0.14 (0.06-0.91)[a]

Value represents median and range. Different letter in each column indicates statistically significant differences ($p<0.05$)

The pattern of root filling materials penetration into isthmuses areas revealed that in type I isthmus, each group of the three obturation techniques had similar pattern. The root filling materials were massive at the wide and short isthmuses level and the presence of voids increased at the central part of the isthmuses in narrow and long isthmuses (Figure 8).

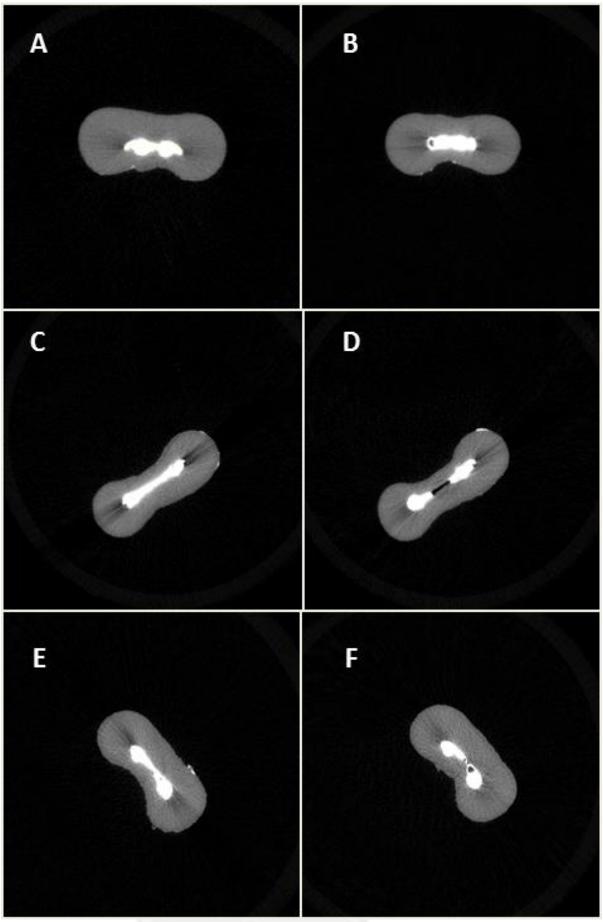


Figure 8 Two-dimensional (2D) slices of root canal representative for the pattern of root filling materials penetration into type I isthmuses at different level according to the morphology of isthmuses by three obturation techniques. (A-B) Continuous wave, (C-D) Lateral condensation, (E-F) Matched-taper single cone. The root filling materials were massive at the wide and short isthmuses (A,C,E), the presence of voids increased at the central part of the narrow and long isthmuses (D,F).

In type II, III and IV lateral compaction group had a higher presence of voids in the main root canals. All obturation techniques, demonstrated the increased of voids and variation in the volume of voids and distribution in the narrow isthmuses area (Figure 9). All the voids present were internal void.

