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# COMPARISON OF MICROLEAKAGE OF TWO RETROFILLING TECHNIQUES

Flight Lieutenant Tippawan Inthararith



A Thesis Submitted in Partial Fulfillment of the Requirements

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Department of Operative Dentistry

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Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master 's Degree

W. Tasachan ..... Dean of the Faculty of Dentistry

(Associate Professor Wacharaporn Tasachan)

THESIS COMMITTEE

Sirivimol Srisawasdi ..... Chairman

(Assistant Professor Sirivimol Srisawasdi, Ph.D.)

Piyanee Panitvisai ..... Thesis Advisor

(Associate Professor Piyanee Panitvisai, M.D.Sc (Endodontics))

..... Examiner

(Associate Professor Mansuang Arksornnukit, Ph.D.)

Peraya Sur ..... External Examiner

(Assistant Professor Peraya Surapipongpuntr, Ph.D.)

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วัตถุประสงค์ของงานวิจัยนี้คือเพื่อศึกษาเปรียบเทียบการรั่วซึมของเทคนิคการอุดย้อนปลายรากชนิดใช้วัสดุเอ็มทีเอกับชนิดใช้วัสดุเรซินคอมโพสิตชนิดไหลแผ่เป็นวัสดุอุดย้อนปลายรากฟัน ทั้งกรณีที่มีและไม่มีการปนเปื้อนด้วยเลือด โดยใช้ฟันหน้าบนของมนุษย์ 68 ซี่ แบ่งรากฟันออกเป็น 4 กลุ่มการทดลอง (กลุ่มละ 15 ราก) และ 2 กลุ่มควบคุม (กลุ่มละ 4 ราก) สองกลุ่มการทดลองแรกเตรียมปลายรากฟันด้วยหัวอัลตราโซนิกส์ลึก 3 มิลลิเมตร แล้วอุดย้อนปลายรากด้วยวัสดุเอ็มทีเอ ทั้งแบบที่มีและไม่มีการปนเปื้อนด้วยเลือด อีกสองกลุ่มการทดลองเตรียมปลายรากฟันด้วยหัวกรอรูปกลมกรอปลายรากฟันให้มีรูปร่างเว้าเล็กน้อย อุดย้อนปลายรากฟันด้วยวัสดุเรซินคอมโพสิตชนิดไหลแผ่ ทั้งแบบที่มีและไม่มีการปนเปื้อนด้วยเลือด ประเมินการรั่วซึมโดยใช้สีเมทธีลีนบลูในระบบสุญญากาศและวิธีดายเอ็กซแทร็กชั่น วิเคราะห์ผลโดยใช้สถิติวิเคราะห์ความแปรปรวนสองทางที่ระดับนัยสำคัญ 0.05 ผลการศึกษาพบว่ากลุ่มการทดลองที่ใช้เทคนิคเตรียมปลายรากฟันให้มีรูปร่างเว้าเล็กน้อย แล้วอุดย้อนปลายรากฟันด้วยวัสดุเรซินคอมโพสิตชนิดไหลแผ่ แบบที่ไม่มีการปนเปื้อนด้วยเลือด มีการรั่วซึมของสีน้อยกว่ากลุ่มทดลองอื่นอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 การรั่วซึมของการอุดย้อนปลายรากฟันด้วยวัสดุเอ็มทีเอและวัสดุเรซินคอมโพสิตชนิดไหลแผ่ ขึ้นกับเทคนิคการอุดย้อนปลายรากและปัจจัยของการปนเปื้อนด้วยเลือด โดยเทคนิคเตรียมปลายรากฟันให้มีรูปร่างเว้าเล็กน้อย แล้วอุดย้อนปลายรากฟันด้วยวัสดุเรซินคอมโพสิตชนิดไหลแผ่ แบบที่ไม่มีการปนเปื้อนด้วยเลือด มีการรั่วซึมน้อยที่สุด



ภาควิชาทันตกรรมหัตถการ ..... ลายมือชื่อนิสิต ..... ทิพวัลย์ อินทรฤทธิ์

สาขาวิชาวิทยาเอ็นโดดอนต์ ..... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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KEYWORDS : BLOOD CONTAMINATION / FLOWABLE RESIN COMPOSITES / MTA: MINERAL TRIOXIDE AGGREGATE / SEALING ABILITY

TIPPAWAN INTHARARITH : COMPARISON OF MICROLEAKAGE OF TWO RETROFILLING TECHNIQUES. THESIS ADVISOR : ASSOCIATE PROFESSOR PIYANEE PANITVISAI, 97 pp.

The purpose of this study is to compare the dye leakage of root-end preparation technique using MTA and root-end preparation technique using flowable resin composite with and without human blood contamination. Sixty-eight human upper anteriors were collected and randomly divided into four experimental (n=15) and two control groups (n=4). Two experimental groups of class I root-end preparation were retroprepared with an ultrasonic tip (KiS-1D) to the depth of 3 mm and filled with MTA with and without blood contamination. Another two groups of concave cavity root-end preparation were filled with flowable resin composite with and without blood contamination. The leakage of dye was evaluated by dye vacuum penetrating test and dye extraction method. Two-way ANOVA was used to test for significant differences of the leakage among the groups of specimens at the significant level of 0.05. The concave cavity root-end preparation filled with flowable resin composite without blood contamination displayed significantly less leakage ($p<0.05$) than all other groups. Leakage of retrofillings using MTA and flowable resin composite was affected by both the root-end preparation techniques and the blood contamination. The concave cavity root-end preparation filled with flowable resin composite without blood contamination displayed the least leakage.

Department : Operative dentistry ..... Student's Signature : Flt. Lt. Tippawan Intharanith

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

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# CHAPTER I

# INTRODUCTION

## Background of present study

The degree of success of root canal therapy has been reported between 45-98.7% [1-3]. Harty et al. [4] reported that the majority of failures of non-surgical endodontic procedures were because of inadequate apical seal. The preferred treatment of failing endodontic cases is non-surgical retreatment. However the procedure may not achieve due to the complexity of root canal systems and the presence of physical barriers such as post and core restoration, separated instruments.

When nonsurgical root canal retreatment has been unsuccessful or is inadvisable, surgical root canal treatment is often carried out to save teeth. Its success is reported to range between 58-96% [5-7]. This procedure routinely consists of root-end exposure of the involved apex, resection of its apical end, a root-end preparation, and the placement of a root-end filling material to seal the root canal system.

The apical seal is the single most important factor affecting success in surgical endodontics [4]. The placement of a root-end filling material during periapical surgery is a procedure of paramount importance to seal the root canal that inhibits the leakage of residual irritants from the root canal into the periradicular tissues [8]. Several materials have been evaluated for used as root-end filling materials. They include amalgam, gutta-percha, zinc oxide-eugenal cements, composite resins, glass ionomers, polycarboxylate cements, ethoxybenzoic acid (EBA) cament, and mineral trioxide aggregate (MTA), but none of them has been demonstrated to be free from limitations [8, 9].

Ideal materials for sealing root-end cavities should be: [9-11]

- Easy to manipulate
- Radiopaque
- Non absorbable
- Well tolerated by periradicular tissue

- Promote healing and regeneration
- Adhere and seal the root canal system in three dimensions
- Nontoxic
- Biocompatibility
- Dimensional stability
- Moisture insensitivity

There are two root-end preparation techniques which were reported in the literature depended on the types of root-end filling materials:

1. **Class I root-end preparation**

The root-end filling materials used with this technique are amalgam, reinforced zinc oxide and eugenol cements (IRM), Super-ethoxy benzoic acid (EBA) cement, mineral trioxide aggregate (MTA) and etc. Originally amalgam was the material of choice for root-end fillings for many years; however, it has several disadvantages are associated with amalgam, including marginal leakage, expansion characteristics, potential of corrosion, moisture sensitivity, electrochemical reactions, staining of the mucosa, scattering of particles, release of free mercury toxicity and the need for an undercut root-end preparation [11-16]

Reinforced zinc oxide and eugenol cements (IRM) and Super-ethoxy benzoic acid (EBA) cement, have gained popularity over the years. IRM is reinforced with polymethyl-methacrylate while Super-EBA cement is reinforced with alumina. Numerous studies have reported favorable results in sealing ability, marginal adaptation, tissue tolerance, and clinical success for both IRM and Super-EBA [5, 17]. One disadvantage of using IRM is the way it handles in clinical situations. IRM must be placed in the root-end preparation in one segment because it does not self-adhere well and cannot be added in small increments. Super-EBA can be placed in the root-end preparation and condensed incrementally because it is self-adhesive. The main disadvantage of using Super-EBA is that mixing is very technique-sensitive. The variations of temperature and humidity alter its manipulative properties [18]. Both IRM and

Super-EBA are soluble in different media, including serum and more soluble than glass ionomer and amalgam [18, 19].

Torabinejad and colleagues have published numerous articles advocating MTA [20-25]. The principal compounds of MTA are tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide, as well as a few other mineral oxides. The powder consists of fine hydrophilic particles, which set in the presence of moisture. MTA's characteristics have been investigated, including its physical and chemical properties, sealing ability, marginal adaptation, cytotoxicity, antibacterial effects, and histologic evaluation in animal studies. Even though mineral trioxide aggregate (MTA) is reputed to possess many ideal characteristics [26], some clinicians claim to have difficulties in handling when filling a root-end prepared root cavity. It is extremely difficult to press inside the root, and it tends to easily be washed out from its seat [27]. Furthermore MTA is expensive and has a very long setting time [28].

2. Concave cavity root-end preparation

The root-end filling materials used with this technique are adhesive materials such as glass ionomer (GI) cement, dentin bonding agent as a sealant, and resin composite with dentin bonding agent. Glass ionomer cement has been proposed as a root-end filling material because of chemical adherence to both enamel and dentin. However, clinically, the plasticity and stickiness of glass ionomer cement impede condensation into the root-end cavity, and it is extremely sensitive to moisture [29-32]. In recent years, new generations of composite resins and dentin bonding agents have been introduced in restorative dentistry. This material has shown remarkable clinical success, however bonding systems are technique and moisture sensitive. It is plausible that a flowable, light-cure composite resin combined with a high output of curing light in a 10-second duration could be used to fill any root-end preparation effectively. Specifically, the short curing time required could minimize the adverse effect of moisture and hemorrhagic contamination [17]. Successful use of resin composite as a root-end filling material is a matter of obtaining permanent good retention without gaps between filling and root substance. A special designed resin composite filling was applied using a concave preparation of the apical root end around the canal, this method may provide

a tight seal of the apical end, giving a higher success rate than that of retrograde amalgam [14, 15].

Guttman and Harrison [33] suggested that, if proper nonsurgical root canal treatment is unfeasible, then root-end cavity depth must be deep enough to eliminate the possible leakage of bacteria or necrotic debris from the main canal or tubules [33]. The use of a more transverse root-end resection combined with surgical ultrasonics to obtain class I root-end preparation results in a high-quality, root-end preparation. However, Abedi et al. [34] demonstrated that root-end preparations with ultrasonic tips 3 mm deep create more microfractures than root-end preparation with burs. Another obvious concern is the decreased crown-root ratio following the class I root-end preparation. A concave cavity root-end preparation filled with resin composite as root-end filling material would decrease this possibility of microfractures while preserving the crown-root ratio [35-37]. In addition, the preparation technique offers treatment to otherwise inaccessible or fragile roots with root-end filling and is also easier to perform clinically than the commonly used root-end prepared cavities [38].

Resin composite is readily available in clinical practice, less expensive than MTA, and provide better seal than other root-end filling materials [39]. When a concave cavity root-end preparation was performed, resin composite may be considered as alternative root-end filling material to MTA.

## Research questions

- Are there differences on leakage of root-end preparation techniques using MTA and root-end preparation technique using flowable resin composite with and without human blood contamination?

## Research objectives

- To **compare** the leakage of root-end preparation technique using MTA and root-end preparation technique using flowable resin composite by dye penetration method and dye extraction method.

- To **compare** the leakage of root-end preparation technique using MTA and root-end preparation technique using flowable resin composite after contamination with human blood by dye penetration method and dye extraction method.

## Hypothesis

- The null hypothesis is that there are **no differences on leakage** between the two root-end preparation techniques.

- The null hypothesis is that there are **no differences on leakage** between the two root-end preparation techniques after contamination with human blood.

## Experimental Design

- **Sample:** Human radicular dentin surface of root-end preparation in straight, single root upper anterior teeth which have complete root formation, no crack or fracture, no root caries, no root resorption.

- **Variables & Outcome Measurement**

1. Independent variables

*Techniques of root-end preparation:*

- Perpendicular root-end resections for 3 mm., 3 mm. deep of ultrasonic root-end preparation and retrofill with Mineral trioxide aggregate (ProRoot™, Dentsply, Tulsa, OK, USA)
- Perpendicular root-end resections for 3 mm., slightly concave cavity root-end preparation and retrofill with flowable resin composite (Tetric® flow, Ivorclar Vivadent, Shaan, Liechtenstein)

*Blood contamination*

2. Dependent variables:

Dye penetration observed under stereomicroscope and dye absorbance measured on a spectrophotometer at 580 nm.

- **Outcome Measurement**

1. Dye penetration method

A vertical sectioning technique is used to visualize the amount of dye penetration. Dye penetration depth is evaluated in each root using a stereomicroscope x30 magnification. The greatest depth of dye penetration was used to determine. The results in each group are calculated and compared statistically with a significance level of $p<0.05$.

2. Dye extraction method

The specimens are dissolved in acid and the dye absorbance is recorded by the use of a spectrophotometer. The average absorbance in each group is calculated and compared statistically with a significance level of $p<0.05$.

## Limitations of research

The experimental design is an *in vitro* study using human extracted teeth. Effects of the interventions in this experiment cannot be completely represented to the populations.

## Keywords

Blood contamination, Flowable resin composites, MTA: Mineral Trioxide Aggregate, Sealing ability

## Benefits

- To evaluate the sealing ability of two root-end preparation techniques and the sealing ability of two root-end preparation techniques after contamination with human blood

- To obtain basic knowledge for further studies in clinical situation.

## Research design

Laboratory experimental research

## Ethical consideration

There was no ethical problem because human teeth were obtained from upper anterior teeth which extracted for clinical reason with patient's informed consent at the Department of Oral Surgery, Faculty of Dentistry, Chulalongkorn University.

## Steps to propose research

1. Preparing the study
    a. Literature review and study data
    b. Planning and design research
    c. Pilot study
    d. Form proposal
    e. Present proposal
2. Conduct research and collect data
3. Analyze data and assess results
4. Report the results
    a. Prepare report
    b. Present the results to the research committee

# CHAPTER II

## LITERATURE REVIEW

As most endodontic failures are due to inadequate cleaning and shaping and/or insufficient obtulation, the placement of root-end filling materials in the root which require root-end resection is recommended [40-42]. The purpose of inserting a root-end filling material is to provide an apical seal that inhibits the leakage of irritants from the root canal system into the periradicular tissues [43]. Numerous materials have been suggested as root-end filling materials including:

- Gutta percha
- Zinc oxide eugenol
- Cavit®
- Resin composite
- Gold foil
- Glass ionomer
- Amalgam
- Super EBA
- IRM
- Polycarboxylate cement
- Zinc phosphate cement
- Cryanoacrylate
- Diaket
- MTA
- Teflon

Only some materials have been commonly used in clinical practice within the past ten years with class I root-end preparation. These materials are zinc oxide-eugenol cement (IRM and superEBA), mineral trioxide aggregate (MTA). Meanwhile glass ionomer cement, resin composite, resin glass ionomer hybrids were used with

concave root-end preparation. However, none of the materials has most of the ideal properties of a root-end filling material.

## Root-end cavity preparation

The cause of surgical endodontic retreatment is the residual bacteria at apical area of the root. Generally apical resections at 3 mm are most effective for eliminating potential causes of failure, then canals are sealed with retrograde fillings [28].

### Class I root-end preparation

Conventionally, a root-end cavity is prepared by means of a round or inverted cone bur in a micro-handpiece. However, this technique of apical preparation has a number of limitations. The axis of preparation may not parallel to root canal, risk of perforation of lingual dentin wall, insufficient depth of root-end cavity, requires a root-face bevel of $45^{o}$ or more which enlarged area of patent dentinal tubules and reduced surgical site visibility [44]. Alternative root-end preparation techniques like slot preparation using a fissure bur or reverse instrumentation using modified K-files or Hedstrom files in special holders or hemostats were advocated in the 1980s [45-47]. These methods have circumvented some of the limitations inherent to the conventional technique, but have not become standard procedures in periradicular surgery [44].

Richman [48] reported the first documented application of ultrasound in periradicular surgery in 1957 using an ultrasonic chisel to cut bone and resects apical tooth tissue. The first root-end preparation by means of modified ultrasonic inserts following apicoectomy is attributed to Bertrand in 1976 [49]. Flath and Hicks [50] also reported the application of ultrasonics for root-end cavity preparation in two case presentations. Ultrasonic instrumentation was found to produce less enlargement of canal than the conventional technique. Preparation were well centered and thicker dentin walls were maintain in ultrasonically prepared teeth compared to bur preparations [51, 52].

O'Conner et al. [16] investigated the apical leakage of amalgam and EBA root-end fillings with two methods of root-end preparation. Teeth were randomly placed into four groups. Two groups received perpendicular root-end resections, 3 mm deep ultrasonic root-end preparations, and either amalgam or Super EBA root-end fillings. The

other two groups received beveled root-end resections, 3 mm deep micro-handpiece preparations, and either amalgam or Super EBA root-end fillings. Microleakage was assessed at 4 months using methylene blue dye. Statistical analysis showed that, regardless of technique, Super EBA leaked significantly less than amalgam. The ultrasonic preparations showed less leakage than bur preparations, however, there was no significant difference between the two root-end resection and preparation techniques.

Lloyd et al. [53] analyzed the apical seal of amalgam and Daiket as root-end filling materials following two root-end preparation techniques. Amalgam fillings exhibited significantly more linear dye leakage than Daiket irrespective of the preparation techniques. No significant difference in leakage was observed between the root-end preparations made with ultrasonic retrotips compared to cavities prepared by burs.

The study by Chailertvanitkul et al. [54] evaluated the coronal leakage of EBA root-end fillings following two root-end preparation techniques using bacterial leakage model. After 90 days, the ultrasonic group showed significantly fewer specimens with complete leakage than the bur group. This study demonstrated significantly less (coronal) leakage of root-end fillings following ultrasonic root-end preparation compared to bur preparation. Other leakage studies [16, 53] found no statistically significant differences between root-end preparation techniques within the limits of apical dye penetration studies.

Class I ultrasonic root-end preparation is popular and has been widely studied. However, there are some disadvantages of the ultrasonic root-end preparation technique. They are less canal debridement, cracking, chipping, difficult to have an ideal preparation and fracture of instrument.

*Less canal debridement*

Two studies reported difficulty in removing gutta-percha with ultrasonic instrumentation resulted in less canal debridement with some residual gutta-percha remaining on the wall [51, 55]. Gutta-percha remnants were detected on the walls of both

large and thin roots in most of the ultrasonically prepared cavities, regardless of the type of tip (Fig. 1) [56].

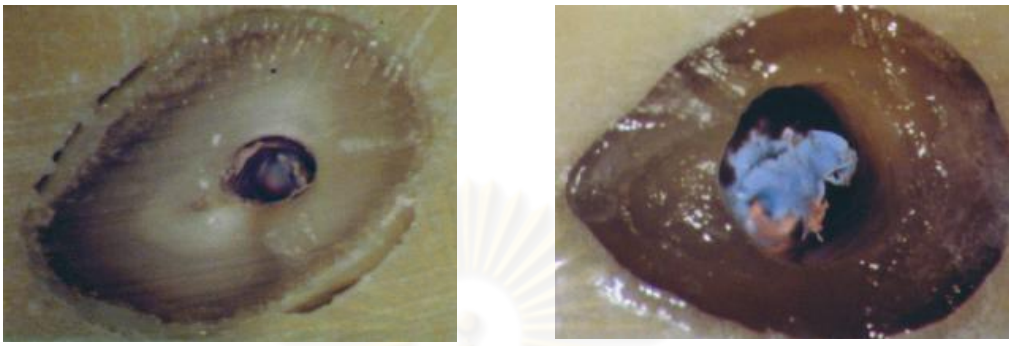


Fig. 1 Debris found in the cavities consisted of superficial dentinal chips and gutta-percha remnants.[56]

*Cracking*

The specific problem of root-end cracking as a result of ultrasonic preparation was first noted by Saunders et al. [57]. Dye leakage studies with teeth that have been cleared chemically have suggested that the use of ultrasonic instrumentation may result in an increased incidence of root face cracking, which could increase the possibility of apical leakage [54]. Another study [34] has illustrated that the cracking, especially in thin fragile areas, may be more prevalent with ultrasonic instrumentation (CT-2 and EIE ultrasonic tips) than with conventional rotary preparation. Cracking correlated with the remaining width of dentin walls, with 95% of cracks found in the thinnest part of cavity wall. When the remaining dentin walls were thinner than 1 mm 75% of ultrasonically prepared root-ends developed cracks. Whereas this phenomenon was never observed in bur preparations [34]. The different types of ultrasonic root end instruments, as well as the time of cavity preparation, are indicated to monitor whether these factors may cause root end fracture [34]. The experiments involving the ENAC system have reported that root-end cavity preparation with the ultrasonic tips required additional time and effort compared with the use of a bur; additional hand instrumentation was sometimes necessary [55, 58]. The significance of root-end cracking would seem to be increased susceptibility to root fracture, the inability to adequately

seal the root-end preparation, and the likelihood of additional sites of bacterial contamination [59].

