THE NOVEL CYANOACRYLATE BASED FLUORIDE VARNISH

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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นวัตกรรมไซยาโนอะคริเลตฟลูออไรด์วาร์นิช

นายณรงค์ฤทธิ์ ลาภบุญผล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ณรงค์ฤทธิ์ ลาภบุญผล : นวัตกรรมไซยาโนอะคริเลตฟลูออไรด์วาร์นิช . (THE NOVEL CYANOACRYLATE BASED FLUORIDE VARNISH) อ.ที่ปรึกษาหลัก : รศ. ทพ. ดร. นิยม ธำรงค์อนันต์สกุล

ปัจจัยสำคัญที่ส่งผลต่อประสิทธิภาพของการให้การรักษาด้วยฟลูออไรด์วาร์นิชคือความเข้มข้นของฟลูออไรด์ไอออนที่ปลดปล่อยออกมาและระยะเวลาที่ฟลูออไรด์วาร์นิชคงอยู่บนผิวฟัน แต่ฟลูออไรด์วาร์นิชส่วนใหญ่ที่มีในท้องตลาดในปัจจุบันมีความสามารถในการต้านทานต่อการขัดสีเชิงกลที่ต่ำ ทำให้ผู้ป่วยต้องได้รับคำแนะนำหลังการรักษาให้หลีกเลี่ยงการใช้งาน การกินและดื่ม ในบริเวณที่ได้รับการทาด้วยฟลูออไรด์วาร์นิชเป็นเวลาอย่างน้อย 12-24 ชั่วโมง ซึ่งทำให้เกิดความไม่สะดวกสบายและความกังวลแก่ผู้ป่วย ผู้วิจัยจึงได้คิดค้นฟลูออไรด์วาร์นิชที่มีสารไซยาโนอะคริเลตเป็นองค์ประกอบขึ้นเพื่อแก้ปัญหาเหล่านี้ การศึกษานี้เปรียบเทียบการปลดปล่อยฟลูออไรด์ไอออนรายวัน ความทนทานต่อการขัดสีจากการแปรงฟัน และความเป็นพิษต่อเซลล์ไฟโบรบลาสต์เหงือกมนุษย์ ระหว่างนวัตกรรมไซยาโนอะคริเลตฟลูออไรด์วาร์นิชและดูราแฟตวาร์นิช ผลการทดลองแสดงให้เห็นว่า นวัตกรรมไซยาโนอะคริเลตฟลูออไรด์วาร์นิชมีการปลดปล่อยฟลูออไรด์ที่สูงกว่าอย่างมีนัยสำคัญทางสถิติ เป็นเวลา 9 วันหลังการทาวาร์นิช สามารถทนทานต่อการขัดสีจากการแปรงฟันที่สูงกว่าอย่างมีนัยสำคัญทางสถิติ และมีความเป็นพิษต่อเซลล์ไฟโบรบลาสต์เหงือกมนุษย์ที่ต่ำกว่าเล็กน้อยเมื่อเทียบกับดูราแฟตวาร์นิช นวัตกรรมไซยาโนอะคริเลตฟลูออไรด์วาร์นิชอาจเป็นฟลูออไรด์วาร์นิชทางเลือกชนิดใหม่ในการป้องกันฟันผุ

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The important factors contributing to the effectiveness of fluoride varnish are the amount of fluoride ion release and the retention time of the varnish on the tooth surface. Commercial fluoride varnishes are susceptible to mechanical removal; therefore, patients are informed to avoid eating for a few hours and refrain from tooth brushing for 12–24 h, which results in patient inconvenience. However, the novel cyanoacrylate based fluoride varnish would not have these disadvantages. This study compared the daily fluoride ion release, abrasion resistance to brushing, and toxicity to human gingival fibroblasts (hGFs) between a newly-developed cyanoacrylate based fluoride varnish and Duraphat varnish. The results demonstrated that the cyanoacrylate varnish had a significantly higher fluoride release for 9 d after application, higher abrasion resistance to brushing, and slightly less toxicity to hGFs compared with Duraphat varnish. This novel cyanoacrylate varnish could be an alternative fluoride varnish for preventing dental caries.

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CHAPTER 1: INTRODUCTION

Background and significance of problem

Fluoride application has been introduced in clinical dentistry for dental caries prevention and remineralization since 1940 and it was approved by the U.S. Food and Drug Administration (FDA) in 1994. In the beginning, topical fluoride products were aqueous solution including sodium fluoride gels and stannous fluoride solutions. In 1964, non-aqueous fluoride product (varnish) was developed to increase the retention time on tooth surface. The first commercial fluoride varnish was Duraphat varnish (5% NaF, Colgate-Palmolive, Canton). Moreover, Duraphat varnish has been the most widely used and extensively studied fluoride varnish. Several studies show that Duraphat varnish was the effective topical fluoride in preventing caries (1-4). In clinical dentistry, Duraphat varnish is considered to be a fluoride varnish of choice. The concentration of sodium fluoride in Duraphat varnish is 5% (22,600 ppm fluoride or 50 mg NaF/ml) in resin carrier which promotes longer retention time on tooth surface.

Mechanism of topical fluoride in preventing caries is the concentration of released fluoride ion from varnish induced forming fluoroapatite in demineralized tooth structures and globules of CaF_2-like material on tooth surface. CaF_2 globules are stabilized by phosphate-binding protein from saliva and serve as reservoirs of fluoride

which is important for caries prevention. During the cariogenic challenge, lower pH induces dissolution of CaF_2 from the globules. Subsequently, the released fluoride ions promote remineralization in demineralized tooth structures (5) and calcium ions neutralize the acid and increase pH.

The anti-caries effect of fluoride varnish cannot be based on the amount of released fluoride ion in a few hour after application because they can be rinsed out by water and saliva but the important part is the formation of fluoride reservoir (CaF_2 globules) on the tooth surface (6) which are responsible for sustained provision of fluoride during demineralization and remineralization on enamel. Several studies reported that the amount of CaF_2 formation was related to retention time of topical fluoride on tooth surface (7-9) and fluoride ion concentration in saliva and plaque (6, 10). Moreover, remineralization on enamel is time dependent and improves overtime (7, 8, 11). Furthermore, it has been found that most of NaF in varnish remain insoluble after 24 hours of application, thus the longer retention time will allow remaining NaF to dissolve and promote more CaF_2 globules formation which serve as fluoride reservoirs for caries prevention.

Unfortunately, commercial fluoride varnishes in the market are susceptible to mechanical removal. To increase retention time of varnish on tooth surface, the mechanical removal of fluoride varnish should be postponed including refraining from eating hard food for a few hours after application and refraining from tooth brushing between 4-24 hours depend on the manufacturer recommendation but the clinical

protocols recommend refraining from tooth brushing 12-24 hours after fluoride varnish application (12-14).

In fact, the mechanical removals of fluoride varnish are not only eating or tooth brushing but also oral tissue scrubbing (cheek, tongue). Thus, it is impossible to avoid mechanical removal of the varnishes unless they are stronger, faster setting time, and self progressively flake off (to serve as fluoride varnish, not permanent restoration). Incorporating cyanoacrylate adhesive could solve these problems because it is strong, immediately set when contact with moisture, biocompatible, self progressively flake off and able to create strong bond to the tooth surface. The aim of this study was to compare the fluoride ion release, resistance to tooth brushing and cytotoxicity to human gingival fibroblast (hGFs) of novel cyanoacrylate fluoride varnish and Duraphat varnish.

Research Questions

A. Is novel cyanoacrylate based fluoride varnish effective on daily fluoride ion releasing compare to Duraphat varnish?
B. Can novel cyanoacrylate based fluoride varnish withstand the mechanical removal from tooth brushing compare to Duraphat varnish?
C. Is novel cyanoacrylate based fluoride varnish toxic to human gingival fibroblast?

Research Objectives

A. To compare the concentration of daily fluoride ion releasing between novel cyanoacrylate based fluoride varnish and Duraphat varnish.
B. To compare the numbers of brushing strokes that varnish can withstand the mechanical removal from tooth brushing between novel cyanoacrylate based fluoride varnish and Duraphat varnish.
C. To evaluate the cytotoxicity to human gingival fibroblast of novel cyanoacrylate based fluoride varnish.

Hypothesises

A. H_0: There is no statistically significance difference in the concentration of daily fluoride ion releasing between novel cyanoacrylate based fluoride varnish and Duraphat varnish.

   H_a: There is statistically significance difference in the concentration of daily fluoride ion releasing between novel cyanoacrylate based fluoride varnish and Duraphat varnish.
B. H_0: There is no statistically significance difference in the numbers of brushing strokes that varnish can withstand mechanical removal from tooth

brushing between novel cyanoacrylate based fluoride varnish and Duraphat varnish.

H_a: There is statistically significance difference in the numbers of brushing strokes that varnish can withstand mechanical removal from tooth brushing between novel cyanoacrylate based fluoride varnish and Duraphat varnish.

C. H_0: Novel cyanoacrylate based fluoride varnish is not toxic to human gingival fibroblast.

H_a: Novel cyanoacrylate based fluoride varnish is toxic to human gingival fibroblast.

CHAPTER 2: REVIEW LITERRATURE

Fluoride varnish

Fluoride has been proved to be an effective agent for caries prevention. The mechanism of topical fluoride in preventing caries is the concentration of fluoride in varnish induced forming fluoroapatite in enamel and globules of CaF_2-like material. CaF_2 globules are stabilized by phosphate-binding protein from saliva and precipitated into protective CaF_2 layers over tooth surface. CaF_2 layers serve as reservoirs of fluoride which is important for caries prevention. During the cariogenic challenge, lower pH induces dissolution of CaF_2 from the globules. This CaF_2 is the source of fluoride ion for forming fluoroapatite which is higher resistance to acid attack than hydroxyapatite. Furthermore, the dissolution of CaF_2 globules increase calcium ion concentration which will decrease the solubility constant of calcium and phosphate ion from tooth structure as well as increase the rate of remineralization of lost mineral tooth structure (5). The mechanisms of topical fluoride are shown on Fig. 1 (15).

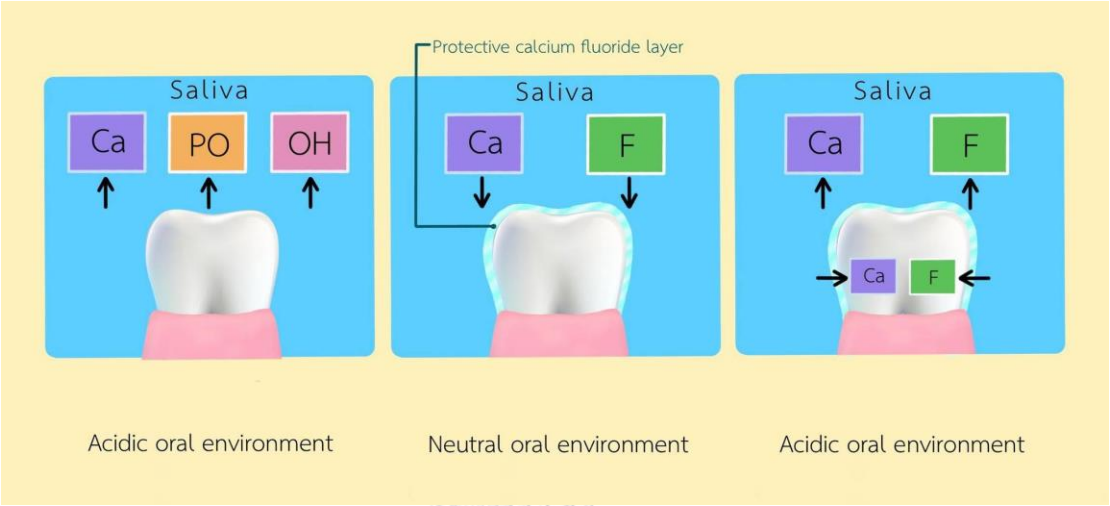

Fig. 1 - The mechanisms of topical fluoride to prevent dental caries
(Modified from Scientific Documentation Fluor Protector)

1. At acidic oral environment, demineralization of enamel released calcium (Ca_2^+) and phosphate ions (HPO_4^{2-}) into the saliva.
2. Topical fluoride treatment induced forming of (CaF_2) globules and protective calcium fluoride layers over tooth surface.
3. Acidic oral environment dissolved calcium ion (Ca_2^+) and fluoride ion (F^-) ions from protective calcium fluoride layers instead of tooth structure. Thus, tooth structure was protected.

In dental profession, there are 2 types of topical fluoride agent. First is aqueous topical fluoride including fluoride mouth rinse and fluoride gel. Second is fluoride varnish. Varnish was invented to increase the retention time of fluoride on tooth surface. WHO has claimed that fluoride varnishes have significant potential to reduce caries (16). *In vitro* and *vivo* studies showed that fluoride varnish has higher efficiency for caries reduction than other topical fluoride agents (17, 18). Moreover, the

bioavailability of fluoride when using fluoride varnish is relatively lower than fluoride gel because fluoride varnish is sticky (hard to flush out by water or saliva, low risk of ingestion) and slow dissolution of fluoride from varnish. Thus, fluoride varnish is considered to be safe and preferable.