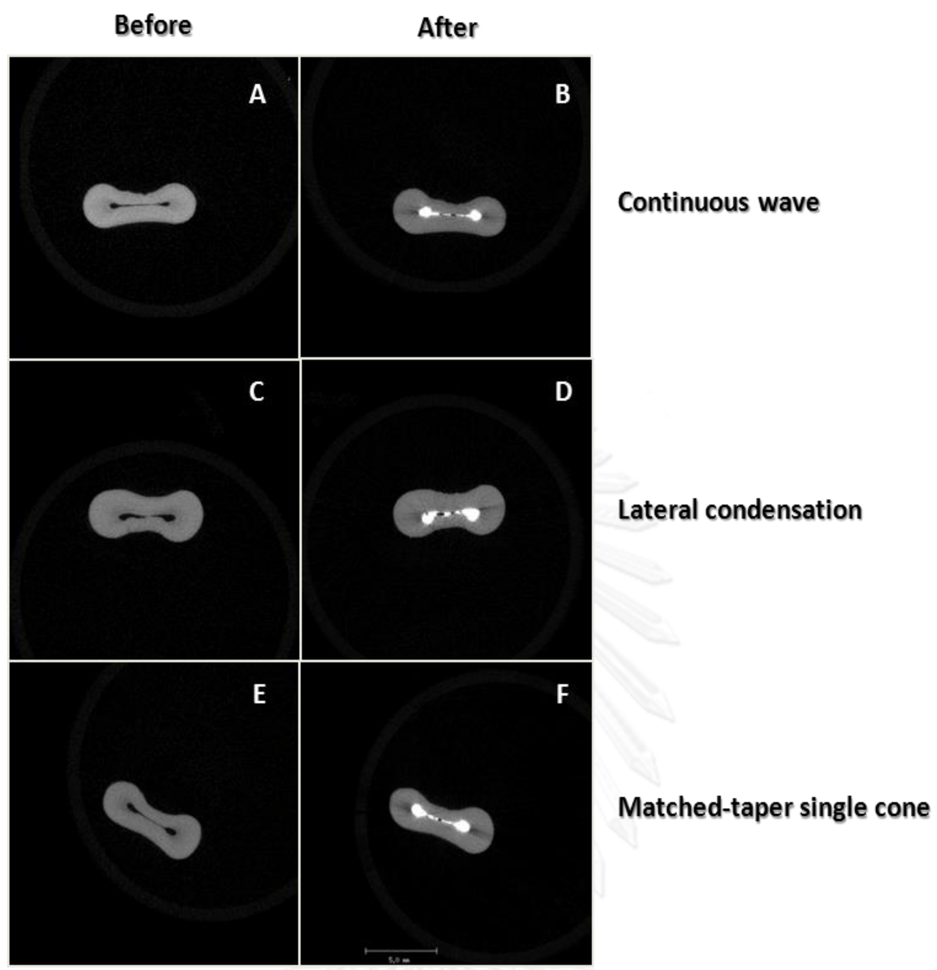


Figure 9 Two-dimensional (2D) slices of root canal representative for of a sample root canal system representative for type II, III, IV isthmuses: (A, C, E) Before instrumentation , (B, D, F) the pattern of root filling materials penetration into type II, III IV narrow isthmuses areas at the middle third level of three obturation techniques. (A, B) Continuous wave, (C, D) Lateral condensation, (E, F) Matched-taper single cone. The presence of voids, variation in the volume of voids and distribution in isthmuses area can observed in all techniques, lateral compaction had a higher presence of voids in the main root canal.

Glucose Filtration Leakage Test

No leakage was detected in any of the negative controls throughout the observation period. For the positive controls, gross leakage was detected on the first day, and increased rapidly over time (Figure 10).

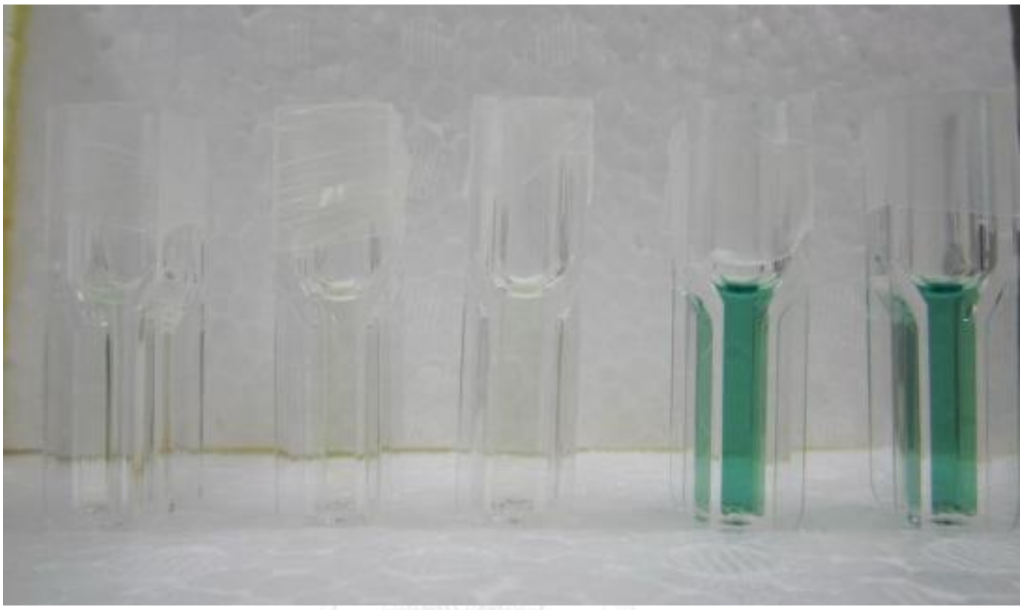


Figure 10 The three cuvettes on the left demonstrated detected color of the negative control and the two cuvettes on the right demonstrated detected color of positive control in glucose infiltration test

