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PHYSICAL PROPERTIES AND BIOLOGICAL RESPONSES OF A VARNISH CONTAINING POTASSIUM AND APATITE.

Mr. Yossakit Lochaiwatana

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Dental Biomaterials Science
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ยศกฤต หล่อชัยวัฒนา : คุณสมบัติทางกายภาพและการตอบสนองทางชีวภาพของวาร์นิชที่มีส่วนผสมของโพแทสเซียมและอะพาไทต์. (PHYSICAL PROPERTIES AND BIOLOGICAL RESPONSES OF A VARNISH CONTAINING POTASSIUM AND APATITE.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ทพ. ดร.สุชิต พูลทอง, หน้า.

วัตถุประสงค์: เพื่อพัฒนาและทดสอบวาร์นิชชนิดใหม่สำหรับใช้เป็นสารลดเสียวฟันในแง่ของสมบัติทางกายภาพและความเป็นพิษต่อเซลล์ วิธีการทดลอง: สังเคราะห์ผงอะพาไทต์ด้วยวิธีการตกตะกอน พิสูจน์เอกลักษณ์ด้วยเอกซเรย์โฟโตอิเลคตรอนสเปกโทรสโกปี, ฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปกโทรเมตรี และกล้องจุลทรรศน์อิเลคตรอนชนิดส่องกราด สังเคราะห์วาร์นิชที่มีส่วนผสมของโพแทสเซียมและอะพาไทต์ วัดการปล่อยโพแทสเซียมไอออนโดยอินดักทีฟคัปเปิลพลาสมาออพติคอลอีมิสชันสเปกโทรสโกปี วัดการลดสภาพซึมผ่านได้ของเนื้อฟันด้วยด้วยชุดเครื่องมือสำหรับการวัดความนำชลศาสตร์และวัดการอุดปิดท่อเนื้อฟันด้วยกล้องจุลทรรศน์อิเลคตรอนชนิดส่องกราดโดยเปรียบเทียบกับฟลูออไรด์วาร์นิชและวาร์นิชสูตรต่างๆ วัดความเป็นพิษต่อเซลล์ด้วยวิธีการสัมผัสโดยตรงและวิธีเอ็มทีทีโดยทดสอบกับเซลล์ไฟโบรบลาสต์ที่ได้จากเนื้อเยื่อเหงือกของมนุษย์และเนื้อเยื่อในของฟันมนุษย์ ผลการทดลอง: วาร์นิชที่สังเคราะห์ขึ้นมีการปล่อยโพแทสเซียมไอออนอย่างต่อเนื่องใน 6 ชั่วโมงแรก หลังจากการทาวาร์นิชบนผิวชิ้นฟัน กลุ่มทดลองมีค่าร้อยละของสภาพซึมผ่านได้ลดลงมากกว่ากลุ่มควบคุมและฟลูออไรด์วาร์นิชอย่างมีนัยสำคัญทางสถิติ, พบการอุดปิดท่อเนื้อฟันในกลุ่มทดลอง, ไม่พบความเป็นพิษต่อเซลล์ไฟโบรบลาสต์ที่ได้จากเนื้อเยื่อเหงือกของมนุษย์และเนื้อเยื่อในของฟันมนุษย์เมื่อเทียบกับฟลูออไรด์วาร์นิช สรุป: วาร์นิชมีส่วนผสมของโพแทสเซียมและอะพาไทต์มีสมบัติทางกายภาพที่เหมาะสมสำหรับการพัฒนาเป็นสารลดเสียวฟันและไม่เป็นพิษต่อเซลล์ไฟโบรบลาสต์ที่ได้จากเนื้อเยื่อเหงือกของมนุษย์และเนื้อเยื่อในของฟันมนุษย์

สาขาวิชา ทันตชีววัสดุศาสตร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2556 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

5287812220 : MAJOR DENTAL BIOMATERIALS SCIENCE
KEYWORDS: VARNISH / POTASSIUM CHLORIDE / FLUORIDATED HYDROXYAPATITE / DENTIN HYPERSENSITIVITY / DENTIN PERMEABILITY

YOSSAKIT LOCHAIWATANA: PHYSICAL PROPERTIES AND BIOLOGICAL RESPONSES OF A VARNISH CONTAINING POTASSIUM AND APATITE.. ADVISOR: ASST. PROF. DR.SUCHIT POOLTHONG, Ph.D., pp.

Objective: The purpose of this study was to evaluate the potential of a novel varnish containing potassium chloride and fluoridated hydroxyapatite in terms of physical properties for DH management and cytotoxicity in vitro. Method: Fluoridated hydroxyapatite was synthesized by precipitation method. The chemical analysis of the powder was performed by X-ray photoelectron spectroscopy and Fourier transmission infrared spectroscopy. The morphology was characterized by scanning electron microscopy. The novel varnish containing potassium chloride and fluoridated hydroxyapatite was formulated. Potassium ions released from the varnish were measured by inductively coupled plasma-optical emission spectrometry. Dentin permeability measurement was conducted and permeability reduction percent from the varnish was calculated and compared to fluoride and control varnishes. Dentinal tubule occlusion was observed by scanning electron microscopy. Direct contact test and MTT assay were performed for cytotoxic evaluation. Results: The results showed that the novel varnish could release potassium ions over 6 h. Specimens applied by the novel varnish demonstrated statistically higher dentin permeability reduction to fluoride varnish and placebo. Scanning electron microscopy observation exhibited dentinal tubule occlusion in the novel varnish. Direct contact test and MTT assay showed its biocompatibility to gingival and pulpal fibroblasts with respect to fluoride varnish. Conclusion: This study suggested the potential of the novel varnish as a promising biomaterial for dentin hypersensitivity management with no cytotoxicity to human gingival and pulpal fibroblasts.

Field of Study: Dental Biomaterials Science

Academic Year: 2013

Student's Signature .....

Advisor's Signature .....

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CHAPTER I
Introduction

Problem statement

Discomfort from dentin hypersensitivity (DH) is a common finding in adult populations. A wide range of DH prevalence was reported from 4 to 74 percent in the general population[1-3]. These variations may be due to differences in the study population and methods used. The escalation of geriatric population with higher number of remaining teeth leads to the higher risk of developing cervical DH as a result of physiological gingival recession and exposure of cervical dentin[4]. DH exerts as a short sharp pain arising typically in response from the exposed dentin[5]. It mostly affects the daily life and leads to quality of life impairment[6]. Thus it should be considered as an important problem.

Two main methods for DH treatment are alteration of pulpal sensory nerve activity and occlusion of dentinal tubules. Development of desensitizing agents is based on these mechanisms. Nowadays, these agents are known in terms of either the oral care products for personal use, such as toothpastes and mouthrinses, or the in-office products for professional use. Ideal requirements of dentin desensitizing agents were described as: rapidly acting with long-term effects, non-irritant to pulp, painless and easy to apply and should not stain the tooth[7-9].