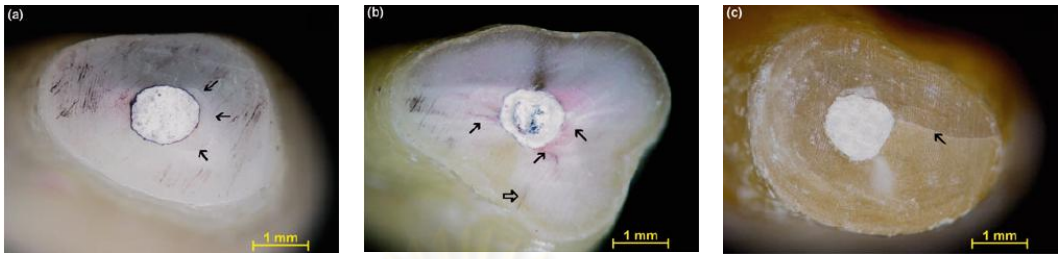



Fig. 2 (a) Intracanal crack with a semilunar pattern (thin arrow) originated within the canal and extends into dentin. (b) Extracanal crack (thick arrow) developed on the root surface and extends into the dentin. Synchronically three intracanal cracks (thin arrow) are also seen in a branching pattern. (c) Communicating crack (thin arrow) extends from the root surface to the canal. (Magnification x40) [60]

*Chipping*

Chipping of the cavity margins appeared to increase with power setting, this is likely to be the prominent factor in the degree and distribution of chipping observed (Fig. 3A). The significance of the cavity margin finish is unclear but it may effect the marginal seal produced when a root end filling material is placed [58]. Lloyd et al. [53] reported that bur preparations had significantly less chipping of the cavity margins than ultrasonic preparations. The possibility of a chipped, ragged margin increasing the likelihood of marginal flaking of the filling material and subsequent leakage must also be considered.

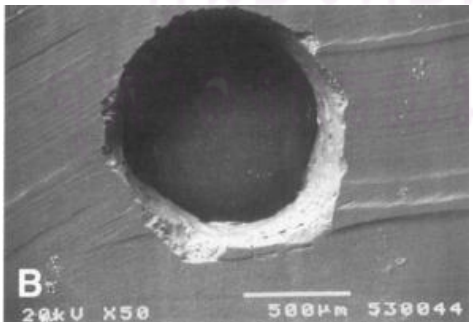



Fig. 3A Marginal chipping after ultrasonic preparation (magnification x50)

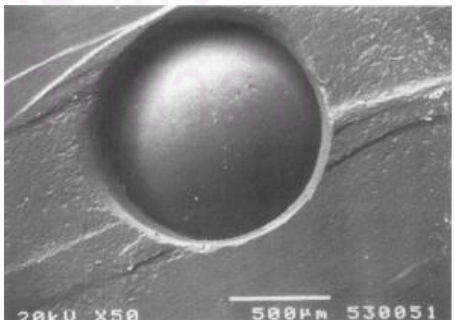



Fig. 3B Rotary bur prepared cavity that is lack of marginal chipping [58] (magnification x50)

*Sometimes difficult to have an ideal preparation*

According to Arens [61], the class I apical preparation must be parallel to the long axis of the root, 3 mm deep, centered in the root throughout its depth, and should include the entire apical root canal system. To accomplish these goals a significant amount of the apical bone is removed and the roots are resected and beveled toward the operator. This procedure is sometimes difficult or even impossible to execute for several reasons: the anatomical complexity of the apical portion of the root canal, root location, the flexibility of instruments, and the unavailability of enough apical bone or root structures.

*Fracture of instrument*

The problem of instrument fracture when ultrasonically driven microsurgical instruments were used for root-end cavity preparation is always occured. Angulation of retrotips increases the transverse ascillation and decreases the longitudinal ascillation, putting the greatest strain on the bend of the instrument. It is therefore recommended not to put excessive load on the activated retrotips [44]. Walmsley et al. [62] also suggested reducing the angulated design and producing thicker instruments to resist breakage. However the straighter design will restrict access to difficult to reach areas, and thicker instruments prevent instrumentation of fine root canals and isthmuses.

**Concave cavity root-end preparation**

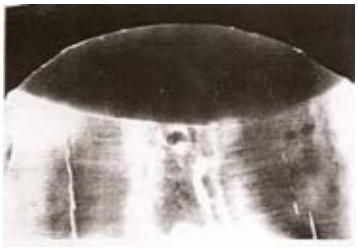
The cavity design is a slightly concave dissection of the apical part of the root. This root-end preparation technique using an adhesive material especially resin composite with dentin bonding agent as recommended by Rud et al. [63, 64]. The bonding capability permits the filling to be retained on an almost flat root surface without further preparation to a class I other cavity, as the one needed, when amalgam is used as filling material. It is an advantage that a class I other cavity preparation can be avoided. The filling may thus be placed on otherwise inaccessible roots in molars. Furthermore, gap formation, often found in a class I other cavity filled with resin composite, is avoided when the composite is placed on an almost flat surface [63].

The resected root surface was made slightly concave by using a round bur. This facilitated precise application of the composite to the central aspect of the resected root surface, without contaminating the surrounding tissues [64].

A concave cavity root-end preparation filled with resin composite as root-end filling material would decrease this possibility of microfractures while preserving the crown-root ratio [35-37]. In addition, the preparation technique offers treatment to otherwise inaccessible or fragile roots with root-end filling and is also easier to perform clinically than the commonly used root-end prepared cavities [38].

Clinically, in teeth receiving a previous root resection or teeth with short roots and long posts, where a 3 mm root-end cavity depth cannot be achieved the concave cavity preparation might be a better choice for root-end preparation than Class I root-end preparation. Insufficient length of remaining gutta-percha root filling has been shown to provide an un predictable seal [65]. A concave root-end preparation technique using a suitable adhesive material as root-end filling could also be less effective when the remaining length of the root filling apical of the post is short [66].

Ambus and Munksgaard [38] evaluated the ability of five different commercial dentin bonding agents to bond composite to concave root-end preparations. They concluded that all five dentin bonding systems yielded gap-free specimens. It has been shown that the size of marginal contraction gaps of composite fillings decreases by increasing the cavosurface angle from $90^{\circ}$ to $160^{\circ}$. Thus, the slightly curved surface (Fig. 4) with a cavosurface angle close to $180^{\circ}$ will prevent formation of contraction gaps between composite retrograde filling and bonding agent treated dentin [38]. This configuration produces far less interfacial stresses during polymerization contraction than that which would occur in a class I root-end preparation [67].



[38]

Fig. 4

The study of Platt and Wannfors which compared the treatment outcome of concave root-end preparation with a root-end filling of bonded compomer and dentin adhesive to a class I root-end preparation with a root-end filling of glass ionomer at 1 year reported that concave root-end preparation with a root-end filling of bonded compomer improved significant healing than another group. A concave root-end preparation technique using an adhesive material covering the whole surface of the denuded root could reduce apical leakage and enhance the possibility of healing [66].

Among the availability of root-end filling materials, composite resins and MTA have been shown to have superior sealing characteristics [68].

**Mineral Trioxide Aggregate (MTA)**

Mineral Trioxide Aggregate (MTA) was described for the first time in 1993 by Lee [69] and 1995 by Torabinejad [24]. MTA is a derivative of Portland cement used at Loma Linda University as a root-end filling material in surgical endodontic treatment [9, 20, 70]. Gray MTA has been patented and approved by Federal Drug Administration (FDA) in the USA. In 2002 a white version replaced the gray MTA in order to solve esthetic concern [71, 72].

The MTA powder consists of fine hydrophilic particles. The main constituents of this material are calcium silicate, bismuth oxide, calcium carbonate, calcium sulfate, and calcium aluminate. When mixed with sterile water, hydration of the MTA powder results in a colloidal gel that solidifies into a hard structure consisting of discrete crystals in an amorphous matrix. The crystals are composed of calcium oxide and the amorphous matrix which has the composition of 33% calcium, 49% phosphate, 2% carbon, 3% chloride, and 6% silica [21].

The physical and chemical properties of MTA has been investigated which pH, radiopacity, setting time, compressive strength and solubility of the material were compared with amalgam, superEBA and IRM [21]. The pH of MTA immediately after mixing was 10.2, rising to 12.5 at 3 hours after which it remained constant. Because MTA has high pH similar to calcium hydroxide cement, it is possible that the induction of hard tissue formation may occur following the use of MTA as a root-end filling material. The radiopacity was equivalent to 7.17 mm of aluminum which is more radiopaque than

conventional gutta-percha and dentin. It should be easily distinguishable on radiographs when used as a root-end filling material. The mean setting time was 2 h 45 min (± 5 min). The mean compressive strength was 40.0 ± 4.4 MPa at 24 h and 67.3± 6.6 MPa after 21 days. MTA initially had the lowest compressive strength among materials tested, but its value increased with time. The increase in compressive strength of MTA required the presence of moisture. However, the compressive strength value obtained for MTA is similar to those obtained for Super-EBA, IRM, and zinc phosphate. There was no significant in solubility among MTA and the others. [20].

The biocompatibility assessment of MTA encompassed *in vitro* cell culture techniques using either established cell lines or primary cell cultures. The results showed MTA to be biocompatible [73-78]. *In vivo* usage testing revealed less periradicular inflammation with MTA compared with amalgam [25, 79]

The sealing ability of MTA was also investigated using fluorescent dye and confocal microscopy [9], methylene blue dye [80] and bacterial marker [24]. Its marginal adaptation was assessed using electron microscopy [25]. The long term seal was measured over a 12-week [81] and 12-month period [82] using different fluid filtration methods. They all reported good results with MTA when ranked with other materials which mostly were amalgam, IRM and superEBA.

MTA originally designed for application in endodontic surgery as a root-end filling material and to seal the communications between the root canal system and the periodontium [9, 80, 83]. The other indications are vital pulp therapy [84-86], apexification [87, 88], reparation of root perforation [83, 89-91], internal bleaching and resorption repair.

*Advantages of MTA:*

- MTA is the hydrophilic cement which can set in the presence of water and calcium in its hydroxide stage can release when MTA mixed with water [92].
- MTA has been shown to be superior to other materials in terms of biocompatibility *in vitro* and *in vivo* [23, 76, 77, 79, 93, 94].
- MTA has good sealing ability [9, 24, 25, 82, 95-97]
- MTA enhancing regeneration of the periradicular tissues [87], including antimicrobial effect [22, 98].

- MTA has the radiopacity and dimensional stability.

  *Disadvantages of MTA:* [71].

- MTA has the extended setting time
- Expensive
- Difficulty in handle

Grey MTA (GMTA) has been associated with the occasional staining of teeth [99]. As a result, white MTA (WMTA) has recently been introduced as a more esthethically appealing material. The structure and physical characteristics of GMTA and WMTA that may account for the observed differences in cell growth, the surface of GMTA and WMTA may differ slightly in chemical composition. According to the manufacturer, the compositions of GMTA and WMTA are similar except WMTA does not contain tetracalcium aluminoferrite [100]. However, a recent study that analyzed the chemical differences of GMTA and WMTA found that WMTA contained significantly less borundum ($Al_2O_3$), periclase (MgO), and especially FeO compared to GMTA [101].

Holland et al. [102] subcutaneously exposed rat connective tissue to dentin tubes filled with GMTA and WMTA. They found that GMTA and WMTA had very similar biocompatibility. In a different in vitro study it was found that primary osteoblasts initially bound to the surface WMTA, but did not remain bound and visible by the end of a 13-day incubation period [103]. In this same study, cells growth on GMTA remained bound and visible at this 13-day endpoint. However, it was observed that the surface morphology of GMTA and WMTA are similar and human osteosarcroma cells grow equally well on these materials [71]. Parirokh et al. [104] examined the dental pulp responses in dogs to both types of MTA used as a pulp capping agent. Histological analysis was performed one and two weeks after treatment. Calcified bridge could be seen one week after treatment with both types of MTA, with no significant differences between the two treatments. The study of Oviir et al. [100] that evaluated and compared the effects of GMTA and WMTA on the proliferation of epithelial cells and cementoblasts. Cells were grown for 72 h on GMTA or WMTA that had been cure for 24 h or 12 days. The results indicated that WMTA (24-h and 12-day-cure) is more biocompatible than

GMTA (24-h and 12-day-cure). The high degree of biocompatibility with cementoblasts demonstrated in this study suggested that GMTA (12-day-cure) and WMTA (24-h and 12-day-cure) may significantly contribute to the regeneration of tissue after root perforation repair [100]. An in vitro study concerning repair of furcal perforations with MTA concluded that the two types of MTA showed no significant difference in preventing bacteria leakage [91]. In addition, the study of Stefopoulos et al. [105] compared the root canal sealing efficiency of white and grey MTA when used as apical barriers in teeth with open apices demonstrated that no statistically significant differences between the two types of MTA in sealing ability when tested with dye leakage model (basic fuchsine).

Resin composites

Resin composite is a blend of aromatic and/or aliphatic dimethacrylate monomer such as bis-GMA, triethylglycol dimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA). Resin composite materials have some desirable properties and may be considered for use as root-end filling materials. From systematic reviews of *in vitro* leakage of root-end filling materials, the results indicated that the most effective root-end filling material when measured by dye/ink penetration is resin composite which is more effective than glass ionomer cement, amalgam, and superEBA [39]. While *in vivo* review of effectiveness determined by reduction in periapical radiolucency, the results showed that resin composite and superEBA were more effective than amalgam [106].

An in vitro study indicated less bacterial microleakage with dentine-bonded composite resin root-end fillings when compared with amalgam and Super EBA [107]. In a clinical and radiographic study of 388 operated teeth, Rud et al. (1989) compared amalgam and composite resin with a bonding agent as root-end seals. After 1 year the total number of healed cases increased 16% (statistically significant) using bonded composite rather than amalgam root-end seals.

The restorative composites have a relatively high modulus of elasticity, and it has been suggested that this high stiffness contributes to their inability to compensate for contraction stress during polymerization. This can lead to bond failure, resulting in microleakage [108].

Flowable resin composite was introduced to the dental profession in late 1996. These flowable composites had as their principal characteristic a viscosity that allowed them to be injected into a cavity preparation [109]. It is purported to offer higher flow, better adaptation, easier insertion and greater elasticity than restorative composite. Depending on the type of filler used, the majority of flowable resin composite are filled between 41-53% by volume which translates into 56-70% by weight [110]. The filler content was found to be 20% to 25% less than the universal composite materials [108]. Because of its low filler content, the flowable resin composite presents the flow characteristics compared to a restorative resin composite. As a result, enhanced wetting of the tooth surface and a low modulus of elasticity can be achieved. Two clinical benefits are expected: reduction of marginal microleakage in the short term as a result of its stress-reduction-by-flow property and reduction of marginal microleakage in the long term because of improved durability under flexural load [111].

According to Hooke's law, the higher the elastic modulus and the polymerization shrinkage of the composite, the higher the contraction stress will be [112]. Some in vitro studies have shown that use of flowable composites reduces restoration microleakage and the occurrence of voids [113-115]. It has been suggested that favorable effects are due to the improved cavity adaptation and stress-absorbing ability. Microleakage studies comparing flowable with nonflowable composite materials have shown better results with the flowable material [114, 116].

Flowable resin composite has been introduced in surgical endodontics as a root-end filling material with the desirable property of ease in placement. It can also be used in areas that are difficult to access. Their viscosity, consistency, handling characteristics and delivery system allows the material to readily adapt to the prepared tooth structure. A study of flowable resin composite as root-end filling material, using scanning electron microscopy (SEM) showed that there was no interface gap formation when used in combination with dentin bonding agent (Fig. 5A, 5B) [17].

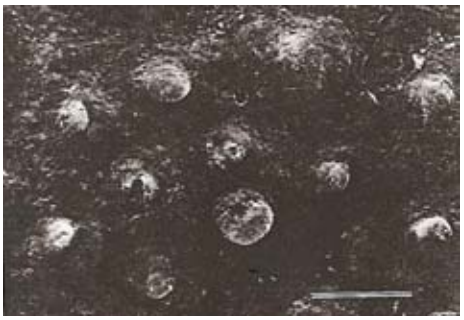


Fig. 5A Scanning photomicrograph of dentinal tubules filled with composite resin. (Original magnification, 3,500X). Bar: 5 μm.

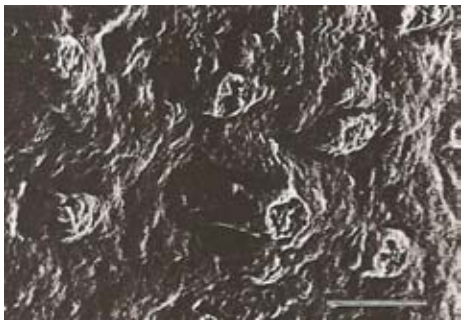


Fig. 5B Scanning photomicrograph of the side view of root-end preparation shows dentinal tubules filled with composite resin. (Original magnification, 3,500X). Bar: 5 μm. [17]

The biological tissue compatibility is also the utmost concern when evaluating materials to be used in periapical tissue. A 1 year histological evaluation of periapical tissue surrounding the Gluma bonding and Retroplast root–end fillings placed in monkeys revealed the absence of inflammatory cells around the filling and a close contact between the filling and fibroblasts with collagenous fibers, including the formation of cementum and Sharpey's fibers in contact with the filling [63]. Similarly, Andreason and colleagues [35] reported that in vivo study, testing tissue reactions to retrograde bonded composite resin filling in monkeys that the roots were treated by apicoectomy and a retrograde dentin-bonded composite filling. Periapical healing was observed a few months later by a histological and scanning electron microscopic examination showed reformation of periodontium adjacent to the resin composite, including deposition of cementum and insertion of new Sharpey's fibres entering a newly formed apical lamina dura. These pointed to tissue regeneration, including cementogenesis, and consequently forms a biological closure of the root canal. The study of Zhu [94] which adhesion of human osteoblasts to root-end filling materials (mineral trioxide aggregated (MTA), IRM, resin composite, and amalgam) was observed by scanning electron microscope (SEM) showed adhesion and the spreading of human osteoblasts on resin composite that was used as root-end filling material (Fig. 6A, 6B).

The risk of biological harm is from the degraded or unpolymerized monomers [117]. Because all polymerized resins contain a surface layer of oxygen-inhibited monomer, this layer should be removed by swapping with a 70% alcohol gauze before wound closure [37].

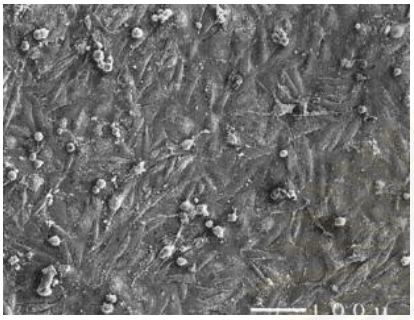


Fig. 6A Scanning electron microscopy of osteoblasts cultured on composite resin for 24 hr (original magnification X100).

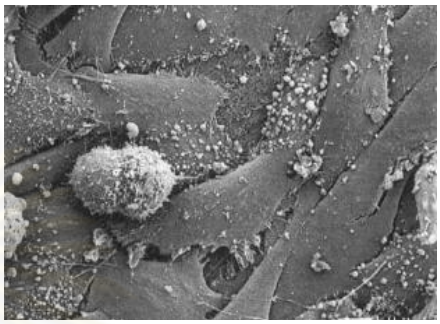


Fig. 6B Cell morphology of osteoblasts on composite resin (original magnification X1000). [94]

The study which evaluated the pulpal response and in vivo microleakage of a flowable resin composite bonded with self-etching adhesive compared with a glass ionomer cement and amalgam reported that there was no inflammatory cell infiltration detected in flowable resin composite group used in cervical cavity and no bacterial penetration along the cavity walls was detected in the flowable resin composite after 30 days [118]. The histological analysis of pulp reaction to Z100 resin composite and Scotchbond Multi-Purpose adhesive system shown that no inflammatory response was observed in the pulp at 4 week after restoration [119].

*Advantages of resin composite:*

- Resin composite has good sealing ability [39, 107].
- Resin composite has biocompatibility and tissue regeneration [35, 64, 94].
- Easy to manipulate [120]
- Inexpensive

*Disadvantages of resin composite:*

- Moisture sensitivity

Retroplast is a chemically cured flowable resin composite developed in 1984. It is a BISGMA/TEGDMA-based resin composite in combination with a dentine-bonding agent (GLUMA) which has been developed for root-end filling during endodontic surgery [63]. Retroplast is a two paste system. Paste A is composed of Bis-GMA/TEGDMA 1:1, benzoyl peroxide N, N-di-(2-hydroxyethyl)-p-toluidine. This is mixed in equal parts with paste B, which is composed of resin ytterbium trifluoride and ferric oxide. A Gluma-based dentin bonding agent is used to adhere the material to root-end surface. The working time is 1 ½ to 2 minutes, and the radiopacity (due to the ytterbium trifluoride) is equivalent to 6 mm of aluminum.

Retroplast is not condensed into an apical cavity, but applied onto a slightly concave resection surface to seal both root canals and exposed dentinal tubules that does not involve preparation of an apical cavity as for other root-end filling materials.