The concentration of NaF in fluoride varnish is usually 5%. The first commercial fluoride varnish was Duraphat varnish (Colgate-Palmolive, Canton). There are a lot of studies proved that Duraphat varnish is effective in caries prevention (1-4). Moreover, It has been the most widely used fluoride varnish since 1980 until now.

There are a lot of commercial fluoride varnishes in the market and these are some of most common fluoride varnish including Duraphat varnish (Colgate), Fluor Protector (Ivoclar Vivadent), MI Varnish (GC America), Kolorz ClearShield (DMG America), Vanish Varnish (3M). The composition and detail are shown in Table 1 (19).

Fluoride varnish	Active ingredient	note
Duraphat varnish (Colgate)	5% NaF in ethanol + Colophonium, shellac	- first varnish on the market in 1964

Fluor Protector S (Ivoclar Vivadent)	1.5% ammonium fluoride (NH_4F) in ethanol + water +polymer + additives	- After the solvent evaporates, the fluoride concentration is up 4x. - Polymer and additive promote adhesion to the tooth surface
MI Varnish (GC America)	5% NaF + CPP-ACP + ethanol + hydrogenated rosin + ethoxyethanol (combination of casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP))	- ACP crystallizes on tooth surface in the form of new enamel when combined with water - CPP stabilizes ACP molecules until they are applied to teeth and bind them to plaque, bacteria, soft tissue, and dentin.
Kolorz ClearShield (DMG America)	5% NaF + ethanol +rosin	- Clear color for esthetic - Great tasting flavors and taste: watermelon, bubblegum, mint, cookie dough and caramel. - Higher acceptance and patient satisfaction

Vanish Varnish (3M)	5% NaF and tri-calcium phosphate (TCP) + Pentaerythritol glycerol ester of colophony resin + n-HEXANE + ethanol + flavor enhancer + thickener	- Invisible and durable - Continuously releasing fluoride up to 24 hours until brush off - Easy to apply, no drying needed - Can be applied to tooth surfaces where plaque is present

Table 1 - Some of common fluoride varnish (2020) and details

Effect of retention time of fluoride varnish on tooth surface

CaF_2 layer over tooth surface is the important factor in caries prevention. Several studies reported that the amount of CaF_2 were related to retention time of topical fluoride on tooth surface (7-9) and remineralization on enamel is time dependent and improve overtime (7, 8, 11). Moreover, it has been found that most of NaF in varnish still remain insoluble after 24 hours of application, thus the longer retention time will allow remaining NaF to dissolve and promote more CaF_2 globules formation which serve as fluoride reservoirs for caries prevention.

Unfortunately, commercial fluoride varnishes in the market are susceptible to mechanical removal. To increase retention time of varnish on tooth surface, the mechanical removal of fluoride varnish should be postponed to increase retention time including refraining from eating hard food for a few hours after application and refraining from tooth brushing between 4-24 hours depend on the manufacturer recommendation but the clinical protocols recommend refraining from tooth brushing 12-24 hours after fluoride varnish application (13, 14, 20). These protocols cause inconvenience to the patients, which are considered to be the disadvantages of fluoride varnishes in the market.

However, Rodrigo A. Giacaman, et al. reported that fluoride uptake level increased significantly after 18 hours after fluoride varnish application (11). While, Fernández CE, et al. reported that the amount of CaF_2 forming reached maximum concentration at 24 hours (8).

Cyanoacrylate adhesive

Cyanoacrylate, known as super glue, is a strong fast setting glue. It was invented by scientists of Kodak, company named Harry Coover Jr. and Fred Joyner in 1949 (21) and first sold as an adhesive named "Eastman #910". Cyanoacrylate polymers are polar and linear molecules. Cyanoacrylate monomers can create a strong covalent bond to high surface energy substances such as animal tissue, skin, wood, leather, metal, glass,

plastic etc (22). and polymerize rapidly in the presence of water, –OH group and any weak base on substance. In the form of monomer, cyanoacrylate monomer is a clear liquid with low viscosity (1-3 mPa). Common Derivatives of cyanoacrylate monomer are alky-cyanoacrylate such as methyl 2-cyanoacrylate (MCA), ethyl 2-cyanoacrylate (ECA, known as superglue) which are commonly used for industry and the home. N-butyl cyanoacrylate (n-BCA), octyl cyanoacrylate , and 2-octyl cyanoacrylate are more biocompatibility and used in medical and veterinary surgery as tissue adhesives to replace using sutures (23-26). The advantages of cyanoacrylate adhesive are high adhesion force, fast curing, oil resistivity, non-toxic, and anti-microbial properties, in contrast, the disadvantages are poor water resistance, poor thixotropic property, fragility, high polymerization shrinkage, poor wear resistance and self-progressively flake off when contact with water (22).

Cyanoacrylate polymerization

Cyanoacrylate can polymerize through 2 mechanisms including free radical and anionic polymerization mechanisms (22) but the anionic reaction is energetically more favorable than the radical reaction.

Anionic polymerization

The strong electron-withdrawing nitrile group (CN) and ester group (CO_2R) of cyanoacrylate monomer are high reactivity. When cyanoacrylate monomer contacts with the moisture, alcohol and weakly basic or alkali surface, they will initiate the anionic polymerization reaction and proceed until no available monomer or be terminated by proton or acid (27). The reaction mechanisms are shown in Fig. 2 (28). The duration of polymerization depends on the amount of basic or moisture on the surface of substances.

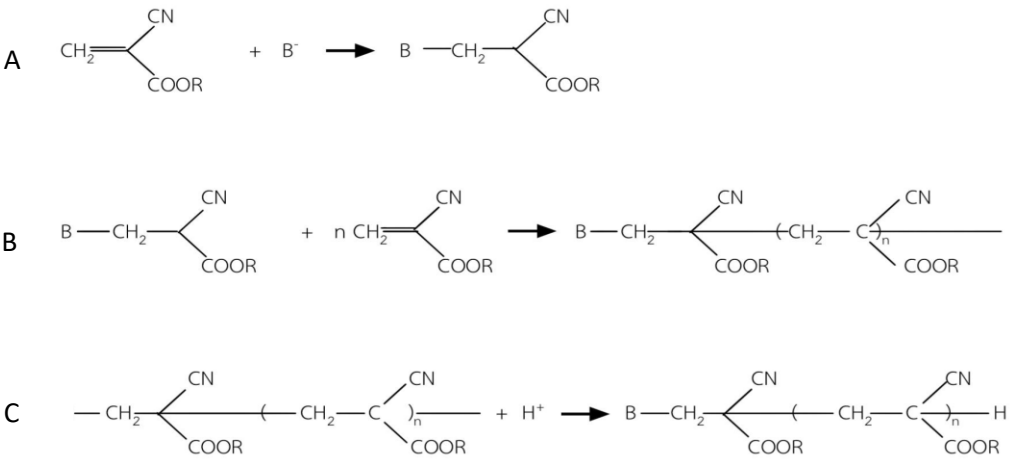

Fig. 2 - A-initiation, B-propagation, and C-termination
(Modified from ZHU Y-H, et al. 2011)

Radical polymerization

This mechanism requires initiator to give a pair of radical, which will react with cyanoacrylate monomer to form a propagating radical. The polymerization proceed until no available monomer or be terminated by coupling and disproportionation of free radical. The reaction mechanisms are shown in Fig. 3 (27).

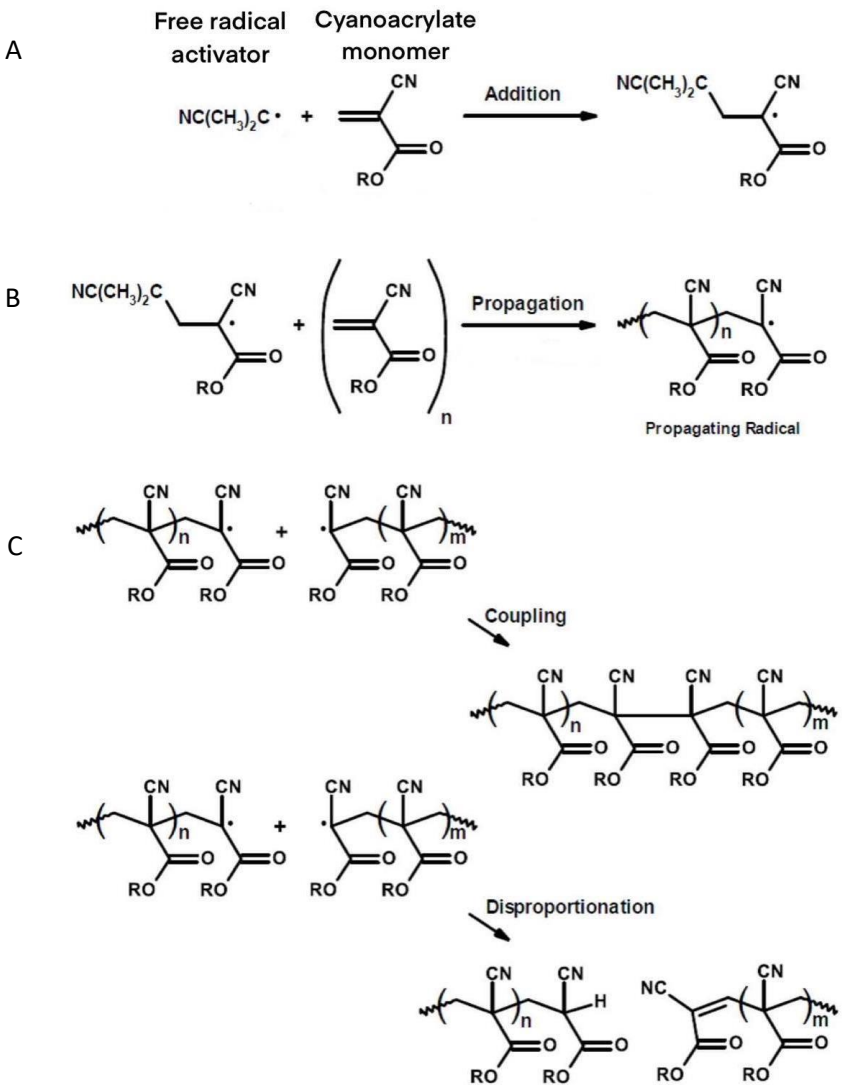

Fig. 3 - A-initiation (initiator: cyanoisopropyl), B-propagation and C-termination (Modified from Duffy C, et al. 2018)

Before polymerization, the chemical interactions between cyanoacrylate monomers are van der Waals forces and turn into covalent bonds after polymerization which are shorter than van der Waals, thus result in volumetric shrinkage in the polymers (29). The polymerization shrinkage of methyl 2-cyanoacrylate is 16.3% and ethyl 2-cyanoacrylate is 14.1%.

In general, short and straight side chain cyanoacrylates form tighter and stronger bond as well as faster setting and degradation time than the longer and more complexity side chain cyanoacrylate. In contrast, short and straight cyanoacrylates tend to be more brittle and higher polymerization shrinkage, which lead to lower tensile strength of adhesive film. In addition, the longer alkyl chain cyanoacrylates have lower vapor pressure and fewer odors.

Degradation

Poly-cyanoacrylates have 2 degradation mechanisms [Fig. 4] including enzyme dependent (esterase, superoxide dismutase, indomethacin and acetyl-salicylic acid) and hydrolysis in the presence of water. The common degradation process of cyanoacrylate polymer is hydrolysis (30-33), therefore poly-cyanoacrylates are very susceptible to degradation when contact with water. Temperature and basic substances are the catalysts of hydrolysis (31, 34).

The products of enzyme degradation are poly (2-cyanoacrylic acid) and alcohol, which are not toxic to human cell. In contrast, the products from hydrolytic degradation are formaldehyde and alkyl-cyanoacetate that exhibit some toxicity (31). Therefore, the toxicity of poly-cyanoacrylate is largely related to the rate of common hydrolytic degradation process. Factors that affect the rate of hydrolytic degradation are temperature, pH and the length of alky chain. The lower the temperature, pH and the longer alkyl chain, the slower the rate of degradation and less toxicity. The reaction mechanisms are shown in Fig. 4 (33).

