EFFECT OF FLUORIDE VARNISH AND FLUORIDE-CONTAINING SEALANT ON ARTIFICIAL CARIES ON PRIMARY TEETH

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ABSTRACT

The purpose of this in vitro study was to compare the effectiveness of fluoride varnish and fluoride-containing resin sealant on remineralization of artificial white spot lesions on the smooth surface of deciduous teeth. **Materials and Methods:** Forty-five caries free primary incisor teeth were selected. Artificial enamel caries were created on two separated opened window areas (1x1 mm) at the buccal surface, and then teeth were sectioned bucco-lingually into 2 pieces, the one on the left was used for the control group while the right side was the experimental group. The matched pair specimens were divided into 3 groups: group 1- tested specimens were coated with Duraphat; group 2- tested specimens were coated with Enamel Pro varnish (for 24 hours before being wiped off); and group 3- tested specimens were coated with Clinpro sealant. All groups were thermocycled at 500 cycles and pH-cycled for 7 days. Specimens were then sectioned bucco-lingually at the middle of the window area (one piece per window). A polarized light microscope was used to evaluate the mean lesion depth. **Results**: The mean lesion depth of all tested groups showed a significant reduction as compared to their control groups (p=.00). Clinpro^{TM} sealant had the lowest mean lesion depth compared to the other groups (p=.00). The mean lesion depth reduction between fluoride varnish groups was not statistically significantly different (p>.05).

KEY WORDS: FLUORIDE VARNISH / FLUORIDE SEALANT / ARTIFICIAL CARIES

48 pages

ผลของฟลูออไรด์วาร์นิชและวัสดุเคลือบหลุมร่องฟันที่ผสมฟลูออไรด์ต่อรอยผุระยะเริ่มแรกในฟันน้ำนม
EFFECT OF FLUORIDE VARNISH AND FLUORIDE-CONTAINING SEALANT ON ARTIFICIAL CARIES ON PRIMARY TEETH

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บทคัดย่อ

การวิจัยนี้เป็นการศึกษาในห้องปฏิบัติการเพื่อเปรียบเทียบประสิทธิภาพของฟลูออไรด์วาร์นิช 2 ชนิดและวัสดุเคลือบหลุมร่องฟันเรซินที่ผสมฟลูออไรด์ในการส่งเสริมการคืนกลับของแร่ธาตุบนรอยผุจำลองที่ผิวเคลือบฟันด้านเรียบในฟันน้ำนม

วิธีการศึกษา: ฟันตัดน้ำนมจำนวน 45 ซี่ นำไปทำให้เกิดรอยผุระยะเริ่มแรก 2 ตำแหน่ง และถูกตัดแบ่งครึ่งในแนวแก้มลิ้นเป็น 45 คู่ เพื่อเป็นชิ้นควบคุมและชิ้นทดลอง แล้วแบ่งออกเป็น 3 กลุ่ม กลุ่มละ 15 คู่ คือ กลุ่มที่ 1 ทาอีนาเมลโปรวาร์นิช กลุ่มที่ 2 ทาดูราแฟต โดยทั้งสองกลุ่มนี้ทาฟลูออไรด์วาร์นิชทิ้งไว้ 24 ชั่วโมงก่อนเช็ดออก และกลุ่มที่ 3 ทาด้วยวัสดุเคลือบหลุมร่องฟันเรซินที่ผสมฟลูออไรด์ แล้วนำฟันทุกกลุ่มไปผ่านกระบวนการจำลองการเปลี่ยนแปลงอุณหภูมิ 500 รอบและการเปลี่ยนแปลงความเป็นกรดด่างในช่องปาก เป็นเวลา 7 วัน จากนั้นนำชิ้นฟันมาตัดที่ตำแหน่งกึ่งกลางรอยโรคในแนวแก้มลิ้นและนำมาคำนวณค่าเฉลี่ยความลึกของรอยผุด้วยกล้องจุลทรรศน์แสงโพลาไรซ์ด้วยกำลังขยาย 50 เท่า

จากผลการศึกษาพบว่าค่าเฉลี่ยความลึกของรอยผุในชิ้นทดลองของทั้งสามกลุ่มมีค่าลดลงมากกว่าในชิ้นควบคุมอย่างมีนัยสำคัญทางสถิติ (p=.00) โดยพบว่ากลุ่มที่ทาด้วยวัสดุเคลือบหลุมร่องฟันเรซินที่ผสมฟลูออไรด์มีความลึกของรอยผุน้อยที่สุดซึ่งแตกต่างจากกลุ่มฟลูออไรด์วาร์นิชอย่างมีนัยสำคัญทางสถิติ (p=.00) โดยการใช้ฟลูออไรด์วาร์นิชทั้งสองกลุ่มไม่มีความแตกต่างกัน ทางสถิติ (p>.05)

48 หน้า

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LIST OF ABBREVIATIONS

Abbreviations	Description
%	Percent
ACP	Amorphous calcium phosphate
ECC	Early childhood caries
dmft	decayed, missing or filled teeth
PATF	Professionally applied topical fluoride
APF	Acidulated phosphate fluoride
ppm	parts per million
NaF	Sodium fluoride
wt	weight
F	Fluoride
FDA	Food and Drug Administration
SnF_2	Stannous fluoride
Na_2PO_3F	Sodium monofluorophosphate
CPP-ACP	Casein phosphopeptide-amorphous calcium phosphate
Bis-GMA	Bisphenol-A-glycidylmethacrylate
MMA	Methyl-methacrylate
TEGDMA	Triethyleneglycol dimethacrylate
nm	nanometer
mm^2	square millimeter
ºC	Celsius
ml	milliliter
mM	millimolar
$CaCl_2$	Calcium chloride
KH_2PO_4	Potassium dihydrogen phosphate
M	Molar

LIST OF ABBREVIATIONS (cont.)

Abbreviations	Description
KOH	Potassium hydroxide
µm	micrometer, micron
$NaHCO_3$	Sodium bicarbonate
NaH_2PO_4	Sodium dihydrogen phosphate
KCl	Potassium chloride
ANOVA	Analysis of variance
50x	50 times
mg	milligram
SD	Standard deviation

CHAPTER I
INTRODUCTION

A clinical appearance of early carious lesion is a white opaque spot lesion. This lesion area is slightly softer and more porous than the surrounding sound enamel and increases in whiteness when dried with air.

White spot lesion can be detected easily by direct visual. The lesion can be reverse by proper preventive program because enamel caries has the potential to be arrested or even remineralized [(1)]. The common preventive treatment of enamel caries comprises application of fluorides as well as oral hygiene care and proper diet. However, this regimen has some limitations in high-risk and noncompliant patients. New concepts in restorative dentistry concentrate more on preserving the integrity of the tooth rather than filling a cavity. A non-invasive alternative therapy for the arrest of caries lesions might be superficial sealed by various materials. Since the outer surface zone is more porous than sound enamel, a porosity of enamel caries act as diffusion pathways for acids and dissolved minerals, infiltration of these lesions with resins might occlude the pathways, thus leading to an arrest of caries progression [(2)]. Several studies have demonstrated that artificial porous lesions in permanent teeth can be infiltrated by commercially available adhesives and fissure sealants [(3-7)]. However, there are few studies in caries inhibiting potential of fluoride containing sealant on demineralization and remineralization of incipient enamel caries in primary teeth.

Conventional treatment of incipient enamel caries is fluoride varnish application especially in the primary teeth. Many products have been developed to prevent demineralization of the enamel surface. Fluoride varnish containing ACP formula is one type of varnish which claims that it can provides 2 times more enamel fluoride uptake when compared with 5% conventional sodium fluoride varnish according to a manufacturer.

The main purpose of this research was to evaluated the remineralization of incipient artificial caries in primary teeth after application of three materials: Fluoride varnish, ACP formula (Enamel Pro® Varnish), conventional fluoride varnish (Duraphat®) and fluoride containing resin sealant (Clinpro™ Sealant).

CHAPTER II
OBJECTIVES

Objective

To compared the effect of a fluoride varnish ACP formula (Enamel Pro® Varnish), a conventional fluoride varnish (Duraphat®) and a fluoride containing sealant (Clinpro™) on demineralization and remineralization of artificial initial carious lesions on primary incisor teeth.

Expected benefits and applications

This *in vitro* study was demonstrated the effect of fluoride varnish and fluoride containing resin sealant on artificial caries by measure the mean lesion depth reduction. From the results of the experiment, dentists can consider the most effective material to prevent and inhibit early dental caries progression in young children.