A successful radiograghic healing rate of 74-92% using Retroplast as root-end filling material was reported by Rud et al. after a 6 month to 12 year follow up period [36, 63, 64, 121-123].

Previous studies on dentine-bonded Retroplast for root-end filling

| Study | No. of patients | No. of roots | Length of follow-up (years) | Radiographic classification (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Complete healing | Incomplete healing | Uncertain healing | Unsatisfactory healing |
| Rud *et al.* (1991b) | 388 | No information | ½–1 | 74 | 4 | 15 | 7 |
| Rud *et al.* (1996) | No information | 347 | 2–4 | 89 | 0 | 1 | 9 |
| Rud *et al.* (1997) | No information | 551 | 2–4 | 86 | 8[b] | | 7 |
| Rud *et al.* (2001) | No information | 834 | ½–12 | 92 | 0 | 1 | 7 |
| Jensen *et al.* (2002) | 60 | 77 | 1 | 78 | 4 | 18 | 0[a] |

[a]Six patients re-operated and excluded from the radiographic classification.
[b]Incomplete healing and uncertain healing.

Table 1 [124]

The present long-term study indicates that Retroplast used as a root-end filling material is associated with a successful treatment outcome (Table 1) [124]. However, in the cases which were poor hemostasis during surgery, there was an absence of complete healing, possibly because of bond failure between the Retroplast and root dentin [63, 124]. Therefore, avoidance of contamination of the resection surface

with blood and saliva during the conditioning procedure and application of Retroplast is important for a successful treatment outcome [64].

Although Retroplast has shown remarkable clinical success, unfortunately Retroplast is not yet available in Thailand. Therefore other available flowable resin composite might be considered as candidates for this procedure.

Tetric® Flow (Ivoclar Vivadent, Liechtenstein) (Fig. 7) is flowable, light-curing, radiopaque fine-particle hybrid composite for the restorative therapy which widely available in clinical practices. Tetric® Flow's compositions are the monomer matrix (contains Bis-GMA, urethane dimethacrylate, and triethylene glycol dimethacrylate), the inorganic filler particles (comprise barium glass, ytterbium trifluoride, Ba-Al-fluorosilicate glass, highly dispersed silicon dioxide, and spheroid mixed oxide), catalysts, stabilizers, and pigments (are additional contents). The total content of inorganic fillers is 64.6% wt, or 39.7% vol. The particle size is between 0.04 and 3.0 µm. The mean particle size is 0.7 µm, the density is 1.83-1.96 g/cm$^3$ and the solubility in water is less than 0.1%.


Fig. 7 Tetric® Flow (Ivoclar Vivadent, Liechtenstein)

(http://www.ivoclarvivadent.com/content/products/detail.aspx?id=prd-t1.1399779828&product=Tetric+Flow)

| Standard-Composition: | Tetric Ceram | Tetric Ceram HB | Tetric Flow | | Tetric Flow Chroma |
|---|---|---|---|---|---|
| | | | Cavifil | Syringe | |
| Dimethacrylates | 20.2 | 18.4 | 13.1 | 35.1 | 32.6 |
| Bariumglass filler, Ba-Al-Fluorosilicate-glass | 55.6 | 55.4 | 47.9 | 45.2 | 50.0 |
| Ytterbiumtrifluoride | 17.0 | 12.0 | 14.6 | 13.7 | 12.0 |
| Mixed oxide, High dispersed silica | 6.0 | 13.0 | 5.3 | 4.9 | 5.0 |
| Additives | 0.9 | 0.9 | 0.7 | 0.7 | - |
| Catalysts and Stabilizers | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 |
| Pigments | < 0.1 | < 0.1 | <0.01 | <0.01 | <0.03 |

(in weight %)

Table 2

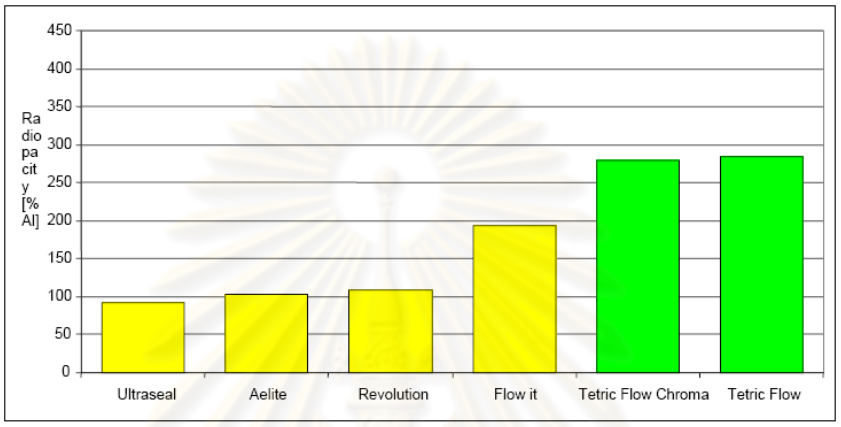
(http://www.ivoclarvivadent.com/content/products/detail.aspx?id=prd-t1.1399779828&product=Tetric+Flow)

| Physical properties: | Unit | Tetric Ceram | Tetric Ceram HB | Tetric Flow | | Tetric Flow Chroma |
|---|---|---|---|---|---|---|
| | | | | Cavifil | Syringe | |
| Flexural strength | MPa | 130 | 150 | 110 | 110 | 110 |
| Flexural modulus | MPa | 9400 | 12000 | 5300 | 5300 | 5300 |
| Compressive strength | MPa | 230 | 290 | 230 | 230 | 230 |
| Vickers hardness | MPa | 600 | 740 | 400 | 350 | 350 |
| Water absorption | µg/mm$^3$ | 21.5 | 16.0 | 24.3 | 24.3 | 24.3 |
| Water solubility | µg/mm$^3$ | 1.0 | 0.5 | 1.0 | 1.0 | 1.0 |
| Radiopacity | % Al | 400 | 330 | 280 | 280 | 280 |
| Depth of cure | mm | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 1.5 |
| Total filler content | wt% | 79 | 81 | 68.1 | 64.6 | 64.6 |
| Total filler content | vol% | 60 | 63 | 43.8 | 39.7 | 39.7 |
| Density (unpolymerised) | g/cm$^3$ | 2.25 | 2.25 | 1.96 | 1.83 | 1.83 |
| Bleach I: | | 2.06 | | | | |

Table 3

(http://www.ivoclarvivadent.com/content/products/detail.aspx?id=prd-t1.1399779828&product=Tetric+Flow)

The minimum radiopacity of restorative materials has been defined to comply with the radiopacity of 250% of aluminium (%Al). Only a few composite restoratives demonstrate radiopacity higher than 250% Al. By adding ytterbium trifluoride to Tetric® Flow makes it achieve both fluoride release and a high radiopacity (Table 2, Fig. 8).




Internal investigation, Ivoclar-Vivadent, Schaan, Liechtenstein

**Conclusion:** When using the definition of minimum radiopacity of more than 250 % Al by Lutz (1980), Tetric Flow and Tetric Flow Chroma are currently the only radiopaque flowable restorative materials.

Fig. 8

(http://www.ivoclarvivadent.com/content/products/detail.aspx?id=prd-t1.1399779828&product=Tetric+Flow)

Bouschlicher et al. [125] suggested that composite materials should have radiopacity similar to or greater than that of enamel for optimal radiographic diagnosis. Their study determined the radiopacity of dentin, enamel, and 20 resin composite materials. Four of the six flowable composites tested complied with ISO Standard 4049, but were less radiopaque than enamel (165% Al in this study). If “acceptable radiopacity” is defined as greater than enamel, then two flowable composites, Flow-It and Tetric® Flow, demonstrated adequate radiopacity (Table 4) [125].

| Material | Densitometer Reading (SD) | Al Equivalent (mm) | % Al |
|---|---|---|---|
| All-Bond 2 D/E Resin | 3.05 (0.07) | 0.30 | 15 |
| OptiBond FL Adhesive | 2.34 (0.29) | 1.88 | 94 |
| Ultraseal XT | 2.31 (0.07) | 1.96 | 98 |
| DENTIN | 2.30 (0.07) | 1.99 | 100 |
| FloRestore | 2.17 (0.07) | 2.35 | 118 |
| AeliteFlo | 2.12 (0.07) | 2.49 | 125 |
| Revolution | 2.08 (0.07) | 2.61 | 131 |
| ENAMEL | 1.87 (0.05) | 3.29 | 165 |
| Bis-Fil-2B | 1.80 (0.05) | 3.54 | 177 |
| Charisma | 1.78 (0.04) | 3.61 | 181 |
| Heliomolar | 1.75 (0.04) | 3.73 | 187 |
| Hytac Aplitip | 1.73 (0.03) | 3.80 | 190 |
| Flow-It | 1.70 (0.06) | 3.92 | 196 |
| Herculite XRV Dentin | 1.63 (0.06) | 4.20 | 210 |
| Prodigy | 1.63 (0.04) | 4.20 | 210 |
| Herculite XRV Enamel | 1.59 (0.06) | 4.37 | 219 |
| Restorative Z-100 | 1.53 (0.04) | 4.63 | 232 |
| Dyract | 1.52 (0.04) | 4.68 | 234 |
| Compoglass | 1.50 (0.04) | 4.77 | 239 |
| TPH | 1.48 (0.03) | 4.86 | 243 |
| Tetric-Flow | 1.39 (0.04) | 5.31 | 266 |
| Pertac II | 1.25 (0.04) | 6.09 | 305 |

Table 4 [125]

The study of Murchison et al. [126] suggested that the radiopacity of 2 mm of aluminum lies between reported values for enamel and dentin. The flowable composites Tetric® Flow displayed a radiopacity greater than an aluminum equivalent of 2 mm and had the highest radiopacity, above enamel and other flowable resin composites. Similary Sabbagh et al. [127] compared the radiopacity of 41 resin-based materials using conventional dental x-ray film (Ultraspeed-D) and a digital system (Digora) demonstrated that Tetric® Flow had the highest radiopacity above other flowable resin composites.

| Material (2 mm) | (n) | Mean aluminum equivalent (mm) |
|---|---|---|
| Tetric Flow | 5 | 4.70 mm |
| Flow-It LF | 5 | 3.35 mm |
| Herculite XRV | 5 | 3.15 mm |
| Crystal-Essence | 5 | 2.30 mm |
| AeliteFlo | 5 | 1.75 mm |
| Revolution | 5 | 1.88 mm |
| VersaFlo | 5 | 1.75 mm |
| UltraSeal XT plus | 5 | 1.65 mm |
| FloRestore | 5 | 1.63 mm |
| Aluminum | 3 | 2.00 mm |
| Enamel | 5 | 2.50 mm |
| Dentin | 5 | 1.50 mm |

[126]

Table 5

The advantages of Tetric® Flow are hygienic single-dose delivery form, long thin application tip, easy access to even the smallest cavities, bubble-free direct application, accurate and economic application, possibility of gentle, minimally invasive preparation, and excellent retention even in preparations without undercuts. This material completely fills the smallest of cavities without entrapping air. The paste is easy to model without flowing away.

Tetric® Flow is a material that requires etching and use of bonding agent before placement. Placement is taken approximately 2 minutes. As Tetric® Flow is a flowable composite, placement is easy. Furthermore, little amount of material is required and it exhibits a contrasting shade to the root [120].

Bonding agent

Contemporary dentin adhesives are classified into three-step, two-step, and single-step systems depending on how the three cardinal steps of etching, priming, and bonding to dentin are accomplished [128]. The three-step systems require acid etching, rinsing, priming, and application of an adhesive. The two-step systems are subdivided into self-priming adhesives that require a separate etching step and the self-etching primers that require an additional bonding step. The single-step adhesive systems contain a mixture of acidic monomers capable of acid-etching dentin, mixed with hydrophilic monomers that can both prime and bond dentin, all in a single application [129].

Conventional bonding systems for the direct placement of resin composites usually utilize a three-step bonding procedure, combining a separate surface acid etching and rinsing step, a priming step, and placement of the bonding agent.

Advances in dentin bonding have been significant since 1982, with the introduction of hybridization concepts and hydrophilic adhesives by Nakabayashi [130]. The interlocking of resin monomer with the collagen network of decalcified dentin, obtained after acid application to form a hybrid layer has been largely accepted as the bonding mechanism [131]. Due to the relative complexity, technique sensitivity, and time

consumption of total-etch adhesive systems [132, 133], new adhesive systems have been introduced onto the market in an effort to simplify clinical procedures [131, 134]. Among these new materials, there are one-bottle adhesive systems, which combine primer with their corresponding adhesive monomers in one single bottle, demanding basically two clinical steps (acid etching and bonding) [135].

In an effort to simplify clinical bonding procedures, self-etching adhesives were developed, eliminating separate acid-etching and primer-application steps. Dentin hybridization is obtained in only one step [131] through acidic monomers (primer) which can dissolve the smear layer, promoting superficial dentin demineralization [136]. Therefore, there is a simultaneous incorporation of resin monomers into the remaining smear layer and subjacent dentin [133, 136]. It is important to note that the application of self-etching primers reduces technique-sensitive factors, such as rinsing and drying steps, which are difficult to standardize under clinical conditions. Thus, the use of self-etching primers reduces the possibility of surface overwetting and overdrying, which could compromise bond strength [131, 136].

The development of bonding systems has been toward improved quality and ease of manipulation.

*The advantages of self-etch bonding systems over phosphoric acid based systems* [137, 138]

1.Mild acid gentle on tooth structure

Self-etch bonding system does not excessively decalcify and weaken dentin tissue and collagen fibers (Fig. 9A, 9B).

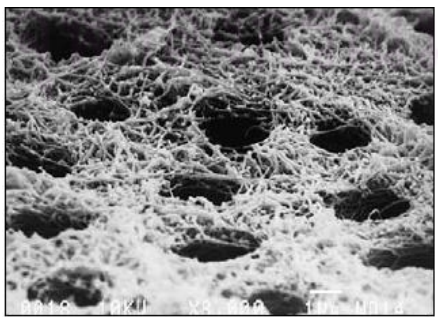


Fig. 9A Phosphoric acid decalcification zone

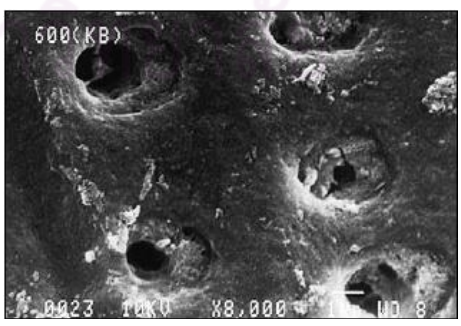



Fig. 9B Self-etching primer (Clearfil SE Bond) decalcification zone [138]

Treating prepared dentin with phosphoric acid can result in excessive decalcification and exposure of the collagen fiber network because it is a relatively strong acid. The collagen network may collapse upon itself, creating a physical barrier to the penetration of the bonding agent to the full depth of the decalcified zone, and resulting in weak, unsupported dentin. This in turn may result in decreased strength within the dentin tissue and impaired overall adhesion durability.

2.Formation of a uniform hybrid layer

With conventional bonding systems, primers and/or bonding agents are introduced into the dentinal tissue following phosphoric acid etching and water rinsing. However, in many cases the phosphoric acid may have decalcified the tooth structure deeper than the primer and bonding agent are able to penetrate. The full penetration of the monomer may also be inhibited should the unsupported collagen fibers collapse upon themselves. Any area within the tooth structure that are not fully penetrated by the bonding monomer will become weak links in the overall restoration.

Self-etching primer decalcifies only as deeply as it penetrates. And because the acid is polymerizable phosphate monomer, complete support for the decalcified tissue is provided by its co-polymerization with the bonding component. In this way, self-etch bonding system eliminates the problem of void formation, and creates a very uniform hybrid layer.

3.Reduced technique sensitivity

Self-etch bonding system does not employ the technique sensitive “wet bonding” procedure. The majority of bonding systems employing phosphoric acid etching specify the use of the “wet bonding” technique, which requires critical attention to the amount of moisture left on the preparation surface after water rinsing. Accurate moisture control is a requirement for success with the “wet bonding” technique systems because water acts as the wetting agent to allow permeability. If the preparation is dried excessively after water rinsing, the primer and bond will not sufficiently penetrate the tooth structure and weak, unsupported voids will result. Alternatively, if excessive water

is allowed to remain, this will dilute the bonding monomer excessively and reduce adhesion. Clinically, however, it is extremely difficult to precisely control the amount of moisture remaining on the preparation surface due to variables such as the strength and duration of the air stream used for drying, or the amount of moisture blotted with sponges. The depth of the preparation will also affect the amount of moisture removed with such procedures. With self-etch bonding system, the preparation may be dried extensively following application of the primer; there is very little possibility of over- or under-drying the preparation. With the consistent surface treatment afforded by this priming procedure, far greater adhesion consistency can also be obtained, regardless of the clinical case or operator. The main advantage of self-etch bonding agent in apical surgery is that its conditioning primer, used to etch the dentin, does not require washing, and thus there is less chance of contamination [139].

When bonded to dentin, it is evident that two-step self-etch adhesives are able to compete with etch-and-rinse adhesives, not only in terms of early bonding effectiveness, but also in terms of durability [140, 141]. Taking into account the lower technique sensitivity [142], the faster application procedure and the lower risk to nanoleakage [143], the two-step self-etch approach may become the future standard of adhesion.

Thirawat and Edmunds [144] found that the use of bonding agent (Scotch-bond; etch & rinse adhesive) provided the better seal than super EBA and amalgam with varnish. Hardy et. al. [145] who investigated the ability of One-Up Bond (One-step self-etch adhesive) alone and MTA with and without a secondary seal of One-Up Bond or Super EBA to seal saucer-shaped perforation defects in human molars. The integrity of the seal was evaluated by fluid filtration. It was shown that MTA alone leaked significantly more than One-Up Bond alone or MTA with either secondary seal or Super EBA at 24 hours. A study of Adamo [97] which MTA, Super-EBA, composite with ProBond (two-step self-etching adhesive) and amalgam as root-end filling materials were tested for their ability to prevent microleakage in a bacterial assay system for 12 weeks. The results showed no statistically significant differences in the rate of microleakage among all groups. Fogel and Peikoff [146] evaluated the microleakage of amalgam, Super EBA,

IRM, MTA and dentin bonding resin (Clearfil Liner Bond 2; self-etching adhesive system) using a fluid filtration system and root-end preparations were made to a depth of 3 mm using ultrasonic. MTA seemed to perform as well as Super EBA, IRM, and Clearfil Liner Bond 2.

Some studies have shown that using self-etching adhesive with a resin composite did not provide as good a bond to the enamel surface as a phosphoric etching system [147]. However, several studies reported good bonding performance of the self-etching adhesive system by Kuraray Co., Clearfil SE Bond on enamel and dentin surfaces [118]. The study of Munck et al. [148] determined the bonding effectiveness and the interaction with enamel/dentin of three contemporary one- and two-step self-etch adhesives by microtensile bond strength testing (μTBS) when compared to a control two-step self-etch and a three-step etch-and-rinse adhesive. The one-step self-etch adhesive, Adper Prompt (3M ESPE), scored the lowest μTBS of all experimental and control adhesives tested. Conversely, the two-step self-etch adhesives Clearfil SE Bond (Kuraray) and OptiBond Solo Plus Self-Etch (Kerr) approached the values obtained by the three-step etch-and-rinse control (OptiBond FL, Kerr) when bonded to enamel and dentin. A TEM study revealed that thick smear layers (up to 4.1 μm) did not hinder Clearfil SE Bond to hybridize dentin [149].

Mitsui et al. [150] evaluated the influence of occlusal load cycling on cervical microleakage of proximal slot restorations located in dentin, using two self-etching (Experimental EXL 547, Clearfil SE Bond) and two one-bottle dentin adhesive systems (Single Bond, Optibond Solo Plus) and restored following the manufacturers' instructions. All specimens were subsequently immersed in a 2% methylene blue solution (pH 7), and sectioned to examine the extent of dye penetration under a stereomicroscope. The results showed that the Experimental self-etching adhesive EXL 547 presented the lowest microleakage scored, however no statistically significant difference was observed in comparison with the other adhesive systems.

Nikaido et al. [151] evaluated the in vitro dentin bond strengths of two dentin bonding systems (Clearfil SE Bond; self-etching bonding system and Single Bond; two-step etch-and-rinse bonding system) in class I cavities following fatigue load

cycling with thermal cycling. Clearfil SE Bond produced excellent dentin bonds that were much stronger than those produced by Single Bond, which was shown to be a technique-sensitive bonding system, most probably due to defects created by the overwet phenomenon. From the clinical standpoint, a self-etching primer system should provide more reliable results in various complex cavity preparations [151].

Air-blowing is important to eliminate substances that could influence polymerization and to ensure a good distribution of the adhesive on the dentin surface. Since the introduction of self-etching primers, gentle air-blowing was generally required for removing the excess primer solution. It is commonly recommended by the manufacturer that gentle blowing should be performed in order to achieve higher bond strengths. Strong air-blowing may have caused over-removal of the adhesive resin causing incomplete enveloping of the collagen fibrils leading to adhesive failure [152]. It is speculated that when strong air-blowing is used, water and solvents are evaporated quickly resulting in a viscous resinous on the dentin surface. This would lead to weaker mechanical properties, resulting in lower bond strengths. Spresfico et al. [153] assessed the effect of gentle air-blowing and strong air on the bond strength of one 2-step self-etching system: Clearfil SE Bond and three all-in-one systems: G-Bond, Adper Prompt L-Pop, and SSB-200. The purpose of the study was to evaluate the effect of technique sensitive on microtensile bond strength of four different self-etching adhesive, focus on the air-blowing step. Clearfil SE Bond showed the highest bond strengths regardless of gentle and strong air-blowing during the application of the primer. For all materials, the gentle air-blowed group showed more complex failures (at different plane), while failures in the strong air group showed the presence of many voids in the adhesive. The authors speculate that these voids are pockets of air, which could be stress raisers within the cured adhesive resin during testing. This may be a big reason for the poor adhesion [153]. However, the fact that most of the fractures occurred within the adhesive layer of Clearfil SE Bond suggests that this system is less technique sensitive to the air-blowing step.