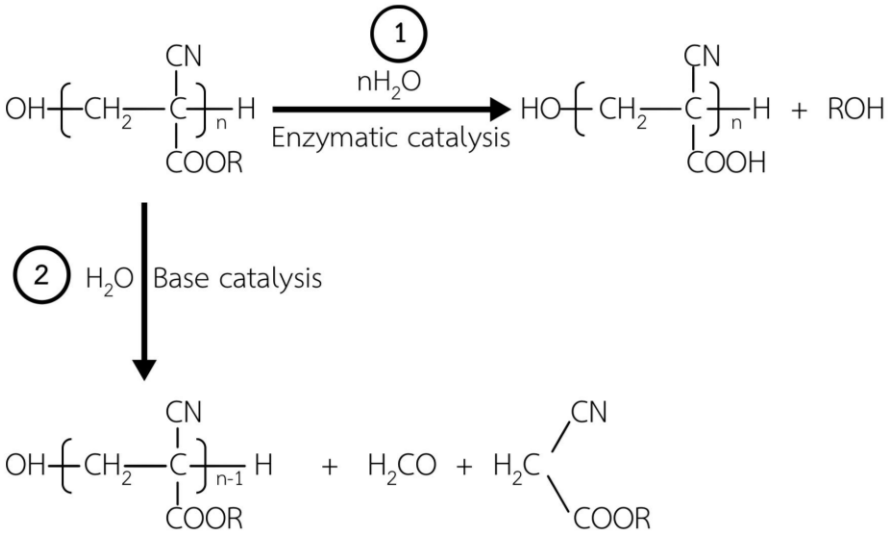

Fig. 4 - Poly-2-cyanoacrylate s biodegradation pathways: (1) enzyme-dependent biodegradation; (2) hydrolytic degradation.
(Modified from Rustamov I, et al. 2014)

Due to linear molecular structure, poly-cyanoacrylate adhesives are susceptible to shear stress and have low cohesive strenght (35). Moreover, poly-cyanoacrylate can progressively flakes off within 5-10 days (24).

Cyanoacrylate in medical

In medical, cyanoacrylate has been used as the soft and hard tissue adhesive (36-38). The U.S. Food and Drug Administration (FDA) aprroved its use as a medical adhesive since 1976 (39). Cyanoacrylates that are considered relatively non-toxic including *n*-butyl cyanoacrylate (n-BCA), octyl cyanoacrylate and 2-octyl cyanoacrylate because they have longer alkyl chains which exhibit more hydrophobic properties than short alky chain that lead to slower hydrolytic degradation process and less toxicity. The significant degradation of butyl- and octyl-cyanoacrylate can take months or even year (40). These degradation products are excreted via exhalation, in the feces and urine (41).

The advantages of cyanoacrylate tissue adhesives are quick, painless, simple, no need for removal and good esthetic result. The disadvantages of cyanoacrylate tissue adhesives are high cost and limited to low tension and short laceration or surgical incision (42, 43). Several studies reported that cyanoacrylate improved cosmetic results compared to sutures (36, 44-47). Mertz et al. reported that

cyanoacrylates have antimicrobial properties especially against gram positive organism (48). The adhesive films can form antimicrobial barrier to protect the wound and reduce infection.

Recently, cyanoacrylate adhesives were used as local drug delivery devices (49, 50). Their ability to fast and strong adhere to tissue, self progressively flake off and biodegradation are very useful in local drug delivery system by traping the drugs in adhesive film and they will be slowly released from adhesive film for local treatment (50). There are 3 drug released mechanisms of poly-cyanoacrylate adhesives. 1. Drugs release via biodegradation of cyanoacrylate by enzymes from blood or tissue 2. Drugs release via disolution through surface roughness or void of cyanoacrylate film 3. Drugs release via artificially introduced defects in adhesive matrix by adding hydrophilic materials into the matrix which will dissolve when contact with water and leave the passages or pores behind (28).

CHAPTER 3: NOVEL CYANOACRYLATE BASED FLUORIDE VARNISH'S FORMULA

Novel cyanoacrylate fluoride varnish's formula (from pilot study)

1 ml of varnish contain:

N-butyl cyanoacrylate	0.40 ml
Acetone	0.35 ml
Vegetable oil	0.20 ml
Sodium lauryl sulfate(SLS)	0.05 ml
NaF (powder)	50 mg
Fumed silica	35 mg

Properties of novel cyanoacrylate fluoride varnish (from pilot study)

1. Mixing time 1 min and working time 7-8 min.
2. When contact with water, the varnish immediately set from the surface. (sticky turn into non-sticky surface), turn into rubbery consistency within 30 sec and complete set (solid) in 1 min. Therefore, patients can drink, eat or even tooth brushing after 1 min of cyanoacrylate fluoride varnish application.
3. Release fluoride for 8-9 days

Use n-butyl cyanoacrylate as active ingredient in varnish

N-butyl cyanoacrylate (n-BCA) and 2-octyl cyanoacrylate (2-OCA) are biocompatible and used in medical treatment. In this study, the researcher used n-butyl cyanoacrylate (n-BCA) as the active ingredient in varnish composition because the glass transition temperature (Tg) of n-BCA is appropriate for the human body temperature (Table 2). Tg of n-BCA is 130 ºC which means at 37 ºC human body temperature, n-BCA polymer is in the glass phase while 2-OCA polymer (Tg 10 ºC) is in the plastic phase. Thus, n-BCA has greater strength to withstand mechanical removal in oral cavity such as tooth brushing, food, tongue and cheek scrubbing.

Poly Cyanoacrylate	Tg (ºC)	Ref
Methy Cyanoacrylate	160	(51)
Ethyl Cyanoacrylate	150	(52)
N- butyl Cyanoacrylate	130	(53)
2-octyl Cyanoacrylate	10	(54)

Table 2 - Glass transition temperature of poly-cyanoacrylate

In this study, the researcher used 0.4 ml/ml of n-BCA in varnish composition. From pilot study, researcher found that when the concentration of n-BCA is higher than 0.4 ml/ml (0.5 ml/ml) the accumulative dissolved fluoride ion was lower than 0.4 ml/ml of n-BCA. It could be the dense polymer structure inhibited dissolution of NaF form the varnish film and also decreased working time of varnish. In contrast, when the concentration of n-BCA is lower than 0.4 ml/ml (0.3 ml/ml), some specimens showed spontaneous cohesive failure of varnish.

NaF powder 50mg/ml (particle size <45 μm)

NaF is a basic salt which can initiate polymerization of cyanoacrylate monomer. From pilot study, NaF powder gave the longer working time compare to NaF solution which has water as solvent (water can cause the higher rate and more exothermic

polymerization of cyanoacrylate). 50mg/ml of NaF is the same concentration of NaF in Duraphat varnish. Researcher used small particle size to promote equal distribution of NaF in varnish.

Acetone as solvent

Unfortunately, the only one best and biocompatible solvent of NaF is water (NaF can disslove 4.3 g/100 ml of water at 25 ℃), which is also the best initiator for anionic polymerization of cyanoacrylate. Researcher used acetone as solvent to disolve NaF powder and cyanoacrylate together as well as increase working time of cyanoacrylate varnish because acetone isn't the initiator of cyanoacrylate polymerization and acetone is the only one biocampatible solvent that can disolve NaF and cynoacrylate but the solubility to NaF is significantly lower than the water and ethanol (Ethanol also initate polymerization of cyanoacrylate). As a result, most of NaF remained insoluble in the varnish.

Fumed silica (increase viscosity, thixotropic properties)

Cyanoacrylate monomer is clear liquid with low viscosity. To be a fluoride varnish, the viscosity of novel cyanoacrylate varnish needs to be increased. Fumed silica is the common filler for increase viscosity and thixotropic properties of

cyanoacrylate monomer. Zhengwei et al. reported that the addition of fumed silica can increases viscosity of cyanoacrylate monomer. Moreover, silica can create the hydrogen bonds with polar groups of cyanoacrylates lead to increase thixotropic properties as well as reduce internal stress and polymerization shrinkage which are the cause of debonding (55). In addition, the increased viscosity also reduced precipitation of NaF particles after mixing.

In this study, we use 35mg/ml of fumed silica in varnish composition. From pilot study, researcher found that when amount of fumed silica was more than 35 mg/ml (40 mg/ml), the viscosity was too thick and some specimens showed spontaneous cohesive failure in varnish. When amount of fumed silica was less than 35 mg/ml (30 mg/ml), the viscosity was too thin which led to precipitation of NaF after mixing.

Sodium lauryl sulphate (SLS)

Unequal distribution of NaF powder in varnish was the big problem that researcher found in pilot study because NaF can only dissolve in strong polar solvent such as water, hydrogen peroxide, hydrogen fluoride etc. Unfortunately, the only one best and biocompatible solvent of NaF is water (NaF can disslove 4.3 g/100 ml of water at 25 °C) which is also the best initiator for anionic polymerization of

cyanoacrylate thus, acetone was used as a solvent for NaF, which has low solubility to NaF. Most of NaF remained insoluble in the varnish and can precipitate. Food grade SLS in the varnish promote equal distribution and prevent precipitation of NaF in the varnish. Moreover, SLS also increased the working time of the varnish.

Vegetable oil

Another problem that researcher found in pilot study was adding SLS to promote distribution of NaF in varnish greatly increased the dissolution rate of NaF. (all of NaF dissolved from varnish within 3-4 days) Vegetable oil increased hydrophobicity of varnish and also reduced the dissolution rate of NaF, thereby extending the duration of fluoride releasing from cyanoacrylate varnish (all of NaF dissolved from varnish within 8-9 days) as well as increased working time.

Fluoride releasing mechanism from cyanoacrylate varnish

The completed setting of cyanoacrylate fluoride varnish has the matrix composed of cyanoacrylate polymer, fumed silica and vegatable oil. The NaF particles were entraped inside the micelles of SLS and dispersed in cyanoacrylate varnish's film. In the oral environment, water can penetrate and disolve NaF from the cyanoacrylate varnish and also leave the empty space behind which could be the passage for

disloution of the inner NaF thus cyanoacrylate varnish can serve as the fluoride varnish for caries prevention and remineralization on enamel (Fig. 5).

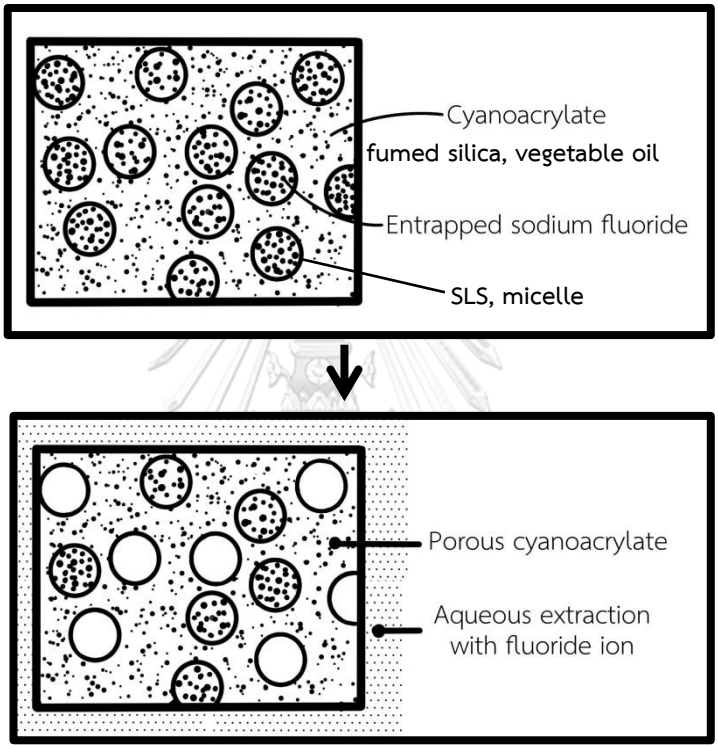

Fig. 5 - Fluoride ion release from cyanoacrylate varnish

CHAPTER 4: MATERIALS AND METHODS

Sample size

Sample size of fluoride release test and brushing test were calculated by using Thabane L. formula

$$n_i = \frac{2\left[z_{\frac{\alpha}{2}} + z_{\beta} \right]^2 \sigma^2}{(\mu_1 - \mu_2)^2}$$

n = number of sample

$Z\alpha_{/2}$ = Z critical value at 95% confidence from Z table

$Z\beta$ = Z power chosen at 80% from Z table

σ = standard deviation control group from pilot study

μ_1 = mean value of control group from pilot study

μ_2 = mean value of sample group from pilot study

From pilot study

1. Sample size of fluoride release test: standard deviation was 4.7108 and $\mu_1 - \mu_2$ was 14.753 The result of **n is 1.598**
2. Sample size of brushing test: all samples of Duraphat varnish (control group) showed residual no varnish left after 20 strokes of brushing thus, the result of n is 0

Sample size of cytotoxic test: followed ISO 10993-5:2009(E), that a minimum of three replicates shall be used for test samples and controls. Moreover, the study of BT Hoang-Dao et al. 2008 (56) also had 3-20 samples in their study. In addition, the pilot study demonstrated that the results of indirect contact test when using 3 samples were satisfactory and consistent with the research hypothesis.