CHAPTER III
LITERATURE REVIEW

Dental caries prevalence

Dental caries is the most common oral disease. Early childhood caries (ECC) is still a problem in Thailand. According to the 2006-2007 National Oral Health Survey in Thailand has shown that oral health problem among children tend to be on the rise, among children at 3 years of age group, prevalence of dental carries is 61.37% (mean dmft 3.21) and at 6 years of age group**,** prevalence of dental carries is 80.64% (mean dmft 5.43) [(8)].

The early carious lesion

Dental caries is a dynamic process with phases of demineralization and remineralization. These phases cycle back and forth as intraoral conditions change; demineralization is a process that some of the calcium and phosphate which enamel is largely composed are dissolved out of the enamel into the plaque. However, once the plaque acid has been neutralised the minerals can return to the enamel surface - a process called remineralization. Both of the de- and remineralization phases can be occurring at the same time in different parts of the mouth. Lesions develop if the balance of the equilibrium shifts towards demineralization for extended periods of time.

Demineralization of the tooth surface was occurred caused by multifactors**.** Factors that affect the phase of mineralization include the oral pH, the contents and concentration of saliva, the oral bacteria present, frequency of sucrose ingestion, presents of fluoride or other chemicals and the duration of time all of these factors are present [(9)].

Different names and definitions have been used to refer to the presence of dental caries among very young children. Currently, ECC is defined as "the presence of one or more decayed (cavitated or not cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger" [(10)]. Like other types of caries, ECC is caused by mutans streptococci that ferment dietary carbohydrates to produce acid attacks on susceptible teeth over a period of time. The early implantation of mutans streptococci, the use of a feeding bottle containing sugary solutions and prolonged breast-feeding, especially at night, are important predisposing factors. The upper front teeth are typically the most severe to damaged.

Human dental enamel is a unique mineralized substance that is composed of hydroxyapatite (92-94%), carbonate (2%), trace element (1%), fluoride (0.01-0.05%), proteins and lipid (<1%) [(11)]. The initial formation of the lesion is due to the dissolution of hydroxyapatite from the enamel prisms forming the enamel surface. The first signs of demineralization (or decalcification) are chalky white spots or lines across the surface of teeth with a relatively intact surface layer (initial caries or incipient caries). As the lesion continues to demineralize, it can turn brown but will eventually turn into a cavitation.

Because of there are differences between the composition and structure of permanent teeth and primary teeth such as the enamel of primary teeth is less-mineralized and less hard than and only half as thick as that of permanent teeth, the diffusion coefficient and porosity are greater in primary enamel than in permanent enamel so that the primary enamel is more susceptible to acid attack and more rapidly progression than permanent enamel [(12)]. So the caries prevention for initial caries in primary teeth is very important.

Histopathology of the early carious lesion

The early carious lesion consists of 4 zones (figure 1) of alternating level of mineralization; 1. The surface zone is intact with a 10 to 50 times more porous than sound enamel [(12)] and porosity of this surface zone amounts to 1-2%. This porous enamel may enhance remineralization or caries progression; depend on oral hygiene practices and fluoride exposure. 2. The body of lesion is porous and pores volume is

excess of 5%. 3. The dark zone has a pore volume of 2-4% and 4. The translucent zone has a pore volume of 1% [13].

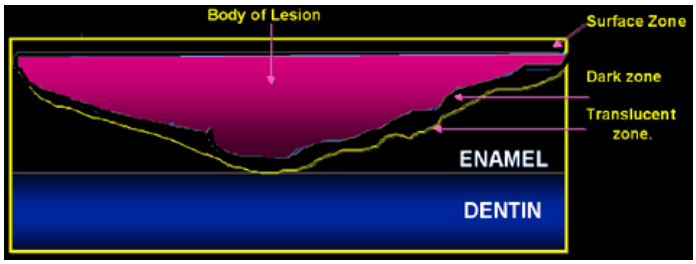


Figure 1: zone of enamel lesion

Dental caries prevention: Fluoride and fluoride varnish

Dental caries in young children can be prevented. A recent report showed that at least 51% of white spot lesions had disappeared, 36% showed no change and only 13% had progressed to frank caries [14]. One of the common effective methods is to use fluoride for promoting remineralization. The efficacy of fluoride in caries prevention has been well documented since the 1930s. The relationship between fluoride and tooth decay is complex and probably not yet fully understood. However, it is known that fluoride interferes with the process of tooth decay in at least four ways: 1. Fluoride encourages remineralization and ensures that the enamel crystals that are laid down are of improved quality.

2. Fluoride alters the structure of the developing enamel making it more resistant to acid attack.

3. Fluoride reducing the ability of the plaque bacteria to produce acid.

4. Probably minor effect of fluoride is that, if sufficient fluoride is ingested during childhood when the teeth are developing, it affects the depth of the fissures (grooves) on the occlusal surfaces of the teeth tend to be shallower [15].

Over the past 15 years topical fluoride effects appear to have dominated in preventing caries development. The topical fluoride such as solutions, gels, toothpaste and mouthrinses have been used to deliver fluoride onto the tooth surface, resulting in various levels of fluoride uptake and clinical efficacy [16,17].

The use of professionally applied topical fluoride (PATF) is one means of preventing caries that is frequently used in private practice and public health settings. Clinical trials conducted during 1940-1970 demonstrated that professionally applied fluorides effectively reduce caries experience in children [18]. In terms of the relative reduction in decayed and filled surfaces, PATF is more effective against smooth surface caries than occlusal caries [19].

Commonly used fluoride gels include acidulated phosphate fluoride (APF), which contains 1.23% or 12,300 parts per million (ppm) fluoride ions, and 2% sodium fluoride (NaF), which contains 0.90% or 9,050 ppm fluoride ion. In the 1990s, fluoride foam was introduced into dental practice. However, there are few clinical studies of the effectiveness of these foams [20].

Applications of PATF are relatively infrequent, generally at 3 to 12 month intervals. However, fluoride gel poses little risk for enamel fluorosis, especially in young children be able to swallow the gel during application.

The contact time between the tooth surface and a topical fluoride agent is a factor of crucial importance in the efficacy of this preventive measure. In fact, after a topical fluoride application, most of the acquired fluoride fails to react with the enamel and is washed away by saliva [19-21].

To reduce fluoride loss and toxicity, fluoride varnishes were first developed and introduced into dental practice in some European countries. Fluoride varnish is a thin coating of resin that is applied to the tooth surface to protect it from decay. The purpose of applying fluoride varnish is to retard, arrest, and reverse the process of cavity formation [22].

Fluoride varnish was first introduced by Schmidt in 1964, under the trade name Duraphat® (Woelm Pharma Co., Eshwege, Germany). Duraphat® is a natural resin, a neutral colophonium base, containing 5% wt NaF (2.26% F^-) dissolved in ethanol. Arends and Schuthof introduced a second product in 1975 under the trade name Fluor Protector® (Vivadent, Schaan, Liechtenstein) [23] this is a polyurethane based transparent resin, containing 0.1%. F^- as difluorosilane (0.9% wt) dissolved in ethyl acetate and isoamylpropionate solution. The different dissolving agents used in the two lacquers greatly influenced not only the adhesive properties, but also the

bioavailability of fluoride ions. The acidic properties of Fluor Protector®, like those of APF gel, increased the fluoride uptake of enamel [24,25].

The others fluoride varnishes have been introduced were Duraflor (5% NaF/2.26 % F^-), Pharmascience Inc.), Bifluorid 12Ò (Voco Chemie GmbH, Cuxhafen, Germany) (containing 2.71% wt as NaF and 2.92 as CaF_2) [26] and CarexÒ (containing 1.80% of NaF) [27].

Fluoride varnishes are now widely accepted in Europe and their use is on the increase. In 1994, Duraphat® was the first fluoride varnish cleared by the U.S. Food and Drug Administration, or FDA. Under these regulations, the FDA has cleared these products as medical devices to be used as cavity liners and for the treatment of hypersensitive teeth. Laboratory evidence suggests that both Duraphat® and Fluor Protector® have properties equivalent to other dentinal tubule sealants [28,29] but because caries prevention is considered a drug claim, manufacturers would have to submit appropriate clinical trial evidence for review by the FDA before they could be cleared as anticaries agents.

Mechanism of action of fluoride varnish

-Promotes remineralization:

Fluoride varnish painted on to enamel to form a slightly yellowish film. It acts by a slow release of fluoride to the underlying tooth surface. After applied fluoride varnish found that increased fluoride ion concentration, actually enhances mineral deposition, so that reduces the rate of demineralization and promotes remineralization. The greater fluoride concentrations attainable with varnishes produce deposits of calcium fluoride in the porous structure of the enamel. These reservoirs gradually release fluoride into dental plaque, saliva and tooth structure when the pH drops.