*Clearfil SE Bond*

Clearfil SE Bond is a light-curing bonding system, and consists of a self-etching primer and a bonding agent, with the principle ingredients of each shown. The primer offers simultaneous treatment of both dentin and enamel.


Fig. 10

| Product Name | CLEARFIL SE BOND | CLEARFIL LINER BOND 2V |
|---|---|---|
| Components |  Single-liquid type primer<br>Single-liquid type bond |  Dual-liquid type primer<br>Single- or dual- liquid type bond |
| Procedural Time | 1. Priming for 20 seconds (no mixing required)<br>2. Bond application and light curing for 10 seconds (30 seconds in total) | 1. Priming for 30 seconds (mixing required)<br>2. Bond application and light curing for 20 seconds (50 seconds in total) |
| Polymerization Method | Light-cure only | Light-cure, chemical-cure or dual-cure |
| Clinical Indications | • Direct filling restorations using light-curing composite or compomer<br>• Cavity sealing as a pretreatment for indirect restorations<br>• Treatment of hypersensitive and/or exposed root surfaces<br>• Intraoral repairs of fractured facing crowns made of porcelain, hybrid ceramics or composite resin using light-curing composite<br>• Surface treatment of prosthetic appliances made of porcelain, hybrid ceramics or cured composite resin  | • Direct filling restorations using light-curing and chemical-curing composite<br>• Cavity sealing as a pretreatment for indirect restorations<br>• Treatment of hypersensitivity<br>• Intraoral repairs of fractuted porcelain restorations using light-curing composite<br>• Cementing porcelain or composite inlays/onlays using resin cement<br>• Bonding to metals and porcelain when used with CLEARFIL PORCELAIN BOND ACTIVATOR and ALLOY PRIMER<br>• Bonded amalgam restorations  |
| Basic Components | Primer : MDP, HEMA, dimethacrylate monomer, water, catalyst<br>Bond : MDP, HEMA, dimethacrylate monomer, microfiller, catalyst | |
| Technical Characteristics | CLEARFIL SE BOND :<br>• Faster priming procedure due to lower pH<br>• Faster light cure time due to modified catalysts<br>• Single liquid components<br>CLEARFIL LINER BOND 2V:<br>• Dual cure bond capability allows for use in indirect restorations<br>• Dual liquid primer component | |

Table 6 Components and user guide of Clearfil SE Bond

Clearfil SE Bond Primer contains an acidic phosphate monomer which penetrates dentinal tissue well and dissolves the smear layer created during cavity preparation. This replaces the need for a separate phosphoric acid etching and water rinsing procedure as use with traditional bonding systems. Clearfil SE Bond Primer is mildly acidic, with a pH of 2.0. The acidity of the Primer has been optimized to allow simultaneous treatment of enamel and dentin: it etched enamel enough to ensure good bond adaptation, while not excessively decalcifying the collagen structure of the dentin. Importantly, the primer remains diffused throughout the dentin tissue after treatment: it is not rinsed away. Therefore the collagen network does not collapse, but remains fully supported, which allows excellent penetration of the bond component. The Primer reverts to a neutral pH after treatment of enamel and dentin with demineralization. Together they create a strong, stable bonding layer with excellent dentinal sealing.

Clearfil SE Bond is a simplified, light-cure bonding system containing a water-based primer. The procedure time with Clearfil SE Bond is significantly reduced, while providing enhanced bond strength and sealing properties.

In biological aspect, the study of Lu et al. [154] that evaluated the pulp response of beagles following direct pulp capping with Clearfil SE Bond, a self-etching adhesive. Clearfil SE Bond showed good biocompatibility with pulp, most pulp tended to show normal histological features over 30 and 90 days.

## Blood contamination

Clinically, during endodontic surgery, it is often difficult to maintain proper isolation, and blood contamination may occur. When root-end preparations are made using ultrasonics with magnification, the root-end preparations must be filled with a restorative material, such as Super EBA, MTA, resin composite, or others. This is a technique sensitive procedure that requires a dry field for ideal working conditions [129]. In the study of Torabinajad [80] which compared the amount of dye leakage in the presence versus absence of blood in root-end cavities filled with amalgam, Super EBA, IRM, and MTA. The results shown that IRM and Super EBA did not seal well in the

presence of blood and MTA leaked significantly less than other materials tested with or without blood contamination of the root-end cavities.

One possible way to simplify the procedure would be to seal the resected root surface, without a preparation or slightly concave preparation, with self-etching adhesives [129]. The complexity of dentin-bonding procedures and their sensitivity to blood contamination [155, 156] has limited their use to well-isolated sites. Kaneshima et al. suggested some reaction between the exposed collagen network and the blood protein components that could inhibit primer infiltration into dentin [157]. Although most studies indicate that blood contamination causes a significant decrease in bond strengths. However, several studies have shown that dentin surfaces contaminated by blood can be decontaminated relatively easily [156]. Kaneshima et al. [157] reported that the effect of blood contamination varies greatly, according to the surface condition of the adherent. If blood contamination occurred before collagen fibers were exposed by either phosphoric acid etching or self-etching primer application, the contamination presented almost no influence on bond strength. This result indicated that the effect of blood contamination of nontreated dentin surface was eliminated, together with the smear layer, by acid etching or self-etching primer application. Blood contamination of the dentin surface where collagen fibers had been exposed decreased the bond strength. In this situation, blood contamination might permit some reaction between the superficial organic layer of exposed dentinal collagen and the blood protein components. This reaction might inhibit primer infiltration into the dentin or subsequent resin penetration and polymerization. However, when the contaminated collagen fibers were dissolved or when the contamination occurred after the exposed collagen fibers were dissolved, the bond strength was maintained. The bond strength was markedly decreased when the contamination occurred after the primer application, but was restored by reapplication of the self-etching primer [157].

Eiriksson et al. [158] evaluated the effect of blood contamination on microtensile bond strength between resin interface and to determine the best decontamination method to re-establish the original resin-resin bond strength. The adhesive systems used in this study were Single Bond, One-Step, Clearfil SE Bond, and

Prompt L Pop. The results demonstrated that blood contamination significantly reduced the bond strengths between resin composite increments regardless of the materials evaluated. However rinsing with water restored the bond strength significantly. Rinsing and application of a dentin adhesive appears to be necessary whenever blood contamination exists on a resin surface to ensure better interfacial bonding of the next increment.

The in vitro study of Miles et al. [159] that evaluated the effect of blood contamination on the tensile bond strength of adhesive resins. The teeth were contaminated with human blood for 5 min before applied the adhesive system. The results showed no significant difference between the contaminated and control groups of Amalgambond [159].

Vignaroli et al. [37] measured the sealing ability of four dentin bonding agents on the resected root end. The bonding systems evaluated were Amalgam bond (AMB), Scotchbond Multi-Purpose (SMP), Prisma Universal Bond 3 (PUB 3), and All-Bond 2 (AB 2). All materials were applied directly to the resected root-end which were contaminated with human blood for 5 min before bonding. Microleakage was measured using fluid filtration at various time intervals from 1 to 24 weeks. The results indicated that blood contamination did not adversely affect the sealing or bonding of several dentin bonding agents.

Rud [63] reported that when applying the dentin bonding agent Gluma and Retroplast composite as a root-end sealant, a significantly lowered bond strength occurred if blood or saliva contamination occurred at the time of placement. This is interesting because it implies that, even though the bond strength of some agents may be compromised by blood contamination, the seal may not be adversely affected. As the periapical region is an area not subjected to significant dislodging forces, the microleakage of these agents may be more important to the long term success than bond strength [37]. If used in endodontic surgery, sealing the apical preparation rather than obtaining a strong bond would be the primary objective [159].

## Sealing ability assessment

To assess sealing ability, there are a few methodologies in vitro. Generally sealing quality is estimated by measuring microleakage that allows the tracer agent to penetrate the filled canal. Commonly used tracers are dyes, radioisotopes, bacteria and their products, such as endotoxin.

1.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 [160]. Then, the teeth are sectioned longitudinally, transversely, or cleared and the linear penetration of the dye is recorded.

The longitudinal sectioning method enables examination on the exposed filling material and any dye penetration into the material and at the interface of the dentinal wall on one side. The disadvantages of longitudinal dentinal sectioning seem to be the random choice of the cut axis and the very low probability of the section being made through the deepest dye penetration point, with consequently underestimation of the leakage and recording of unreliable data.

Transverse root sectioning results in loss of part of the dentinal tissue and dye to the saw thickness, and only allows one to determine whether or not there is penetration in each section [161].

The clearing technique recommended by Okumura in 1927, in which the teeth become transparent after a process of demineralization, dehydration and immersion in methyl salicylate, provides a three-dimensional view of the internal anatomy of root canal without the loss of dental substance, making it easier to view the leakage area. It is simple, fast, performed with substances low in toxins and does not require complex equipment. It was affirmed that the technique makes it easier to observe the lateral and accessory canals, and clearly reflects the relation between the sealing material and apical foramen [162]. However, some of the samples submitted to

clearing may present deficient demineralization, which would compromise the final transparency of the specimen [163]. Furthermore, the demineralization times differs, as greater the weight of the dentinal part, the greater the mineral content present and the longer it would take to complete the process. The endpoint of this step could be easily assessed by inserting a thin needle in an unimportant area of the crown or by radiography [164]. Another potential problem is that incomplete dehydration will leave opaque areas in the teeth, but this can be corrected by additional dehydration in 100% ethyl alcohol [165]. Immersion in acids such as nitric and alcohol for a long period may cause dye dissolution in this technique [161]. Clearing technique was more precise than the transverse section for detecting apical leakage, as it allows the leakage to be visualized in tenths of millimeters, while transverse sectioning only determines whether or not leakage has occurred in each section [162].

There are various types of dyes: methylene blue, India ink, silver nitrate, etc. Particle molecule size, pH and chemical reactivity of dyes are expected to affect the degree of penetration [162]. A large number of studies used methylene blue as dye because it is inexpensive, easy to manipulate, has a high degree of staining. Methylene blue presents the same leakage as butyric acid, which is a cytotoxic bacterial metabolite with low molecular weight (mol. wt 88), [166] and has greater penetration than Indian ink. Thus, methylene blue may serve as an adequate indicator of leakage of microorganisms and toxic agents of low molecular weight. However methylene blue presents a few disadvantages such as dissolution during the demineralization and clearing process [167].

Indian ink is comprised of carbon particles in suspension in an organic solvent with shellac, a resinous material. Indian ink particles with diameter smaller than or equal to 10 μm are also widely used [161]. Indian ink is used as the leakage detector because of its broadly similar size to bacteria. If clearing tooth technique is used, Indian ink is indicated as acid is used to demineralize the obturated teeth. However, its large particles may have blocked the tubules openings and limited further ingress. Another possible reason for reduced ink penetration is that the shellac/amyl acetate solvent is too viscous to allow tubular penetration [168].

Silver nitrate is the salt of a strong acid and weak base, silver nitrate is acidic when prepared (pH 3.04). Despite its smaller molecular size, silver nitrate was found to have less dentinal penetration power. It can be postulated that upon contact with the dentin, insoluble silver phosphate crystals precipitated within the tubules which may have limited further penetration. Another limiting factor with the use of this tracer is the degree to which the developer can enter the tubules to reduced the silver to its elemental state [169].

One of the major considerations with respect to dye penetration studies is air entrapped in voids along the root canal filling may hinder fluid movement. It has been recommended that dye penetration should be performed under reduced pressure , incorrectly referred to as vacuum [170]. The vacuum method resulted in significantly more dye penetration than fluid filtration and passive dye penetration [171]. 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 [172]. No significant differences between a horizontally positioned experimental group under reduced pressure and groups in passive immersion, but when the apices were in an upright position, the mean leakage was significantly higher under reduce pressure [173]. Tooth positioning had a significant effect on linear dye penetration under reduced pressure. It was emphasized the need to standardize factors that may influence penetration when assessing the methodology of leakage studies.

One possible variable in leakage studies is the pH of the dye solution. In a study of demineralization of enamel, Theuns et al. [174] found that the pH had a significant effect on the rate of demineralization. The acidic pH of methylene blue dye was noted by Antoniazzi et al. [175], who reported that the inorganic portion of the tooth may be dissolved and that adjustment of the pH is important in assessing leakage. In that study, the dye solution was buffered to pH 7. Wu et al. [170] indicated that methylene blue is most commonly used in a 2% aqueous solution in coronal and endodontic microleakage studies. This concentration of methylene blue was observed to be acidic when prepared (pH 3.45) although it proved possible to raise the pH to 6.96 by the

addition of a phosphate buffer. Using the neutral buffered methylene blue would show the true penetrability of the canal by an aqueous solution and not by an acid. The India ink, as obtained from the manufacturer, had a pH of 7.37 and was therefore unnecessary to buffer this tracer.

2.Fluid filtration or transportation methodology

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. in 1993 for use in root canal. 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 [170].The results are generally expressed in µl / min [176].

The fluid filtration method presents many advantages in comparison with dye penetration method. As the samples are not destroyed, therefore it allows both the apical and coronal sealing to be assessed after along 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 [177]. 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 20psi 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 pressures, 15 cm by $H_2O$ would appear to be sufficient when highly sensitive equipment is used. The pressures should included in the results and should be expressed as µl/min cm $H_2O$ instead of µl/min [176].

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 [176].

A new computerized fluid filtration meter based on light refraction at starting and ending positions of air bubble movement inside micropipette was developed [178]. 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.

3.Dye Extraction Method

This method developed by Zakariasen et al. [179]. 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 endodontics 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 [160].

There was no correlation between dye penetration and the fluid filtration which determine microleakage. However, the dye extraction method and the fluid filtration were statistically correlated. The dye extraction method presents an advantage over the fluid filtration method, because the filtration values tend to diminish over time, as the water penetrates all the irregularities until a plateau is reached [160].

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 that penetrated the apex, thereby avoiding the limitations of sectioning the root. The

fluid filtration technique gives results similar to the dye extraction technique because, like the dye extraction technique, 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 [160].

4.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 [180]. Many different strains of bacteria have been used to assess marginal leakage and this has lead 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 [181]. 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 [182, 183].

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 [183, 184].

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 [182]. It has been reported that endotoxin preceded bacterial penetration of canal system [185].

A new method for analysis of endodontic microleakage based on the filtration rate of glucose along the root canal filling was introduced [186]. The model consists of a tube containing concentrated glucose solution that is connected to the coronal aspect of the tooth, whilst the apical region in water. Glucose that accumulates in apical chamber is measure with a spectrophotometer, following an

enzymatic reaction. The amount of leakage was quantified with spectrophotometry. Glucose was selected as the tracer because of its small molecular size (MW=180 Da) and as it is a nutrient for bacteria. So, if glucose could enter the canal from the oral cavity, bacteria that survive root canal preparation could multiply.

5. Scanning electron microscope (SEM)

The SEM provided a means of direct visual observation of the adaptation of restorative materials to the margins of cavity preparations. It has the depth of focus and magnification required for this type of study. Limiting factors related to this method for the study of marginal discrepancies attending adaptation procedures including the technique is restricted to the evaluation of teeth outside of the mouth environment and is not oriented to diffusion and penetration as most previous studies have been. Data analysis of photomicrographs is based on measurements of the fissure size between the restoration and the tooth, and the technique has a potential for the introduction of artifacts during specimen preparation, through SEM viewing, or through the disruption and shifting of the restorative material during sectioning procedures [187].

Among the various methods which have been used to assess sealing quality, dye penetration is the most widely used method [188]. It has been suggested that the reduced air pressure method whereby all entrapped air is removed is the most reliable method for dye penetration studies [189]. Dye extraction provides more reliable results because it measures all of the dye taken up in the root and it is a quantitative measurement.

The leakage occurred in Class I root-end preparation is around the margin of root-end filling material which is direct to the root canal system and can leak through the exposed dentinal tubules at the resected area, but the leakage of concave cavity preparation filling with bonding agent and resin composite is at the cavosurface margin of the preparation.

In apical surgery, it is often very difficult to maintain proper isolation, and blood contamination may occur. Therefore, consideration should be made in choosing

root-end filling material and it would be advantageous to find a material that may inhibit leakage both from the root canal and the resected dentinal tubules.

Flowable resin composite that is available in Thailand are Tetric® Flow (Ivorclar Vivadent, Liechtenstein), Filtek Z350 Flow (3M ESPE, St.Paul, USA), Revolution2 (Kerr, Orange, CA, USA), Estht X flow (Densply, Tulsa, OK, USA), Alite Flo (Bisco, Schaumburg, Illinois, USA) and Flow-It ALC (Pentron, Chicago, Illinois, USA). Tetric® Flow is widely used. It was manufactured in 1997 and imported in Thailand in 1998. Its placement is easy. Only little amount of material is required, and it exhibits a contrasting shade to the root. Tetric® Flow had the highest radiopacity, above enamel and other flowable resin composites, allowing the clinician to evaluate root-end filling integrity at subsequent recall appointments on radiographs. It is much less expensive than MTA and has good biocompatibility to periodontium in both elution and direct contact. It also demonstrated good attachment of cells when contacting to Tetric® Flow [120, 190]. To consider Tetric® Flow as an alternative root-end filling material to MTA, its sealing ability should be investigated.

# CHAPTER III

# MATERIALS AND METHODS

## Population & Sample

**-Target population:** Human radicular dentin surface of root-end preparation

**-Study population:** Human radicular dentin surface of root-end preparation in straight rooted teeth

**-Sample:** Human radicular dentin surface of root-end preparation in straight, single root upper anterior teeth which have complete root formation, no crack or fracture, no root caries, no root resorption.

## Materials

1) Tetric® Flow (Ivorclar Vivadent, Liechtenstein)
2) MTA ProRoot™ (Dentsply, Tulsa, OK, USA)
3) Clearfil SE-bond (Kuraray medical INC., Japan)
4) K-file (SybronEndo, Orange, California, USA)
5) ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland)
6) Glyde™ file prep (Dentsply, Tulsa, OK, USA)
7) Sodium hypochlorite (NaOCl)
8) Ethylenediaminetetraacetic acid solution (EDTA)
9) Gutta-percha (Hygienic Corp, Akron, OH, USA)
10) AH-Plus root canal sealer (Dentsply De Trey, Konstanz, Germany)
11) Cavit (3M ESPE, St.Paul, USA)
12) #701 fissure bur (Kerr, Romulus, MI, USA)
13) KiS-1D retrotips (Obtura-Spartan, Fenton, MO, USA)
14) MTA Gun (Dentsply Maillefer, Ballaigues, Switzerland)
15) Microbrush (Kerr Corporation, Orange, CA, USA)
16) #27 Round carbide bur (Komet, Lemgo, Germany)
17) Nail varnish (Revlon, New York, USA)

18) Methylene blue (Sigma Chemical, St. Louis, MO, USA)
19) Potassium phosphate monobasic-sodium hydroxide (0.05M) buffer solution (Fisher Chemical, Fisher Scientific, Pittsburgh, PA)
20) Polishing disc (Sof-Lex Pop-on, 3M, St Paul, Minn, USA)
21) Syringe filter (Millipore, Billerica, MA, USA)
22) 65% Nitric acid
23) Paper point
24) Blade No.15 (Swann-morton LTD., England)
25) Pasteur pipette (Micropipette, Pipetman, U.S.A.)
26) Pipette tip 200, 1000 µl

## Equipment

1) Light curing unit (VLC, Astralis 5, Vivadent, Shaan, Liechtenstein)
2) Low speed saw (IsoMet®, Buehler Ltd, Lake Bluff, IL, USA)
3) Operating microscope (Carl Zeiss Surgical, Inc.,Thornwood, NY)
4) High-speed handpiece
5) Slow-speed handpiece
6) Spartan ultrasonic unit (Obtura-Spartan, Fenton, MO, USA)
7) Radiometer (Model 100, Demetron/Kerr, Danbury, CT, USA)
8) Accumet model 950 pH/Ion meter (Fisher Scientific, Pittsburgh, PA)
9) Vacuum pump (Bio-Rad Laboratories,3300 Regatta Boulevard, Richmond, CA 94804)
10) Stereoscopic microscope (Model XT, Olympus, Tokyo, Japan)
11) Centrifuged machine (Universal 16R; Hettich Zentrifugen, Tuttlingen, Germany)
12) Automatic spectrophotometer (SLT Spectra II; Labinstruments A-5082, Salzburg, Austria)
13) Incubator (Contherm series five, Contherm Scientific Ltd., New Zealand)

## Data collection

### Tooth selection, storage and root canal preparation

68 extracted, single-rooted, single-canals of upper anterior teeth were collected. All teeth had canal curvatures less than 10°C [191] and the canals were patent. No previous root canal treatment or restorations had been performed on the teeth. Teeth that present any root fractures, immature apices, resorption, root caries were excluded from the study. Only teeth which had initial apical file (IAF) equal file number 20 or 25 were used. Attempt to standardize the size of the roots was made by measuring the circumference (15mm.) of each tooth at the level of 3 mm from the apex. Teeth were then stored in a 0.5% chloramine solution for at least 1 week. Thereafter, they were stored in distilled water in a refrigerator at 4°C according to the guidelines of ISO [192]. In order to reduce deterioration, the storage medium was replaced once a week.