Materials and methods

Duraphat varnish (5% NaF, Colgate-Palmolive, Canton, MA, USA) (Fig. 6) was used as a control because it is fluoride varnish of choice, which has been the most widely used and extensively studied fluoride varnish. There are several studies proved that Duraphat varnish was the effective topical fluoride in preventing caries (1-4). The cyanoacrylate fluoride varnish was prepared by mixing 35 mg/ml fumed silica (Cab-O-Sil® M-5P, Cabot Corporation, Billerica, Massachusetts) and 50 mg/ml Sodium fluoride

(NaF, particle size <45 μm, EMPROVE®, Merck KGaA, Darmstadt, Germany) together (this powder part can be pre-mixed), added 0.2 ml/ml Vegetable oil (King® Rice Bran oil, Thai Edible Oil Co., Ltd, Nakhon Ratchasima, Thailand), 0.05 ml/ml Sodium lauryl sulfate (SLS, Sulfopon® 1630, BASF SE, Ludwigshafen, Germany), and 0.35 ml/ml acetone into the powder part and mix for 40 sec, then adding 0.4 ml/ml n-butyl cyanoacrylate (Vetbond® tissue adhesive, 3M, St. Paul, MN, USA, 99% by weight n-butyl cyanoacrylate, <1% by weight Hydroquinone, and 0.01% by weight blue dye) (Fig. 7) and continue mixing for 20 sec. The ingredients were mixed in a closed vessel on a stirrer at room temperature. The total mixing time was 1 min.

Active ingredient: 50 mg NaF/ 1 ml of varnish

Solvent: ethanol

Base: Colophonium, mastix, shellac

Fig. 6 - Duraphat varnish and composition

N-butyl cyanoacrylate 99% by weight

Hydroquinone < 1% by weight

Blue dye 0.01% by weight

Fig. 7 - Vetbond® tissue adhesive and composition

Viscosity test

The varnish viscosity was measured using a viscometer (HAAKE™ MARS 60™ Rheometer, Thermo Fisher Scientific, Karlsruhe, Germany) (Fig. 8). Parallel plates (35 mm diameter) were used as the measuring apparatus. One milliliter of each fluoride varnish formulation was gently placed on the lower plate surface to avoid air bubbles. The upper plate was connected to the rotor. The space between the upper and lower plate was 1 mm. The tests were performed in 2 modes. The first mode was the viscosity test of the varnishes by increasing the shear rate from 0.0–150 1/s and 30 points of data were collected. The second mode was the viscosity test of the varnish over time when the shear rate was constant at 10 1/s and 100 points of data were collected. The tests were performed at 37°C.

Fig. 8 - HAAKE™ MARS 60™ Rheometer

In vitro fluoride release test

Seven samples for each fluoride varnish group were made by loading Duraphat varnish and the cyanoacrylate fluoride varnish in an 8 mm diameter and 1 mm deep polyvinyl siloxane mold (Fig. 9). Each sample was immersed in 3 ml artificial saliva (3.90 mmol Na_3PO_4, 4.29 mmol NaCl, 17.98 mmol KCl, 1.10 mmol $CaCl_2$, 0.08 mmol $MgCl_2$, 0.50 mmol H_2SO_4, 3.27 mmol $NaHCO_3$, and distilled water, at pH 7.2) in a plastic container (polystyrene, PS) and kept in an incubator (Memmert®, 100-800, Memmert GmbH+Co, Schwabach, Germany) at 37°C for 14 d (Fig. 10). Each day, the samples were removed from the artificial saliva immersion solution and rinsed with deionized water for 30 sec, and dried with blotting paper, then placed in a new plastic container with 3 ml artificial saliva. The released fluoride ion concentration in the immersion solution was measured from Day 1–14. 300 µL TISAB III solution (Sigma-Aldrich®, Merck KGaA, St. Louis, Missouri, USA) was added to the immersion solution and stirred on a stirrer for 30 sec. A fluoride ion selective electrode (Orion®, 9609BNWP, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was placed in the solution for 2 min and the amount of released fluoride (ppm) was measured using an electrochemistry meter (Orion®, VERSASTAR, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the data were recorded (Fig. 11). The electrode and meter were calibrated before each use.

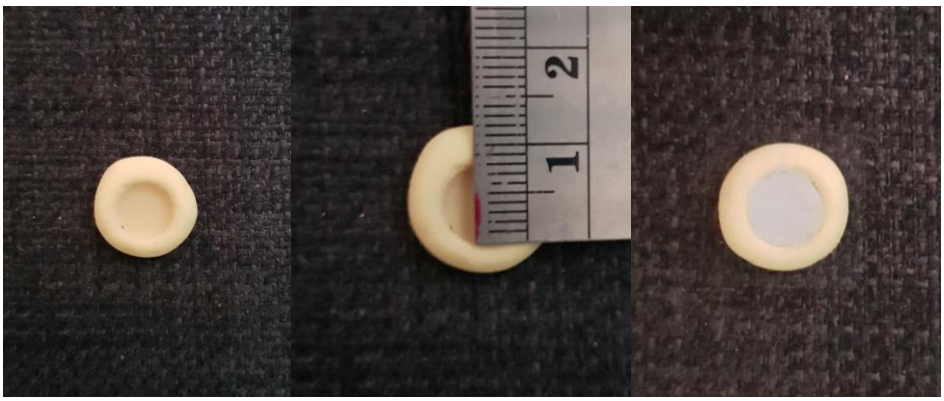

Fig. 9 - Fluoride release test's specimen

Fig. 10 - Incubator (Memmert®, 100-800, Memmert GmbH+Co. KG, Schwabach, Germany)

Fig. 11 - The fluoride ion selective electrode (Orion®, 9609BNWP, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and electrochemistry meter (Orion®, VERSASTAR, Thermo Fisher Scientific, Waltham, Massachusetts, USA)

Brushing test

This test measured the number of brushing strokes that the varnish could resist being mechanically removed by tooth brushing. Prior studies found that 20 brushing strokes (10 strokes/area for tooth brushing with tooth brushing being performed twice a day) are equal to the effective daily number of brushing strokes to brush a specific tooth area usually recommended by dentist (57, 58). The average human brushing force is 1.6 ± 0.3 N (59).

Forty human lower incisors and premolars with smooth and non-carious enamel on the buccal surface of the teeth were obtained from patient's treatment planned for tooth extraction at the Department of Oral Surgery. The method was approved by The Human Research Ethics Committee of the Faculty of Dentistry,

Chulalongkorn University (HREC-DCU 2021-057). The samples were cut below the cemento-enamel junction to reduce the tooth size and embedded in acrylic in a 12x18x6 mm epoxy mold. To control the position of the teeth in the acrylic, the flat buccal surfaces of the teeth were attached to a glass slab with 2-sided thin adhesive tape, and the glass slab was placed on the top of the mold that was filled with acrylic to 2/3 of the mold's depth. After the acrylic was set, the samples were removed and the surfaces of the teeth were cleaned with acetone. The samples were immersed in 37°C artificial saliva for 24 h. After immersion, the samples were blown dry with oil-free air for 30 sec. The samples were randomly divided into 8 groups (n=5), 4 groups were applied with Duraphat varnish (D group) and the other 4 groups were applied with cyanoacrylate fluoride varnish (C group). The area and thickness of the applied varnish were controlled using adhesive tape (100 μm thick) with a 2 mm diameter hole (Fig. 12). The fluoride varnish was applied to the tooth surface and was covered with a glass slide for 30 sec. The glass slide was taken off, the excess varnish was removed with micro brush and the adhesive tape was removed. The samples were immediately immersed in 37°C artificial saliva for 4 h before performing the brushing test (per the Duraphat varnish manufacturer's recommendation). The brushing test details are described in Table 3.

Fig. 12 - Brushing test specimen

Group C (Cyanoacrylate), D (Duraphat varnish)	Strokes
C1, D1	20
C2, D2	200
C3, D3	400
C4, D4	600

Table 3 - Brushing test specimen

The samples were examined using a stereomicroscope (SZ 61, Olympus, Japan) at 30x magnification to capture the images of the applied varnish before testing. The brushing test was performed at room temperature using a V-8 cross brushing machine (SABRI Dental Enterprises, Inc., USA) at 90 strokes/min and a 1.6 N brushing force with soft bristle toothbrushes in the dentifrice slurry (ISO 11609:1995) 40 ml/specimen at a ratio of 25 mg toothpaste/40 ml deionized water (Colgate® Great regular flavor,

Colgate-Palmolive Ltd., Chonburi, Thailand). As shown in Fig. 13. After the brushing test, the sample's image was captured using a light stereomicroscope at 30x magnification. The percent area loss of varnish was calculated using the ImageJ program. The distance between the samples and the microscope lens before and after the brushing test was fixed at 10.7 cm to control the accuracy of the surface area measurement for each sample.

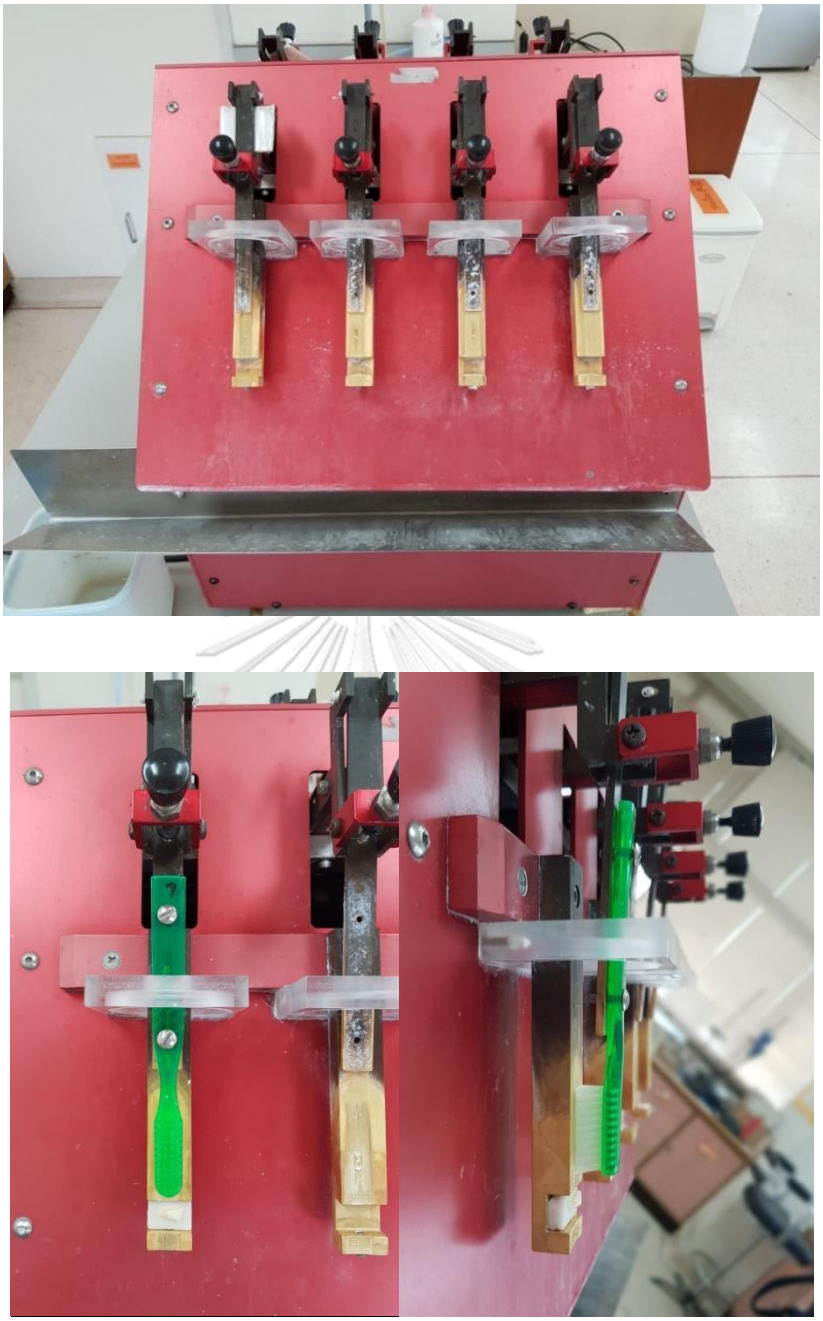

Fig. 13 - V-8 cross brushing machine and specimen

Cytotoxicity test

In this study, hGFs obtained from the gingival tissues of 3 healthy donors were used to evaluate the cytotoxicity of the Duraphat and cyanoacrylate fluoride varnishes. The donors provided informed consent before undergoing the gingivectomy procedure. The method was approved by The Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2021-057).