Even though plenty of desensitizing agents were introduced, none of them possesses all ideal properties, such as technique sensitivity of dentin adhesives, application for dentin desensitizing method and tissue irritation by glutaraldehyde desensitizing based system. Many desensitizing agents have short term desensitizing effect. Thus, novel biomaterials are expected to be introduced, in order to meet patient's needs regarding DH management, biocompatibility with oral tissues and convenient method of use.

Research purpose

The purpose of this study was to evaluate the potential of a novel varnish containing potassium chloride (KCl) and fluoridated hydroxyapatite (FHA) in terms of physical properties for DH management and cytotoxicity *in vitro*.

Hypothesis

1. Varnish containing KCl and FHA can release potassium ions *in vitro*.
2. Varnish containing KCl and FHA can occlude dentinal tubules *in vitro* better than fluoride varnish.
3. Varnish containing KCl and FHA can reduce dentin permeability *in vitro* better than fluoride varnish.
4. Varnish containing KCl and FHA has equivalent or less cytotoxicity to human gingival fibroblasts and human pulpal fibroblasts than fluoride varnish.

Expected outcome

This study will introduce a novel biomaterial for DH treatment which can release potassium ions for nerve desensitization and occlude the dentinal tubules.

CHAPTER II

Literature Review

Dentin hypersensitivity

DH symptom is a short sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical[5]. DH is one of the most common oral problems found among adult. A wide range of DH prevalence is reported from 4 to 74 percent in the general population[1-3]. These variations may be due to differences in the study population and methods used. DH affects women more often than men and mostly occurs in patients of 30 to 40 years old. It can also occur in younger or older patients[10]. DH can affect any tooth but canines and premolars are reported to be affected the most often[11]. Cervical DH is found more often and affects a large percentage of the population. This is because life expectancy increases and patients retain their natural teeth longer due to more effective treatments for dental caries and periodontal disease[4]. The relationship between intake of dietary acids and timing of toothbrushing may be very important in the etiology of DH[12]. As a result of greater oral health awareness and higher frequency of acidic food and beverage intake, DH may occur earlier and in younger populations than reported previously[12]. Subsequent prevention of the condition should involve not only modification of toothbrushing technique, but also advice on reducing the combined effects of toothbrushing and dietary acids on the dental tissues[12].

DH presents the dentin as a vital and sensitive tissue. There are numerous etiological and predisposing factors to DH. However, no prime cause can be identified. By definition, DH may arise as a result of loss of enamel and/or root surface denudation with exposure of underlying dentin[13]. In general, dentin is covered by the hard tissue either enamel or cementum which may be removed or denuded as a result of abfraction and/or tooth wear which may result from abrasion, attrition or erosion. In reality, it is a combination of these but often with differing proportional effects[13].

Abfraction or cervical stress lesion describes the deep V-shape cervical notch. It is thought to involve eccentric occlusal loading leading to cusp flexure. This leads to compressive and tensile stresses at the cervical fulcrum area of the tooth with the

resultant weakening of the cervical tooth structure. The process may be co-destructive with abrasion and/or erosion[13].

Abrasion describes the wear of teeth caused by objects other than tooth, such as toothbrush, toothpaste, pipe smoking or other similar habits. Typical toothbrush abrasion lesions are side dependent. For instance, the right-handed individual tends to have greater abrasion of teeth on the left-side quadrants. The buccal cervical area of the teeth is the site of predilection. Furthermore, canines and premolars are most affected because of their position within the dental arch where they receive the most attention during toothbrushing[13].

Attrition describes the wear of teeth at sites of direct contact between teeth. Attrition is associates with occlusal function and may be exaggerated by habits or parafunctional activities, such as bruxism.

Erosion is currently believed to be an important factor involved in tooth wear and had been defined as the dissolution of tooth surfaces by acids which are not of bacteria origin. Erosion may be a result from either intrinsic or extrinsic acids. Intrinsic erosion may result from gastric reflux. When erosion is caused by gastric regurgitation, the palatal aspects of upper incisors and the occlusal and buccal aspects of lower posterior teeth are primarily affected. Extrinsic erosion can be resulted from two major causes. Firstly, dietary erosion may result from foods or drinks containing acids, such as citrus fruits, juices, carbonated drinks wines and ciders. Secondly, environmental erosion results from occupational exposure to acids, such as workers in battery manufacture, swimmers who trained in poorly maintained pools[13].

Alternatively, in some individuals the cementum and enamel which normally cover the dentin do not meet and result in dentin exposure as a result of a developmental anomaly[3].

Dentin is sensitive due to its anatomy and physiology. It is a porous, mineralized connective tissue with an organic matrix of collagenous proteins and inorganic component, hydroxyapatite. The porous structure is consisted of abundant dentinal tubules which are the micro-canals that radiate outward through the dentin from the pulp cavity toward the dentin surface, with different configurations and diameters in different teeth. Moreover, the myelinated Aδ fibers are located within the dentinal tubules which responsible for the sensation of DH. This complicated structure is called dentin-pulp complex[14]. Each dentinal tubule contains an odontoblastic process of an odontoblast. The odontoblastic processes are nourished

by specific fluid called dentinal fluid. It helps dentin-pulp complex to communicate between the dentin surface and the pulp.

Several theories have been proposed to explain the mechanism of DH. The direct innervation theory explains that nerve endings penetrate dentin and extend to the dentino-enamel junction[15]. This concept advocates that thermal or mechanical stimuli directly affect nerve endings within the dentinal tubules through direct communication with pulpal nerve fibers. Direct mechanical stimulation of these nerves will initiate an action potential. Developmental studies have shown that the plexus of Rashkow and intratubular nerves do not establish themselves until the tooth has erupted. But the newly erupted tooth is sensitive[14]. Moreover, pain inducers such as bradykinin fail to induce pain when applied to dentin. Bathing dentin with local anesthetic solutions does not prevent pain but it does when applied to skin[9]. While this theory has been supported by the observation of the presence of unmyelinated nerve fibers in the outer layer of root dentin and the presence of putative neurogenic polypeptides, this theory is still considered theoretical with little solid evidence to support it[3].

The odontoblastic transduction theory states that odontoblasts acts as receptors by themselves and relay the signal to a nerve terminal[9]. An exposed dentin surface leads to an excitation of odontoblastic processes by a variety of chemical and mechanical stimuli. As a result, stimulation neurotransmitters are released and impulses are transmitted towards the nerve endings. However, no neurotransmitters have been found to be produced or released by odontoblastic processes[3]. Moreover, majority of studies have shown that odontoblasts are matrix forming cells and hence they are not considered to be excitable cells, and no synapses have been demonstrated between odontoblasts and nerve terminals[16].