The roots of all teeth were cleaned by hand scaling to remove adherent soft tissue, and calculus. The crowns were removed with low speed saw (IsoMet®, Buehler Ltd, Lake Bluff, IL, USA) to give approximately 15 mm. of root length from the coronal surfaces to the apex of the roots. An operating microscope (Carl Zeiss Surgical, Inc.,Thornwood, NY, USA) was used to inspect the roots for cracks under 25× magnification. A size 15 endodontic K-file was placed into the canal until the tip protrudes past the apical foramen. The file was drawn back into the tooth until it just appeared at the level of the apical foramen and then the length was measured. The working length was determined to be 1 mm. short of this length.

All teeth were instrumented with modified crown-down technique, using a set of ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) as follows: $S_1$ (tip diameter 0.17 mm; variable taper) was taken into the canal just short of depth at which hand file was taken previously. The other files were used to working length in the following sequence, $S_2$ (tip diameter 0.20 mm; variable taper), $F_1$ (tip diameter 0.20 mm), $F_2$ (tip diameter 0.25 mm), $F_3$ (tip diameter 0.30 mm), $F_4$ (tip diameter 0.40 mm), and $F_5$ (tip diameter 0.50 mm). The purpose of this preparation regimen was to create a uniform size of canal and to overcome the variation in natural

morphology. Each set of file was discarded after instrumented 20 root canals [193]. During mechanical instrumentation, each file was coated with Glyde™ file prep (Dentsply, Tulsa, OK, USA) to act as a lubricant, and apical patency was confirmed with a small file size 15 (K-Flex-o-files, Dentsply Maillefer, Ballaigues, Switzerland) throughout the procedures after each larger file size. Irrigant was delivered in the canals by means of a 5-ml disposable syringe with a 23-gauge needle. After the use of each file, canal was irrigated with 3.0 ml. of 2.5% sodium hypochlorite (NaOCl) which was delivered to 2-3 mm from working length. After instrumentation, the smear layer was removed by rinsing with 5.0 ml. of 17% ethylenediaminetetraacetic acid solution, follows by a final rinse with 5.0 ml. of sterile water [194]. The canals were dried with sterile paper points and obturated using lateral condensation of gutta-percha (4% taper) and AH-Plus root canal sealer.

At least coronal 4 mm. opening for each canal was sealed with Cavit (3M ESPE, St.Paul, USA) and subsequently, all teeth were stored at 37°C and 95% humidity for 24 hr to allow complete setting of the sealer.

All procedures were conducted by one experienced endodontist.

**Root-end preparation and root-end filling**

The samples were divided randomly into four groups (n=15) according to the technique of root-end preparation, root-end filling material and blood contamination on the resected root surfaces:

Group A: Class I root-end preparation and filled with MTA.

Group B: Class I root-end preparation and blood contamination before filled with MTA.

Group C: Slightly concave root-end preparation, treated the resected surface with the self-etching adhesives (Clearfil SE Bond) and filled with flowable resin composite (Tetric® Flow).

Group D: Slightly concave root-end preparation, treated the resected surface with the self-etching adhesives (Clearfil SE Bond) and blood contamination before filled with flowable resin composite (Tetric® Flow).

The blood sample used as the contamination substance was obtained from one volunteer and immediately inserted in a vacutainer tube containing 50 I.U. Heparin per ml blood. The collected blood sample was used within 24 hr.

*MTA group* (n=34)

Three millimeters of the root apex was resected ($0^{\circ}$ bevel) using #701 fissure bur (Kerr, Romulus, MI, USA) in a high-speed handpiece [18]. Root-end preparation were made with KiS-1D retrotips (Obtura-Spartan, Fenton, MO, USA) driven with the Spartan ultrasonic unit (Obtura-Spartan, Fenton, MO, USA) at the intensity prescribed by the manufacturer to the depth of 3 mm. The specimens were divided into two groups of 15.

Group A: The preparations were dried with paper points. White MTA (ProRoot®, Dentsply, Tulsa, OK, USA) was mixed according to manufacturer's recommendations and placed with a MTA Gun (Dentsply Maillefer, Ballaigues, Switzerland). Microball burnisher and micro-plugger were used to gently condense MTA into the root-end cavity. A moistened gauze was used to gently clean the resected surface and to remove any excessive MTA from the cavity.

Group B: The preparations were dried with paper points and contaminated with human blood for 15 seconds using a microbrush (Kerr Corporation, Orange, CA, USA)[195]. Care was taken that the contaminant completely covered the preparation. White MTA ProRoot® (Dentsply, Tulsa, OK, USA) was filled with the same technique as group A.

The radiographs were exposed to evaluate the quality and density of the root canal obturations and the root-end fillings. If the poor quality was seen, it was refilled.

The material was allowed to completely set for 24 hr. as recommended by the manufacturer. Then three layers of nail varnish (Revlon, New York, USA) were applied to the external surfaces of all roots except at the resected root surfaces.

The teeth in the negative control of the MTA group (n=2) were prepared and fill with the same techniques as described. The entire root surfaces and the apical foramens were covered with three layers of nail varnish to prevent any dye penetration.

Two instrumented roots which were root-end prepared with the same techniques as described (no obturation and root-end fillings placed) were served as positive controls (n=2).

*Composite group* (n=34)

Three millimeters of the root apex was resected ($0^{\circ}$ bevel) using #701 fissure bur (Kerr, Romulus, MI, USA) in a high-speed handpiece [18]. The resected root apex was prepared slightly concave using a large, round carbide bur (#27, Komet, Lemgo, Germany). Each bur was replaced after preparing each root. The prepared surface was dried with a small sponge. The specimens were divided into two groups of 15.

Group C: The resected surface was treated with the two-step self-etching adhesive system (Clearfil SE Bond, Kuraray Medical Co., Ltd., Osaka, Japan) following the manufacturer's instructions. The prepared surface was filled with Tetric® flow (Ivorclar Vivadent, Shaan, Liechtenstein). The average height of the resin composite filling was 2 mm measured from root-end preparation surface to the outer surface of Tetric® flow [38]. The composite root filling was polymerized under 40 seconds light cured. The light intensity was measured periodically during the restorative procedures using a radiometer (Model 100, Demetron/Kerr, Danbury, CT, USA) that ranged from 720 to 800 $mW/cm^2$. Care was taken to perpendicular light cure the restorative area from a distance of 10 mm. Oxygen inhibited layer was removed by swabbing with 70% alcohol and distilled water soaked cotton pellet as recommended by Rud et al. [64].

Group D: The resected surface was conditioned with the self-etching primer (Clearfil SE Bond Primer) and left for 20 seconds as recommended by the manufactured. The treated surface was gentle dried carefully by 10 mm oil-free compressed air to obtain the dried and shiny appearance which ensuring complete removal of the solvent. The conditioned surface was contaminated with human blood for 15 seconds using a microbrush (Kerr Corporation, Orange, CA, USA). No rinsing or re-conditioning was performed in order to investigate the variables under the harshest possible condition. Then the bonding agent (Clearfil SE Bond) was applied and light-cure for 10 seconds using a visible light-curing unit (VLC, Astralis 5, Vivadent, Shaan,

Liechtenstein) and filled with Tetric® flow (Ivorclar Vivadent, Shaan, Liechtenstein) in the same technique as group C. Three layers of nail varnish were applied to all external root surfaces except the resected root surfaces.

The teeth served as negative control in composite group (n=2) were prepared and filled with the same techniques as described, but the entire root surfaces were covered with three layers of nail varnish to prevent any dye penetration. Two instrumented roots which were root-end prepared with the same techniques as described but no obturation and root-end fillings placed were served as positive controls (n=2).

All specimens were stored at 37°C and 95% humidity for 24 hr before leakage test.

**Dye vacuum penetrating test**

Two percent methylene blue dye solutions (0.062 M) were prepared by mixing 2 g of methylene blue powder (MW 319.9; Sigma Chemical, St. Louis, MO, USA) with 100 ml of a standardized potassium phosphate monobasic-sodium hydroxide (0.05 M) buffer solution (Fisher Chemical, Fisher Scientific, Pittsburgh, PA, USA) of pH 7. The pH of the solution was measured prior to the addition of methylene blue with an Accumet model 950 pH/Ion meter (Fisher Scientific, Pittsburgh, PA, USA).

Each root sample was connected to a glass tube and sealed with sticky wax. The assembly was then placed in a centrifuge tube with a rubber cap. Two centimeters of the neutral buffered 2% methylene blue solution was dispensed into the centrifuge tube. The root was suspended in the dye, leaving 2 mm of the root apex in the dye [189] (Fig 11). The glass tube was connected to the vacuum pump (Bio-Rad Laboratories, Richmond, CA, USA) by using a solid glass plug. Pressure in the system was measured by a manometer that was connected to the vacuum pump and stabilized at 20 inch Hg for 15 minutes [173]. The vacuum was released slowly and the root remained in the 2% methylene blue. All specimens were stored in the incubator at 37 ˚C and 95% humidity for 24 hr.

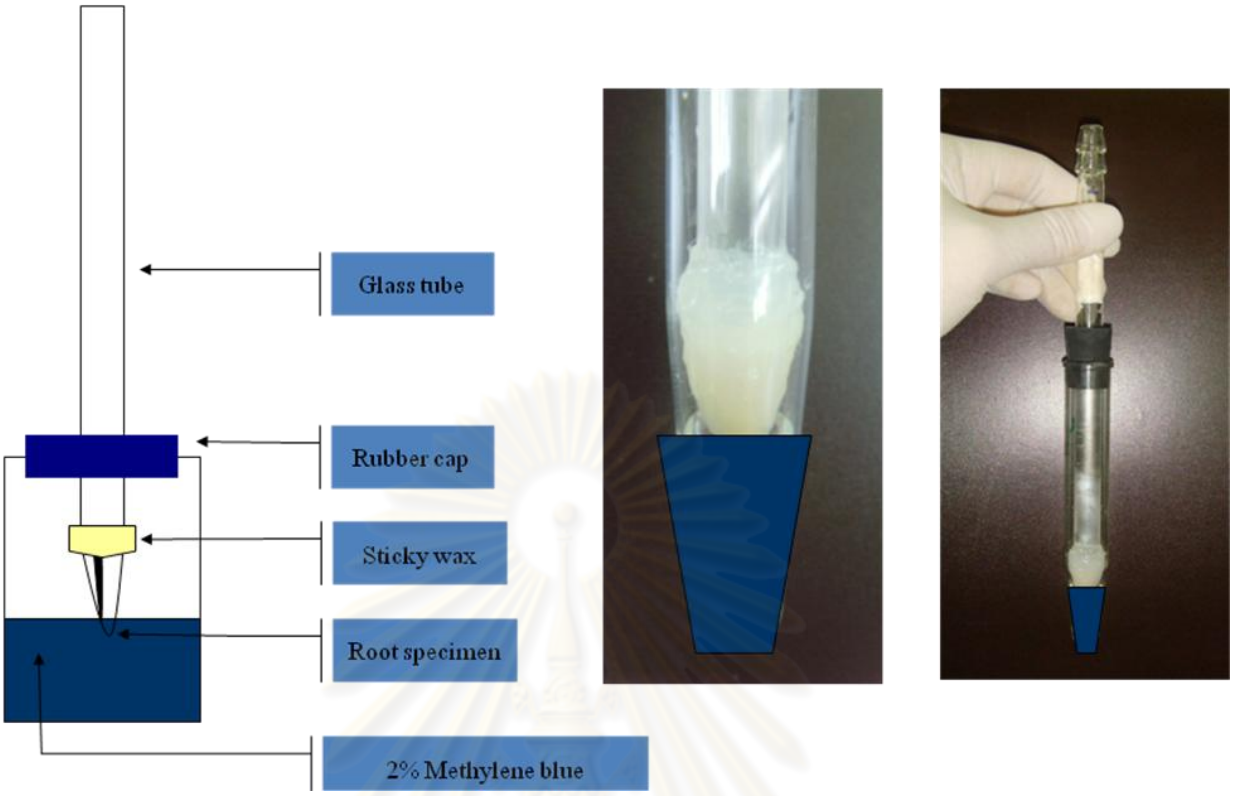



Fig 11 Dye penetrating model

The roots were removed and thoroughly rinsed with running water for 30 min. The nail varnish and sticky wax were scraped away from the teeth with blades #15 (Swann-Morton, Sheffield, England) and the polishing disc (Sof-Lex Pop-on, 3M, St Paul, Minn, USA) on a rotary slow-speed handpiece. Care was taken not to remove tooth structure.

The roots were sectioned vertically along the long axis using the low speed saw (IsoMet®, Buehler Ltd, Lake Bluff, IL, USA). The two halves of each tooth were mounted with the repositioned pin for viewing and recording under a stereoscopic microscope (Model XT, Olympus, Tokyo, Japan) at x30 magnification and evaluated by UTHSCSA Image tool program version 3.0. The maximum distance of dye penetration was measured by tracing the leakage from the beginning point at the interface of root surface and root-end filling material to the furthest point in the root canal system. The measurements were made by a blinded investigator. Reliability of the investigator was tested using the Intraclass correlation coeffient. (SPSS for windows, version 15.0; SPSS

Inc., Chicago, IL, USA). The data were recorded for statistical analysis. These same two root halves were then subjected to quantitative dye extraction.

### Dye Extraction test

The teeth were stored in a vial containing 1ml of concentrated (65%) nitric acid for 3 days. The solution was transferred to the centrifuged tube and then centrifuged (Universal 16R; Hettich Zentrifugen, Tuttlingen, Germany) at 14,000 rpm for 5 min to separate debris from the extracted dye. Nine hundred microliters of supernatant was taken from each centrifuged tube and was filtrated using syringe filter (Millipore, Billerica, MA, USA). The clear liquid solution was transferred to the glass cuvette. Sample absorbance was determined using an automatic spectrophotometer (SLT Spectra II; Labinstruments A-5082, Salzburg, Austria) at 580 nm using 65% nitric acid as the blank [160]. Pilot study had indicated that this was the optimal wavelength.

## Statistic Analysis

The data were analyzed by the Two-Way ANOVA followed by post hoc multiple comparison (Dunnett T3) test. The level of significance was set at $\alpha$ = 0.05 (SPSS for windows, version 15.0, SPSS Inc., Chicago, IL, USA). The Pearson correlation coefficient was used for statistical analysis of the difference in leakage outcome between the dye penetration method and the dye extraction method.

The reliability level of the investigator was estimated using Intraclass correlation coefficient at statistically significant level 0.05.

# CHAPTER IV

# RESULTS

## Dye vacuum penetrating test

The Intraclass correlation coefficient is 0.95 at statistically significant level 0.05 indicating excellent reliability of the blind investigator.

All positive control teeth exhibited 100% dye leaked into the root canal system. In contrast, no leakage was observed in the negative control group.

Dye leakage degree can be widely divided as complete leakage and incomplete leakage. Complete leakage was determined by dye leakage through root-end filling material into the root canal system. Incomplete leakage was determined by dye leakage through root-end filling material but not into the root canal system (Fig 12).

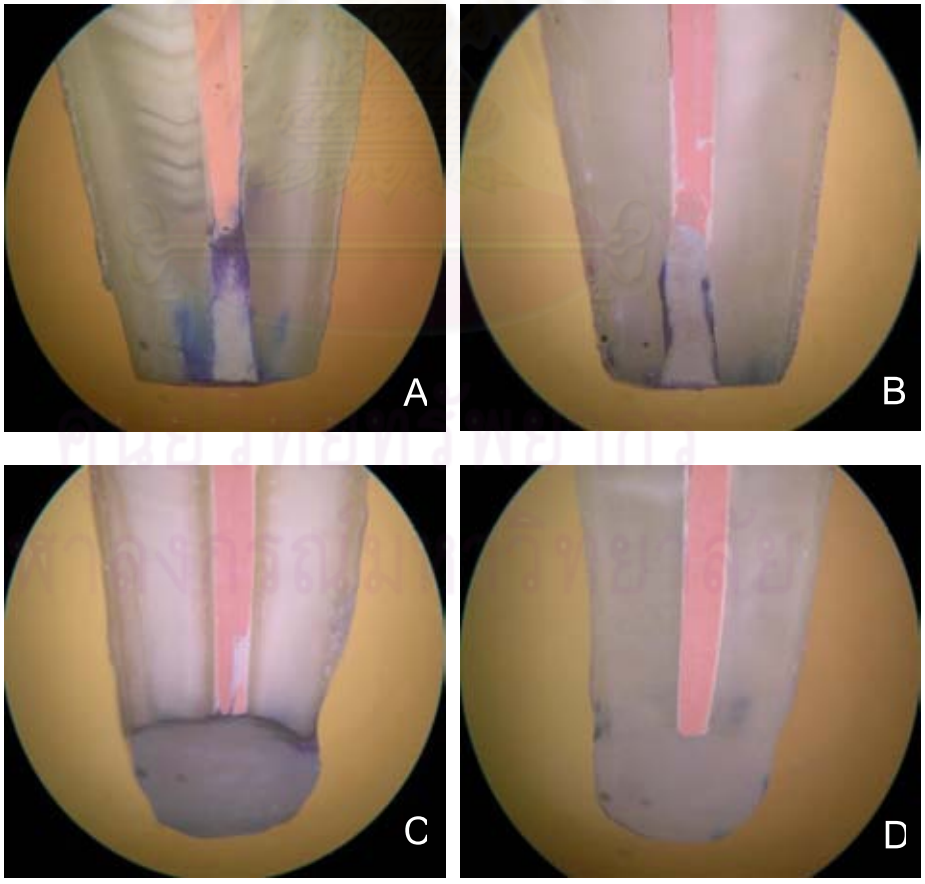


Fig 12 Stereo micrographs at magnification X30 of MTA groups show complete dye leakage (A) and incomplete dye leakage (B) and Tetric Flow groups show complete dye leakage (C) and incomplete dye leakage (D)

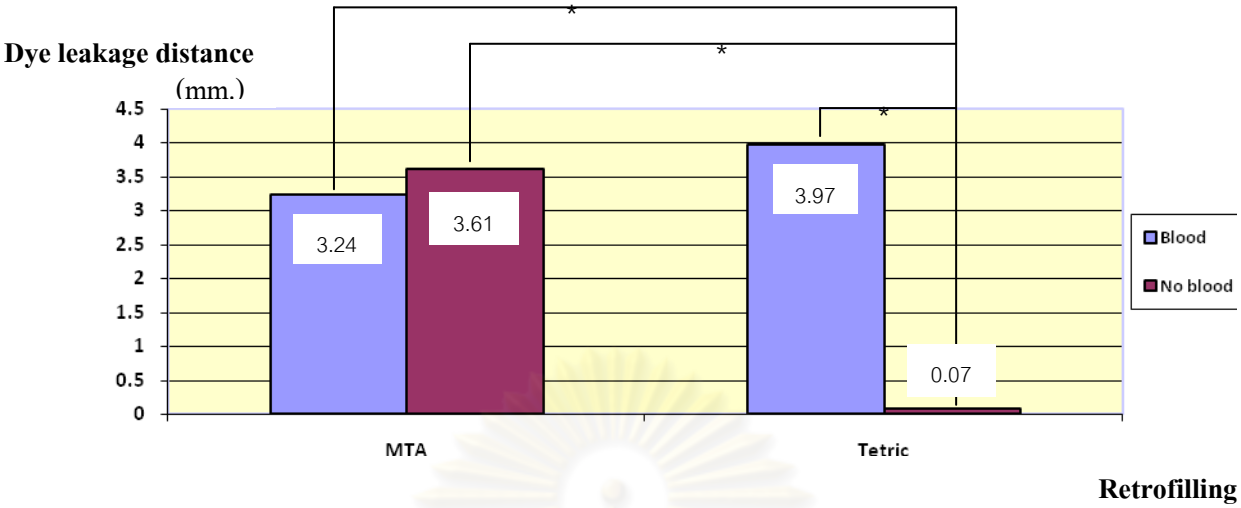
Complete leakage was observed in 14 roots (93.33%) of the contaminated MTA group, 15 roots (100%) in the uncontaminated MTA group, and 14 roots (93.33%) in the contaminated Tetric Flow group. Incomplete leakage was observed in 15 roots (100%) of the uncontaminated Tetric Flow group and 1 root (6.67%) both in the contaminated MTA and the contaminated Tetric Flow group.

Means and standard deviation of the dye leakage distance (mm.) are summarized in table 7

| Technique | Blood contamination | N | Mean leakage (mm.) | Std. Deviation |
|---|---|---|---|---|
| MTA | No blood | 15 | 3.61 | 1.36 |
| | Blood | 15 | 3.24 | 1.19 |
| Tetric Flow | No blood | 15 | 0.07 | 0.08 |
| | Blood | 15 | 3.97 | 1.64 |

Table 7 Mean and standard deviation of the methylene blue leakage into root canal space (mm.)

A double classification analysis of variance was performed to determine the significance of the main effects (root-end filling techniques and blood contamination) and their interaction. Analysis of the data indicated that the main effects and their interaction were significantly related to dye leakage in the tooth. (0.05 level of significance).