Several in vivo studies have been performed over the last 20 years to evaluate fluoride uptake and caries preventive effect of fluoride varnishes. The study compared the effect of various forms of topical fluoride treatments, such as 2% NaF solution, 8% SnF_2, 5% Na_2PO_3F, 2% APF gel and Duraphat®. Fluoride gradients in sound enamel taken from homologous human teeth were analyzed in a depth range from 40 to 200 μm. The highest fluoride uptake was observed with Duraphat®, while

the fluoride uptake from conventional topical applications was low and limited to the outer enamel layer [30].

A comparison of the concentration of permanently bound fluoride in teeth treated with two fluoride varnishes (Duraphat® and Fluor Protector®) was performed by Seppa *et al* in 1982 [31]. The enamel fluoride concentration of the Fluor Protector® treated subjects was higher than that of the Duraphat® group. Six months later, enamel fluoride content was determined again. In spite of the fact that the fluoride concentration of Fluor Protector® is lower than that of Duraphat®.

The inhibiting effect of both Duraphat® and Fluor Protector® were studied by Holmen *et al* in 1986 [32] and de Bruyn and Buskes in 1988, they demonstrating that the demineralization of enamel induced by cariogenic challenge could be reduced of 53–75% by a fluoride varnish.

Autio-Gold JT in 2001 [33] evaluated the effect of fluoride varnish on early enamel carious lesions in the primary dentition of preschool-aged children and found the varnish group 81.2% of active enamel lesions were inactive after nine months ($p<.0001$) compared with 37.8% of active enamel lesions in the control group. The results of this study indicated that two applications of fluoride varnish may be effective in arresting early active enamel lesions in the primary dentition.

A several number of clinical trials have confirmed the caries preventive effect of fluoride varnishes. Data show an overall reduction in caries increment following fluoride varnish applications, ranging approximately from 18 to 70% compared to untreated controls [34].

Tewari *et al* in 1990 [35] compared Duraphat® with a 2% NaF solution, a 1.23% APF, gel and a negative control. They reported that after 2.5 years, the varnish resulted in a higher percentage of caries reduction (74%) than did the NaF solution (28%) and the APF gel (37%).

Some clinical trials have evaluated the caries prevention efficacy of fluoride varnishes on the primary dentition. Weinstein *et al* in 1994[36] investigated the possibility of preventing baby bottle tooth decay with fluoride varnish application to the maxillary incisors; they found that caries development in the fluoride varnish treated children was 14% lower than children in the similarly aged group. Results indicated a significant decrease in decalcification from 35% to 21%.

Castillo *et al* in 2001 [37] evaluated amount of fluoride release between Duraphat® and Duraflor® in primary molar teeth, they found that the release of fluoride from both varnish products maintain in low level over sixth months. Duraphat® and Duraflor® released 67% and 56% (respectively) of the total of the total fluoride applied.

Twetman and Petersson in 1996 [38] studied the effect of fluoride varnish on caries incidence in 4- to 5 year-old children, using the World Health Organization or WHO criteria, and found that the fluoride-silane varnish had a cariostatic effect in the primary dentition.

-Antibacterial effect:

The recent study has tested the antibacterial effect of fluoride varnishes. In that study, Zikert and Emilson in 1982 [39] found that Duraphat® did not significantly affect the levels of *Streptococcus mutans* in saliva and pooled dental plaque from children receiving varnish treatment. It seems, therefore, that the main cariostatic effect of fluoride varnish probably is caused by the remineralization of early carious lesions. Recently, new fluoride varnishes was introduced and will be use in this study: Enamel Pro varnish® (Premier Dental, Canada) is a 5% sodium fluoride varnish. The only varnish formulated to deliver Amorphous Calcium Phosphate or ACP. The claims of advantage were dried to an esthetic white appearance, deposits ACP to stimulate remineralization of tooth enamel and prevent the loss of enamel, provides 3 times more available fluoride to all tooth surfaces, including proximal, cervical and occlusal and delivers 2 times more fluoride uptake.

Dental caries prevention: Amorphous calcium phosphate

Over the past several years the interest in calcium phosphate based remineralization technologies has grown significantly. Early studies demonstrated that dairy products have anti-caries activity. The dairy products that were determined to be responsible were milk, casein, and cheese. The concept of casein phosphopeptides amorphous calcium phosphate (CPP-ACP) as a remineralizing agent was first introduced in 1998 [40], a bioactive agent based on milk products have been also developed in order to release elements that enhance remineralization of the enamel and

dentine, under cariogenic conditions. So, this agent in a paste formula has become commercially available. In nature, the enzymes in the mouth and stomach produce peptides from milk protein. There is a group of peptides called casein phosphopeptides that stabilized calcium and phosphate, maintaining them in an amorphous or soluble form known as ACP. Calcium and phosphate are the building blocks of tooth enamel. Normally they are highly insoluble, but in the presence of these peptides they remain soluble and bioavailable. When this peptide-calcium phosphate complex is delivered to the teeth in a chewing gum, paste or mouthwash, or even potentially in food, the peptides bind to the tooth surface and effectively provide a reservoir of soluble calcium and phosphate at the surface of the tooth.

A multifactorial anticariogenic mechanism has been proposed for CPP-ACP. It has been claimed that it promotes remineralization of the carious lesions by maintaining a supersaturated state of enamel mineral [41]. CPP-ACP has been shown to prevent enamel demineralization and to promote remineralization of subsurface enamel lesions in animal and human in situ caries models [41-43].

One of the latest products in preventative care is amorphous calcium phosphate. A products containing ACP to aid in tooth desensitization as well as remineralized early carious lesion in enamel and dentin. Tung MS in 1993 [44] found that ACP can remineralized the tooth and decreased the dentin permeability.

ACP is inorganic amorphous calcium phosphate, made by combining soluble salts of calcium and phosphorous. ACP has fast formation and dissolution rates, its works by enhancing the natural healing process of saliva, helping to prevent tooth decay**.** A teeth surface are almost entirely made up of calcium and phosphate so that when the calcium and phosphate crystals in ACP are delivered to the tooth, they dissolve before re-crystallizing as hydroxyapatite and repair early lesions in the tooth's surface, literally converting to the tooth mineral.

ACP technology was developed by Tung MS. This system was first introduced in 1999 as a toothpaste product (Enamelon). Currently, ACP is available in a several dental products such as bleaching gel, polishing paste, sealant and also fluoride varnish [45]. The action of ACP system came from mixing of calcium sulfate salt and dipotassium phosphate salt to form ACP that can precipitate onto the tooth surface for remineralization.

Currently, there are no clinical studies that support the use of fluoride varnish containing ACP and there are no FDA approvals for the product at this time. However, none of the fluoride varnishes are FDA approved as caries preventive agents. They are used "off-label" for preventing dental caries.

Dental caries prevention: Dental sealant

A definition of dental sealant is a material that is placed in the pits and fissures of teeth in order to prevent or arrest the development of dental caries [(46)].

Dental sealants play a significant role in preventing dental caries by providing a protective barrier on the teeth, particularly when used during a child's formative years. The concept of pit and fissure sealants was introduced when Dr. Michael Buonocore in 1955 [(47)] demonstrated the ability to acid etch technique as a means of bonding self-curing methylmethacrylate resin to the tooth structure. The first adaptation of the etching technique that included pits and fissures was reported in 1967, Ceuto and Buonocore [(48)] were used a 50% phosphoric acid and methylcyanoacrylate as a dental sealant, but was not marketed. The first commercial sealant was available in 1970 by Bowen and co-workers who had introduced "Bowen's Resin" consisting of a difunctional epoxy molecule (Bis-GMA), which was the chemical reaction product of bisphenol A and glycidyl methacrylate. This resin is used commonly in most of sealants nowadays [(49)]. Alternative resins used in sealant materials are urethane dimethacrylate and other dimethacrylates [(50)].