The most widely accepted theory to explain DH is the hydrodynamic theory that first introduced by Gysi in 1900[17] and proposed by Brannström and co-workers in 1964[18]. This theory is described based on the presence of dentinal fluid and its movement in response to stimuli. These fluid changes or movements stimulate a baroreceptor which leads to activation of the nerve endings, Aδ fibers surrounding the odontoblast, at the end of the dentinal tubules or at the dentin-pulp complex[16]. The movement is as a result of the stimuli, such as cooling, drying, evaporation and application of hypertonic chemical substances[19]. Studies performed *in vivo* revealed that the response of the pulpal nerves was proportional to the pressure and the rate of fluid flow[20]. Interestingly, stimuli which cause an outward

flow of dentinal fluid from the pulp, such as cold, produce more rapid and greater pulpal nerve responses than stimuli which cause an inward flow, such as heat[20]. This could explain the rapid and severe response to cold stimuli compared to the slow and dull response to heat[20].

The exact mechanism by which the fluid flow stimulates pulpal nerves is not known with any certainty[13]. However, from animal experiments a mechanoreceptor is suggested[20]. Thus the pressure change across dentin distorts the pain receptors at the pulp dentin border[13]. This would be similar to the activation of touch sensitive nerves around hair skin follicles by the application of light pressure to the protruding hair[13]. The role of the odontoblast and the odontoblastic process in DH has been reviewed[16]. Essentially, the odontoblastic process is thought to extend only a short distance into dentinal tubules and thereby cannot be directly involved in stimulus transmission across dentin as explained by the odontoblastic transduction theory[13]. Nevertheless, the large shear forces created by fluid flow in dentinal tubules could damage odontoblasts resulting in a local neurogenic inflammation of the pulp[13].

For DH to occur, not only does the dentin need to become exposed (lesion localization) but the tubules also need to be patent to the pulp (lesion initiation)[21]. Many people have dentin exposed to the oral environment owing to loss of cementum and/or enamel, but clinical experiences indicate that only a proportion of those people suffer from DH[21]. Lesion localization occurs by exposure of dentin, either by loss of enamel or by gingival recession[22]. Attrition, abrasion, erosion, abfraction, gingival recession from aggressive tooth brushing or flossing, crown preparation or periodontal disease and/or periodontal treatment may play a role in the etiology of this phase. However, not all exposed dentin is sensitive. Lesion initiation occurs when the smear layer or tubular plugs are removed, which opens the outer ends of the dentinal tubules[13]. The hypersensitive dentin appeared to have more widely open tubules and thin or under calcified smear layer as compared with normal dentin. The wider dentinal tubules increase the fluid movement and thus the pain starts[23, 24]. *In vitro* studies indicate the erosion from acidic soft drinks causes rapid loss of the smear layer resulting in the wide opening tubules, and similarly most toothpastes readily remove the smear layer to expose tubules[21]. However, toothbrushing can also replace the smear layer, creating a dynamic environment[21].

Dentin hypersensitivity management

An accurate diagnosis is essential before starting the DH treatment. DH has features which are similar to other dental conditions like dental caries, fractured or chipped enamel/dentin, pain due to irreversible pulpitis and post dental bleaching sensitivity. DH diagnosis starts with a thorough clinical history and examination. The other causes of dental pain should be excluded before a definite diagnosis of DH is made[9]. Some of these techniques include pain response upon the pressure of tapping teeth (to indicate pulpitis/periodontal involvement), pain on biting a stick (suggests fracture), use of transilluminating light or dyes (to diagnose fractures) and pain associated with recent restorations[25]. Patients generally complain that pain arising from DH is usually rapid in onset, sharp in character and short in duration[25].

Managements of DH are so numerous and diverse. They can be categorized according to desensitizing mechanisms. The hydrodynamic theory prompts two basic approaches for DH treatment, namely reduction of intradental nerve excitability and occlusion of patent dentinal tubules, which lead to the development of desensitizing agents based on these mechanisms[26]. Moreover, they can be summarized according to delivery and therapeutic aims into professional (in-office) and home use treatments[13]. The choice of professional, home use treatments or indeed both combined depends on the dentist. Currently, there are no tried and tested regimens proven superior to others[13].

Clinical experiences suggest that the professional approach to treating DH has been based on results of treatment rather than addressing the etiological and predisposing factors that created the problem. Hence, an array of products is available to professionals formulated to treat DH, many showing equivocal efficacy[21]. A few of the investigations have compared one professional treatment with another or one home use product with another. But mostly comparisons are with a control, minus active ingredient, or placebo treatment. There appear to have been no comparisons of professional treatments with home use products. Studies reveal that an unusually large number of very diverse agents or formulations are apparently effective in reducing the DH symptoms[13].

Potassium ion is widely accepted as agent for nerve desensitization. Meanwhile, dentinal tubule occlusion can be achieved by either endogenous desensitization from mineralized tissue formation, such as intratubular dentin, peritubular dentin, tertiary dentin, *etc.* or exogeneous desensitization by means of tooth restoration or application of desensitizing agents[27].

Treatment of DH can be administrated professionally or for use at home depending on the degree of the problem and the dentist/patient preference. Home treatment usually involves toothpastes and mouthwashes and is by far the earliest method of administering treatment. It is also fairly inexpensive. A wide range of commercially available products are manufactured for self-treatment. Current products in the market include potassium, strontium, oxalate and fluoride salts combined in toothpastes and mouthrinses[21].

According to professionally applied products, a wide range of desensitizing agents is available for DH treatment. In-office treatments are considered for the patients who have received preventive advice and tried at-home products but found them to be ineffective[21].

Desensitizing agent

Although desensitizing agents are continuously developed with more advanced technologies, they are still based on two mechanisms mentioned. The former method seems to have only potassium ion even if it appears in different compound, such as KCl, potassium citrate ($K_3C_6H_5O_2$), potassium nitrate (KNO_3), *etc*. On the other hand, the latter method has a variety of agents developed, *e.g.*, bioactive glass, calcium carbonate, calcium phosphate, dentin bonding agent, fluoride varnish, glass ionomer cement, glutaraldehyde, oxalic acid, potassium oxalate, resin composite, silver nitrate, sodium fluoride, stannous fluoride, strontium chloride, strontium chloride hexahydrate, zinc chloride. These agents occlude the dentinal tubules and reduce dentinal fluid movement. However, occluding materials can be washed from the tubule or may be acid labile. Wear can also occur, abrading the surface of, for example, a dentin-bonding agent or a glass ionomer after a couple of months[21]. Etching before applying the desensitizing agents has been reported to enhance the tubule occlusion due to the increase of free calcium ions from etched peritubular dentin[28]. Moreover, the dentin desensitizing agents should meet the ideal requirements, such as rapidly acting with long-term effects, non-irritant to pulp, painless and easy to apply and should not stain the tooth[7-9].

Reviewing clinical trials of DH helps to explain the equivocal efficacy of desensitizing agents. Even though there are several clinical trials published, the protocols for comparing different agents are not standardized resulting in numerous variables to be compared. A paste with an active ingredient may be tested against its base paste, a conventional fluoride paste or another paste with an active ingredient.