* = statistically significance

Fig. 13 Methylene blue leakages of two retrograde filling techniques with and without blood contamination.

The ANOVA showed a statistical difference among the experimental groups ($p<0.05$). Because of the variance of the data were unequal the Dunnett T3 test was used to determine which pair of the experimental groups that were statistically significant differences. Fig 13 showed that the uncontaminated Tetric Flow group displayed the least leakage ($p<0.05$), whereas no difference was observed among the three other experimental groups.

## Dye Extraction test

| Technique | Blood contamination | N | Mean leakage absorbance (580 nm) | Std. Deviation |
|---|---|---|---|---|
| MTA | No blood | 15 | .037 | .024 |
| | Blood | 15 | .030 | .018 |
| Tetric Flow | No blood | 15 | .009 | .006 |
| | Blood | 15 | .080 | .037 |

Table 8 Mean and standard deviation of the leakage absorbance

Results of the spectrophotometric readings for each group were summarized in Table 8. The highest dye absorbance was seen in the blood contaminated Tetric Flow group (0.08) followed by the uncontaminated (0.037) and blood contaminated MTA groups (0.03). The uncontaminated Tetric Flow group (0.009) had the least dye absorbance. The blood contaminated Tetric Flow group showed significantly higher dye absorbance than the other three experimental groups. The two groups of MTA were not significantly different but had higher dye absorbance than the uncontaminated Tetric Flow group.

A double classification analysis of variance was performed to determine the significance of the main effects (root-end filling technique and blood contamination) and their interaction. Analysis of the data indicated that blood contamination and the interaction between the main effects (root-end filling technique and blood contamination) were significantly related to the dye absorbance (0.05 level of significance) (Table 9, Fig 14).

| Effects | df | F | Sig |
|---|---|---|---|
| Retrofilling tech | 1 | 2.953 | .091 |
| Blood contamination | 1 | 26.908 | .000 * |
| Tech x Blood | 1 | 40.104 | .000 * |

* = statistically significance

Table 9 Double classification analysis of variance


Fig 14 Profile plots of Between-Subjects Effects show interaction between the main effects (root-end filling techniques and blood contamination)

The Dunnett T3 significance test was performed on the data to determine where differences if any occurred within the interaction between the main effects (Table 10).

Dependent Variable: Ext

Dunnett T3

| (A) type | (B) type | Mean Difference (A-B) | Sig. |
|---|---|---|---|
| MTA-B | MTA-NB | -.007067 | .923 |
| | Tet-B | -.049667* | .001 |
| | Tet-NB | .021400* | .002 |
| MTA-NB | Tet-B | -.042600* | .006 |
| | Tet-NB | .028467* | .002 |
| Tet-B | Tet-NB | .071067* | .000 |

*. The mean difference is significant at the .05 level.

Table 10 The Dunnett T3 significance test

The Dunnett T3 indicated that there were no significant differences between the blood contaminated and uncontaminated MTA groups, but differences did exist when comparing the blood contaminated Tetric Flow group with the blood contaminated and uncontaminated MTA groups. The uncontaminated Tetric Flow group displayed significant difference from the three other experimental groups (Table 10).

# CHAPTER V

# DISCUSSION AND CONCLUSION

## Discussion

Leakage study methodologies and their results, which are often contradictory, are being questioned. Factors such as the choice of storage solution, choice of dye and their pH as well as a great deal of other variables, can influence the outcome in these in vitro studies. The most popular method for leakage assessment, namely, linear measurement of tracer or dye penetration, should be considered as a semiquantitative technique which does not provide any information about the volume or tracer that penetrates a root filling [196]. The limitations of dye penetration technique have been discussed in previous publications [176, 188, 197]. Methylene blue solution penetrating through the root-end filling does not indicate the passage of bacteria, however the leakage of bacterial by-products, like metabolites, toxins, and degradation products smaller than bacteria may occur and play a decisive role in periapical disease [166]. Torabinejad et al. [9] stated that a material that is able to prevent the penetration of small molecules (dye) should be able to prevent larger substances like bacteria and their byproducts. Although bacterial microleakage models can represent the reality of bacteria leakage in clinical [180] but it should also be taken into account that common procedures for sterilizing specimens used in bacterial microleakage models (autoclaving and ethylene oxide) may alter the structure of dentin [198]. In addition, this method is not suitable for testing leakage of anti-bacterial materials like MTA [199].

Quantitative measurements include fluid filtration technique and dye extraction technique. The methodology of using dye extraction technique to analyze leakage was presented in previous studies [179, 200, 201]. The dye extraction technique involves recovering all of the dye that penetrated the interfaces between the root-end filling material and the root, thereby avoiding the limitations of sectioning the root. In addition, this technique is based on quantitative measurements of the passage of a liquid within these interfaces [160]. This technique has one advantage over the linear

method in that linear measurements do not allow for differentiation in density of leakage, provide only a two-dimensional view of a three-dimensional body, and do not allow for leakage that penetrates laterally into the dentinal tubules [179, 201]. In endodontics, bacteria are entrapped within the dentinal tubules and the infection potential they represent is likely proportional to their number [202]. This is estimated better by the quantitative methods; the dye extraction technique and the fluid filtration technique than the qualitative method; dye penetration method [160]. Dye leakage degree can be widely divided as complete leakage and incomplete leakage. Complete leakage was determined by dye leakage through root-end filling material into the root canal system. Incomplete leakage was determined by dye leakage through root-end filling material but not into the root canal system (Fig 12). Most of specimens in each experimental group showed complete leakage except the uncontaminated Tetric Flow group which all specimens showed incomplete leakage. In clinic, complete leakage indicates the failure of sealing ability. Therefore, this study chosen to determine the leakage using dye penetration method and dye extraction method. The data provided both the pattern of leakage and the quantitative leakage of the same tooth. The results from both measurement techniques were in the same way but dye extraction method was better classification. The contaminated Tetric Flow group was significant higher dye absorbance than the contaminated and uncontaminated MTA groups but from dye penetration study these three groups were not significant differences. This may indicate that the dye penetration did not provide enough discrimination to find any difference between the two MTA groups and the contaminated Tetric Flow group.

The means of dye leakage distances from this study (the contaminated MTA group = 3.24 mm and the uncontaminated MTA group = 3.61 mm) are higher than the study of Torabinejad [80] which mean distance of the contaminated MTA group was 0.28 mm and the uncontamination MTA group was 0.31 mm. This study tested the leakage of methylene blue under 20 inches Hg vacuum pressure for 15 minutes to simulate the severe condition that may occur. The pilot study found that 20 inches Hg pressure was the appropriate reading and could exhibit 100% dye leaked into the root canal system (positive control group). The pH and chemical reactivity of dye might also

influence the degree of dye penetration [170, 175]. The 2% concentration of methylene blue was observed to be acidic when prepared (pH 3.45) [170, 203]. If use without adjustment to the neutral pH, it would cause too much leakage than the reality as the inorganic part of dentine was dissolved by acid [170, 175, 204]. The other factor that affects leakage is the root position suspended in dye. If the root was upright position and tested under vacuum systems, leakage would be more than testing under atmospheric pressure and horizontal position under vacuum [173]. In this study the neutral pH of methylene blue was adjusted to get the actual leakage of root-end filling whereas the study of Torabinejad [80] tested under atmospheric pressure and only 1% concentration of methylene blue was used. No information about pH of dye and root suspended position which might cause the different leakage values.

Ambus and Munksgaard [38] evaluated the ability of dentin bonding agents to bond composite to concave root-end preparations. They concluded that the slightly curved surface will prevent formation of contraction gaps between composite retrograde filling and bonding agent treated dentin. This is consistent with the data from this study which did not find any gap between the dentinal wall and root-end filling material in the uncontaminated Tetric Flow group under x30 magnification microscope. However gaps were found in most of specimens in the contaminated Tetric Flow group due to loss of adhesion on the root surfaces when contaminated with blood [155-159].

In clinical endodontic surgery, it is often difficult to avoid blood contamination. Torabinejad et al. [80] reported that blood contamination did not affect the sealing ability of MTA. This is in consistent with the results of this study which the contaminated MTA group was not statistically significant different from the uncontaminated MTA group. It was notable that rood-end fillings in the contaminated Tetric Flow group had lower bond strength than the uncontaminated Tetric Flow group. As in the process of cutting the roots longitudinally to assess the dye leakage, composite root-end filling materials of most of specimens in the contaminated Tetric Flow group were dislodged immediately when contacting with the cutting disc while all of the uncontaminated Tetric Flow group were intact. When blood contamination occurred on the dentin surface after dentinal collagen had been exposed, the bond

strength was decreased. The inhibition of adhesion by blood contamination on the dentin is strongly associated with the superficial layer of exposed collagen. Blood contamination might permit some reaction between the superficial organic layer of exposed dentinal collagen and the blood protein components. This reaction might inhibit resin penetration into the dentin and polymerization. The residues or reactants can become a strong mechanical inhibitor to the adhesion between the dentin and the adhesive resin cement [157]. Therefore, blood contamination on the adherent surface during the root-end filling is likely to be one of the main causes of leakage.

The different root-end filling techniques created different pattern of leakage. This study found that both the root-end preparation techniques and the blood contamination influenced the leakage of root-end filling materials. Only the uncontaminated Tetric Flow group was the least leakage significantly. Three millimeters deep ultrasonic root-end preparation is difficult to get an ideal preparation. The axis of the preparation may not parallel to the root canal and often left some residual gutta-percha remaining on the root canal wall [56]. In the process of root-end preparing in this study, the same problem occurred which may affect the occurrence of leakage. The slightly concave root-end preparation filled with flowable resin composite was easy and simple to perform. The short time required when performing the slightly concave root-end preparation could minimize the adverse effect of moisture and hemorrhagic contamination [37].

In cases which are difficult to maintain proper isolation from blood contamination, ultrasonic root-end preparation filled with MTA is more suitable than slightly concave root-end preparation filled with flowable resin composite. If blood contamination can control properly the slightly concave root-end preparation filled with flowable resin composite seems to be another alternative technique which is effectively resistance to leakage.

## Conclusion

Factors affecting dye leakage of the two root-end filling techniques are both the techniques and the blood contamination. The concave cavity root-end preparation filled with Tetric Flow without blood contamination has the least dye leakage.

# REFERENCES


(1) Hession, R.W. Long-term evaluation of endodontic treatment: anatomy, instrumentation, obturation--the endodontic practice triad. Int Endod J. 14(1981):179-84.

(2) Meeuwissen, R., Eschen, S. Twenty years of endodontic treatment. J Endod. 9(1983):390-3.

(3) Sjogren, U., Hagglund, B., Sundqvist, G., Wing, K. Factors affecting the long-term results of endodontic treatment. J Endod. 16(1990):498-504.

(4) Harty, F.J., Parkins, B.J., Wengraf, A.M. Success rate in root canal therapy. A retrospective study of conventional cases. Br Dent J. 128(1970):65-70.

(5) Dorn, S.O., Gartner, A.H. Retrograde filling materials: a retrospective success-failure study of amalgam, EBA, and IRM. J Endod. 16(1990):391-3.

(6) Frank, A.L., Glick, D.H., Patterson, S.S., Weine, F.S. Long-term evaluation of surgically placed amalgam fillings. J Endod. 18(1992):391-8.

(7) Zesis, A., Lin, S., Fuss, Z. [Endodontic surgery (apicoectomy)--success rate of more than 90% using dental operating microscope and ultrasonic tips] [Abstract]. Refuat Hapeh Vehashinayim. 22(2005):33-41, 86.

(8) Torabinejad, M., Pitt Ford, T.R. Root end filling materials: a review. Endod Dent Traumatol. 12(1996):161-78.

(9) Torabinejad, M., Watson, T.F., Pitt Ford, T.R. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod. 19(1993):591-5.

(10) Poggio, C., Lombardini, M., Alessandro, C., Simonetta, R. Solubility of root-end-filling materials: a comparative study. J Endod. 33(2007):1094-7.

(11) Gartner, A.H., Dorn, S.O. Advances in endodontic surgery. Dent Clin North Am. 36(1992):357-78.

(12) Moodnik, R.M., Levey, M.H., Besen, M.A., Borden, B.G. Retrograde amalgam filling: a scanning electron microscopic study. J Endod. 1(1975):28-31.

(13) Abdal, A.K., Retief, D.H. The apical seal via the retrosurgical approach. I.A. preliminary study. Oral Surg Oral Med Oral Pathol. 53(1982):614-21.

(14) Kaplan, S.D., Tanzilli, J.P., Raphael, D., Moodnik, R.M. A comparison of the marginal leakage of retrograde techniques. Oral Surg Oral Med Oral Pathol. 54(1982):583-5.

(15) Stabholz, A., Friedman, S., Abed, J. Marginal adaptation of retrograde fillings and its correlation with sealability. J Endod. 11(1985):218-23.

(16) O'Connor, R.P., Hutter, J.W., Roahen, J.O. Leakage of amalgam and Super-EBA root-end fillings using two preparation techniques and surgical microscopy. J Endod. 21(1995):74-8.

(17) Tam, A., Yu, D.C. A new approach to restoring root-end cavity preparation in surgical endodontic procedures--a scanning electron microscopy study. Compend Contin Educ Dent. 23(2002):1123-6, 8, 30; quiz 32.

(18) Bondra, D.L., Hartwell, G.R., MacPherson, M.G., Portell, F.R. Leakage in vitro with IRM, high copper amalgam, and EBA cement as retrofilling materials. J Endod. 15(1989):157-60.

(19) Bruce, G.R., McDonald, N.J., Sydiskis, R.J. Cytotoxicity of retrofill materials. J Endod. 19(1993):288-92.

(20) Torabinejad, M., Hong, C.U., Lee, S.J., Monsef, M., Pitt Ford, T.R. Investigation of mineral trioxide aggregate for root-end filling in dogs. J Endod. 21(1995):603-8.

(21) Torabinejad, M., Hong, C.U., McDonald, F., Pitt Ford, T.R. Physical and chemical properties of a new root-end filling material. J Endod. 21(1995):349-53.

(22) Torabinejad, M., Hong, C.U., Pitt Ford, T.R., Kettering, J.D. Antibacterial effects of some root end filling materials. J Endod. 21(1995):403-6.

(23) Torabinejad, M., Hong, C.U., Pitt Ford, T.R., Kettering, J.D. Cytotoxicity of four root end filling materials. J Endod. 21(1995):489-92.

(24) Torabinejad, M., Rastegar, A.F., Kettering, J.D., Pitt Ford, T.R. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endod. 21(1995):109-12.

(25) Torabinejad, M., Smith, P.W., Kettering, J.D., Pitt Ford, T.R. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endod. 21(1995):295-9.

(26) Schwartz, R.S., Mauger, M., Clement, D.J., Walker, W.A., 3rd Mineral trioxide aggregate: a new material for endodontics. J Am Dent Assoc. 130(1999):967-75.

(27) Chng, H.K., Islam, I., Yap, A.U., Tong, Y.W., Koh, E.T. Properties of a new root-end filling material. J Endod. 31(2005):665-8.

(28) Kim, S. Retrofilling materials and techniques. In: Philadelphia, editor. Color atlas of microsurgery in endodontics: W.B. Saunders; 2001. p. 115-24.

(29) Barkhordar, R.A., Pelzner, R.B., Stark, M.M. Use of glass ionomers as retrofilling materials. Oral Surg Oral Med Oral Pathol. 67(1989):734-9.

(30) Olson, A.K., MacPherson, M.G., Hartwell, G.R., Weller, R.N., Kulild, J.C. An in vitro evaluation of injectable thermoplasticized gutta-percha, glass ionomer, and amalgam when used as retrofilling materials. J Endod. 16(1990):361-4.

(31) Aktener, B.O., Pehlivan, Y. Sealing ability of cermet ionomer cement as a retrograde filling material. Int Endod J. 26(1993):137-41.

(32) Pissiotis, E., Sapounas, G., Spangberg, L.S. Silver glass ionomer cement as a retrograde filling material: a study in vitro. J Endod. 17(1991):225-9.

(33) Guttman, J.L., Harrison, J.W. . Surgical endodontics. Boston: Blackwell Scientific Publications.; 1991. p. 230-63.

(34) Abedi, H.R., Van Mierlo, B.L., Wilder-Smith, P., Torabinejad, M. Effects of ultrasonic root-end cavity preparation on the root apex. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 80(1995):207-13.

(35) Andreasen, J.O., Munksgaard, E.C., Fredebo, L., Rud, J. Periodontal tissue regeneration including cementogenesis adjacent to dentin-bonded retrograde composite fillings in humans. J Endod. 19(1993):151-3.

(36) Rud, J., Rud, V., Munksgaard, E.C. Long-term evaluation of retrograde root filling with dentin-bonded resin composite. J Endod. 22(1996):90-3.

(37) Vignaroli, P.A., Anderson, R.W., Pashley, D.H. Longitudinal evaluation of the microleakage of dentin bonding agents used to seal resected root apices. J Endod. 21(1995):509-12.

(38) Ambus, C., Munksgaard, E.C. Dentin bonding agents and composite retrograde root filling. Am J Dent. 6(1993):35-8.

(39) Theodosopoulou, J.N., Niederman, R. A systematic review of in vitro retrograde obturation materials. J Endod. 31(2005):341-9.

(40) Rud, J., Andreasen, J.O., Jensen, J.F. A multivariate analysis of the influence of various factors upon healing after endodontic surgery. Int J Oral Surg. 1(1972):258-71.

(41) Lin, L., Skribner, J., Shovlin, F., Langeland, K. Periapical surgery of mandibular posterior teeth: anatomical and surgical considerations. J Endod. 9(1983):496-501.

(42) Friedman, S. Retrograde approaches in endodontic therapy. Endod Dent Traumatol. 7(1991):97-107.

(43) Aqrabawi, J. Sealing ability of amalgam, super EBA cement, and MTA when used as retrograde filling materials. Br Dent J. 188(2000):266-8.

(44) von Arx, T., Walker, W.A., 3rd. Microsurgical instruments for root-end cavity preparation following apicoectomy: a literature review. Endod Dent Traumatol. 16(2000):47-62.

(45) Serota, K.S., Krakow, A.A. Retrograde instrumentation and obturation of the root canal space. J Endod. 9(1983):448-51.

(46) Reit, C., Hirsch, J. Surgical endodontic retreatment. Int Endod J. 19(1986):107-12.

(47) Amagasa, T., Nagase, M., Sato, T., Shioda, S. Apicoectomy with retrograde gutta-percha root filling. Oral Surg Oral Med Oral Pathol. 68(1989):339-42.

(48) Richman, M.J. The use of ultrasonics in root canal therapy and root resection. J Dent Med. 12(1957):12-8.

(49) Bertrand, G. Use of ultrasound in apicoectomy. Quintessence Int. 4(1976):9-12.

(50) Flath, R.K., Hicks, M.L. Retrograde instrumentation and obturation with new devices. J Endod. 13(1987):546-9.

(51) Engel, T.K., Steiman, H.R. Preliminary investigation of ultrasonic root end preparation. J Endod. 21(1995):443-5.

(52) Lin, C.P., Chou, H.G., Kuo, J.C., Lan, W.H. The quality of ultrasonic root-end preparation: a quantitative study. J Endod. 24(1998):666-70.

(53) Lloyd, A., Gutmann, J., Dummer, P., Newcombe, R. Microleakage of Diaket and amalgam in root-end cavities prepared using MicroMega sonic retro-prep tips. Int Endod J. 30(1997):196-204.

(54) Chailertvanitkul, P., Saunders, W.P., Saunders, E.M., MacKenzie, D. Polymicrobial coronal leakage of Super-EBA root-end fillings following two methods of root-end preparation. Int Endod J. 31(1998):348-52.

(55) Gutmann, J.L., Saunders, W.P., Nguyen, L., Guo, I.Y., Saunders, E.M. Ultrasonic root-end preparation. Part 1. SEM analysis. Int Endod J. 27(1994):318-24.

(56) Khabbaz, M.G., Kerezoudis, N.P., Aroni, E., Tsatsas, V. Evaluation of different methods for the root-end cavity preparation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 98(2004):237-42.

(57) Saunders, W.P., Saunders, E.M., Gutmann, J.L. Ultrasonic root-end preparation, Part 2. Microleakage of EBA root-end fillings. Int Endod J. 27(1994):325-9.

(58) Waplington, M., Lumley, P.J., Walmsley, A.D. Incidence of root face alteration after ultrasonic retrograde cavity preparation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 83(1997):387-92.

(59) Gray, G.J., Hatton, J.F., Holtzmann, D.J., Jenkins, D.B., Nielsen, C.J. Quality of root-end preparations using ultrasonic and rotary instrumentation in cadavers. J Endod. 26(2000):281-3.

(60) Tobon-Arroyave, S.I., Restrepo-Perez, M.M., Arismendi-Echavarria, J.A., Velasquez-Restrepo, Z., Marin-Botero, M.L., Garcia-Dorado, E.C. Ex vivo microscopic assessment of factors affecting the quality of apical seal created by root-end fillings. Int Endod J. 40(2007):590-602.

(61) Arens, D. Surgical endodontics. In: Cohen, S., Burns R., editors. Pathways of the pulp. St. Louis: CV Mosby; 1991. p. 594-6.

(62) Walmsley, A.D., Lumley, P.J., Johnson, W.T., Walton, R.E. Breakage of ultrasonic root-end preparation tips. J Endod. 22(1996):287-9.