The basic sealant system is a combination of an organic matrix of a polymer resin and inorganic fillers. The polymer may contain some coloring agents and fluoride-releasing components may be present in the fillers [(49)]. The organic matrix of sealant consists of Bis-GMA resin. To obtain a low viscous sealant, usually three parts of the viscous Bis-GMA are mixed with one part of methyl methacrylate (MMA) or other low viscous diluents such as triethyleneglycol dimethacrylate (TEGDMA). The matrix portion may also contain a light sensitive photoinitiator such as camphoquinone which has peak sensitivity at a wavelength of approximately 460 nm [(50)]. Many sealant materials include inorganic fillers which vary in percentage by weight, volume, particle size, or particle shape. The fillers contribute to increasing

strength, better wear resistance, and also decrease polymerization shrinkage. However, filled sealants penetrate poorly into the fissure system and will require occlusal adjustment, while unfilled sealant will abrade rapidly within 24 to 48 hours without occlusal adjustment [51]. Fillers, frequently used in sealants, are strontium glass, barium and lithium aluminum silicates, and zirconia. The amount of filler particle in sealant materials varies from 3% to 60% by weight. Titanium dioxide is added to made sealant tinted and easily to visualized during placement and recall visit [49].

Classification of sealant

The development of pit and fissure sealants has progressed from the first generation (activated with ultraviolet light), through second (autopolymerized) and third (activated by visible light), to fourth generation (contains fluoride).

Sealant materials can classify by various properties, such as mode of polymerization, addition of filler particles, fluoride releasing, and visibility. For fluoride releasing property, sealant can divided into 2 major groups, one is the conventional non-fluoride containing sealant and the other is fluoride containing sealant.

1. **Non-fluoride containing sealants**

2. **Fluoride containing sealants**

Fluoride containing sealants can divided into 2 subgroups

2.1 Fluoride-containing resin sealant

2.2 Glass-ionomer sealants

Fluoride-containing resin sealants

The addition of fluoride into sealants to improve caries prevention cause fluoride inhibit demineralization, promote remineralization and form a fluoroapatite crystal which more resistance to acid was considerated more than 25 years ago [52,53]. There are two methods of fluoride incorporation into fissure sealants. Firstly, soluble fluoride salt is added to unpolymerized resin, the salt dissolves and fluoride ions are released after the sealant is applied to the tooth. Secondly, an organic fluoride

compound is chemically bound to the resin and the fluoride is released by exchange with other ions (anion exchange system).

Regarding physical and mechanical properties, the addition of fluoride to a fissure sealant does not affect mechanical bonding or clinical retention. In a vitro study, no significant difference in microleakage and shear bond strength between non-fluoride-containing resin sealant (PrismaShield) and fluoride-containing resin sealant (FluroShield) [54]. Furthermore Cooley *et al* [55] reported that there was no significant difference in microleakage between non-fluoride-containing resin sealant (HelioSeal) and fluoride-containing resin sealant (FluroShield). Clinical trials showed good retention rate of fluoride-containing resin sealants. Another study of 48 months 162 teeth between F-releasing filled sealant (Fluroshield) and non-F one (Delton), 124 teeth (77%) in the first group and 144 (89%) in the second were fully sealed, and 23 (14%) compared to 9 (6%) were partly sealed. The caries development, 14 teeth (9%) had developed caries in the first group and 19 (10%) in the second. However, total sealant loss and caries increment was similar in both groups. In a 2-year clinical study of 204 teeth of 6 to 8 year-old patients with 431 sealants placed in first permanent molars, complete retention of 77% and partial retention of 22% were reported [56].

Hicks *et al* in 2000 [6] evaluated the effects of a fluoride-containing resin sealant and a non-fluoride-containing resin sealant on caries-like lesion formation in human enamel, they found that mean surface lesion depths were significant difference between two groups, in a fluoride-containing resin sealant groups mean surface lesion depths were less than a non-fluoride-containing resin sealant. Similar to several studies demonstrated that artificial caries lesion in permanent teeth can be infiltrated and reduced depth of lesion by commercially available adhesives and fissure sealant.

Recently, the product (Clinpro^{TM}) is a light-cured, low viscosity, sealant with a unique patented color change feature. Clinpro^{TM} sealant is pink when applied to the tooth surface, and changes to an opaque off white color when exposed to light. Clinpro^{TM} sealant contains a patented soluble organic fluoride source. The fluoride is released from the sealant in a diffusion-limited process by exchange of hydroxide for the fluoride ion.

Rawls and Zimmerman in 1983 [57] showed that a fluoride exchanging resin decreased lesion body and increased thickness of dark zone when applied over artificial carious lesion. However, there is no investigator study demineralization and remineralization of incipient enamel caries in primary teeth by apply fluoride varnish containing ACP compare with fluoride sealant before.

The purpose of this in vitro study was to evaluated and compared the effect of a fluoride varnish ACP formula (Enamel Pro® Varnish), a conventional fluoride varnish (Duraphat®) and a fluoride containing sealant (Clinpro™) on demineralization and remineralization of artificial initial carious lesion on primary incisor teeth.

CHAPTER IV
MATERIALS AND METHODS

1. Samples selection

45 sound extracted primary incisor teeth with macroscopic caries-free were collected in 0.9% normal saline solution in incubator at 37° C to prevent dehydration. The buccal and lingual surfaces were evaluated under a stereoscopic microscope (Nikon, Model Eclipse E400, Nikon, Japan) and inspected for the possible enamel defects and caries. If any defects or caries was detected, the tooth was then excluded.

2. Experimental procedure

2.1 Cleaning and storage of teeth

The teeth were cleaned under deionized water using a soft brush to remove plaque and tissue debris following by polished with fluoride free prophylaxis paste by rubber cup that attached to slow-speed handpiece for a few seconds. After that, thoroughly rinsed with deionized water, then the cleaned teeth were immersed in deionized water at 37° C until further use.

2.2 Preparation of artificial white spot lesion

- After cleansing the teeth, artificial initial carious lesions were created on their sound buccal enamel surfaces. First the tooth was apically sealed with melted sticky wax and two pieces of tape size approximately 1x1 mm^2 were applied to sound, intact surface of the buccal enamel surface at same level then the entire tooth crown was covered with two layers of acid resistant nail varnish (Revlon, USA). The nail varnish was allowed to dry at room temperature for 20 minutes before the second application. Following additional 20 minutes of drying at room temperature the tapes were removed to reveal the windows of sound enamel. After that the samples were placed in deionized water at 37° C until further use.

Figure 2: Windows (exposed enamel) on middle third of buccal surface

- After that the windows of each tooth were exposed to 5 ml of demineralizing solution containing 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4 and 0.05 M acetic acid (the pH was adjusted with 1 M KOH to be pH 4.4) for 96 hours [58] at 37° C to produced initial artificial carious lesions approximate 100-150 µm [59] in depth in each window. At the end of a 96 hours demineralization period all teeth were removed and rinsed with deionized water for 10 seconds and wiped off carefully with a paper tissue. The samples were placed in deionized water at 37° C until further use.

2.3 Tooth separation

Each tooth was bucco-lingual longitudinally sectioned in half for separated the window into 45 pairs by using a hard tissue microtome (Accutom-50, Struers Co. Denmark) with slow speed diamond saw under copious water spray creating 45 pairs left and right windows. The left side window was used as the control specimen and the right side was used as the tested specimen. After that the exposed enamel and dentin were covered with two layers of acid resistant nail varnish as described above. The samples from each tooth were placed in deionized water at 37° C until further use.

Figure 3: Tooth separation

2.4 Surface treatment of the artificial white spot lesion

The teeth were randomly assigned into 3 groups of equal number.

The tested specimens (right side) windows were applied as described below

Group A: applied Enamel Pro® Varnish 5% Sodium Fluoride Varnish, ACP Formula (Premier Dental, Canada). The varnish was coated for 24 hours, and then the varnish was subsequently removed.

Group B: applied Duraphat® (Colgate, Canton, MA). The varnish was coated for 24 hours, and then the varnish was subsequently removed.

Group C: applied light cured fluoride containing sealant (Clinpro™, 3M ESPE, USA) according to respective manufacturer's instructions.

2.5 Thermocycling and pH-cycling model

All specimens were thermocycled in artificial saliva (20 mM $NaHCO_3$, 3 mM NaH_2PO_4, 1 mM $CaCl_2$, pH 7.0) for 500 cycles between 5º C and 55º C with a dwell time of 20 seconds, then the specimens were removed, rinsed with deionized water, dried with paper tissue and then the specimens were placed in a 7-day pH-cycling model to stimulating a high caries challenge.

pH-cycling model

All of the specimens were underwent pH-cycling for 7 days in the following manner:

- Each cycle was involved three hours of demineralization twice a day with two hours of remineralization in between.

- Each specimen was immersed in the demineralizing solution (5 ml per specimen) for three hours followed by thorough rinsing with deionized water and dried with paper tissue.

- After that all of the specimens were immersed in remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl and the pH were adjusted to 7.0) for two hours then all teeth were rinsed in deionized water and dried with paper tissue.