Treatments rarely take into account etiological factors, and the measurement of pain is difficult to standardize between individuals owing to its subjective nature[21]. Furthermore, complicating factors, such as the placebo effect, Hawthorn effect, regression to the mode and control product effect, compound the interpretation of clinical findings and hide the true effect of the treatment[21].

Potassium salt

Potassium salts were used over 100 years ago as obtundents prior to the introduction of local anesthetics. However, these agents fell out of favor for nearly 100 years. In a largely anecdotal account, topical applications of 1 to 15% KNO_3, saturated solutions of KNO_3 or a paste containing 10% KNO_3 were effective in reducing DH[26].

Potassium ions reduce intradental nerve excitability by diffusion along the tubules, thereby, raising the concentration of local extracellular potassium ion concentration. When a sufficient level of potassium ions is reached in the dentinal fluid, nerve conduction is blocked. Therefore, the nerves do not respond to the stimulus-evoked fluid movement in the tubules[29]. Wanachantararak *et al.*[30] explained the possibility that normal dentinal fluid may contain a higher concentration of potassium ion than extracellular fluid elsewhere. However, the exact concentration of normal intact teeth is not known. Once the dentin is exposed and the dentinal tubules are opened, the potassium concentration of the tubular fluid is expected to fall and may lead to DH. Potassium ions presumably depolarize the nerve fiber membrane, eliciting an initial increase of action potentials. After the initial depolarization, the nerve fibers cannot repolarize due to the maintained high levels of extracellular potassium ions and thus sustained depolarized state occurs. Few or no action potentials can be evoked during this state. This phenomenon is sometimes called axonal accommodation in physiological terms[31].

In 1986, Kim[31] reported that the 0.052 M $K_3C_6H_5O_2$ caused a 57% reduction sensory nerve activity in animals, while 0.0629 M concentration caused an even greater reduction of 87% of the control value. However, the higher concentration of 0.126 M was less effective than the lower concentration of 0.0629 M. Several agents at a 0.189 M concentration were reported to be effective in reducing sensory nerve activity, such as potassium bicarbonate ($KHCO_3$), potassium oxalate ($K_2C_2O_4$) and KCl. KNO_3 was also found to be very effective in sensory nerve activity reduction. A comparison of potassium ion and sodium ion compounds clearly demonstrates that

potassium ion containing agents are the most effective in reducing sensory nerve activity in animals.

In vivo studies by Markowitz *et al*.[29, 31] found the effect of 756 mM KCl solution in decreasing intradental nerve in the cat with potassium ions. In human subjects, Ajcharanukul *et al*.[32] introduced the effect of 500 mM KCl solution in reduction of DH under 150 mmHg pressure. Likewise, Noparatkailas *et al.*[33] reported the reduction by using only 250 mM KCl solution under atmospheric pressure for 10 min.

Potassium ions applied to outer dentin will have to diffuse along the dentinal tubules to achieve and sustain a potassium concentration of at least 8 mM to inactivate intradental nerves at the pulpal ends of dentinal tubules or even in the peripheral pulp. However, it is technically very difficult to measure the potassium concentration in dentinal tubules[26].

There are several forms of potassium salt developed for DH management, such as potassium containing toothpastes and mouthrinses as oral care products for DH relief at home, potassium containing gels and solutions for in-office treatment. Even though a great number of clinical trials concerning potassium containing toothpastes have been published, a systematic review reported no evidence confirmed that potassium salts is the key ingredient for DH reduction. Potassium concentration in saliva only increased minimally even if subjects brushed their teeth everyday with KNO_3 toothpastes. It is possible that the DH reduction may be due to dentinal tubule occlusion of other ingredients in those toothpastes[26]. Moreover, a Cochrane review revealed no clear evidence is available for the support of 5% KNO_3 toothpastes for DH management[34]. A clinical study on 5% KNO_3 solution also found no DH reduction[35]. Meanwhile, a study on 10% KNO_3 gel reported to relieve DH at 35% within 48-96 h[36] and another study on 35% KNO_3 gel reported to immediately reduce DH at 91% after application[37].

Calcium phosphate

Dentinal tubule occlusion was described to relieve DH by reducing any stimulus-evoked fluid flow with a wide range of agents[26]. The desensitizing products may be in the form of topically applied agents, such as resins, varnishes, dentin bonding agents. But recently, calcium phosphate is a promising agent for DH management. This compound is bioactive, biocompatible and similar to the major

composition of human bone and tooth structure. Thus, it seems to be the desirable material for dentinal tubule occlusion[38, 39].

Apatite is a very famous form of calcium phosphate and is widely used in dental material developments, such as hydroxyapatite and fluorapatite. The calcium phosphate containing materials are potentially transformed to hydroxyapatite as a final product, which is the principal material in teeth. Human saliva contains an abundance of calcium and phosphate. Hence, the supersaturation of salivary fluid with respect to hydroxyapatite is expected to contribute to further growth in size of hydroxyapatite crystals formed in the oral environment. Therefore, a calcium phosphate compound may have the potential to keep the dentinal tubules occluded in long-term[40]. According to degree of saturation with respect to synthetic saliva, fluorapatite was superior to hydroxyapatite and CaF_2 respectively[41]. Both apatites were highly supersaturated that could easily deposit on tooth surface, meanwhile CaF_2 could dissolve easily in saliva[41].

Powdered apatite glass ceramic was reported to contribute to the immediate occlusion of dentinal tubules, when combined with application of acidic solutions of fluoride followed by a paste of $LaCl_3$, and had a superior resistance against toothbrush abrasion[42, 43]. A sequential application of disodium phosphate and calcium chloride on patent dentinal tubules immediately yielded calcium phosphate crystals *in situ*, thus occluding dentinal tubules and yielding immediate relief from DH in 84% of patients[44]. An *in vitro* study was demonstrated that the application of calcium phosphate containing desensitizers effectively reduced dentin permeability even after artificial saliva immersion[40]. In another *in vitro* study, the toothpastes containing nano-carbonate apatite were effective in occluding dentinal tubules in short-term use, such as toothbrushing for 500 strokes and less[45]. Clinically, precipitating the exposed dentin surfaces with hydroxyapatite powder was reported to significantly reduce the hypersensitive symptoms and scanning electron microscopy (SEM) images confirmed the obliterated dentinal tubules[39].

In terms of remineralization, the different nano-hydroxyapatite toothpastes revealed similar remineralizing capacities with enamel and dentin lesions. The nano-hydroxyapatite in dental products might help to promote remineralization *in vitro*[46]. It is also preferable for open dentinal tubules to be occluded with calcium phosphate which does not inhibit the spontaneous remineralization of the tooth surface[38].