(63) Rud, J., Munksgaard, E.C., Andreasen, J.O., Rud, V., Asmussen, E. Retrograde root filling with composite and a dentin-bonding agent. 1. Endod Dent Traumatol. 7(1991):118-25.

(64) Rud, J., Munksgaard, E.C., Andreasen, J.O., Rud, V. Retrograde root filling with composite and a dentin-bonding agent. 2. Endod Dent Traumatol. 7(1991):126-31.

(65) Abramovitz, L., Lev, R., Fuss, Z., Metzger, Z. The unpredictability of seal after post space preparation: a fluid transport study. J Endod. 27(2001):292-5.

(66) Platt, A.S., Wannfors, K. The effectiveness of compomer as a root-end filling: a clinical investigation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 97(2004):508-12.

(67) Davidson, C.L., de Gee, A.J., Feilzer, A. The competition between the composite-dentin bond strength and the polymerization contraction stress. J Dent Res. 63(1984):1396-9.

(68) Johnson, B.R. Considerations in the selection of a root-end filling material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 87(1999):398-404.

(69) Lee, S.J., Monsef, M., Torabinejad, M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod. 19(1993):541-4.

(70) Abdullah, D., Ford, T.R., Papaioannou, S., Nicholson, J., McDonald, F. An evaluation of accelerated Portland cement as a restorative material. Biomaterials. 23(2002):4001-10.

(71) Camilleri, J., Montesin, F.E., Brady, K., Sweeney, R., Curtis, R.V., Ford, T.R. The constitution of mineral trioxide aggregate. Dent Mater. 21(2005):297-303.

(72) Song, J.S., Mante, F.K., Romanow, W.J., Kim, S. Chemical analysis of powder and set forms of Portland cement, gray ProRoot MTA, white ProRoot MTA, and

gray MTA-Angelus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 102(2006):809-15.

(73) Balto, H.A. Attachment and morphological behavior of human periodontal ligament fibroblasts to mineral trioxide aggregate: a scanning electron microscope study. J Endod. 30(2004):25-9.

(74) Bonson, S., Jeansonne, B.G., Lallier, T.E. Root-end filling materials alter fibroblast differentiation. J Dent Res. 83(2004):408-13.

(75) Keiser, K., Johnson, C.C., Tipton, D.A. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. J Endod. 26(2000):288-91.

(76) Koh, E.T., Torabinejad, M., Pitt Ford, T.R., Brady, K., McDonald, F. Mineral trioxide aggregate stimulates a biological response in human osteoblasts. J Biomed Mater Res. 37(1997):432-9.

(77) Koh, E.T., McDonald, F., Pitt Ford, T.R., Torabinejad, M. Cellular response to Mineral Trioxide Aggregate. J Endod. 24(1998):543-7.

(78) Osorio, R.M., Hefti, A., Vertucci, F.J., Shawley, A.L. Cytotoxicity of endodontic materials. J Endod. 24(1998):91-6.

(79) Torabinejad, M., Pitt Ford, T.R., McKendry, D.J., Abedi, H.R., Miller, D.A., Kariyawasam, S.P. Histologic assessment of mineral trioxide aggregate as a root-end filling in monkeys. J Endod. 23(1997):225-8.

(80) Torabinejad, M., Higa, R.K., McKendry, D.J., Pitt Ford, T.R. Dye leakage of four root end filling materials: effects of blood contamination. J Endod. 20(1994):159-63.

(81) Bates, C.F., Carnes, D.L., del Rio, C.E. Longitudinal sealing ability of mineral trioxide aggregate as a root-end filling material. J Endod. 22(1996):575-8.

(82) Wu, M.K., Kontakiotis, E.G., Wesselink, P.R. Long-term seal provided by some root-end filling materials. J Endod. 24(1998):557-60.

(83) Ford, T.R., Torabinejad, M., McKendry, D.J., Hong, C.U., Kariyawasam, S.P. Use of mineral trioxide aggregate for repair of furcal perforations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 79(1995):756-63.

(84) Aeinehchi, M., Eslami, B., Ghanbariha, M., Saffar, A.S. Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. Int Endod J. 36(2003):225-31.

(85) Menezes, R., Bramante, C.M., Letra, A., Carvalho, V.G., Garcia, R.B. Histologic evaluation of pulpotomies in dog using two types of mineral trioxide aggregate and regular and white Portland cements as wound dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 98(2004):376-9.

(86) Ford, T.R., Torabinejad, M., Abedi, H.R., Bakland, L.K., Kariyawasam, S.P. Using mineral trioxide aggregate as a pulp-capping material. J Am Dent Assoc. 127(1996):1491-4.

(87) Shabahang, S., Torabinejad, M., Boyne, P.P., Abedi, H., McMillan, P. A comparative study of root-end induction using osteogenic protein-1, calcium hydroxide, and mineral trioxide aggregate in dogs. J Endod. 25(1999):1-5.

(88) Hayashi, M., Shimizu, A., Ebisu, S. MTA for obturation of mandibular central incisors with open apices: case report. J Endod. 30(2004):120-2.

(89) Nakata, T.T., Bae, K.S., Baumgartner, J.C. Perforation repair comparing mineral trioxide aggregate and amalgam using an anaerobic bacterial leakage model. J Endod. 24(1998):184-6.

(90) Main, C., Mirzayan, N., Shabahang, S., Torabinejad, M. Repair of root perforations using mineral trioxide aggregate: a long-term study. J Endod. 30(2004):80-3.

(91) Ferris, D.M., Baumgartner, J.C. Perforation repair comparing two types of mineral trioxide aggregate. J Endod. 30(2004):422-4.

(92) Fridland, M., Rosado, R. Mineral trioxide aggregate (MTA) solubility and porosity with different water-to-powder ratios. J Endod. 29(2003):814-7.

(93) Mitchell, P.J., Pitt Ford, T.R., Torabinejad, M., McDonald, F. Osteoblast biocompatibility of mineral trioxide aggregate. Biomaterials. 20(1999):167-73.

(94) Zhu, Q., Haglund, R., Safavi, K.E., Spangberg, L.S. Adhesion of human osteoblasts on root-end filling materials. J Endod. 26(2000):404-6.

(95) Fischer, E.J., Arens, D.E., Miller, C.H. Bacterial leakage of mineral trioxide aggregate as compared with zinc-free amalgam, intermediate restorative material, and Super-EBA as a root-end filling material. J Endod. 24(1998):176-9.

(96) Yatsushiro, J.D., Baumgartner, J.C., Tinkle, J.S. Longitudinal study of the microleakage of two root-end filling materials using a fluid conductive system. J Endod. 24(1998):716-9.

(97) Adamo, H.L., Buruiana, R., Schertzer, L., Boylan, R.J. A comparison of MTA, Super-EBA, composite and amalgam as root-end filling materials using a bacterial microleakage model. Int Endod J. 32(1999):197-203.

(98) Stowe, T.J., Sedgley, C.M., Stowe, B., Fenno, J.C. The effects of chlorhexidine gluconate (0.12%) on the antimicrobial properties of tooth-colored ProRoot mineral trioxide aggregate. J Endod. 30(2004):429-31.

(99) Glickman, G.N., Koch, K.A. 21st-century endodontics. J Am Dent Assoc. 131 Suppl(2000):39S-46S.

(100) Oviir, T., Pagoria, D., Ibarra, G., Geurtsen, W. Effects of gray and white mineral trioxide aggregate on the proliferation of oral keratinocytes and cementoblasts. J Endod. 32(2006):210-3.

(101) Asgary, S., Parirokh, M., Eghbal, M.J., Brink, F. Chemical differences between white and gray mineral trioxide aggregate. J Endod. 31(2005):101-3.

(102) Holland, R., Souza, V., Nery, M.J., Faraco Junior, I.M., Bernabe, P.F., Otoboni Filho, J.A., et al. Reaction of rat connective tissue to implanted dentin tubes filled with a white mineral trioxide aggregate. Braz Dent J. 13(2002):23-6.

(103) Perez, A.L., Spears, R., Gutmann, J.L., Opperman, L.A. Osteoblasts and MG-63 osteosarcoma cells behave differently when in contact with ProRoot MTA and White MTA. Int Endod J. 36(2003):564-70.

(104) Parirokh, M., Asgary, S., Eghbal, M.J., Stowe, S., Eslami, B., Eskandarizade, A., et al. A comparative study of white and grey mineral trioxide aggregate as pulp capping agents in dog's teeth. Dent Traumatol. 21(2005):150-4.

(105) Stefopoulos, S., Tsatsas, D.V., Kerezoudis, N.P., Eliades, G. Comparative in vitro study of the sealing efficiency of white vs grey ProRoot mineral trioxide aggregate formulas as apical barriers. Dent Traumatol. 24(2008):207-13.

(106) Niederman, R., Theodosopoulou, J.N. A systematic review of in vivo retrograde obturation materials. Int Endod J. 36(2003):577-85.

(107) Dharananidhikul, A.N., R. Rosenberg, PA. Boylan, RJ. . Comparative bacterial assay of microleakage of three retrograde filling materials. Journal of Endodontics. 21(1995):225 (abstract).

(108) Attar, N., Tam, L.E., McComb, D. Flow, strength, stiffness and radiopacity of flowable resin composites. J Can Dent Assoc. 69(2003):516-21.

(109) Moon, P.C., Tabassian, M.S., Culbreath, T.E. Flow characteristics and film thickness of flowable resin composites. Oper Dent. 27(2002):248-53.

(110) Bayne, S.C., Thompson, J.Y., Swift, E.J., Jr., Stamatiades, P., Wilkerson, M. A characterization of first-generation flowable composites. J Am Dent Assoc. 129(1998):567-77.

(111) Ziskind, D., Adell, I., Teperovich, E., Peretz, B. The effect of an intermediate layer of flowable composite resin on microleakage in packable composite restorations. Int J Paediatr Dent. 15(2005):349-54.

(112) Braga, R.R., Hilton, T.J., Ferracane, J.L. Contraction stress of flowable composite materials and their efficacy as stress-relieving layers. J Am Dent Assoc. 134(2003):721-8.

(113) Payne, J.H.t. The marginal seal of Class II restorations: flowable composite resin compared to injectable glass ionomer. J Clin Pediatr Dent. 23(1999):123-30.

(114) Ferdianakis, K. Microleakage reduction from newer esthetic restorative materials in permanent molars. J Clin Pediatr Dent. 22(1998):221-9.

(115) Malmstrom, H.S., Schlueter, M., Roach, T., Moss, M.E. Effect of thickness of flowable resins on marginal leakage in class II composite restorations. Oper Dent. 27(2002):373-80.

(116) Fruits, T.J., VanBrunt, C.L., Khajotia, S.S., Duncanson, M.G., Jr. . Effect of cyclical lateral forces on microleakage in cervical resin composite restorations. Quintessence Int. 33(2002):205-12.

(117) Bouillaguet, S. Biological Risks of Resin-Based Materials to the Dentin-Pulp Complex. Crit Rev Oral Biol Med. 15(2004):47-60.

(118) Shimada, Y., Seki, Y., Sasafuchi, Y., Arakawa, M., Burrow, M.F., Otsuki, M., et al. Biocompatibility of a flowable composite bonded with a self-etching adhesive compared with a glass Ionomer cement and a high copper amalgam. Oper Dent. 29(2004):23-8.

(119) Goracci, G., Mori, G., Bazzucchi, M. Marginal seal and biocompatibility of a fourth-generation bonding agent. Dent Mater. 11(1995):343-7.

(120) Jenkins, S., Kulild, J., Williams, K., Lyons, W., Lee, C. Sealing ability of three materials in the orifice of root canal systems obturated with gutta-percha. J Endod. 32(2006):225-7.

(121) Rud, J., Rud, V., Munksgaard, E.C. Effect of root canal contents on healing of teeth with dentin-bonded resin composite retrograde seal. J Endod. 23(1997):535-41.

(122) Rud, J., Rud, V., Munksgaard, E.C. Periapical healing of mandibular molars after root-end sealing with dentine-bonded composite. Int Endod J. 34(2001):285-92.

(123) Jensen, S.S., Nattestad, A., Egdo, P., Sewerin, I., Munksgaard, E.C., Schou, S. A prospective, randomized, comparative clinical study of resin composite and glass ionomer cement for retrograde root filling. Clin Oral Investig. 6(2002):236-43.

(124) Yazdi, P.M., Schou, S., Jensen, S.S., Stoltze, K., Kenrad, B., Sewerin, I. Dentine-bonded resin composite (Retroplast) for root-end filling: a prospective clinical and radiographic study with a mean follow-up period of 8 years. Int Endod J. 40(2007):493-503.

(125) Bouschlicher, M.R., Cobb, D.S., Boyer, D.B. Radiopacity of compomers, flowable and conventional resin composites for posterior restorations. Oper Dent. 24(1999):20-5.

(126) Murchison, D.F., Charlton, D.G., Moore, W.S. Comparative radiopacity of flowable resin composites. Quintessence Int. 30(1999):179-84.

(127) Sabbagh, J., Vreven, J., Leloup, G. Radiopacity of resin-based materials measured in film radiographs and storage phosphor plate (Digora). Oper Dent. 29(2004):677-84.

(128) Haller, B. Recent developments in dentin bonding. Am J Dent. 13(2000):44-50.

(129) Murray, M.J., Loushine, R.J., Weller, R.N., Kimbrough, W.F., Pashley, D.H. Use of self-etching adhesives to seal resected apices. J Endod. 30(2004):538-40.

(130) Nakabayashi, N., Kojima, K., Masuhara, E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res. 16(1982):265-73.

(131) Cardoso, P.E., Carrilho, M.R., Francci, C.E., Perdigao, J. Microtensile bond strengths of one-bottle dentin adhesives. Am J Dent. 14(2001):22-4.

(132) Van Meerbeek, B., Perdigao, J., Lambrechts, P., Vanherle, G. The clinical performance of adhesives. J Dent. 26(1998):1-20.

(133) Hannig, M., Reinhardt, K.J., Bott, B. Composite-to-dentin bond strength, micromorphology of the bonded dentin interface and marginal adaptation of Class II composite resin restorations using self-etching primers. Oper Dent. 26(2001):157-65.

(134) Nakajima, M., Sano, H., Urabe, I., Tagami, J., Pashley, D.H. Bond strengths of single-bottle dentin adhesives to caries-affected dentin. Oper Dent. 25(2000):2-10.

(135) Kanca, J. One-step bond strength to enamel and dentin. Am J Dent. 9(1997):5-8.

(136) Nakabayashi, N., Pashley, D.H. Hybridization of dental hard tissues. Quintessence. First ed. Tokyo1998.

(137) Nakatsuka, K., Arikawa, K. Adhesive Characteristics of a New Bonding System "KBN". J Dent Res. 78(1999):2291.

(138) Inoue, H., Inoue, S., Uno, S., Komatsu, H., Sano, H. Adhesion of new simplified adhesive system to human dentin. J Dent Res. 78(1999):2998.

(139) Sutimuntanakul, S., Worayoskowit, W., Mangkornkarn, C. Retrograde seal in ultrasonically prepared canals. J Endod. 26(2000):444-6.

(140) Miyazaki, M., Sato, M., Onose, H., Moore, B.K. Influence of thermal cycling on dentin bond strength of two-step bonding systems. Am J Dent. 11(1998):118-22.

(141) Sano, H., Yoshikawa, T., Pereira, P.N., Kanemura, N., Morigami, M., Tagami, J., et al. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res. 78(1999):906-11.

(142) Van Meerbeek, B., Vargas, M., Inoue, S., Yoshida, Y., Peumans, M., Lambrechts, P. Adhesives and cements to promote preservation dentistry. Oper Dent. 26(2001):119-44.

(143) Sano, H., Takatsu, T., Ciucchi, B., Horner, J.A., Matthews, W.G., Pashley, D.H. Nanoleakage: leakage within the hybrid layer. Oper Dent. 20(1995):18-25.

(144) Thirawat, J., Edmunds, D.H. Sealing ability of materials used as retrograde root fillings in endodontic surgery. Int Endod J. 22(1989):295-8.

(145) Hardy, I., Liewehr, F.R., Joyce, A.P., Agee, K., Pashley, D.H. Sealing ability of One-Up Bond and MTA with and without a secondary seal as furcation perforation repair materials. J Endod. 30(2004):658-61.

(146) Fogel, H.M., Peikoff, M.D. Microleakage of root-end filling materials. J Endod. 27(2001):456-8.

(147) Hara, A.T., Amaral, C.M., Pimenta, L.A., Sinhoreti, M.A. Shear bond strength of hydrophilic adhesive systems to enamel. Am J Dent. 12(1999):181-4.

(148) De Munck, J., Vargas, M., Iracki, J., Van Landuyt, K., Poitevin, A., Lambrechts, P., et al. One-day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. Oper Dent. 30(2005):39-49.

(149) Tay, F.R., Sano, H., Carvalho, R., Pashley, E.L., Pashley, D.H. An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. J Adhes Dent. 2(2000):83-98.

(150) Mitsui, F.H., Bedran-de-Castro, A.K., Ritter, A.V., Cardoso, P.E., Pimenta, L.A. Influence of load cycling on marginal microleakage with two self-etching and two one-bottle dentin adhesive systems in dentin. J Adhes Dent. 5(2003):209-16.

(151) Nikaido, T., Kunzelmann, K.H., Ogata, M., Harada, N., Yamaguchi, S., Cox, C.F., et al. The in vitro dentin bond strengths of two adhesive systems in class I cavities of human molars. J Adhes Dent. 4(2002):31-9.

(152) Miyazaki, M., Platt, J.A., Onose, H., Moore, B.K. Influence of dentin primer application methods on dentin bond strength. Oper Dent. 21(1996):167-72.

(153) Spreafico, D., Semeraro, S., Mezzanzanica, D., Re, D., Gagliani, M., Tanaka, T., et al. The effect of the air-blowing step on the technique sensitivity of four different adhesive systems. J Dent. 34(2006):237-44.

(154) Lu, Y., Liu, T., Li, X., Li, H., Pi, G. Histologic evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide in beagles. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 102(2006):e78-84.

(155) Pashley, E.L., Tao, L., Mackert, J.R., Pashley, D.H. Comparison of in vivo vs. in vitro bonding of composite resin to the dentin of canine teeth. J Dent Res. 67(1988):467-70.

(156) Xie, J., Powers, J.M., McGuckin, R.S. In vitro bond strength of two adhesives to enamel and dentin under normal and contaminated conditions. Dent Mater. 9(1993):295-9.

(157) Kaneshima, T., Yatani, H., Kasai, T., Watanabe, E.K., Yamashita, A. The influence of blood contamination on bond strengths between dentin and an adhesive resin cement. Oper Dent. 25(2000):195-201.

(158) Eiriksson, S.O., Pereira, P.N., Swift, E.J., Heymann, H.O., Sigurdsson, A. Effects of blood contamination on resin-resin bond strength. Dent Mater. 20(2004):184-90.

(159) Miles, D.A., Anderson, R.W., Pashley, D.H. Evaluation of the bond strength of dentin bonding agents used to seal resected root apices. J Endod. 20(1994):538-41.

(160) Camps, J., Pashley, D. Reliability of the dye penetration studies. J Endod. 29(2003):592-4.

(161) Ahlberg, K.M., Assavanop, P., Tay, W.M. A comparison of the apical dye penetration patterns shown by methylene blue and india ink in root-filled teeth. Int Endod J. 28(1995):30-4.

(162) Lucena-Martin, C., Ferrer-Luque, C.M., Gonzalez-Rodriguez, M.P., Robles-Gijon, V., Navajas-Rodriguez de Mondelo, J.M. A comparative study of apical leakage of Endomethasone, Top Seal, and Roeko Seal sealer cements. J Endod. 28(2002):423-6.

(163) McMichen, F.R., Pearson, G., Rahbaran, S., Gulabivala, K. A comparative study of selected physical properties of five root-canal sealers. Int Endod J. 36(2003):629-35.

(164) Tagger, M., Tamse, A., Katz, A., Tagger, E. An improved method of three-dimensional study of apical leakage. Quintessence Int Dent Dig. 14(1983):981-98.

(165) Robertson, D., Leeb, I.J., McKee, M., Brewer, E. A clearing technique for the study of root canal systems. J Endod. 6(1980):421-4.

(166) Kersten, H.W., Moorer, W.R. Particles and molecules in endodontic leakage. Int Endod J. 22(1989):118-24.

(167) Pathomvanich, S., Edmunds, D.H. Variation in the microleakage produced by four different techniques in root fillings in a simulated root canal model. Int Endod J. 29(1996):156-62.

(168) Youngson, C.C., Jones, J.C., Manogue, M., Smith, I.S. In vitro dentinal penetration by tracers used in microleakage studies. Int Endod J. 31(1998):90-9.

(169) Tay, F.R., Pang, K.M., Gwinnett, A.J., Wei, S.H. A method for microleakage evaluation along the dentin/restorative interface. Am J Dent. 8(1995):105-8.

(170) Wu, M.K., De Gee, A.J., Wesselink, P.R. Fluid transport and dye penetration along root canal fillings. Int Endod J. 27(1994):233-8.

(171) Wimonchit, S., Timpawat, S., Vongsavan, N. A comparison of techniques for assessment of coronal dye leakage. J Endod. 28(2002):1-4.