The amounts of the glucose leakage measured in all experimental groups were shown in Table 4. The glucose leakage could not be detected in any of the experimental group. Therefore, the data were not statistically analyzed and the correlation between the glucose filtration measurement and micro CT analysis also could not be computed because the glucose leakage was constant data.

Table 4 Glucose leakages (mM) in three experimental groups

Day	Continuous wave	Lateral condensation	Matched-taper single cone	Negative control	Positive control
1	0.00	0.00	0.00	0.00	*nd
7	0.00	0.00	0.00	0.00	*nd
14	0.00	0.00	0.00	0.00	*nd
21	0.00	0.00	0.00	0.00	*nd
28	0.00	0.00	0.00	0.00	*nd

*nd (not determinded) : over the limit of the observational detection due to high leakage of glucose

CHAPTER V
DISCUSSION

The quality of root canal fillings have been assessed using different methods. The ideal evaluation is to obtain maximal volume of the filling material with minimal voids. Previous studies have shown limitations in measuring the percentage of area occupied by filling materials and voids which were compensated by the analysis of root sections (*9, 20, 77*). This might not be accurate as some filling material might be lost in the process, and sectioning might cause smeared gutta-percha leading to underestimation of voids. Sectioning level is a random choice which may result in different finding at other levels (*77*).

Micro CT is one technique applied in many different fields of dentistry. Its advantages are rapid, accurate and non-destructive. With the help of a 3D-reconstruction, specimens can be both quantitatively and qualitatively examined. Moreover, the evaluation of the quality of root canal filling using the leakage of particles or solutions is considered to be another suitable method. Since it allows the travel of the tracer agent from the coronal part through the apical ends which mimic the clinical situation (*78*). During the experiments temperature and moisture were controlled similar to the oral environment. This study assessed the quality of root canal obturation using micro CT scanner and the determination of leakage to verify the correlation between the void volume and sealing ability after root canal obturaion.

Standardization of the specimens was necessary for comparative purpose. It is difficult to recognize an isthmus in the procedure of nonsurgical root canal treatments. Thus, in this study, configurations of the isthmuses were identified using micro CT analysis and classified according to the classification of Fan et al (*3*). The samples were randomly assigned equally to each experimental group (Table 2). After root canal preparation, there is no statistically significant difference of the root canal volume in each isthmus classification (Table 2). After root canal obturation using different techniques, there was no significant difference of the root filling volume in any obturation technique (Table 3).

At present, there was only one study by Marciano et.al giving the information about obturation of irregular canal with isthmuses (*20*). Their study evaluated the root canal filling in mandibular molars by different technique using 2D sections of roots without classifying isthmus types. The quality of root filling was evaluated both in teeth with and without isthmuses. Gutta-percha, sealer and void were determined to evaluate the quality of root filling. As both gutta-percha and sealer were root filling materials, there is no need to study separately. From the study of the sections, it was found that thermoplaticized gutta-percha techniques gave a higher percentage of filling material (gutta-percha with sealer) and less voids than the single cone and lateral condensation technique. The presence of isthmuses increased the voids only in the lateral condensation group. These results are in concordance with ours. In main root canal, lateral condensation technique gave a higher presence of voids than other techniques. The continuous wave technique gave a similar incidence of voids as the single cone technique in the main canal. This may be due to the root canal anatomy of small, narrow or tear-shaped cross-section of the isthmuses area. After root canal preparation with NiTi rotary file, the root canals were enlarge and rounded in cross-section. When the root canal was obturated using matched-taper cone with according size to the final file, it could produce full and dense filling in main canal (Figure 9). While the lateral condensation technique might produce the presence of void due to the use of spreaders. It could not produce a uniform mass and the spreader tracks were frequently present in the filling (*11, 77, 79*).