- All of the specimens were placed in the remineralizing solution overnight.

- All solutions (demineralizing and remineralizing solution) were freshly prepared for each cycle; separate containers were used for each group throughout the experimental period. The pH of the demineralizing and remineralizing solutions were measured before each cycle.

- After the 7 days cycle the teeth were removed from the solution, rinsed with deionized water and dried with paper tissue. The acid resistant nail varnishes on the specimens were removed carefully with acetone before next step evaluation.

Table 1: pH-cycling procedure

Time period	Procedure
3 hours	Immersed in demineralized solution Rinsed with deionized water and dried with paper tissue
2 hours	Immersed in remineralized solution Rinsed with deionized water and dried with paper tissue
3 hours	Immersed in demineralized solution Rinsed with deionized water and dried with paper tissue
16 hours	Immersed in remineralized solution Rinsed with deionized water and dried with paper tissue

2.6 Tooth sectioning

The teeth were cut through the control window and the experimental window at middle of the lesion area 1 piece/window longitudinally by using a hard tissue microtome and slow speed diamond saw under copious water

spray. After that the sections were polished with a waterproof abrasive paper number 800 to provide approximately 150-200 µm thick and measured thickness by electronic digital caliper (Mitutoyo® model CD-6C, Japan)

2.7 Calculating the mean lesion depth

- The specimens were blinded before the examiner examined for mean lesion depth by using Polarized light microscope (Nikon® model eclipse E400 pol, Japan) 50x magnifications in water imbibitions and Digital images were taken with specific software (Image-Pro Plus, Media Cybernetics, Silver Spring, USA).

- The method to determine the depth of lesion: firstly, calibrate the scale by using a measure tool bar after that converse the scale into a micron unit (1 mm = 1000 µm) as showed in the figure 4

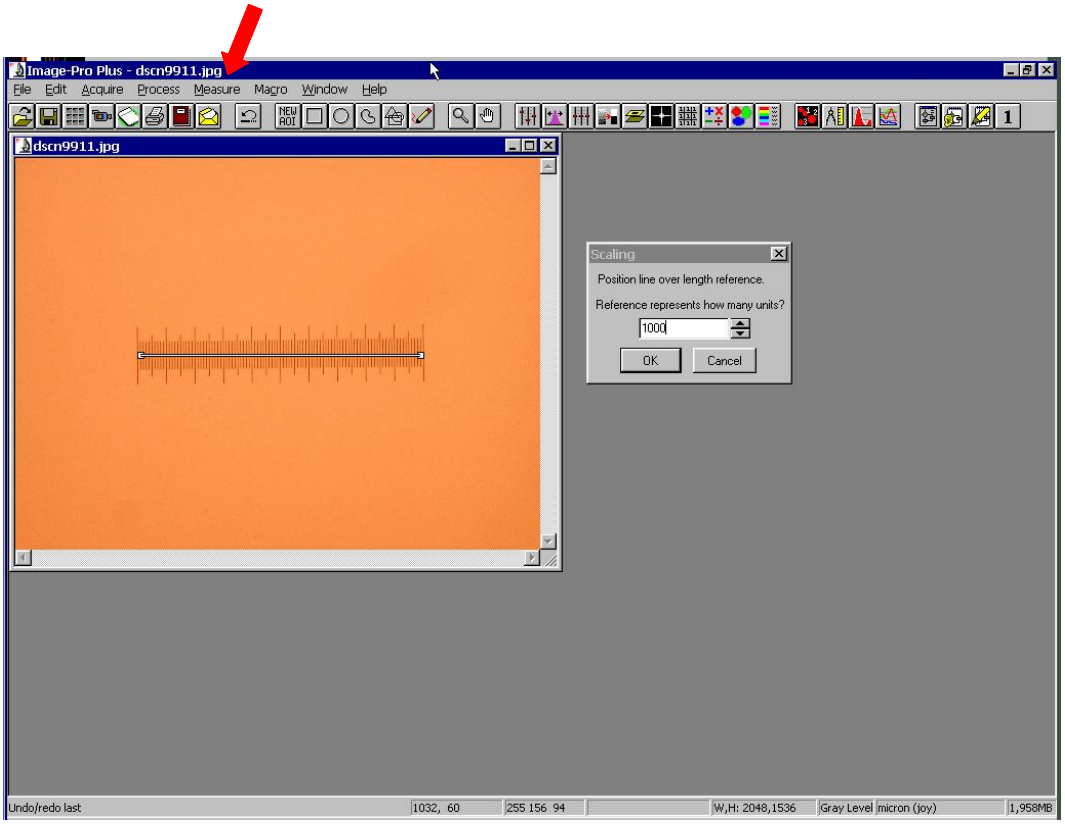


Figure 4: Calibration the scale

- Each specimen was finding out the deepest depth of lesion after that, the deepest lesion depth was determined for three times and the average depth of the lesion was used for analyzed as showed in the figure 5.

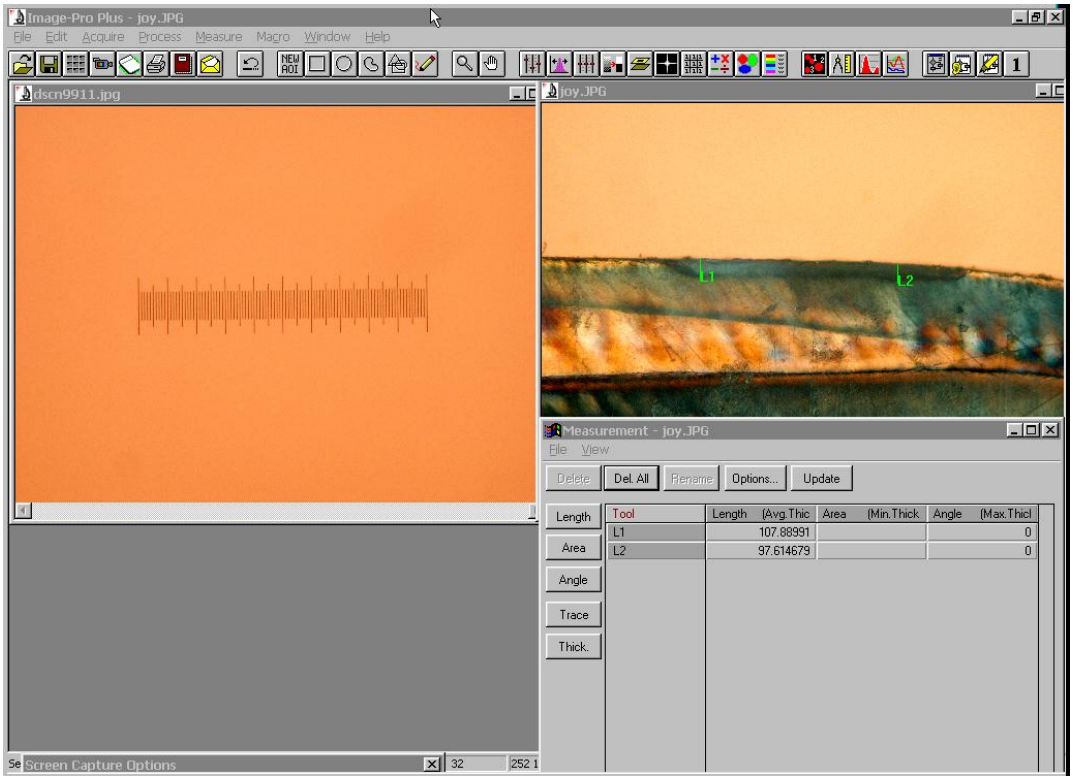


Figure 5: Determined the lesion depth

- Intra-examination reliability

Eighteen specimens (20% of all specimens) were randomly selected and reexamined by the same examiner under the same conditions. The intra-examination reliability was tested by using Spearman rank correlation coefficients. Results from the duplicate examination showed that the intra-examination reliability of the mean lesion depth was 0.983 which showed good reliability.

2.8 Statistical Analysis

- SPSS version 17 was used to analyze the data.

- Descriptive statistics was given for mean difference and standard deviation of polarized light microscopy measurements values.

- Kolmogorov Smirnov test (K-S test) was used to test the distribution of the data. The results showed normal distribution.

- One-way ANOVA at 0.05 level of significance was used to compare the mean lesion depths between control groups. The result found that they were not statistically significant ($p>.05$). Therefore, the experimental group was compared with control group.

- A paired *t*-test was used to reveal a significant difference of mean lesion depth among the experimental and control groups at 0.05 level of significance.

- One-way ANOVA and multiple comparisons at 0.05 level of significance were used to compare groups mean lesion depth reduction.