Varnish

One of the popular dental materials for dentinal tubule occlusion is varnish. The varnish was first introduced for DH treatment in 1986[47]. It was applied on the pulpal floor of tooth cavity before a filling material was restored. This would create thin film to cover the dentin surface and result in DH reduction[48]. Pashley *et al.*[49] also found that applying varnish decreased the dentin permeability between 20 to 50 percent. Furthermore, adding some chemicals, such as strontium chloride or sodium fluoride, were respected to reduce tooth sensitivity[4, 50-54].

Fluoride varnish is a well-known and widely used desensitizing agent. Unique advantage of this material is the convenience of using. It can be applied on any area of dentin exposure even though the surface is not completely dry. After the application, solvent will evaporate within a short period of time and retain a film of dried varnish coated that surface. In this way the insoluble resins, in the shape of a sticky-plastic congealing film, gradually fall out and, for instance, sodium fluoride is thought to then dissolve and deposit on the tooth[21]. Patients are able to rinse their mouths immediately but discouraged to eat or drink for 3-4 h. In clinical situation, it showed the efficacy to reduce DH instantly after painting on the dentin exposure area[52].

Mechanism of action of fluoride varnish is not yet clear but it was believed to be a result of CaF_2 crystal formation by chemical reaction between fluoride ions from fluoride varnish and calcium ions from dentin to occlude the dentinal tubules[22]. Generally, CaF_2 crystal is very small in size, approximately 0.05 μm, hence a single applying may not completely occlude the tubules. The other reason may be the occlusion of dentinal tubules by rosin in the varnish[4].

A clinical study revealed that most of patients tended to perceive the sensitivity at the beginning of the treatment and DH decreased after applying varnish once a week for 2-3 consecutive weeks. However, it was only temporary reliefs because CaF_2 crystal is a soluble substance that can be dissolved in saliva[55, 56]. Observation showed that fluoride varnish reduces the pain of DH for as long as the varnish is on the tooth[21]. Removal of smear layer and varnish in acidic condition enhanced the ion permeability[57]. Fluoride ions were exhibited to be released 56 to 67 percent of total fluoride ions in the varnish for up to 6 months after the varnish application[58].

Dental varnish in dentistry was extensively modified with the addition of several substances with regards to its good properties, for instance, easy applying,

requiring less patient's compliances and sustained releasing of some constituent agents[59, 60]. Chemicals in varnish could be released to its environment in 3 major ways, namely releasing into saliva, adhering to the pellicle to establish a reservoir that can slowly release the chemicals over time and penetrating into tooth substances[61].

CHAPTER III

Materials and Methods

FHA synthesis and analysis

FHA was synthesized by precipitation method as described by Okazaki *et al.*[62]. Apparatus for apatite synthesis was showed in Figure 1. We mixed 0.5 L of 0.2 M $Ca(CH_3COO)_2 \cdot H_2O$ and 0.5 L of 0.12 M $NH_4H_2PO_4$ containing 0.05 M NH_4F, with 1 L of CH_3COONH_4 buffer at 60±1°C and stirred for 3 h. The pH was maintained at 7.4±0.1 by the addition of NH_4OH. The solution was kept at room temperature for 24 h and the resultant slurry was filtered and dried at 60°C for 48 h. The chemical analysis were performed by X-ray photoelectron spectroscopy (XPS) (AXIS-HS, Kratos, Manchester, UK) and Fourier transmission infrared spectroscopy (FTIR) (FT 8400S, Shimadzu, Kyoto, Japan). The morphology of the powder was characterized by SEM (JSM-5410LV, JEOL, Tokyo, Japan).

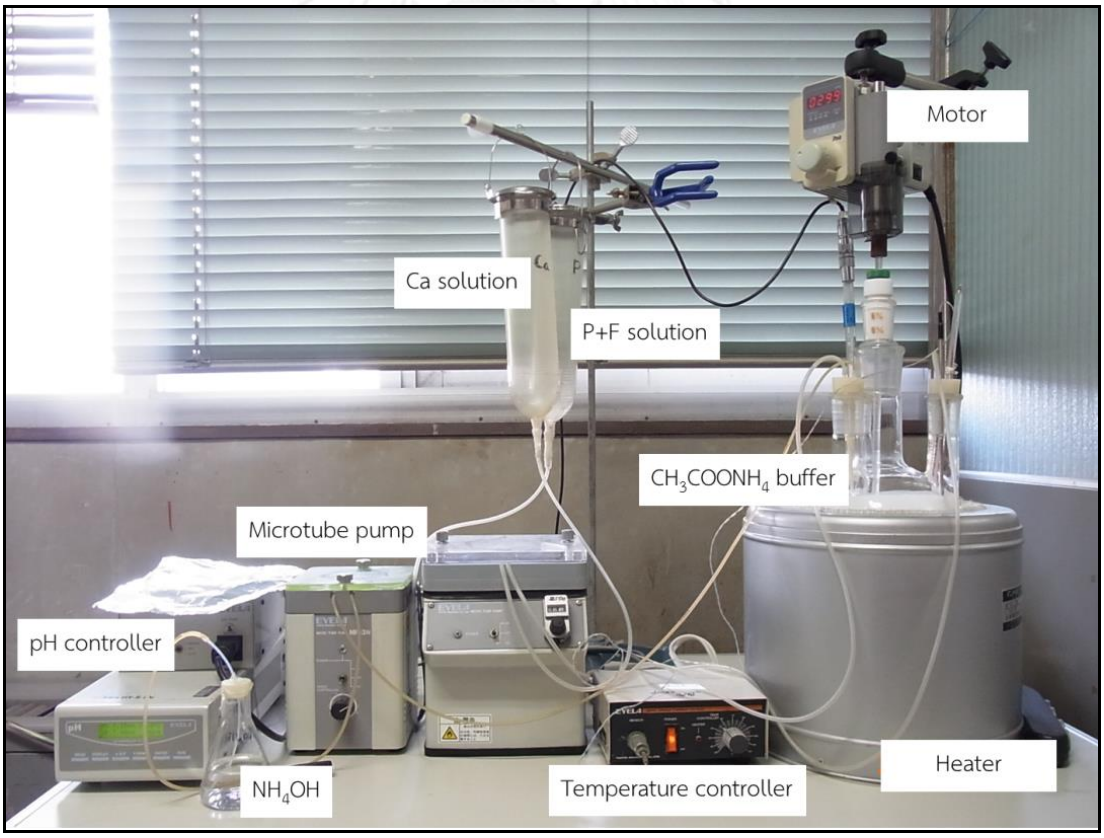

Figure 1 Apparatus for apatite synthesis by precipitation method

Varnish preparation

Analytical grade-chemicals, namely KCl (AnalaR® BDH, VWR International, Poole, England), fully hydrogenated rosin (Foral™ AX-E, Eastman Chemical BV, Capelle aan den IJssel, The Netherlands), hydrophilic fumed silica (Aerosil® 200 Pharma, Evonik Industries AG, Rheinfelden, Germany), absolute ethanol (Merck, Merck KGaA, Darmstadt, Germeny) and FHA were prepared. Four varnishes were formulated in order to investigate effects of each ingredient. Percentage of ingredients for each varnish formulation was reported in Table 1.