In isthmuses type I which had sheet connections morphology, similar presence of voids was produced though different obturation techniques were applied. In wide and short isthmuses, filling materials were able to greater penetrate than in narrow and long isthmuses. The presence of voids increased at the central part of the isthmuses with variation in the volume and distribution of voids. For obturation the isthmus type I teeth using continuous wave technique, the root filling materials had greater mass in isthmuses than the other two techniques (Figure 8). In this study, this type of isthmus was found in only three out of seventy specimens. Fan et al. (*3*) classified the morphology of isthmuses in first and second mandibular molars and found isthmus type I at 23% and 46% respectively. The low incidence may have little impact clinically. In non-sheet isthmuses (type II, III, IV), all obturation technique gave the presence of voids which varied in the volume and distribution. Most of isthmuses were narrow and long. It was difficult for the filling material to penetrate into these areas which were considered anatomical limitation that impact all the obturation techniques (Figure 8). These types of anatomy made it difficult to access during instrumentation, produced insufficient exposure to chemical irrigant, and could be obstructed by dentin debris during instrumentation (*27, 49*). These prevented root filling material to fully occupy the space, consequently. At present, the use of ultrasonics was suggested as an adjunct to clean the root canal. However, no technique has been found to achieve complete canal debridement (*35, 39*). However, the presences of voids in all teeth in this study were internal void. The voids inside filling materials could be considered less clinically relevant because they could not allow the passage of leakage to the periapex. (*15, 17*). Furthermore, the results of glucose filtration study were also reported supporting these findings (Table 4).

The method for analysis of endodontic microleakage that represented a modification of the previously reported protocol by Xu et al (*12*) had been used. As the samples are not destroyed, therefore it allows the sealing to be assessed after a long period of time. Glucose had a small molecular size and was a nutrient for bacteria. Bacteria that might survive in the root canal, could use glucose which penetrates into the apical part, multiply and potentially lead to endodontic failure (*12*). The glucose concentration assayed by glucose oxidase had a highly sensitive enzymatic reaction. Previous studies could detect the lowest glucose level in several levels for example, 0.003 mM (*65*), 0.2 mM (*80*). In this study, the lowest glucose level that could detect was 0.005 mM. Below this level, leakages could not be determined by absorbance in visible light region.

Sticky wax was rigid, fragile and might move which lead the leakage to occur. Areas between the experimental teeth and all connections including the root canal orifice were sealed by silicone sealant instead of sticky wax. In negative control, only the root canal orifice was covered, leaving the apical foramen exposed. These were different from other studies which the whole root were covered (*12, 72*). In other words, negative controls in this study could ensure that leakage occurred only through the root canal than via other routes (*81*).

Several studies compared the quality of root canal filling by leakage assessment using single-cone and lateral condensation techniques in round-shape, straight single root canal. They concluded that no significant difference between groups (*18, 19, 71, 72*). While thermoplastic techniques offer the potential of better sealing ability than lateral condensation techniques (*82*). In irregularly-shaped canals, Wu et al. (*17*) evaluated the quality of root filling by fluid filtration method in mesial root canals of lower molar. Single cone techniques with 25/.02 tapered gutta-percha cone and epoxy resin sealer was used to compare with lateral condensation technique. No significant difference during 7 days after obturation was found. Their assessment time was short and no classification of isthmuses was identified. Obturating irregularly canal with isthmuses is more difficult than obturating round, single canal. Thus, a thermoplastic technique was recommended as it offered a better adaptation of gutta-percha to the canal space (*9, 83*), more root filling volumes with minimal sealer which overtime, tend to dissolve (*84*). Up to now, leakage comparison of root filling in irregular canal with isthmuses using thermoplastic technique and other technique has not been reported.

The extent of sealer or gutta-percha into irregular area of canal is the important point in obturating the root canal with isthmuses. AH plus, an epoxy-resin sealer, was used in this study. The epoxy-resin sealer might have contributed to the penetration and sealing ability of these complex anatomy areas (*9, 85, 86*). The epoxy-resin sealer had the good penetration, adaptation, and adhesion properties (*87-91*). The flow was one of the main factors to influence the sealer tubular penetration and affected the seal of the root filling because there was an increase of the contact surface between filling material and dentin (*90*). Sealer placement into the root canal was also the main factor. A volume of sealer should be sufficient throughout the root canal. In this study, a lentulo spiral was used. The sealer was delivered into the root canal until extrusion was observed at the apical foramen to confirm sealer penetration inside the root canal (*92*). These sealer penetrations may provide the similar filling mass to all experimental groups.