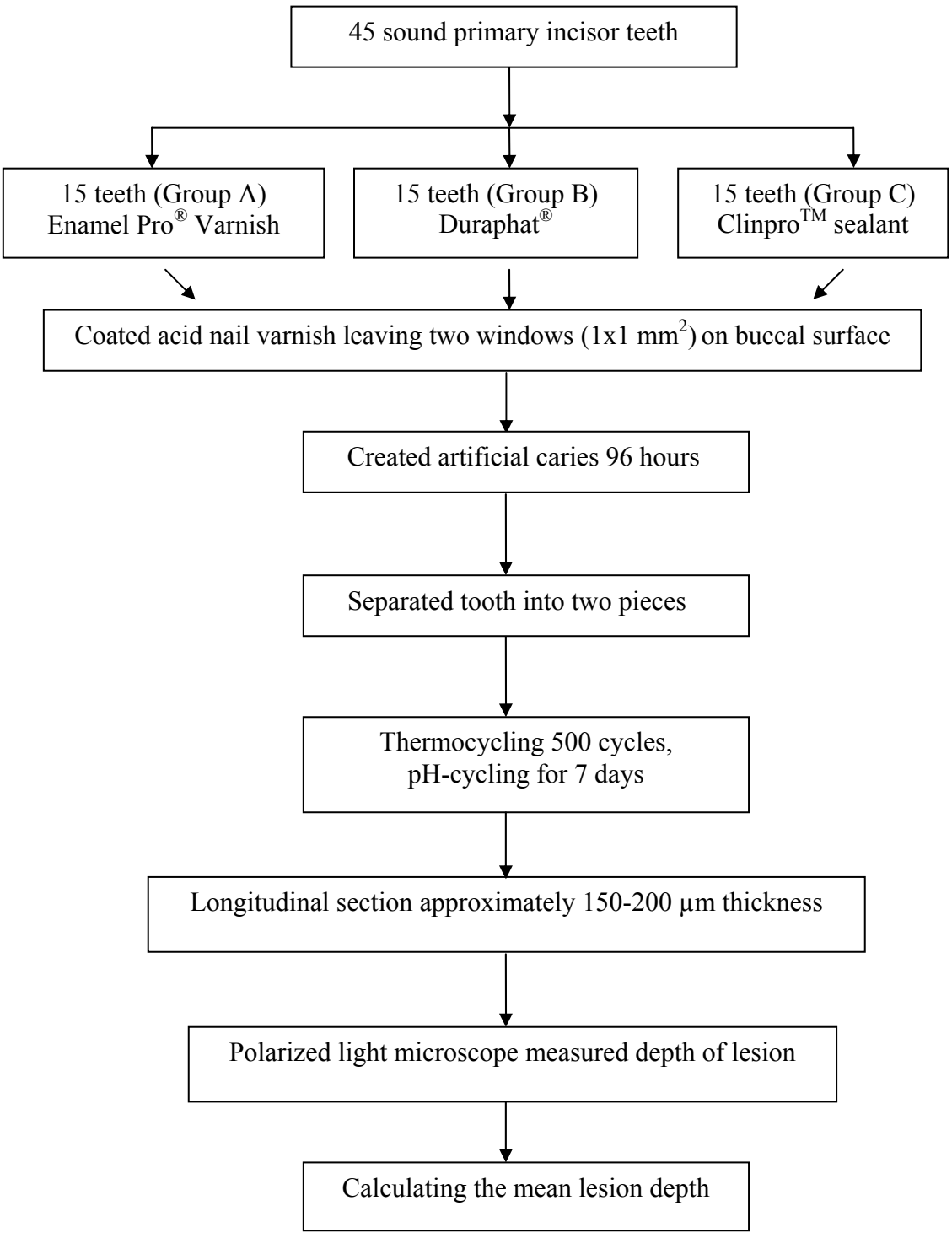


Figure 6: Diagram of the experimental procedure

Table 2: Materials used in the study

Materials	Composition	Procedures
Enamel Pro® Varnish (Premier Dental, Canada) 	- 5% Sodium Fluoride Varnish - Amorphous Calcium Phosphate	Coated for 24 hours, then the varnish was subsequently removed
Duraphat® (Colgate, Canton, MA) 	- 5% Sodium Fluoride Varnish - 50 mg Sodium Fluoride per ml About 22.6 mg Fluoride	Coated for 24 hours, then the varnish was subsequently removed
ClinproTM (a resin-based, light-cured, fluoride-containing pit and fissure sealants, 3M ESPE, USA) 	-Triethylene glycol dimethacrylate -Bisphenol A diglycidyl methacrylate -Ethyl4-(dimethylamino)benzoate -Diphenyliodonium hexafluorophosphate -DL Camphorquinone -Buthylated hydroxytoulene, -Dichorodimethylsilane reaction product withsilica -Tetrabutylammonium tetrafluoroborate -atitranium dioxide -Rose bengal sodium	1. Applied etchant (37 % phosphoric acid) 15 seconds 2. Thoroughly rinsed teeth with air/water spray to removed etchant 15 seconds 3. Thoroughly dried the etchant surface 4. Applied sealant with an explorer 5. Applied light cure 40 seconds

CHAPTER V

RESULTS

The mean and standard deviation (SD) of lesion depth in control and experiment of all groups were given in Table 3. The results showed that the mean lesion depth ± SD of control specimens in each group was not statistically significant ($p>.05$). The experimental group was compared with their control group. The paired *t*-test indicated that the mean lesion depth were statistically significant difference between control and experimental groups ($p=.00$).

Table 3: Mean ± SD of lesion depth of artificial caries in control and test groups

Group	**N**	Depth of lesion (µm) (mean ± SD)	
		Control	Test
A. Enamel Pro® Varnish	**15**	166.52 ± 19.73	97.05 ± 10.69*
B. Duraphat®	**15**	156.18 ± 12.86	98.11 ± 10.00*****
C. Clinpro™	**15**	183.43 ± 16.62	83.28 ± 14.44*

The mean lesion depth of control specimens were not statistically significant (One way ANOVA, $p>.05$)

***** Showed a significant mean difference compared with control (paired t-test, $p<.05$)

The mean lesion depth in Group A, B and C decreased by 41.72 %, 37.18 %, and 54.59 % when compared with their control, respectively as shown in Table 4.

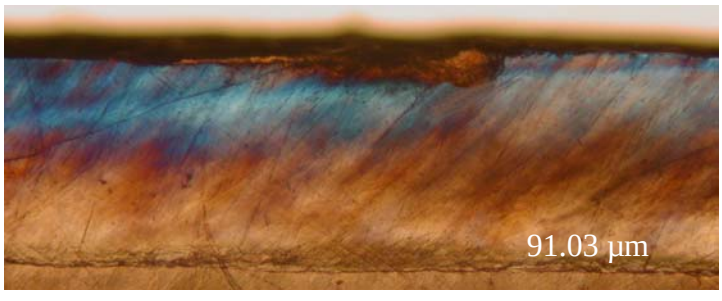
Table 4: Mean ± SD of lesion depth reduction of artificial caries

Group	**N**	Lesion depth reduction (µm) (mean ± SD)	% Mean lesion depth reduction
A. Enamel Pro®	**15**	69.47 ± 17.75	41.72
Varnish	**15**	58.06 ± 13.77	37.18
B. Duraphat®	**15**	100.14 ± 24.04*	54.59
C. Clinpro™			

*Showed a significant mean lesion depth reduction compared with other groups ($p<.05$)

The mean lesion depth in control and tested specimens of Enamel Pro® Varnish group were 166.52 ± 19.73 µm and 97.05 ± 10.69 µm respectively. The mean lesion depth reduction was 41.72 %. The examples of a photograph from the polarizing light microscope in Enamel Pro® Varnish group were shown in Figure 6.

A



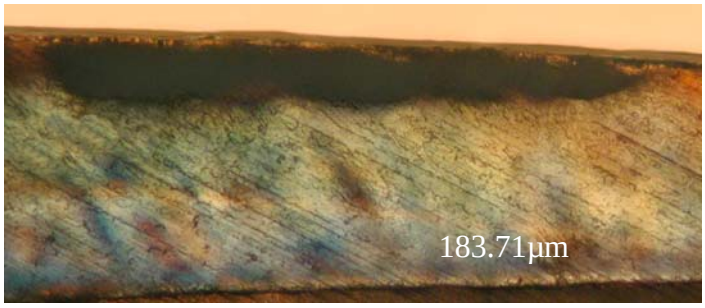
B

Figure 7: Polarized light photomicrograph at 50x magnification of an enamel sample lesion in Enamel Pro® Varnish group.