(172) Spangberg, L.S., Acierno, T.G., Yongbum Cha, B. Influence of entrapped air on the accuracy of leakage studies using dye penetration methods. J Endod. 15(1989):548-51.

(173) Katz, A., Rosenwasser, R., Tamse, A. Root positioning and leakage to dye in extracted teeth using reduced pressure. Int Endod J. 31(1998):63-6.

(174) Theuns, H.M., van Dijk, J.W., Driessens, F.C., Groeneveld, A. Effect of time, degree of saturation, pH and acid concentration of buffer solutions on the rate of in-vitro demineralization of human enamel. Arch Oral Biol. 30(1985):37-42.

(175) Antoniazzi, J.H., Mjor, I.A., Nygaard-Ostby, B. Assessment of the sealing properties of root filling materials. Odontol Tidskr. 76(1968):261-71.

(176) Pommel, L., Camps, J. Effects of pressure and measurement time on the fluid filtration method in endodontics. J Endod. 27(2001):256-8.

(177) Cobankara, F.K., Adanir, N., Belli, S., Pashley, D.H. A quantitative evaluation of apical leakage of four root-canal sealers. Int Endod J. 35(2002):979-84.

(178) Orucoglu, H., Sengun, A., Yilmaz, N. Apical leakage of resin based root canal sealers with a new computerized fluid filtration meter. J Endod. 31(2005):886-90.

(179) Zakariasen, K.L., Douglas, W.H., Stadem, P. Comparison of Volumatric and Linear Measurements of Root Canal Leakage. J Dent Res. 60(1981):627.

(180) Timpawat, S., Amornchat, C., Trisuwan, W.R. Bacterial coronal leakage after obturation with three root canal sealers. J Endod. 27(2001):36-9.

(181) Schafer, E., Olthoff, G. Effect of three different sealers on the sealing ability of both thermafil obturators and cold laterally compacted Gutta-Percha. J Endod. 28(2002):638-42.

(182) Carratu, P., Amato, M., Riccitiello, F., Rengo, S. Evaluation of leakage of bacteria and endotoxins in teeth treated endodontically by two different techniques. J Endod. 28(2002):272-5.

(183) Britto, L.R., Grimaudo, N.J., Vertucci, F.J. Coronal microleakage assessed by polymicrobial markers. J Contemp Dent Pract. 4(2003):1-10.

(184) Chailertvanitkul, P., Saunders, W.P., Saunders, E.M., MacKenzie, D. An evaluation of microbial coronal leakage in the restored pulp chamber of root-canal treated multirooted teeth. Int Endod J. 30(1997):318-22.

(185) Williamson, A.E., Dawson, D.V., Drake, D.R., Walton, R.E., Rivera, E.M. Effect of root canal filling/sealer systems on apical endotoxin penetration: a coronal leakage evaluation. J Endod. 31(2005):599-604.

(186) Xu, Q., Fan, M.W., Fan, B., Cheung, G.S., Hu, H.L. A new quantitative method using glucose for analysis of endodontic leakage. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 99(2005):107-11.

(187) Going, R.E. Microleakage around dental restorations: a summarizing review. J Am Dent Assoc. 84(1972):1349-57.

(188) Matloff, I.R., Jensen, J.R., Singer, L., Tabibi, A. A comparison of methods used in root canal sealability studies. Oral Surg Oral Med Oral Pathol. 53(1982):203-8.

(189) Oliver, C.M., Abbott, P.V. Entrapped air and its effects on dye penetration of voids. Endod Dent Traumatol. 7(1991):135-8.

(190) Trichaiyapon, V., Panitvisai, P. (2008).

(191) Schneider, S.W. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol. 32(1971):271-5.

(192) ISO/TC 106/SC 1/WG 11. Guidance on testing of adhesion to tooth structure. Adhesion test methods and accepted as a Committee Draft at the ISO/TC meeting in Trieste. Dental materials. (1991):October 1991.

(193) Martin, B., Zelada, G., Varela, P., Bahillo, J.G., Magan, F., Ahn, S., et al. Factors influencing the fracture of nickel-titanium rotary instruments. Int Endod J. 36(2003):262-6.

(194) Zehnder, M. Root canal irrigants. J Endod. 32(2006):389-98.

(195) Yoo, H.M., Pereira, P.N. Effect of blood contamination with 1-step self-etching adhesives on microtensile bond strength to dentin. Oper Dent. 31(2006):660-5.

(196) Wu, M.K., De Gee, A.J., Wesselink, P.R., Moorer, W.R. Fluid transport and bacterial penetration along root canal fillings. Int Endod J. 26(1993):203-8.

(197) Barthel, C.R., Moshonov, J., Shuping, G., Orstavik, D. Bacterial leakage versus dye leakage in obturated root canals. Int Endod J. 32(1999):370-5.

(198) White, J.M., Goodis, H.E., Marshall, S.J., Marshall, G.W. Sterilization of teeth by gamma radiation. J Dent Res. 73(1994):1560-7.

(199) Verissimo, D.M., do Vale, M.S. Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review. J Oral Sci. 48(2006):93-8.

(200) Beatty, R.G., Zakariasen, K.L. Apical leakage associated with three obturation techniques in large and small root canals. Int Endod J. 17(1984):67-72.

(201) Johnson, W.T., Zakariasen, K.L. Spectrophotometric analysis of microleakage in the fine curved canals found in the mesial roots of mandibular molars. Oral Surg Oral Med Oral Pathol. 56(1983):305-9.

(202) Peters, L.B., Wesselink, P.R., Buijs, J.F., van Winkelhoff, A.J. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod. 27(2001):76-81.

(203) Starkey, D.L., Anderson, R.W., Pashley, D.H. An evaluation of the effect of methylene blue dye pH on apical leakage. J Endod. 19(1993):435-9.

(204) Greene, H.A., Wong, M., Ingram, T.A., 3rd. Comparison of the sealing ability of four obturation techniques. J Endod. 16(1990):423-8.

# APPENDICES

**Table 1** Reading score data of dye penetration

| MTA-B | Dye penetration (mm) |
|---|---|
| 1 | 2.52 |
| 2 | 2.89 |
| 3 | 6.00 |
| 4 | 2.55 |
| 5 | 2.52 |
| 6 | 2.78 |
| 7 | 2.47 |
| 8 | 2.38 |
| 9 | 2.91 |
| 10 | 2.08 |
| 11 | 3.98 |
| 12 | 2.43 |
| 13 | 5.39 |
| 14 | 3.06 |
| 15 | 4.66 |

| Tetric-B | Dye penetration (mm) |
|---|---|
| 1 | 6.66 |
| 2 | 4.05 |
| 3 | 1.81 |
| 4 | 2.72 |
| 5 | 3.05 |
| 6 | 1.80 |
| 7 | 6.18 |
| 8 | 6.14 |
| 9 | 3.22 |
| 10 | 4.44 |
| 11 | 1.84 |
| 12 | 4.50 |
| 13 | 3.81 |
| 14 | 3.55 |
| 15 | 5.76 |

| MTA-NB | Dye penetration (mm) |
|---|---|
| 1 | 3.31 |
| 2 | 2.33 |
| 3 | 2.39 |
| 4 | 2.76 |
| 5 | 2.81 |
| 6 | 5.41 |
| 7 | 2.69 |
| 8 | 3.64 |
| 9 | 6.04 |
| 10 | 2.63 |
| 11 | 5.53 |
| 12 | 3.12 |
| 13 | 5.88 |
| 14 | 3.03 |
| 15 | 2.54 |

| Tetric-NB | Dye penetration (mm) |
|---|---|
| 1 | 0.00 |
| 2 | 0.10 |
| 3 | 0.20 |
| 4 | 0.25 |
| 5 | 0.00 |
| 6 | 0.00 |
| 7 | 0.05 |
| 8 | 0.11 |
| 9 | 0.00 |
| 10 | 0.09 |
| 11 | 0.11 |
| 12 | 0.09 |
| 13 | 0.00 |
| 14 | 0.00 |
| 15 | 0.08 |

MTA-B = The blood contaminated MTA group

MTA-NB = The uncontaminated MTA group

Tetric-B = The blood contaminated Tetric Flow group

Tetric-NB = The uncontaminated Tetric Flow group

**Table 2** Reading score data of dye penetration for the reliability test of the investigator

*First time*

| MTA-B | Dye penetration (mm) | Tetric-B | Dye penetration (mm) |
|---|---|---|---|
| 1 | 2.74 | 1 | 6.28 |
| 2 | 2.59 | 2 | 7.38 |
| 3 | 2.76 | 3 | 6.97 |
| 4 | 5.87 | 4 | 6.50 |
| 5 | 2.36 | 5 | 5.23 |
| 6 | 5.91 | 6 | 6.63 |
| 7 | 1.71 | 7 | 6.46 |
| 8 | 4.00 | 8 | 6.41 |
| 9 | 5.76 | 9 | 4.03 |
| 10 | 2.88 | 10 | 2.22 |
| 11 | 5.79 | 11 | 7.27 |
| 12 | 2.69 | 12 | 6.12 |
| 13 | 2.84 | 13 | 6.32 |
| 14 | 5.75 | 14 | 4.33 |
| 15 | 6.01 | 15 | 6.37 |
| MTA-NB | Dye penetration (mm) | Tetric-NB | Dye penetration (mm) |
| 1 | 4.42 | 1 | 0.17 |
| 2 | 2.84 | 2 | 0.00 |
| 3 | 2.61 | 3 | 0.53 |
| 4 | 2.69 | 4 | 0.06 |
| 5 | 3.44 | 5 | 0.12 |
| 6 | 6.25 | 6 | 0.00 |
| 7 | 3.88 | 7 | 0.10 |
| 8 | 2.51 | 8 | 0.00 |
| 9 | 3.02 | 9 | 0.30 |
| 10 | 2.50 | 10 | 0.33 |
| 11 | 3.69 | 11 | 0.19 |
| 12 | 6.12 | 12 | 0.36 |
| 13 | 4.73 | 13 | 0.43 |
| 14 | 2.99 | 14 | 0.24 |
| 15 | 2.80 | 15 | 0.00 |

MTA-B = The blood contaminated MTA group

MTA-NB = The uncontaminated MTA group

Tetric-B = The blood contaminated Tetric Flow group

Tetric-NB = The uncontaminated Tetric Flow group

**Table 3** Reading score data of dye penetration for the reliability test of the investigator

*Second time*

| MTA-B | Dye penetration (mm) | Tetric-B | Dye penetration (mm) |
|---|---|---|---|
| 1 | 2.30 | 1 | 7.04 |
| 2 | 3.19 | 2 | 4.05 |
| 3 | 9.24 | 3 | 3.37 |
| 4 | 2.55 | 4 | 2.72 |
| 5 | 2.67 | 5 | 3.05 |
| 6 | 2.78 | 6 | 1.80 |
| 7 | 2.47 | 7 | 5.90 |
| 8 | 3.24 | 8 | 5.87 |
| 9 | 2.91 | 9 | 2.41 |
| 10 | 2.08 | 10 | 6.67 |
| 11 | 2.16 | 11 | 3.59 |
| 12 | 2.17 | 12 | 4.50 |
| 13 | 7.93 | 13 | 3.81 |
| 14 | 3.06 | 14 | 2.78 |
| 15 | 3.31 | 15 | 5.16 |
| MTA-NB | Dye penetration (mm) | Tetric-NB | Dye penetration (mm) |
| 1 | 2.19 | 1 | 0.17 |
| 2 | 1.82 | 2 | 0.20 |
| 3 | 2.19 | 3 | 0.13 |
| 4 | 2.82 | 4 | 0.45 |
| 5 | 2.18 | 5 | 0.12 |
| 6 | 4.56 | 6 | 0.00 |
| 7 | 2.69 | 7 | 0.00 |
| 8 | 4.77 | 8 | 0.22 |
| 9 | 6.04 | 9 | 0.30 |
| 10 | 2.76 | 10 | 0.15 |
| 11 | 7.36 | 11 | 0.03 |
| 12 | 3.12 | 12 | 0.18 |
| 13 | 5.88 | 13 | 0.43 |
| 14 | 3.08 | 14 | 0.24 |
| 15 | 2.28 | 15 | 0.16 |

MTA-B = The blood contaminated MTA group

MTA-NB = The uncontaminated MTA group

Tetric-B = The blood contaminated Tetric Flow group

Tetric-NB = The uncontaminated Tetric Flow group

**Table 4** Quantitative leakage (dye absorbance) data

| MTA-B | Absorbance (580 nm) |
|---|---|
| 1 | 0.031 |
| 2 | 0.023 |
| 3 | 0.032 |
| 4 | 0.017 |
| 5 | 0.032 |
| 6 | 0.040 |
| 7 | 0.027 |
| 8 | 0.006 |
| 9 | 0.025 |
| 10 | 0.004 |
| 11 | 0.026 |
| 12 | 0.036 |
| 13 | 0.069 |
| 14 | 0.064 |
| 15 | 0.019 |

| Tetric-B | Absorbance (580 nm) |
|---|---|
| 1 | 0.110 |
| 2 | 0.085 |
| 3 | 0.035 |
| 4 | 0.043 |
| 5 | 0.022 |
| 6 | 0.075 |
| 7 | 0.116 |
| 8 | 0.027 |
| 9 | 0.086 |
| 10 | 0.123 |
| 11 | 0.071 |
| 12 | 0.129 |
| 13 | 0.060 |
| 14 | 0.084 |
| 15 | 0.130 |

| MTA-NB | Absorbance (580 nm) |
|---|---|
| 1 | 0.027 |
| 2 | 0.007 |
| 3 | 0.015 |
| 4 | 0.032 |
| 5 | 0.040 |
| 6 | 0.073 |
| 7 | 0.006 |
| 8 | 0.043 |
| 9 | 0.014 |
| 10 | 0.024 |
| 11 | 0.088 |
| 12 | 0.033 |
| 13 | 0.059 |
| 14 | 0.050 |
| 15 | 0.046 |

| Tetric-NB | Absorbance (580 nm) |
|---|---|
| 1 | 0.003 |
| 2 | 0.013 |
| 3 | 0.006 |
| 4 | 0.009 |
| 5 | 0.011 |
| 6 | 0.003 |
| 7 | 0.002 |
| 8 | 0.014 |
| 9 | 0.005 |
| 10 | 0.024 |
| 11 | 0.006 |
| 12 | 0.015 |
| 13 | 0.013 |
| 14 | 0.002 |
| 15 | 0.004 |

MTA-B = The blood contaminated MTA group

MTA-NB = The uncontaminated MTA group

Tetric-B = The blood contaminated Tetric Flow group

Tetric-NB = The uncontaminated Tetric Flow group

**Table 5** Statistical analysis using SPSS of dye penetration data for reliability test of the investigator

**Intraclass Correlation Coefficient**

| | Intraclass Correlation | 95% Confidence Interval | | F Test with True Value 0 | | | |
|---|---|---|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Value | df1 | df2 | Sig |
| Single Measures | .904 | .844 | .942 | 19.843 | 59.0 | 59 | .000 |
| Average Measures | .950 | .916 | .970 | 19.843 | 59.0 | 59 | .000 |

Table 6 Statistical analysis using SPSS of dye penetration leakage (ANOVA)

**Descriptive Statistics**

Dependent Variable: mm

| tech | blood | Mean | Std. Deviation | N |
|---|---|---|---|---|
| MTA | no blood | 3.60680 | 1.363821 | 15 |
| | blood | 3.24033 | 1.198413 | 15 |
| | Total | 3.42357 | 1.275148 | 30 |
| Tet | no blood | .07167 | .077520 | 15 |
| | blood | 3.96913 | 1.638950 | 15 |
| | Total | 2.02040 | 2.286521 | 30 |
| Total | no blood | 1.83923 | 2.032944 | 30 |
| | blood | 3.60473 | 1.458584 | 30 |
| | Total | 2.72198 | 1.967121 | 60 |

**Tests of Between-Subjects Effects**

Dependent Variable: mm

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|
| Corrected Model | 144.467[b] | 3 | 48.156 | 32.166 | .000 | 96.499 | 1.000 |
| Intercept | 444.552 | 1 | 444.552 | 296.943 | .000 | 296.943 | 1.000 |
| tech | 29.533 | 1 | 29.533 | 19.727 | .000 | 19.727 | .992 |
| blood | 46.755 | 1 | 46.755 | 31.230 | .000 | 31.230 | 1.000 |
| tech * blood | 68.179 | 1 | 68.179 | 45.541 | .000 | 45.541 | 1.000 |
| Error | 83.837 | 56 | 1.497 | | | | |
| Total | 672.856 | 60 | | | | | |
| Corrected Total | 228.304 | 59 | | | | | |

a. Computed using alpha = .05

b. R Squared = .633 (Adjusted R Squared = .613)

**ANOVA**

mm

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 144.467 | 3 | 48.156 | 32.166 | .000 |
| Within Groups | 83.837 | 56 | 1.497 | | |
| Total | 228.304 | 59 | | | |

**Multiple Comparisons**

Dependent Variable: mm

Dunnett T3

| (I) type | (J) type | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| MTA-B | MTA-NB | -.366467 | .468772 | .964 | -1.68891 | .95598 |
| | Tet-B | -.728800 | .524236 | .664 | -2.21548 | .75788 |
| | Tet-NB | 3.168667* | .310076 | **.000** | 2.23264 | 4.10469 |
| MTA-NB | MTA-B | .366467 | .468772 | .964 | -.95598 | 1.68891 |
| | Tet-B | -.362333 | .550525 | .984 | -1.91719 | 1.19252 |
| | Tet-NB | 3.535133* | .352705 | **.000** | 2.47014 | 4.60013 |
| Tet-B | MTA-B | .728800 | .524236 | .664 | -.75788 | 2.21548 |
| | MTA-NB | .362333 | .550525 | .984 | -1.19252 | 1.91719 |
| | Tet-NB | 3.897467* | .423648 | **.000** | 2.61790 | 5.17703 |
| Tet-NB | MTA-B | -3.168667* | .310076 | **.000** | -4.10469 | -2.23264 |
| | MTA-NB | -3.535133* | .352705 | **.000** | -4.60013 | -2.47014 |
| | Tet-B | -3.897467* | .423648 | **.000** | -5.17703 | -2.61790 |

*. The mean difference is significant at the .05 level.

**Table 7** Statistical analysis using SPSS of dye absorbance (ANOVA)

**Descriptive Statistics**

Dependent Variable: Ext

| tech | blood | Mean | Std. Deviation | N |
|---|---|---|---|---|
| MTA | no blood | .03713 | .023694 | 15 |
| | blood | .03007 | .017854 | 15 |
| | Total | .03360 | .020924 | 30 |
| Tet | no blood | .00867 | .006264 | 15 |
| | blood | .07973 | .036931 | 15 |
| | Total | .04420 | .044537 | 30 |
| Total | no blood | .02290 | .022350 | 30 |
| | blood | .05490 | .038083 | 30 |
| | Total | .03890 | .034910 | 60 |

**Tests of Between-Subjects Effects**

Dependent Variable: Ext

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|
| Corrected Model | .040[b] | 3 | .013 | 23.322 | .000 | 69.965 | 1.000 |
| Intercept | .091 | 1 | .091 | 159.051 | .000 | 159.051 | 1.000 |
| tech | .002 | 1 | .002 | 2.953 | .091 | 2.953 | .393 |
| blood | .015 | 1 | .015 | 26.908 | .000 | 26.908 | .999 |
| tech * blood | .023 | 1 | .023 | 40.104 | .000 | 40.104 | 1.000 |
| Error | .032 | 56 | .001 | | | | |
| Total | .163 | 60 | | | | | |
| Corrected Total | .072 | 59 | | | | | |

a. Computed using alpha = .05

b. R Squared = .555 (Adjusted R Squared = .532)

**ANOVA**

Ext

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .040 | 3 | .013 | 23.322 | .000 |
| Within Groups | .032 | 56 | .001 | | |
| Total | .072 | 59 | | | |

**Multiple Comparisons**

Dependent Variable: Ext

Dunnett T3

| (I) type | (J) type | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| MTA-B | MTA-NB | -.007067 | .007660 | .923 | -.02877 | .01463 |
| | Tet-B | -.049667* | .010592 | .001 | -.08032 | -.01901 |
| | Tet-NB | .021400* | .004885 | .002 | .00704 | .03576 |
| MTA-NB | MTA-B | .007067 | .007660 | .923 | -.01463 | .02877 |
| | Tet-B | -.042600* | .011329 | .006 | -.07491 | -.01029 |
| | Tet-NB | .028467* | .006328 | .002 | .00967 | .04726 |
| Tet-B | MTA-B | .049667* | .010592 | .001 | .01901 | .08032 |
| | MTA-NB | .042600* | .011329 | .006 | .01029 | .07491 |
| | Tet-NB | .071067* | .009672 | .000 | .04206 | .10007 |
| Tet-NB | MTA-B | -.021400* | .004885 | .002 | -.03576 | -.00704 |
| | MTA-NB | -.028467* | .006328 | .002 | -.04726 | -.00967 |
| | Tet-B | -.071067* | .009672 | .000 | -.10007 | -.04206 |

*. The mean difference is significant at the .05 level.

# BIOGRAPHY

Flight Lieutenant Tippawan Inthararith was born on 9th of May 1977 in Udonthani. She graduated with D.D.S. (Doctor of Dental Surgery) from the Faculty of Dentistry, Khon Kaen University in 2001, and became a military service at Royal Thai Air Force. She studied in a Master degree program in Endodontology at Graduate School, Chulalongkorn University in 2007.