This study suggested that different obturation techniques had no impact on the quality of obturation in irregular canals with isthmuses. Matched-taper single cone technique with epoxy-resin sealer (AH plus) is easy to learn, cost efficiency and able to create root filling area comparable to lateral condensation technique and thermoplasticized gutta-percha technique. Matched-taper single cone technique might be used as an alternative technique for root canal obturation in irregular canal with isthmuses.

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APPENDIX

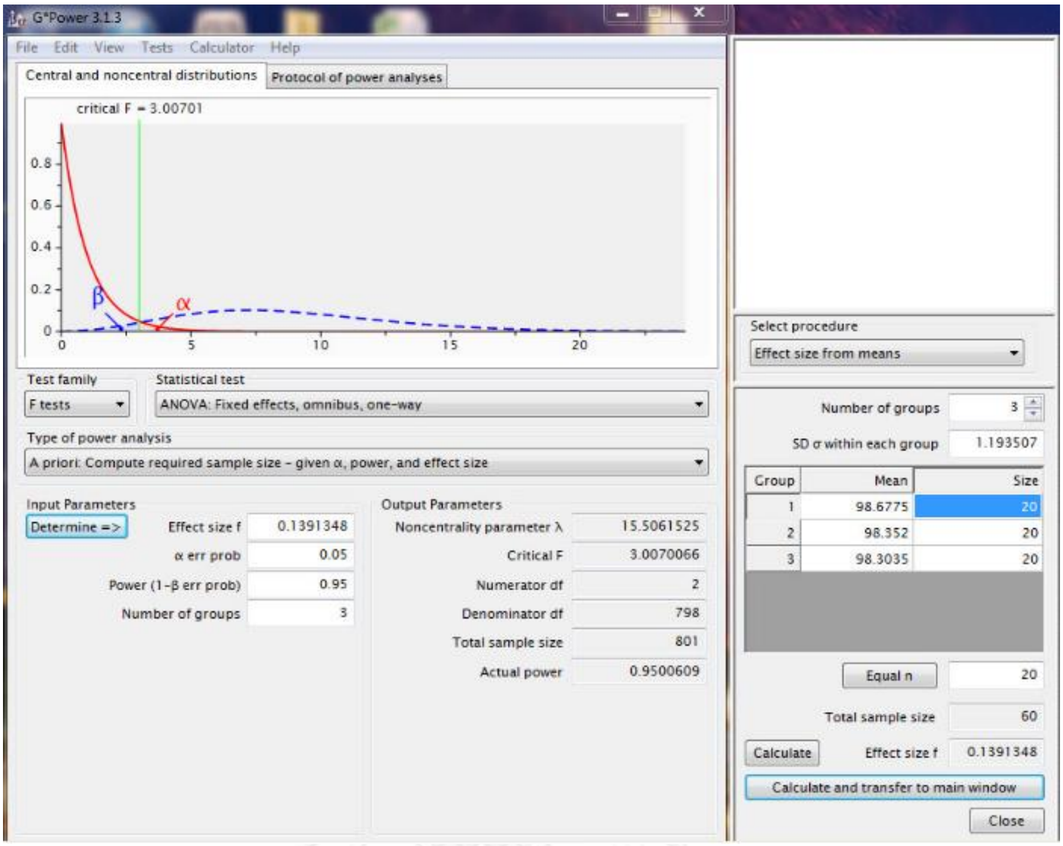

Figure 11 The desired sample size was calculated after root canal obturation because of specimen limitation in the pilot study by using G*Power version 3.1.3 (G*Power, Heinrich-Heine-University, Düsseldorf, Germany). The results showed that the desired sample size were 20 teeth per group.