A = Control specimen B = Test specimen

Duraphat® tested group showed the highest mean lesion depth. The mean lesion depth in control and tested specimens of Duraphat® group were 156.18 ± 12.86 µm and 98.11 ± 10.00 µm respectively. The mean lesion depth reduction was 37.18 %. The examples of photographs from the polarizing light microscope in Duraphat® group were shown in Figure 7.

A



B

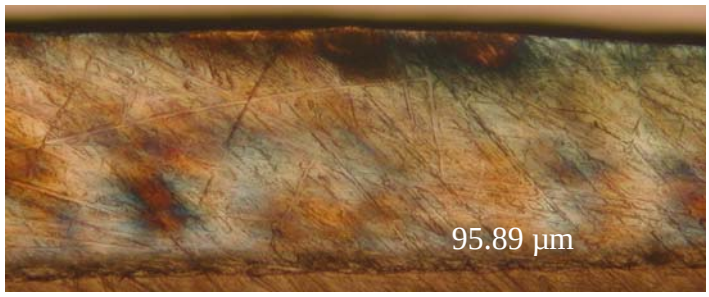
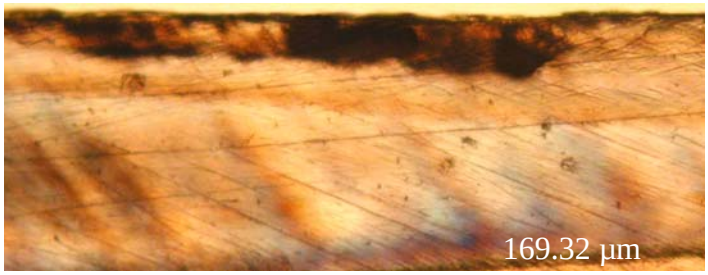


Figure 8: Polarized light photomicrograph at 50x magnification of an enamel sample lesion in Duraphat® group.

A = Control specimen B = Test specimen

Clinpro™ sealant tested group was presented the lowest man lesion depth. The mean lesion depth in control and tested specimens of Clinpro™ sealant group were 183.43 ± 16.62 µm and 83.28 ± 14.44 µm respectively. The mean lesion depth reduction was 54.59 %. The examples of photographs from the polarizing light microscope in Clinpro™ sealant group were shown in Figure 8.

A



B

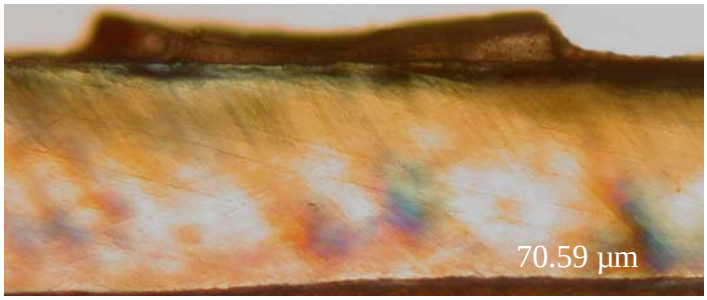


Figure 9: Polarized light photomicrograph at 50x magnification of an enamel sample lesion in Clinpro™ sealant group.

A = Control specimen B = Test specimen

The mean lesion depth reduction between Enamel pro® varnish group and Duraphat® group were not statistic significantly different ($p>.05$). On the other hand, the mean lesion depth reduction between Clinpro™ sealant group and fluoride varnish group were statistic significantly different ($p=.00$).

CHAPTER VI
DISCUSSION

Smooth surface caries remain an oral health problem in young children, particularly in children diagnosed with early childhood caries. White spot lesion is the first sign of initial caries which can easily detect on labial/buccal or lingual tooth surfaces and this lesion may be remineralized [1]. However, children who have inadequate fluoride exposure, improper diet consumption, poor oral hygiene practices and unable to attend regular dental visit, the white spot lesion will continue until cavitated which cannot be revered that when restoration will be needed. Once caries develop in primary teeth, it is more rapid progression into the underlying dentin than occurred in permanent teeth. The reason is the differences in a composition and structure which cause primary enamel more prone to acid attack. Therefore, the caries prevention for initial caries in primary teeth is very important.

Clinicians try to find material that can inhibit the progress and restore the loss of mineral structure. For this reason, the propose of this *in vitro* pH-cycling study was to compared the effect of a fluoride varnish ACP formula (Enamel Pro® Varnish), a conventional fluoride varnish (Duraphat®) and a fluoride containing resin sealant (Clinpro™) on demineralization and remineralization of initial artificial carious lesions on smooth surface of primary teeth. The mean lesion depths in body of the lesion zone were determined by polarized light microscope with water imbibitions because it is a common method for assessing remineralization of carious lesion.

There are several studies demonstrated the remineralization in artificial caries lesion similar to this study [2-7,62-64]. However, there is a few studies in primary incisor teeth and none of these studies used control and experimental window on the same tooth like our study. For our study, the tooth was half sectioned into two pieces after artificial caries formation in order to separated control window and experimental window which can eliminate the possibility of fluoride released from the experimental window contaminate to the control window. The advantage of this designed control

study is that the control and treatment specimens came from the same tooth which can minimize the variation of tooth structure. However, this method resulting in reduced window areas for tooth sectioning.

The American Academy of Pediatric Dentistry (AAPD) has recommended using fluoride to reverse enamel demineralization. Fluoride varnish is recommended to use in very young children. Fluoride varnish is a professionally applied fluoride, which highly concentrated (22,600 ppm) fluoride product. Multiple studies have shown that fluoride varnish can promote enamel remineralization [23,61-64].

Among the fluoride varnish groups, Duraphat® was chosen because it had a high percentage of caries reduction [35], released fluoride better than Duraflor® [37], more fluoride concentration than Fluor-Protector® [31] and more enamel fluoride uptake [30]. Another interesting one is Enamel pro® varnish, a new product of fluoride varnish which delivers ACP to stimulate remineralization of tooth enamel. According to the manufacturer's product instruction (Premier Dental product, Co) it provides 2 times more enamel fluoride uptake when compare with conventional 5% NaF varnish.

The pit and fissure sealant was commonly used to seal the occlusal surface. However, there were several study suggested that pit and fissure sealant materials also useful on smooth enamel surface as well as in pit and fissure areas. Clinpro™ sealant was used in this study because it's widely use in dental clinic.

Based on the result of this study, each of fluoride containing agents had a significant remineralization effect on early carious enamel lesion when compare with control groups.

The present study showed mean lesion depth reduction by Duraphat® was 37.18% similar to other previous studies. This was supported by Øgaard B *et al* in 1996 [63] who found that the usage of a fluoride varnish can reduce lesion depth by 48% as compared to the control similar to Hicks J *et al* in 2001 [64] found lesion depth reductions among the fluoride varnishes were similar and varied between 28%-34%. Furthermore, a several number of clinical trials have confirmed the caries preventive effect of fluoride varnishes. Data showed an overall reduction in caries increment following fluoride varnish applications, ranging approximately from 18 to 70% compared to untreated controls [34,65].

Enamel pro® varnish contains the same amount of 5% sodium fluoride as Duraphat® (22,600 ppm), Furthermore it added ACP which is well known to the stimulation of remineralization and strengthen tooth structure, so that a synergistic cariostatic effect would be expected to occur as a function of this material.

The result from this study showed Enamel pro® varnish reduced lesion depth 41.72% that tend to more remineralized when compare with Duraphat®; a conventional fluoride varnish (37.18%). It may be due to Enamel pro® varnish containing ACP which can enhance the amount of calcium and phosphates available in the enamel surface. The ACP has been shown to rapidly hydrolyze to form apatite, similar to carbonated apatite, the tooth mineral [(44)]. In the presence of fluoride ions condition the unstabilized ACP will bind the free fluoride ion and localizes it, with the calcium and phosphate ions, at the enamel surface and may produce fluorapatite which is more resistant to acid attack.

According to the manufacturer's product instruction Enamel pro® varnish was claimed to increase calcium and phosphate concentrations so that it can delivers 2 times more fluoride uptake into the enamel and provides significantly more available fluoride to tooth surfaces [(66)]. However, the result from this study was showed that the mean lesion depths and lesion depth reduction between Enamel pro® varnish group and Duraphat® group were not statistic significantly different ($p>.05$). Currently, there was no published paper could be found of Enamel pro® varnish treatment yet.