The "plain varnish" was prepared by mixing fully hydrogenated rosin and hydrophilic fumed silica with absolute ethanol in a clean ceramic mortar. The mixed varnish was filled in a capped syringe and stored at room temperature. The plain varnish represented the placebo.

The "KCl varnish" was prepared by mixing 10% (w/w) KCl, fully hydrogenated rosin and hydrophilic fumed silica with absolute ethanol in a clean ceramic mortar. The mixed varnish was filled in a capped syringe and stored at room temperature. This varnish represented a varnish with KCl as the active ingredient for nerve desensitization purpose.

The "FHA varnish" was prepared by mixing 10% (w/w) FHA, fully hydrogenated rosin and hydrophilic fumed silica with absolute ethanol in a clean ceramic mortar. The mixed varnish was filled in a capped syringe and stored at room temperature. This varnish represented a varnish with FHA as the active ingredient for dentinal tubule occlusion purpose.

For the tested varnish, 10% (w/w) KCl and 10% (w/w) FHA were prepared and mixed with fully hydrogenated rosin, hydrophilic fumed silica and absolute ethanol in a clean ceramic mortar. The mixed varnish was filled in a capped syringe and stored at room temperature. This varnish represented a varnish with two active ingredients, KCl and FHA, for dual mechanisms for DH management and was assigned as the "KCl-FHA varnish".

Table 1 Percentage of ingredients for each varnish formulation

Formulation	Rosin	Ethanol	Fumed silica	KCl	FHA
	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)
Plain varnish	62	35	3	0	0
FHA varnish	52	35	3	0	10
KCl varnish	52	35	3	10	0
KCl-FHA varnish	42	35	3	10	10

In addition, varnish formulation with different potassium compounds, different KCl and FHA concentrations were prepared in order to investigate the appropriate ingredient and proportion for this study. Percentage of ingredients for each varnish formulation was reported in Table 2, Table 3 and Table 4.

Table 2 Percentage of ingredients for varnish formulations with different potassium compounds

Formulation	Rosin	Ethanol	Fumed Silica	KCl	KNO_3	$K_3C_6H_5O_2$
	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)
KCl varnish	52	35	3	10	0	0
KNO_3 varnish	52	35	3	0	10	0
$K_3C_6H_5O_2$ varnish	52	35	3	0	0	10

Table 3 Percentage of ingredients for varnish formulations with different KCl concentrations

Formulation	Rosin	Ethanol	Fumed silica	KCl
	% (w/w)	% (w/w)	% (w/w)	% (w/w)
0% KCl varnish	62	35	3	0
5% KCl varnish	57	35	3	5
10% KCl varnish	52	35	3	10

Table 4 Percentage of ingredients for varnish formulations with different FHA concentrations

Formulation	Rosin % (w/w)	Ethanol % (w/w)	Fumed silica % (w/w)	FHA % (w/w)
0% FHA varnish	62	35	3	0
5% FHA varnish	57	35	3	5
10% FHA varnish	52	35	3	10
15% FHA varnish	47	35	3	15

Potassium ion and calcium ion release measurement

Sample preparation and method for measuring potassium ion and calcium ion release were followed the previous study on fluoride ion release from fluoride varnishes[63]. Forty-two 2×4 cm^2 polyester sheets were prepared and assigned to 7 groups (*n*=6/group): plain, 5% KCl, 10% KCl, KNO_3, $K_3C_6H_5O_2$, FHA and KCl-FHA varnishes. The sheets were painted with each varnish on half of the total area on one side (2×2 cm^2) and air dried at room temperature for 5 min. Each strip was weighed before and after varnish application to calculate the varnish weight. Each strip was then immersed in a capped container containing 20 mL of deionized water and changed to a new container at 10 min, 1 h, 3 h, and 6 h after the first immersion. The released potassium and calcium ions were measured from 10 mL of water from each container using inductively coupled plasma-optical emission spectrometry (ICP-OES) (OPTIMA 7300, PerkinElmer, Shelton, CN, USA). The results were normalized to the weight of each sample.

Dentin disc preparation

Dentin discs were obtained from extracted caries-free human third molar subsequent to appropriate informed written consents with an approval from ethical committee, Faculty of Dentistry, Chulalongkorn University (Document Number 033/2012). All teeth were stored in normal saline solution containing 1,000 units/mL penicillin. They were stored not more than 1 month. Coronal part was cut parallelly to the occlusal surface at 2 mm below the cemento-enamel junction and the

second section was made approximately 5 mm above the first section by a low-speed cutting machine (ISOMET 1000, Buehler, Lake Bluff, IL, USA) under water coolant. The pulpal tissue was removed with a pair of tissue forceps and avoided touching the soft surface lining of the pulp chamber. The occlusal surfaces of dentin discs were polished by a polishing machine (NANO 2000, Pace Technologies, Tucson, AZ, USA) with 400, 800, 1000 and 1200 grit paper, 1 and 0.5 μm diamond polishing compound. All dentin discs were cleaned by use of a digital ultrasonic cleaner in deionized water for 30 min and in 70% ethanol for 10 min[64].

Dentin permeability measurement

Seventy-five dentin discs were randomly assigned to 5 groups (*n*=15/group): Group 1: fluoride (F) varnish (Duraphat®, Colgate-Palmolive, New York, NY, USA); Group 2: plain varnish; Group 3: KCl varnish; Group 4: FHA varnish; Group 5: KCl-FHA varnish. The dentin discs were mounted with their occlusal surface up on the center of 3×3×1 cm^3 acrylic squares with a cyanoacrylate adhesive. A hole slightly smaller than the disc pulp chamber was drilled in the center of each acrylic square, and a 3 cm 18-gauge stainless tube was placed through the hole to abut the pulp chamber. The pulp chamber of the dentin disc was filled with sterile normal saline solution and any air bubbles were removed using a 23-gauge needle and syringe.

A 0.3 mm-diameter microcapillary tube was connected to the 18-gauge tube, which fixed with the acrylic square, and a manometer via medical tubing and maintained in a horizontal position. A tiny air bubble was created in the microcapillary tube and 60 mmHg pressure was applied. Apparatus for dentin permeability measurement was showed in Figure 2.