The gingival tissues were cut into 2–3 mm pieces and placed on 35-mm culture dishes (SPL Life Sciences, Pocheon-si, Gyeonggi-do, Korea) and 500 µl complete medium (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% antibiotic-antimycotic solution (Gibco^{TM}, ThermoFisher Scientific, Waltham, Massachusetts, USA)) was added. The gingival tissue cultures were performed at 37°C in the incubator in a humidified 5% CO_2 atmosphere and the culture medium was changed every 2 d until the hGFs reached 95% confluence and 4th–5th passage hGFs were used in the experiments. The cytotoxicity of the fluoride varnishes test was performed using the indirect contact test (ISO 10993-5).

Indirect contact test

Eight mm diameter and 1.5 mm high Duraphat varnish and cyanoacrylate fluoride varnish samples were prepared. The samples were decontaminated using UV light for 30 min before being immersed in the extraction medium. The varnish extraction medium (complete medium) was prepared following ISO 10993-12. The fluoride varnishes samples were immersed in complete medium at ratio of 0.1 g/ml and incubated at 37°C in a humidified 5% CO_2 atmosphere for 24 h. The extraction medium was diluted 1:2 and 1:10 to generate 3 extraction medium groups, undiluted, diluted 1:2, and diluted 1:10. The hGFs ($1.0x10^4$ cells/well) were cultured in 96-well plates (SPL Life Sciences, Pocheon-si, Gyeonggi-do, Korea) at 37°C in a humidified 5% CO_2 atmosphere for 24 h. The culture medium in each well was removed, and the hGFs were cultured in 100 µL extraction medium, and the cell viability was determined after incubating the cells at 37°C for 24, 48, and 72 h. The MTT (3-(4, 5-dimethylthiazolyl-2)-2,5diphenyltetrazolium bromide) assay was performed to evaluate cell viability. At the end of each culture period, the culture medium in each well was removed and 50 µL MTT reagent (1 mg/mL PBS) was added and incubated at 37°C for 4 h. After the incubation period, the solution in each well was removed and 100 µL dimethyl sulfoxide (DMSO) (AMRESCO LLC, Solon, Ohio, USA) solution was added to dissolve the precipitated formazan crystals. The optical density (OD) was

measured at 570 nm (EPOCH, BioTek Instrument, Winooski, Vermont, USA). The percentage cell viability was calculated using the following equation:

Percentage cell viability = (experimental group's OD/control group's OD) x 100

Untreated hGFs, Triton X-100, and DMSO served as the positive control, negative control, and blank group respectively.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistic 28 program. The homogeneity of variances and normal distribution of the *in vitro* fluoride release data and percent area varnish loss from brushing were determined using the Shapiro-Wilk's test. The group means of fluoride release and percent area loss of varnish were compared using Multivariate of Analysis (MANOVA). Significance was determined at $p<0.05$.

CHAPTER 5: RESEARCH RESULT

Viscosity test

The results of viscosity test were plotted into graph on Fig. 14 and Fig. 15. The graph was the sum of forces arising from intermolecular bond strength against the force of the testing machine. In Fig. 14, shear rate was increased from 0.01-150 1/s. The result showed that Duraphat varnish had higher initial viscosity compared with cyanoacrylate fluoride varnish (Duraphat varnish 92,371.86 mPas, Cyanoacrylate varnish 9,151.53 mPas). Both varnishes had shear thinning properties (pseudoplastic) where the viscosity decreased as the shear rate increased. The cyanoacrylate varnish demonstrated shear thinning just prior to the cyanoacrylate setting reaction (period A). When the setting reaction took place, the viscosity of cyanoacrylate varnish began to increase (period B) because to polymerization into cyanoacrylate polymer caused resistance against the force from testing machine. Until the time point that polymerization ceased, the force from the testing machine was greater (period C), resulting in decreased viscosity.

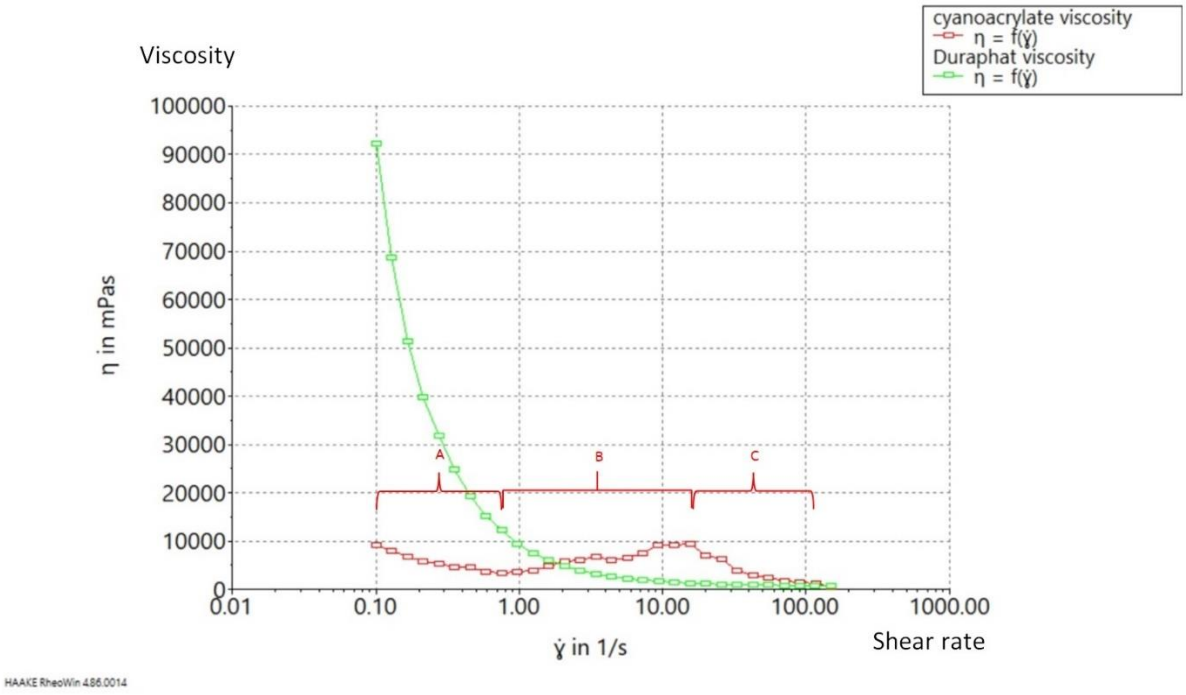

Fig. 14 - Viscosity of Duraphat varnish and novel cyanoacrylate fluoride varnish by varying shear rate from 0.01 up to 150 1/s

Fig. 15 illustrates the viscosity of both varnishes overtime at shear rate of 10 1/s. the results indicated that at 10 1/s shear rate, the viscosity of Duraphat varnish remained almost constant overtime (mean viscosity was 1,560.396 mPas) and Duraphat varnish had higher viscosity compared with the cyanoacrylate varnish until the cyanoacrylate setting reaction occurred. In contrast, the viscosity of cyanoacrylate varnish increased overtime until polymerization ceased, at that point, the viscosity started to decrease. The viscosity curve of the cyanoacrylate varnish can be divided into 4 time periods. Period 1(0-333s), the increase in viscosity was small (from 158.649-

939.345mPas). Period 2 (333–576s), the steep slope of the curve indicated a dramatic increased in viscosity of the cyanoacrylate varnish (from 939.345-39,376.27 mPas) because the high rate of polymerization produced a high resistance force against that of the testing machine. Period 3 (576–774 sec), the change in the slope of the curve fluctuated due to the cyanoacrylate varnish becoming solid and the testing machine concurrently exerted force against the cyanoacrylate polymer formation until the bond between the polymerized monomers was destroyed. These mechanisms alternated, causing the direction of the curve to fluctuate. At the end of this period, the cyanoacrylate varnish reached its maximum viscosity (46,425.64 mPa) and its polymerization reaction ceased. Period 4 (774–900 sec), the curve demonstrated a marked decrease in the viscosity of the cyanoacrylate varnish. This period was the result of the force from the testing machine destroying the bonds between the polycyanoacrylate molecules

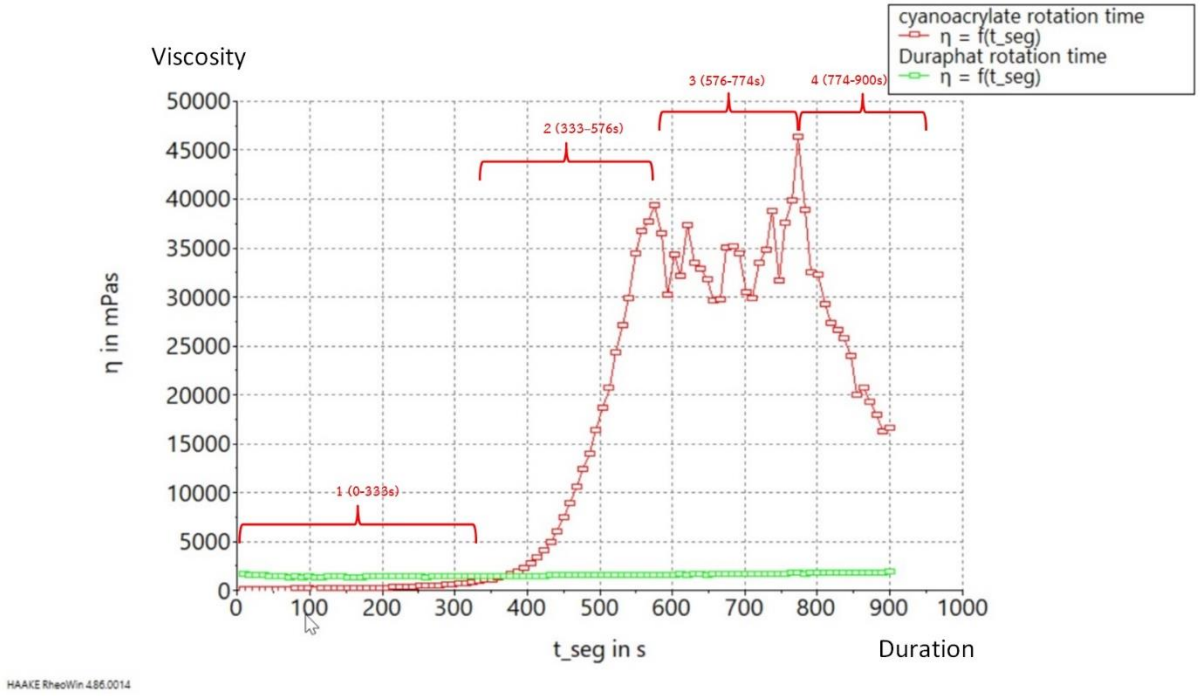

Fig. 15 - Viscosity of Duraphat varnish and novel cyanoacrylate fluoride varnish when the shear rate was 10 1/s

In vitro fluoride release test

The result from *in vitro* fluoride released test was showed in Table 4, 5, 6. Table 4 was fluoride released concentration (ppm) of the novel cyanoacrylate fluoride varnish from day 1–14. Table 5 is fluoride released concentration (ppm) of Duraphat varnish from day 1–14. Table 6 and Figure 16 are the comparison of mean fluoride released concentration (ppm) between the novel cyanoacrylate and Duraphat varnish from day 1–14. The Shapiro-Wilk test indicated that the *in vitro* fluoride ion release data had a normal distribution. The MANOVA of the day 1–14 fluoride release results between the cyanoacrylate and Duraphat varnishes revealed a significant difference in

fluoride release between the cyanoacrylate and Duraphat varnishes (p<0.05, Wilk's lambda=0.000, F=809,130.394).

From table 6 and figure 16, fluoride concentrations released from cyanoacrylate varnish was higher compared with the Duraphat varnish from day 1–9 and then very little fluoride was released after day 9 of immersion. In contrast, although Duraphat varnish released less fluoride, it released fluoride for a longer time compared with the cyanoacrylate varnish. Duraphat varnish released fluoride through day 14 and could be expected to continue to release fluoride over more time. On day 9, which has the minimal different in fluoride released concentration between cyanoacrylate varnish and Duraphat, the result of day 9 independent T-test analysis showed that fluoride concentration of cyanoacrylate varnish still significantly higher compared with the Duraphat varnish. From day 10–14, the fluoride released concentrations of Duraphat were significantly higher compared with cyanoacrylate varnish.