Table 5 Volume of root canal, filling material and voids by continuous wave technique

No	Canal volume (mm^3)	Voids (mm^3)	(%) Filling	(%) Voids
1	12.96	0.07	99.46	0.54
2	8.21	0.17	97.93	2.07
3	13.03	0.20	98.47	1.53
4	13.11	0.14	98.93	1.07
5	13.61	0.34	97.50	2.50
6	14.14	0.18	98.73	1.27
7	9.57	0.02	99.79	0.21
8	17.33	0.04	99.77	0.23
9	8.93	0.05	99.44	0.56
10	8.45	0.06	99.29	0.71
11	9.56	0.18	98.12	1.88
12	10.69	0.08	99.25	0.75
13	13.47	0.10	99.26	0.74
14	12.33	0.24	98.05	1.95
15	6.84	0.05	99.27	0.73
16	16.32	0.22	98.65	1.35
17	11.51	0.12	98.96	1.04
18	6.44	0.04	99.38	0.62
19	8.08	0.50	93.81	6.19
20	7.84	0.04	99.49	0.51

Table 6 Volume of root canal, filling material and voids by lateral condensation technique

No	Canal volume (mm^3)	Voids (mm^3)	(%) Filling	(%) Voids
1	10.51	0.26	97.53	2.47
2	12.90	0.12	99.07	0.93
3	8.99	0.17	98.11	1.89
4	10.95	0.23	97.90	2.10
5	14.06	0.12	99.15	0.85
6	7.40	0.07	99.05	0.95
7	9.59	0.12	98.75	1.25
8	7.01	0.14	98.00	2.00
9	8.06	0.19	97.64	2.36
10	11.37	0.28	97.54	2.46
11	12.90	0.27	97.91	2.09
12	7.95	0.08	98.99	1.01
13	11.00	0.23	97.91	2.09
14	12.39	0.12	99.03	0.97
15	10.23	0.14	98.63	1.37
16	11.59	0.12	98.96	1.04
17	10.23	0.26	97.46	2.54
18	19.91	0.5	97.49	2.51
19	8.55	0.1	98.83	1.17
20	6.60	0.06	99.09	0.91

Table 7 Volume of root canal, filling material and voids by single cone technique

No	Canal volume (mm^3)	Voids (mm^3)	(%) Filling	(%) Voids
1	10.43	0.14	98.66	1.34
2	11.63	0.36	96.90	3.10
3	14.72	0.40	97.28	2.72
4	10.10	0.11	98.91	1.09
5	12.21	0.14	98.85	1.15
6	14.55	0.10	99.31	0.69
7	10.57	0.11	98.96	1.04
8	12.99	0.06	99.54	0.46
9	15.23	0.12	99.21	0.79
10	11.29	0.42	96.28	3.72
11	13.17	0.17	98.71	1.29
12	13.48	0.25	98.15	1.85
13	18.54	0.91	95.09	4.91
14	14.53	0.46	96.83	3.17
15	11.89	0.20	98.32	1.68
16	13.94	0.15	98.92	1.08
17	9.60	0.10	98.96	1.04
18	10.17	0.08	99.21	0.79
19	8.52	0.12	98.59	1.41
20	9.88	0.06	99.39	0.61

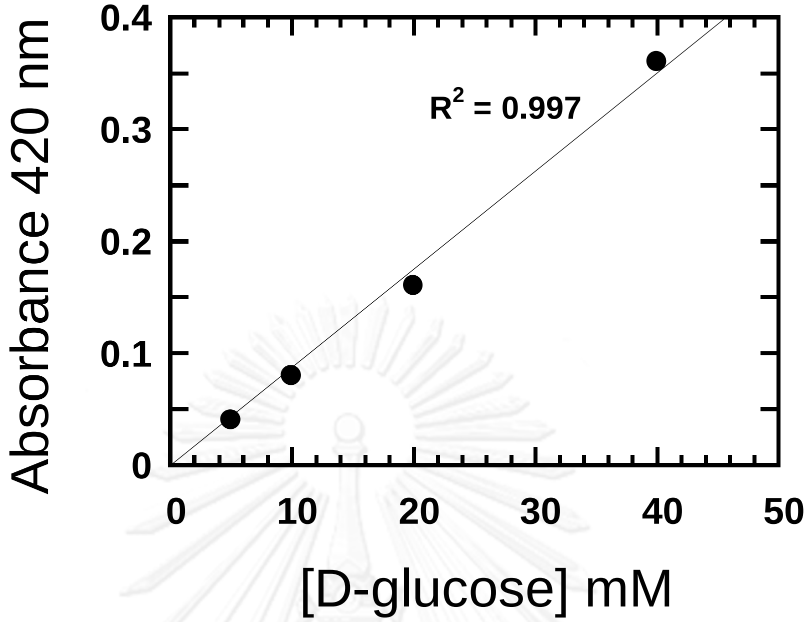

Figure 12 Glucose standard curve plotted between glucose concentration (X axis) and absorbance at 420 mm (Y axis). R^2 = 0.997.