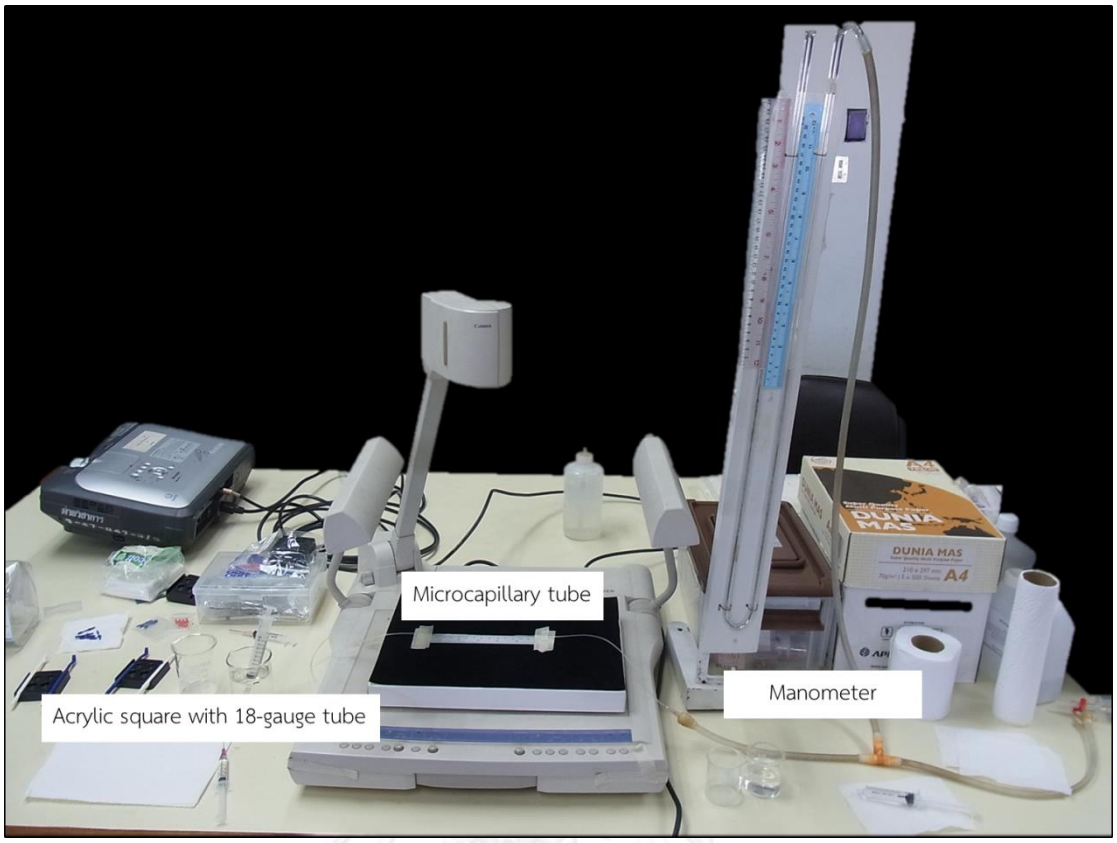

Figure 2 Apparatus for dentin permeability measurement

The movement of the air bubble in the microcapillary tube was recorded over 1 mm as the fluid flow rate (seconds per mm). This procedure was repeated three times for each sample and the average value was then calculated as hydraulic conductance (permeability) using the following equation;

$$Lp = \frac{Q}{A(P_1 - P_2)}$$

where Lp is the hydraulic conductance ($\mu L \cdot cm^{-2} \cdot min^{-1} \cdot cmH_2O$); Q is fluid flow rate ($\mu L \cdot min^{-1}$); P_1 is hydrostatic pressure above chamber (cmH_2O); P_2 is atmospheric pressure (cmH_2O) and A is the surface area (cm^2)[65]. Dentin discs were acid-etched with 37% phosphoric acid for 60 s and rinsed with distilled water for 60 s in order to establish the maximum permeability values of each dentin disc individually. The flow rate was determined as the maximum dentin permeability of each specimen. Once the maximum value was recorded, each specimen was painted by the assigned varnish on etched dentin surface and left for 5 min to evaporate the solvent. The air bubble velocity was measured and considered as the immediate hydraulic

conductance after the varnish application. With respect to the maximum hydraulic conductance, the permeability reduction percent (PR%) was calculated. The results were statistically analyzed by one-way ANOVA and the Bonferroni *post hoc* test using statistical software (PASW® Statistics 18, SPSS Inc., Chicago, IL, USA).

After dentin permeability measurement, each dentin disc was removed from the acrylic square and immersed in a closed container contained 20 mL of artificial saliva. They were placed in an incubator at 37°C for 6 h after varnish application on the tooth surface. Then the varnish layer that covered each dentin disc was removed by brushing with a soft bristle toothbrush and distilled water using cross brushing machine (V-8, SABRI Dental Enterprises Inc., Downers Grove, IL, USA) under brushing pressure of 200 g and strokes speed at 60 rpm for 2 min. Then all specimens were stored in artificial saliva and incubated at 37°C with 100% humidity for 12 h. All specimens were dried by placing in a desiccator for 72 h and gold sputtered with a Fine coater (JFC-1200, JEOL, Tokyo, Japan) for SEM observation.

To observe dentinal tubule occlusion property of 5% FHA varnish, 5 dentin discs were treated and observed by SEM with the similar method.

Cytotoxicity test

The primary explant cultures of human gingival fibroblasts and human pulpal fibroblasts were performed from the gingival and pulp tissues obtained from first premolar extractions due to orthodontic reasons. Informed written consents were requested of and obtained from healthy donor individuals aged between 18-30 years old with an approval from ethical committee, Faculty of Dentistry, Chulalongkorn University (Document Number 033/2012). Gingival and pulp tissues were collected, minced into small pieces and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) L-glutamine and 1% (v/v) antibiotic-antimycotic (Gibco®, Life Technologies, Grand Island, NY, USA). Both gingival and pulpal fibroblasts were maintained in culture dishes in humidified atmosphere of air containing 5% CO_2 at 37°C. The culture medium was changed every 2 days until cells reach their confluence. The cells then were subcultured several times. Cells from the fifth passages were used in this study.

Direct contact test

Fifty-four 5×5 mm^2 cover slips were prepared and washed with 70% and 95% ethanol. They were assigned to 6 groups (*n*=9/group): Group 1: F varnish; Group 2: plain varnish; Group 3: KCl varnish; Group 4: FHA varnish; Group 5: KCl-FHA varnish; Group 6: blank control. Each cover slip was applied with a 2 mm-diameter drop of each varnish and left for 5 min to evaporate the solvent. Samples were then placed on culture plates in direct contact with gingival fibroblasts plated with concentration 1×10^5 cells/mL and incubated in humidified atmosphere of air containing 5% CO_2 at 37°C for 48 h. The culture plates were investigated by an inverted phase contrast microscope (CKX41, Olympus, Tokyo, Japan) and recorded using an imaging software (cellSens Standard software, Olympus, Tokyo, Japan).