Specimen (ppm) / Day	1	2	3	4	5	6	7	Average	SD
1	212.50	223.50	216.00	214.30	226.40	214.30	222.70	**218.53**	**5.52**
2	115.00	108.78	126.30	118.28	131.60	123.65	125.50	**121.30**	**7.74**
3	116.29	102.30	107.90	117.30	119.90	93.50	117.00	**110.60**	**9.78**
4	96.92	97.28	101.20	108.52	91.60	110.70	106.90	**101.87**	**7.06**
5	73.30	85.89	82.12	86.30	76.09	76.20	72.04	**78.85**	**5.88**
6	62.48	52.33	56.09	60.71	59.31	63.80	50.38	**57.87**	**5.11**
7	55.73	41.90	43.20	48.42	49.52	58.27	42.92	**48.57**	**6.47**
8	25.14	19.81	21.32	16.42	18.18	17.31	20.80	**19.85**	**2.95**
9	9.04	7.42	5.13	4.98	2.30	3.42	4.04	**5.19**	**2.33**
10	0.77	0.59	0.29	0.37	0.31	0.15	0.28	**0.39**	**0.21**
11	0.35	0.60	0.27	0.30	0.21	0.23	0.11	**0.30**	**0.15**
12	0.28	0.43	0.11	0.09	0.44	0.08	0.38	**0.26**	**0.16**
13	0.06	0.02	0.03	0.03	0.02	0.06	0.09	**0.05**	**0.02**
14	0.03	0.08	0.07	0.08	0.08	0.08	0.09	**0.07**	**0.02**

Table 4 - Fluoride released concentration (ppm) of novel cyanoacrylate fluoride varnish

Specimen (ppm) / Day	1	2	3	4	5	6	7	Average	SD
1	14.70	11.40	18.87	18.26	14.52	13.60	15.82	**15.31**	**2.61**
2	5.23	3.35	7.09	6.17	1.81	3.28	3.02	**4.28**	**1.91**
3	3.52	2.77	7.73	5.07	2.54	4.08	2.56	**4.04**	**1.87**
4	2.80	2.51	6.19	6.40	2.18	3.98	2.90	**3.85**	**1.76**
5	9.03	2.30	6.53	5.97	2.22	3.42	1.59	**4.44**	**2.78**
6	8.08	3.46	11.59	4.27	1.47	2.60	2.47	**4.85**	**3.66**
7	5.55	3.52	4.63	4.55	1.15	2.76	1.83	**3.43**	**1.60**
8	3.15	2.34	5.47	4.73	1.00	4.61	2.41	**3.39**	**1.60**
9	2.12	1.36	4.14	3.79	2.36	2.89	1.14	**2.54**	**1.14**
10	8.83	1.38	5.24	5.73	3.15	3.49	4.43	**4.61**	**2.36**
11	5.26	1.52	2.85	5.55	2.90	3.02	2.33	**3.35**	**1.50**
12	3.15	1.18	2.09	3.47	6.22	5.69	2.65	**3.49**	**1.84**
13	4.05	1.37	2.92	3.61	7.05	3.22	2.14	**3.48**	**1.81**
14	2.07	1.51	2.93	1.74	4.29	4.20	2.59	**2.76**	**1.12**

Table 5 - Fluoride released concentration (ppm) of Duraphat varnish

Varnish (ppm) / Day	Novel cyanoacrylate varnish	Duraphat varnish
1	218.53	15.31
2	121.30	4.28
3	110.60	4.04
4	101.87	3.85
5	78.85	4.44
6	57.87	4.85
7	48.57	3.43
8	19.85	3.39
9	5.19	2.54
10	0.39	4.61
11	0.30	3.35
12	0.26	3.49
13	0.05	3.48
14	0.07	2.76

Table 6 - Comparison of average fluoride released concentration (ppm) between novel cyanoacrylate and Duraphat varnish

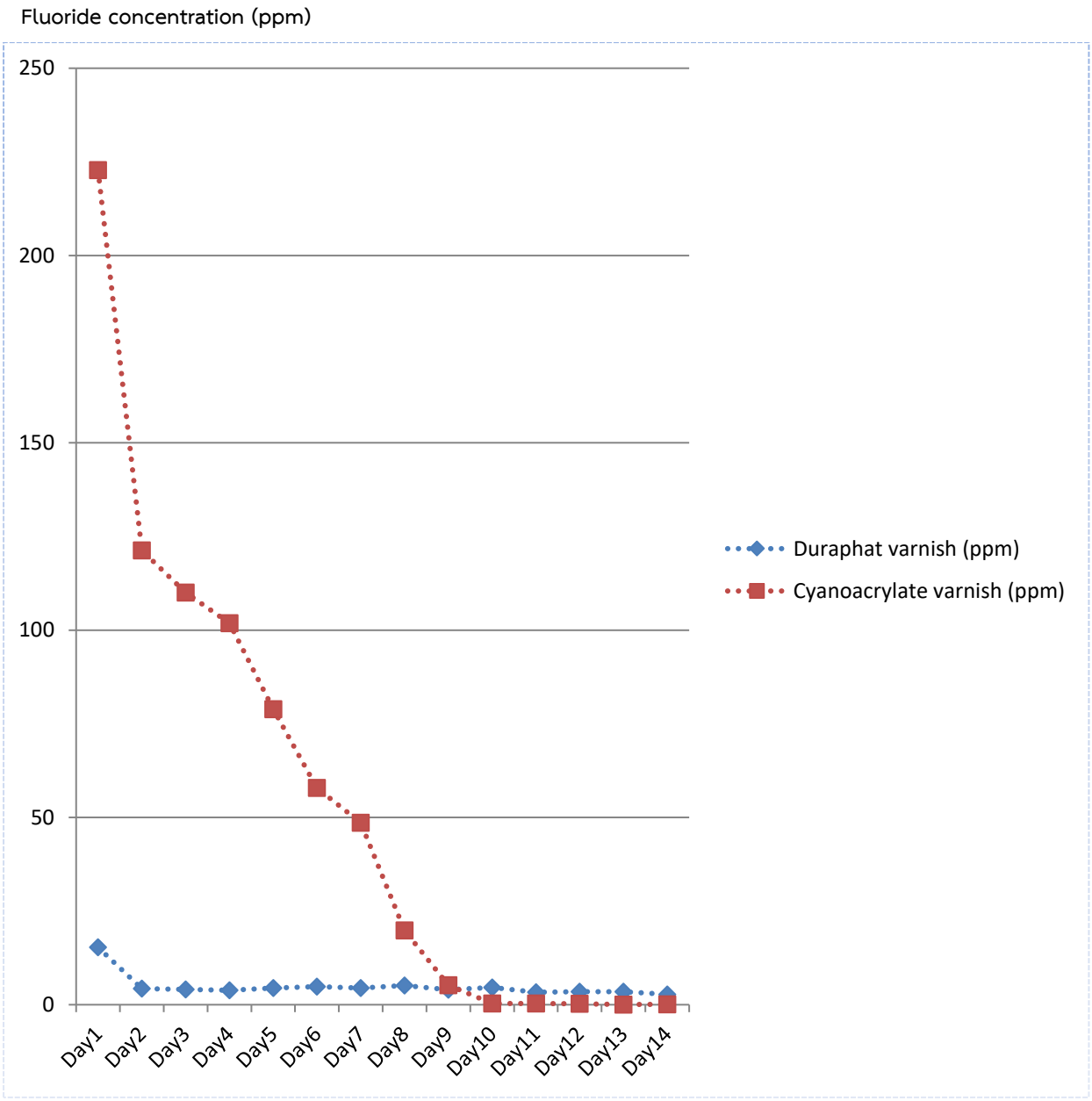

Fig. 16 - Graph of average fluoride released concentration (ppm) between novel cyanoacrylate and Duraphat varnish

Brushing test

The brushing test results demonstrated that none of the Duraphat varnish groups (20, 200, 400, or 600 brushing strokes) had any residual Duraphat varnish on the tooth surface after brushing. In contrast, residual cyanoacrylate varnish was found on the tooth surface in all cyanoacrylate groups after brushing. The percent area loss of the cyanoacrylate varnish on the tooth surface after brushing increased in a stroke-dependent manner, i.e. the greater the number of brushing strokes, the larger the percent area loss of varnish after brushing. The results were shown in Fig. 17, 18 and Table 7.

The results of the brushing test indicated that the cyanoacrylate varnish had a significantly better abrasion resistance compared with Duraphat varnish. The Duraphat varnish was easily removed by abrasion as demonstrated by no residual Duraphat varnish being present on the tooth surface in all groups after brushing, including the 20 brushing stroke group, which is considered a small number of brushing strokes and equivalent to one day of brushing (57, 58).

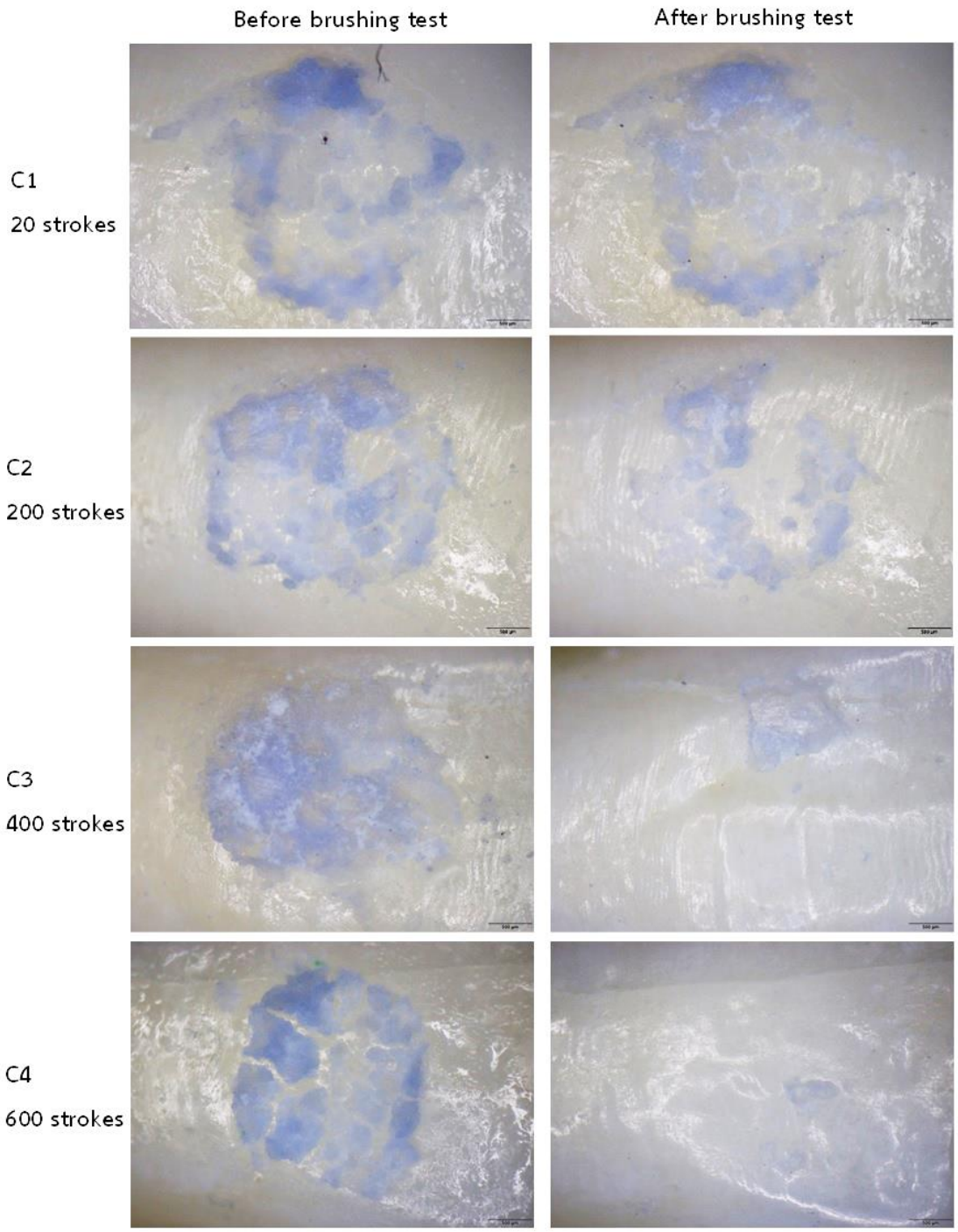

Fig. 17 - Photograph taken with a stereomicroscope at 30x magnification of novel cyanoacrylate fluoride varnish before and after brushing test.