Statistical Analysis

Table 8 Normal distribution test of canal volume using One KS test

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
vertical	.141	20	.200*	.953	20	.408
lateral	.125	20	.200*	.896	20	.034
single	.119	20	.200*	.955	20	.449

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

Lilliefors Significance Correction

The result showed that data was normal distribution (p>0.05) in all obturation techniques. Thus, One-way Anova test was used to analyze in the volume of the prepared root canals.

Table 9 ONEWAY-ANOVA test of canal volume

Oneway

[DataSet1]

Test of Homogeneity of Variances

test

Levene Statistic	df1	df2	Sig.
.884	2	57	.419

ANOVA

test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.887	2	16.443	1.981	.147
Within Groups	473.249	57	8.303		
Total	506.136	59			

Table 10 Data descriptive for filling material by continuous wave technique

			Statistic	Std. Error
ver		Mean	98.6775	.29337
	95% Confidence Interval for Mean	Lower Bound	98.0635	
		Upper Bound	99.2915	
		5% Trimmed Mean	98.8861	
		Median	99.1050	
		Variance	1.721	
		Std. Deviation	1.31198	
		Minimum	93.81	
		Maximum	99.79	
		Range	5.98	
		Interquartile Range	1.22	
		Skewness	-2.912	.512
		Kurtosis	10.326	.992

Table 11 Data descriptive for filling material by lateral condensation technique

			Statistic	Std. Error
lat		Mean	98.3520	.14548
	95% Confidence Interval for Mean	Lower Bound	98.0475	
		Upper Bound	98.6565	
		5% Trimmed Mean	98.3572	
		Median	98.3700	
		Variance	.423	
		Std. Deviation	.65062	
		Minimum	97.46	
		Maximum	99.15	
		Range	1.69	
		Interquartile Range	1.31	
		Skewness	-.109	.512
		Kurtosis	-1.817	.992

Table 12 Data descriptive for filling material by single cone technique

			Statistic	Std. Error
sin		Mean	98.3035	.26795
	95% Confidence Interval for Mean	Lower Bound	97.7427	
		Upper Bound	98.8643	
		5% Trimmed Mean	98.4133	
		Median	98.7800	
		Variance	1.436	
		Std. Deviation	1.19830	
		Minimum	95.09	
		Maximum	99.54	
		Range	4.45	
		Interquartile Range	1.65	
		Skewness	-1.411	.512
		Kurtosis	1.366	.992

Table 13 Normal distribution test of filling material using One KS Test

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
ver	.198	20	.038	.681	20	.000
lat	.180	20	.090	.850	20	.005
sin	.244	20	.003	.829	20	.002

a. Lilliefors Significance Correction

The result showed that data was not normal distribution (p>0.05) in all obturation techniques. Thus, Kruskal Wallis test was used to analyze the percentage of filling material.

Table 14 Kruskal-Wallis Test

Ranks

	treatment	N	Mean Rank
filling_percentage	ver	20	37.63
	lat	20	25.23
	sin	20	28.65
	Total	60	

Kruskal-Wallis Test

	filling_percentage
Chi-Square	5.380
df	2
Asymp. Sig.	.068

a. Kruskal Wallis Test

b. Grouping Variable: treatment

VITA

Miss. Tanawan Manwiwattanakul was born on April 7, 1980 in Chiangmai. She graduated from the Faculty of Dentistry, Chiang Mai University in 2003. After that, she entered the governmental service by taking a position as a dentist at dental unit, Lomkao Crown Prince Hospital, Phetchaboon. After 3 years of service there, she took a position at Pasang Hospital, Lamphun and worked there for 1 year. From 2008 until now Miss. Tanawan Manwiwattanakul has been working for the Department of Dentistry at Lamphun Hospital.