Our study showed that Clinpro™ had the highest lesion depth reduction. It could be explained that the fluoride containing sealant acted as a physical barrier between the carious lesion and the acid challenge resulting in caries arrest and in the same time remineralized the lesion. From the results of this study indicated that more effectiveness of fluoride containing sealant placement for prevention of initial caries in primary teeth than fluoride varnish. This is supported by a 24 months clinical comparison of sealants and fluoride varnish on non-fissured surfaces of first permanent molars resulted in reduction of caries prevalence by 87% compared with 66% for the fluoride varnish [(67)]. However, There was in vitro study showed that the mean lesion area between fluoride varnish and fluoride sealant groups were not statistic significantly different ($p>.05$) [(68)].

Fluoride released from fluoride containing sealant was rapid and the most occurred within the first two days [6,69,70-71]. The amount of fluoride released from Clinpro™ sealant was about 0.665 ± 0.086 ppm [72]. Even though the fluoride released from this material was very small amount when compare with fluoride varnish; but it is sufficient to promote remineralization because only a small concentration (0.1 ppm) of fluoride in dental plaque is required to inhibit enamel demineralization and facilitate remineralization [73]. Furthermore in clinically a major advantage of fluoride containing sealant is the ability to recharge with fluoride source, such that the fluoride can then be re-released [72,74]. So, there are long-term slowly fluoride released into oral cavity. Additional, previous studies have shown that fluoride released from sealants was able to produce an inhibitory effect against S. mutans [75].

Regarding physical and mechanical properties, the addition of fluoride to a fissure sealant does not affect mechanical bonding or clinical retention [55-56,76]. Furthermore, in this study the sealant was suggested to place on labial enamel tooth surface which is non stress bearing area so it might not have a problem from sealant retention. Although, in case of sealed enamel was abraded its may also benefit from retention of residual resin tags and also from incorporation of fluoride released from the resin [6].

Researchers have concluded that well applied pit and fissure sealant can inhibit the progression of decay especially in white spot lesions. In clinically, common treatment of white spot lesion is applying professional topical fluoride, diet advice and oral health motivation. For these methods are need compliance from the patient. Therefore, sometimes the clinician may be found white spot lesions were progress and developed a cavity in recall visit especially in high risk patient. This was showed a failure of preventive program, which may be due to poor oral hygiene practice. So the variable of patient compliance remains the major problem to caries prevention.

The sealant application is non-invasive treatment procedure that requires minimal chair time, little expensive, promote remineralization and also need less compliance in oral health care. For these reasons and based on the results from this study, using fluoride containing sealant may be appropriate for prevent the development of smooth surface caries in primary teeth to those patients with high caries risk and irregular dental visit.

However, the further clinical studies should be tested in order to compare the effectiveness of these materials in remineralizing the smooth surface carious lesion so that the clinician will have the confident in using sealant to prevent and inhibit early dental caries progression in young children.

CHAPTER VII

CONCLUSIONS

The null hypothesis that no difference in lesion depth among fluoride varnish ACP formula, a conventional fluoride varnish and a fluoride containing sealant used in initial artificial carious lesions on primary incisor teeth was rejected.

Within the limitations of this *in vitro* study, it can be concluded that

1. Both fluoride containing sealant and fluoride varnish could remineralize subsurface enamel lesion ($p<.05$).

2. The mean lesion depth reduction between Enamel pro® varnish group and Duraphat® group were not statistically different ($p>.05$).

3. Fluoride containing sealant (Clinpro™) revealed the highest mean lesion depth reduction which statistically different from Enamel pro® varnish group and Duraphat® group ($p=.00$).

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APPENDIX

Table 5: Lesion depth in control and test groups after the 7-day pH-cycling.

No	Group A		Diff A	Group B		Diff B	Group C		Diff C
	Control	Test	(C-T)	Control	Test	(C-T)	Control	Test	(C-T)
1	169.71	91.03	78.68	161.39	107.35	54.04	180.42	80.00	100.42
2	168.74	95.72	73.02	146.91	91.46	55.45	211.19	65.88	145.31
3	139.22	96.39	42.83	146.20	82.46	63.74	183.22	89.41	93.81
4	169.71	85.68	84.03	139.22	99.42	39.80	169.32	70.59	98.73
5	142.27	104.18	38.09	166.93	112.26	54.67	181.82	84.71	97.11
6	182.64	96.39	86.24	161.37	107.51	53.86	176.22	94.11	82.11
7	149.22	85.68	63.53	139.22	108.19	31.03	188.81	61.18	127.63
8	172.94	91.03	81.91	144.68	90.95	53.73	167.03	94.11	72.92
9	155.80	96.39	59.41	166.93	98.22	68.71	179.02	84.71	94.31
10	182.57	83.09	99.47	161.37	93.16	68.21	149.86	98.82	51.04
11	183.08	107.10	75.98	155.80	96.63	59.17	195.80	108.24	87.56
12	180.87	116.26	57.71	158.26	87.72	70.54	208.18	95.72	112.46
13	208.84	118.16	90.68	142.18	87.72	54.46	181.80	89.41	92.39
14	148.27	97.56	50.71	168.50	115.77	52.73	205.98	70.59	135.39
15	143.87	91.03	52.84	183.71	95.89	93.82	172.76	61.78	110.98

Table 6: One-Sample Kolmogorov-Smirnov Test.

		Group A control	Group B control	Group C control
N		15	15	15
Normal Parameters	Mean	166.52	156.18	183.43
	SD	19.73	12.86	16.62
Most Extreme Differences	Absolute	.145	.164	.172
	Positive	.143	.164	.172
	Negative	-.145	-.123	-.113
Kolmogorov-Smirnov Z		.561	.637	.665
Asymp. Sig. (2-tailed)		.911	.812	.769

Test distribution is normal

Table 7: One-Sample Kolmogorov-Smirnov Test. (cont.)

		Group A test	Group B test	Group C test
N		15	15	15
Normal Parameters	Mean	97.05	98.11	83.28
	SD	10.69	9.99	14.44
Most Extreme Differences	Absolute	.214	.157	.144
	Positive	.214	.157	.144
	Negative	-.096	-.156	-.139
Kolmogorov-Smirnov Z		.829	.606	.556
Asymp. Sig. (2-tailed)		.497	.856	.916

Test distribution is normal

Table 8: Paired t-test

	Paired Differences					t	df	Sig. (2-tailed)
Pair Control-Test	Mean	SD	SE	95% CI of the difference				
				lower	upper			
	75.89	25.83	3.85	68.13	83.65	19.71	44	.000

Table 9: one way analysis of variance (ANOVA) of control groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2279.019	2	1139.510	3.163	.053
Within Groups	15130.181	42	360.242		
Total	17409.200	44			

Table 10: one way analysis of variance (ANOVA) of test groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2052.311	2	1026.155	7.28	.002
Within Groups	5919.732	42	140.946		
Total	7972.043	44			

Table 11: Multiple Comparisons of test groups

			Mean Difference (I-J)	SD	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	I	J					
Tukey HSD	A	B	-1.06800	4.335	.967	-11.6000	9.4640
		C	13.76200	4.335	.008	3.2300	24.2940
	B	A	1.06800	4.335	.967	-9.4640	11.6000
		C	14.8300	4.335	.004	4.2980	25.3620
	C	A	-13.76200	4.335	.008	-24.2940	-3.2300
		B	-14.8300	4.335	.004	-25.3620	-4.2980

Table 12: Test of Homogeneous

Group		**N**	**Subset for alpha = 0.05**	
			1	**2**
Tukey	C	15	83.2840	
HSD	A	15		97.0460
	B	15		98.1140
	Sig.		1.000	.967

Table 13: Pearson's Correlation Coefficient Test.

Pearson's Correlation Coefficient Test

		First measure	Repeated Measure
First measure	Pearson Correlation	1.000	.983(**)
	Sig. (2-tailed)	.	.000
	N	18	18
Repeated measure	Pearson Correlation	.983(**)	1.000
	Sig. (2-tailed)	.000	
	N	18	18

** Correlation is significant at the level of .01 (2-tailed).

COE. No. MU-IRB 2009/012.0210

Documentary Proof of Mahidol University Institutional Review Board

Title of Project. Effect of Fluoride Varnish and Fluoride Containing Sealant on Artificial Caries on Primary Teeth

Principle Investigator. Assistant Professor Yuwadee Asvanund

Name of Institution. Faculty of Dentistry

Mahidol University Institutional Review Board is in full compliance with International Guidelines for Human Research Protection such as Declaration of Helsinki, The Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Date of Determination. 2 October 2009

Signature of Chairman. .....

(Professor Rutja Phuphaibul)
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