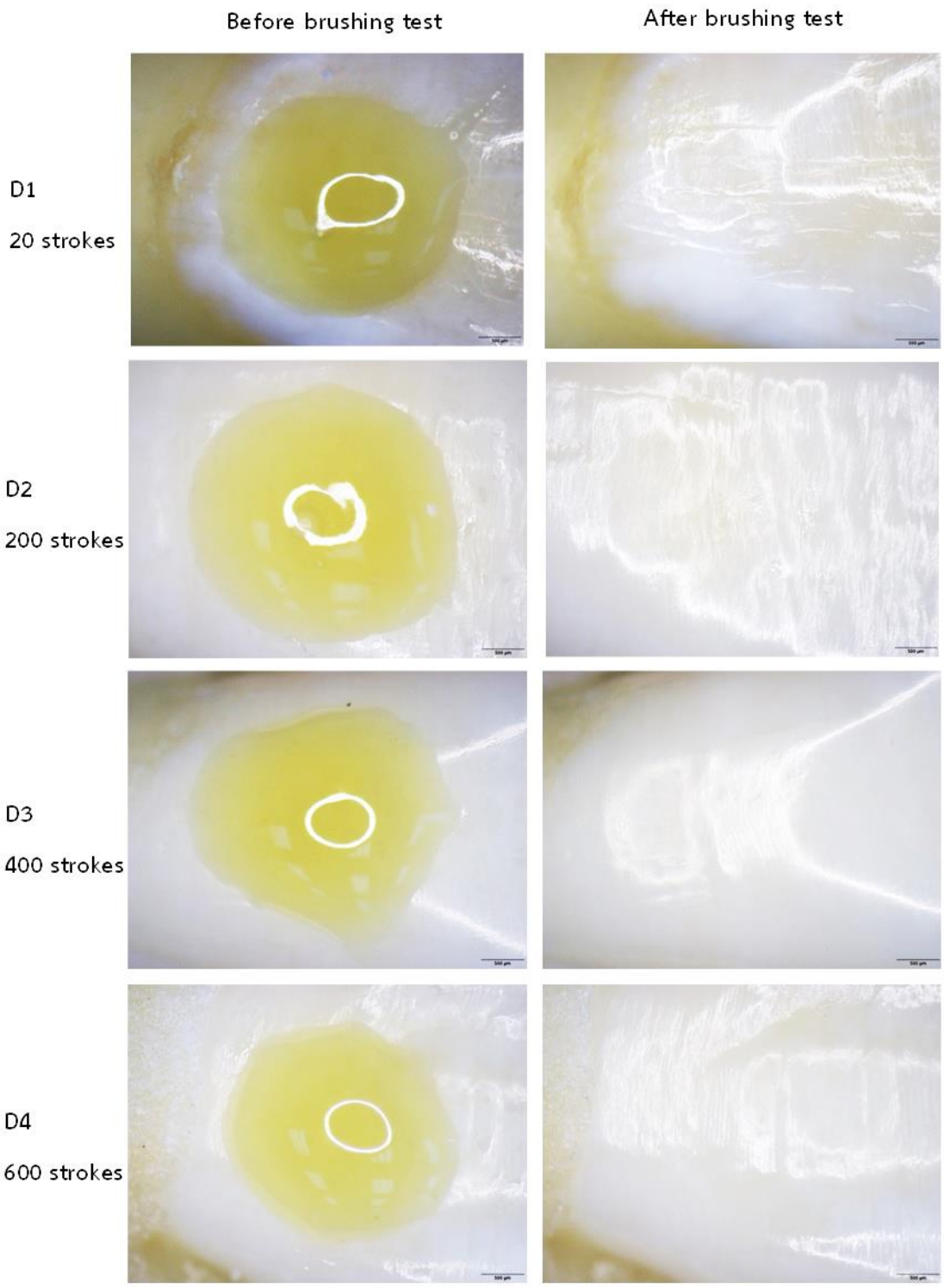

Fig. 18 - Photograph taken with a stereomicroscope at 30x magnification of Duraphat varnish before and after brushing test.

sample / Group	1	2	3	4	5	average	SD
C1	7.26	7.20	15.35	17.78	18.17	13.15	5.51
C2	45.56	34.57	28.11	35.00	41.70	36.99	6.79
C3	79.98	74.68	82.31	72.93	72.42	76.46	4.43
C4	100	92.37	93.51	100	100	97.18	3.89

Table 7 - Percent area loss of cyanoacrylate varnish on specimen using ImageJ program

Cytotoxicity test

The results of cytotoxic test were showed in Table 8, 9, 10 and Fig. 19 (C was cyanoacrylate, D was Duraphat). The results indicated that the undiluted extraction medium of each varnish was toxic to hGFs. When diluted 1:2, the Duraphat varnish extraction medium was toxic to the hGFs at 24, 48, and 72 h (mean percent cell viability 24 h=24.67±3.51%, 48 h=4.67±0.58%, and 72 h=1.67±0.58%). In contrast, the cyanoacrylate varnish extraction medium diluted 1:2 was toxic to the hGFs only at 24 h and not toxic to the hGFs at 48 and 72 h (percent cell viability 24 h=52.67±0.58%, 48 h=112.33±1.15%, 72 h=91.67±0.58%). When the extraction medium of each varnish was diluted 1:10, no toxicity to the hGFs was observed. Moreover, at 48 and 72 h, the

percent cell viability of the higher dilution groups of both varnishes' was found to be higher than 100% due to hGF proliferation.

MTT 24-hour					
Sample	1	2	3	AVG	% viability
C undiluted-N1	-0.011	-0.014	-0.014	-0.013	-5.80
C undiluted-N2	-0.013	-0.016	-0.017	-0.015	-6.70
C undiluted-N3	-0.012	-0.015	-0.012	-0.013	-5.80
C diluted1:2-N1	0.12	0.12	0.12	0.118	53
C diluted1:2-N2	0.12	0.12	0.12	0.118	53
C diluted1:2-N3	0.12	0.12	0.12	0.117	52
C diluted1:10-N1	0.23	0.22	0.23	0.227	101
C diluted1:10-N2	0.22	0.22	0.23	0.225	100
C diluted1:10-N3	0.23	0.22	0.23	0.227	101
D undilute-N1	-0.009	-0.009	-0.009	-0.009	-4.02
D undilute-N2	-0.007	-0.009	-0.008	-0.008	-3.57
D undilute-N3	-0.006	-0.01	-0.005	-0.007	-3.13
D diluted1:2-N1	0.06	0.08	0.05	0.063	28
D diluted1:2-N2	0.05	0.08	0.01	0.047	21
D diluted1:2-N3	0.06	0.05	0.06	0.057	25
D diluted1:10-N1	0.24	0.24	0.26	0.243	108
D diluted1:10-N2	0.25	0.24	0.24	0.240	107
D diluted1:10-N3	0.24	0.24	0.24	0.235	105
CM-Control	0.22	0.23	0.22	0.224	100
TriTonX	-0.079	-0.079	-0.079	-0.079	-35

Table 8 – MTT 24-hour results

MTT 48-hour compared with control 24 hour.					
Sample	1	2	3	AVG	% viability
C undiluted-N1	-0.017	-0.016	-0.015	-0.016	-6.11
C undiluted-N2	-0.012	-0.013	-0.015	-0.013	-4.96
C undiluted-N3	-0.013	-0.015	-0.016	-0.015	-5.73
C undiluted1:2-N1	0.26	0.22	0.26	0.248	111
C undiluted1:2-N2	0.26	0.25	0.25	0.254	113
C diluted1:2-N3	0.26	0.24	0.26	0.254	113
C diluted1:10-N1	0.36	0.32	0.31	0.331	148
C diluted1:10-N2	0.35	0.33	0.32	0.333	149
C diluted1:10-N3	0.33	0.32	0.34	0.330	147
D undiluted-N1	-0.012	-0.014	-0.016	-0.014	-5.34
D undiluted-N2	-0.016	-0.017	-0.016	-0.016	-6.11
D undiluted-N3	-0.014	-0.017	-0.012	-0.014	-5.34
D diluted1:2-N1	0.01	0.01	0.01	0.010	4
D diluted1:2-N2	0.01	0.01	0.01	0.010	5
D diluted1:2-N3	0.01	0.01	0.01	0.011	5
D diluted1:10-N1	0.29	0.3	0.3	0.296	132
D diluted1:10-N2	0.3	0.3	0.3	0.297	133
D diluted1:10-N3	0.3	0.3	0.3	0.298	133
CM-Control	0.26	0.27	0.27	0.262	117
TriTonX	-0.015	-0.015	-0.015	-0.015	-7

Table 9 – MTT 48-hour results

MTT 72-hour compared with control 24 hour.					
Sample	1	2	3	AVG	% viability
C undiluted-N1	-0.035	-0.032	-0.031	-0.033	-12.04
C undiluted-N2	-0.02	-0.019	-0.022	-0.02	-7.3
C undiluted-N3	-0.023	-0.019	-0.02	-0.021	-7.66
C diluted1:2-N1	0.21	0.2	0.2	0.203	91
C diluted1:2-N2	0.2	0.2	0.22	0.206	92
C diluted1:2-N3	0.21	0.21	0.2	0.206	92
C diluted1:10-N1	0.36	0.36	0.36	0.362	162
C diluted1:10-N2	0.36	0.36	0.36	0.361	161
C diluted1:10-N3	0.36	0.36	0.36	0.362	162
D undiluted-N1	-0.019	-0.018	-0.02	-0.019	-6.93
D udiluted-N2	-0.018	-0.022	-0.022	-0.021	-7.66
D undiluted-N3	-0.02	-0.019	-0.022	-0.02	-7.3
D diluted1:2-N1	0.004	0.003	0.0004	0.004	2
D diluted1:2-N2	0.004	0.004	0.004	0.004	2
D diluted1:2-N3	0.002	0.003	0..003	0.003	1
D diluted1:10-N1	0.32	0.32	0.31	0.316	141
D diluted1:10-N2	0.32	0.32	0.32	0.318	142
D diluted1:10-N3	0.32	0.31	0.32	0.317	141
CM-Control	0.28	0.27	0.28	0.274	122
TriTonX	-0.008	-0.008	-0.008	-0.008	-4

Table 10 – MTT 72-hour results

Sample	24 hr. Average % viability	48hr. Average % viability	72 hr. Average % viability
C undiluted	-6.1±0.52	-5.6±0.59	-9±2.64
C diluted1:2	52.67±0.58	112.33±1.15	91.67±0.58
C diluted1:10	100.67±0.58	148±1.00	161.67±0.58
D undiluted	-3.57±0.45	-5.60±0.44	-7.30±0.37
D diluted1:2	24.67±3.51	4.67±0.58	1.67±0.58
D diluted1:10	106.67±1.53	132.67±0.58	141.33±0.58
CM-Control	100	117	122
TriTonX	-35	-7	-4

Table 11 - Summary of MTT results

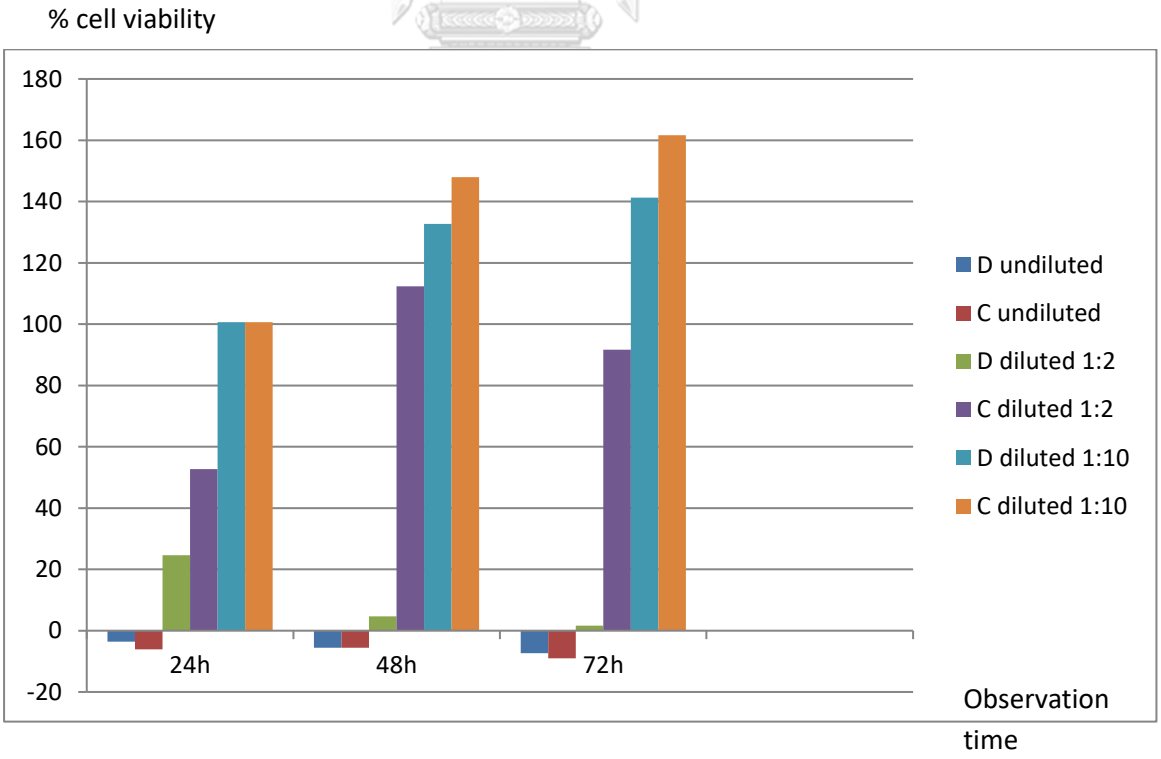

Fig. 19 - Graph of average percent cell viability

CHAPTER 6: DISCUSSION AND CONCLUSION

Discussion

The current commercial fluoride varnishes are susceptible to mechanical removal; therefore, patients are informed to avoid mechanically removing the varnish for at least 12–24 h for the fluoride varnish to be most effective. These protocols lead to inconvenience for the patients. The present study developed a novel cyanoacrylate based fluoride varnish to solve these problems based on the properties of cyanoacrylate, i.e. it immediately sets when contacting moisture, strongly bonds to the tooth surface, and progressively flakes off over 5–10 d, thus, patients can almost immediately eat, drink, and brush their teeth, which is more convenient.

Since there was no study that incorporated cyanoacrylate in fluoride varnish before, the cyanoacrylate varnish's formula, the compositions and mixing guideline were obtained from pilot study via trial-and-error method until we got the acceptable properties of cyanoacrylate varnish. The results of our pilot study demonstrated that the cyanoacrylate fluoride varnish had a mixing time of 1 min and a working time of 7–8 min. More importantly, the cyanoacrylate fluoride varnish solidified at the surface immediately when in contact with water, developed a rubbery consistency in 30 sec, and completely set within 1 min. The cyanoacrylate fluoride varnish was formulated using n-butyl cyanoacrylate as the active ingredient in the varnish rather than 2-octyl

cyanoacrylate, because the glass transition temperature (Tg) of n-butyl cyanoacrylate is appropriate for human body temperature. The Tg of n-butyl cyanoacrylate is 130°C(53), while that of 2-octyl cyanoacrylate polymer is 10°C(54). Therefore, at the 37°C human body temperature, the n-butyl cyanoacrylate polymer is in the glass phase, while the 2-octyl cyanoacrylate polymer is in the plastic phase. Thus, n-butyl has greater strength to withstand mechanical removal, such as tooth brushing, food, and tongue and cheek scrubbing in the oral environment. To control the type and amount of fluoride in the varnish to be equal to that of Duraphat, 50 mg/ml NaF powder was used. NaF is a basic salt that can initiate the polymerization of the cyanoacrylate monomers. Our pilot study revealed that NaF powder gave a longer working time compared with a NaF solution that has water as the solvent, which can cause cyanoacrylate to have a higher polymerization rate that is more exothermic. Small particle size NaF was used to promote the equal distribution of NaF in the varnish. Because water and ethanol can initiate cyanoacrylate polymerization, acetone was used as the solvent for the cyanoacrylate fluoride varnish. Acetone does not initiate cyanoacrylate polymerization and acetone is a biocompatible solvent that can dissolve NaF and cyanoacrylate, however, the solubility of NaF in acetone is lower compared with water and ethanol. Thus, most of the NaF particles were suspended in the varnish. Fumed silica was added to adjust the viscosity and increase the shear thinning property of the cyanoacrylate fluoride varnish and reduce internal stress and polymerization shrinkage, which cause debonding(55). The increased viscosity of the

varnish also reduced the precipitation of the NaF particles after mixing. However, the unequal distribution of NaF particles in the varnish was still an issue. Small amount of SLS was added to the varnish as a surfactant that promoted the equal distribution of NaF in the varnish and also increased the working time of the varnish. Vegetable oil was added to control the fluoride release rate from the cyanoacrylate fluoride varnish by being the water repellent in the varnish film and resulting in decreased dissolution rate of NaF.

The results of the viscosity test demonstrated that the cyanoacrylate fluoride varnish had a lower viscosity compared with the Duraphat varnish, which may be an advantage by being easier to apply, and also had a thinner varnish film thickness than Duraphat varnish, which may result in better esthetics and patient acceptance. Typically, the low viscosity of a varnish makes it easier to rinse off with water or saliva. However, this will not occur with the cyanoacrylate fluoride varnish, because cyanoacrylate solidifies immediately when in contact with water, thus the varnish can remain on the tooth surface. In addition, because the cyanoacrylate monomer is a clear liquid, it is easy to adjust the color of the varnish to match the natural tooth color.

The results of the daily fluoride release experiment indicated that there was a significance difference in the daily fluoride ion release between the cyanoacrylate fluoride varnish and Duraphat varnish, thus the first null hypothesis was rejected. With

the same volume of fluoride varnish, the cyanoacrylate fluoride varnish released significantly more fluoride ions compared with the Duraphat varnish for 9 days of immersion and then very little fluoride was released. In contrast, the Duraphat varnish had a fluoride release pattern characterized by releasing small amounts of fluoride over a longer period of time. This pattern was caused by the hydrophobicity of the rosin (colophony) in Duraphat, which makes it difficult for water to penetrate and dissolve the fluoride from the Duraphat varnish. This finding is consistent with that of Jorge L. Castillo et al. 2001(60), who reported that Duraphat varnish released fluoride for up to 28 weeks. Although the polycyanoacrylate polymer in the cyanoacrylate fluoride varnish is classified as a hydrophobic polymer, the porosity of the cyanoacrylate polymer and the hydrophilicity of the fumed silica, which was added to varnish to adjust its viscosity, promotes water to penetrate and dissolve NaF from the cyanoacrylate varnish, and also leave empty spaces that could allow water to move in and dissolve the inner NaF particles. This mechanism allowed the cyanoacrylate varnish to release higher amounts of fluoride ions and very little fluoride was released after 9 d of immersion.

The fluoride concentration in saliva is an important factor in the degree of remineralization and CaF_2 globule formation. Several studies reported that the effectiveness of fluoride in remineralization and caries prevention was directly related to the fluoride ion concentration(60-76). Fluoride is a very reactive element, less than

0.1 ppm fluoride is sufficient for fluoroapatite formation on the tooth surface(77) and when the fluoride concentration in plaque is more than 10 ppm, it can interfere with the activity of enolase, an enzyme that is important in carbohydrate fermentation by bacteria(78). Moreover, CaF_2 globules can only precipitate when the concentration of fluoride in the plaque and saliva exceeds 100 ppm(6, 10). The higher the fluoride concentration, the more CaF_2 is formed(7, 10, 79). CaF_2 globules can persist on the tooth surface for weeks or months(80, 81) and dissolve when the pH drops(6, 82), which creates a mechanism to prevent dental caries. Thus, applying fluoride varnish only two to three times a year can result in caries reduction.

The brushing test indicated that the number of brushing strokes that the cyanoacrylate fluoride varnish could withstand mechanical removal was significantly higher compared with the Duraphat varnish. Based on these results, the second null hypothesis was rejected. The cyanoacrylate fluoride varnish demonstrated a significantly better abrasion resistance than that of the Duraphat varnish. The cyanoacrylate fluoride varnish withstood up to 600 brushing strokes. In contrast, there was no residual varnish on the tooth surface in any Duraphat varnish brushing number group, including the 20 brushing strokes group, which is equivalent to 1 d of brushing. The cyanoacrylate monomers can form covalent bonds, creating strong adhesion to the hydroxyapatite and collagen fibers on the tooth surface and polymerizes into a polymer that exhibits abrasion resistance. In contrast, the Duraphat varnish, which is

obtained by dissolving colophony with alcohol, is sticky. Duraphat varnish adheres to the tooth surface by Van der Waals forces and solidifies by alcohol evaporation(8), thus, Duraphat has a lower abrasion resistance compared with the cyanoacrylate fluoride varnish.

The brushing test results suggest that there is a high possibility that the cyanoacrylate fluoride varnish could survive the mechanical forces that occur in the oral cavity, including eating, drinking, brushing and oral soft tissue scrubbing, longer than the Duraphat varnish. In addition, in the brushing test, smooth enamel surfaces were used as the varnish bonding sites, which were very difficult for the materials to adhere to. In clinical practice, varnish is applied to prevent dental caries on all areas of the teeth and the fluoride ions released from the varnish are always rinsed out and diluted by water and saliva. Thus, if the varnish can adhere to the most challenging surface of the teeth to bond; it can bond to all other tooth surfaces. However, the retention time of cyanoacrylate varnish and Duraphat varnish on the tooth surface in oral cavity require further investigation.

The remineralization reaction and amount of CaF_2 globules formed are related to the fluoride ion concentration(6, 10) and the retention time of the topical fluoride on the tooth surface, and remineralization increases over time(7-9, 11). The results of fluoride release and brushing test suggest that during the first 1–9 d after varnish application, there is a high possibility that the cyanoacrylate fluoride varnish could

promote a higher degree of remineralization and a higher amount of CaF_2 globule formation on the tooth surface that can serve as fluoride ion reservoirs and play an important role in caries prevention. However, the effect of novel cyanoacrylate fluoride varnish in caries prevention and remineralization still require further investigation.

The cytotoxicity test results demonstrated that both varnish's undiluted extraction mediums were toxic to hGFs. When using 1:2 diluted extraction medium, the cyanoacrylate fluoride varnish was toxic to the hGFs only at 24 h, while at 48 and 72 h, the cyanoacrylate fluoride varnish was not toxic to hGFs. In contrast, the 1:2 diluted Duraphat varnish extraction medium was toxic to the hGFs at all observation times (24, 48, and 72 h). When the extraction medium was diluted 1:10, neither varnish was toxic to the hGFs. BT Hoang-Dao et al. 2008(56) also reported that undiluted Duraphat varnish extraction medium was toxic to hGFs and the toxicity significantly decreased when diluted 1:2. The results of the cytotoxicity test indicate the cyanoacrylate fluoride varnish was slightly less toxic to the hGFs compared with the Duraphat varnish. However, although *in vitro* studies have shown that undiluted and diluted 1:2 Duraphat varnish extraction medium was toxic to hGFs, clinically, Duraphat varnish is considered the fluoride varnish of choice and is the most widely used fluoride varnish since 1980 with few incidences of serious pathology to the patient. This may be due to the dynamics of the oral environment, where substances released from the varnish are constantly rinsed out and diluted by water and saliva, thereby minimizing

the potential toxicity to the gingival tissue. Thus, the third null hypothesis was not rejected; the cyanoacrylate based fluoride varnish is not toxic to gingival fibroblasts

The results of this study demonstrated that the cyanoacrylate fluoride varnish released higher amounts of fluoride ion compared with the Duraphat varnish for 9 d after application, solidified immediately when in contact with water, and was more resistant to abrasion. These properties could improve patient's comfort However, the disadvantages of the cyanoacrylate fluoride varnish are that it has a limited working time of 7–8 minutes and contains highly volatile acetone, which is difficult and complicated for storing and mixing.

Limitation and Further investigation

The present study used the *in vitro* fluoride release test to compare the fluoride release of the cyanoacrylate fluoride varnish and Duraphat varnish. The experiment was based on the principle that greater fluoride release promotes greater remineralization and increased CaF_2 formation. However, clinically, the oral conditions are dynamic. the effect of novel cyanoacrylate fluoride varnish in caries prevention and remineralization still require further investigation. Moreover, fluoride ions released from the varnish are always rinsed away and diluted by water and saliva that may allow the varnish to release more fluoride ions due to the effect of concentration on

the diffusion of the substance compared with the *in vitro* cumulative fluoride release test in this study.

The results of the brushing test demonstrated that the cyanoacrylate fluoride varnish withstood up to 600 brushing strokes, which is equivalent to 30 d of brushing. However, when used clinically there are many uncontrolled and individual factors, such as the tooth brushing technique, eating, drinking, talking, food type, food pH, food composition, and temperature, which can reduce the retention time of the cyanoacrylate varnish on the tooth surface. Moreover, no study has determined how long the varnish remains on the tooth surface after application *in vitro*. This may be because simulating oral cavity is difficult and there are many uncontrolled and individual factors to consider, that can affect the retention time of varnish on tooth surface. Therefore, the brushing test in this study was solely to compare the varnish's abrasion resistance and the actual retention time of cyanoacrylate varnish and Duraphat varnish in oral environment require further investigation.

For the percent area loss measurement, the ImageJ program measured area loss of varnish from the captured image and it can measure the changes that occur only when the varnish is removed in full thickness. It cannot measure the amount of the varnish's loss in the partial thickness of the varnish. However, the aim of the brushing test was to compare the number of brushing strokes that the varnish could

resist being mechanically removed by tooth brushing between the novel cyanoacrylate based fluoride varnish and Duraphat varnish.

In addition, the antimicrobial effects on gram positive organism, the dental plaque accumulation and the fluoride recharge ability of cyanoacrylate fluoride varnish are the interesting topics and should be the further studies.

Conclusion

Based on the results of this study, the novel cyanoacrylate fluoride varnish polymerizes immediately when exposed to water or moisture, releases higher amounts of fluoride, but for a shorter period, has higher abrasion resistance, and is slightly less toxic to the cell hGFs compared with Duraphat varnish. This novel cyanoacrylate fluoride varnish has the potential to be a new alternative fluoride varnish as a topical fluoride treatment that is easy to use and convenient for patients.

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