MTT assay

Cell viability was measured by using an MTT assay. Polyester sheet was trimmed into one hundred and eight 5-mm-diameter discs and washed with 70% and 95% ethanol. The discs were assigned to 12 groups (*n*=9/group): Group 1: F varnish; Group 2: plain varnish; Group 3: KCl varnish; Group 4: FHA varnish; Group 5: KCl-FHA varnish; Group 6: blank control (6 groups for gingival fibroblasts and another 6 groups for pulpal fibroblasts). The upper surface of each disc was applied with the assigned varnish and left for 5 min to evaporate the solvent. Empty discs served as the blank control group. To compare the relative toxicities of different varnishes, 24-well culture plates were seeded with either gingival fibroblasts or pulpal fibroblasts (2×10^4 cells/well) 24 h before exposure to the discs with or without the varnishes. Then the plates were incubated in humidified atmosphere of air containing 5% CO_2 at 37°C for 48 h. A 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, M5655, Sigma-Aldrich, St. Louis, MO, USA) was prepared. After 48 h, the culture medium in each well was aspirated and 5 µL of the MTT solution and 300 µL of DMEM without phenol red were added. The 24-well plates were incubated for 4 h in order to form insoluble purple formazan crystals by functional mitochondria in viable cells. After 4-h incubation, the supernatant in each well was replaced by 1 mL of dimethyl sulfoxide (DMSO). The plates were placed on an orbital shaker for 10 min to thoroughly dissolve the formazan crystals. The absorbance of each well was determined by using a spectrophotometer (Ultrospec 3000, Pharmacia Biotech (Biochrom), Cambridge, England) at wavelength 570 nm.

Each optical density (OD) was compared with respect to the blank control. The results were statistically analyzed by one-way ANOVA and the Bonferroni *post hoc* test using statistical software.

CHAPTER IV
Results

FHA chemical analysis and morphology

The XPS wide spectrum of the FHA powder (Figure 3) and the surface chemical analysis (

Table 5) indicated the existence of calcium, phosphorus, oxygen, carbon and fluorine as elements in FHA powder. The bands of FTIR analysis at 564 cm^{-1}, 601 cm^{-1}, 961 cm^{-1}, 1032 cm^{-1} and 1092 cm^{-1} correspond to the PO_4^{3-} functional group (Figure 4). The peak at 1644 cm^{-1} corresponds to the CO_3^{2-} functional group. SEM images showed typical needle-like crystals of FHA powder approximately 10-50 μm in aggregated globule size and 2-5 μm in particle size (Figure 5a, b).

Table 5 Chemical analysis of the FHA powder

Peak	Position BE (eV)	Atomic Concentration (%)	Mass Concentration (%)
O 1s	528.400	54.79	38.31
Ca 2p	344.700	20.61	36.09
P 2p	130.600	13.29	17.98
C 1s	282.800	5.79	3.04
F 1s	681.500	5.53	4.59

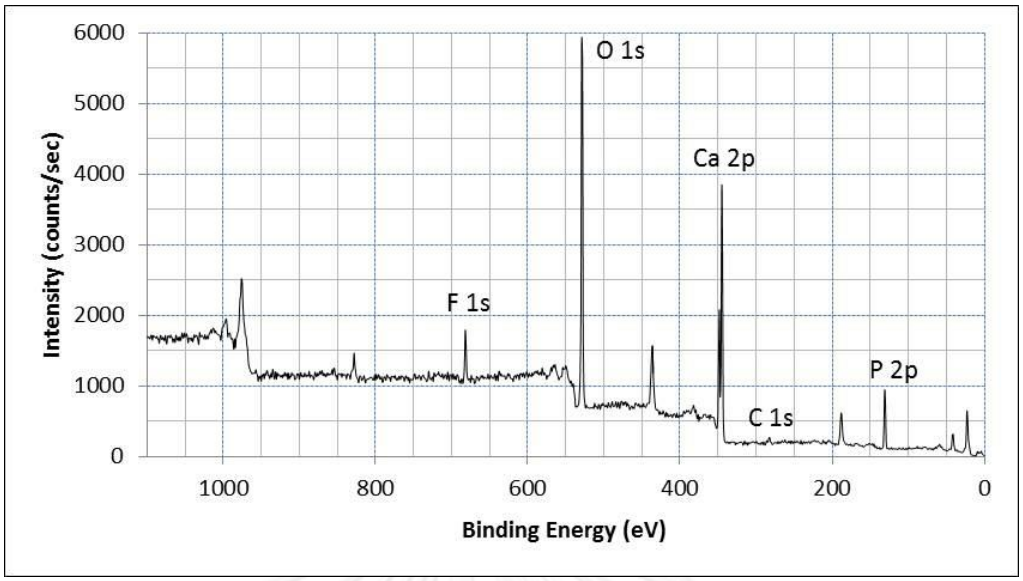

Figure 3 XPS wide spectrum (0-1100 eV) of the FHA powder exhibiting P 2p, C 1s, Ca 2p, O 1s and F 1s peaks at 130, 282, 344, 528 and 681 eV

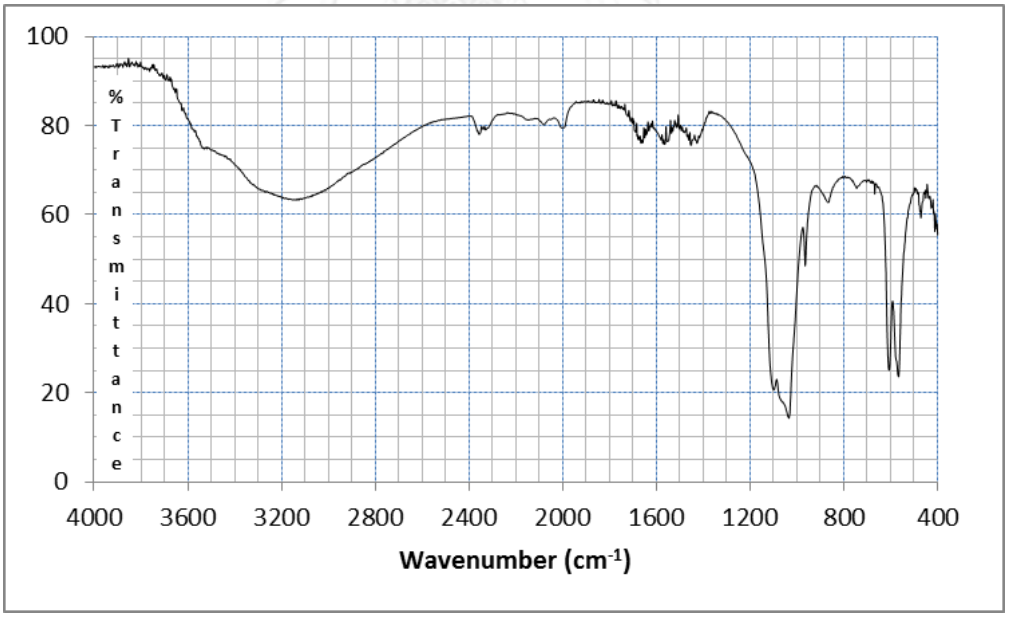

Figure 4 FTIR spectra of the FHA powder exhibiting PO_4^{3-} peaks at 564, 601, 961 and 1032 cm^{-1}

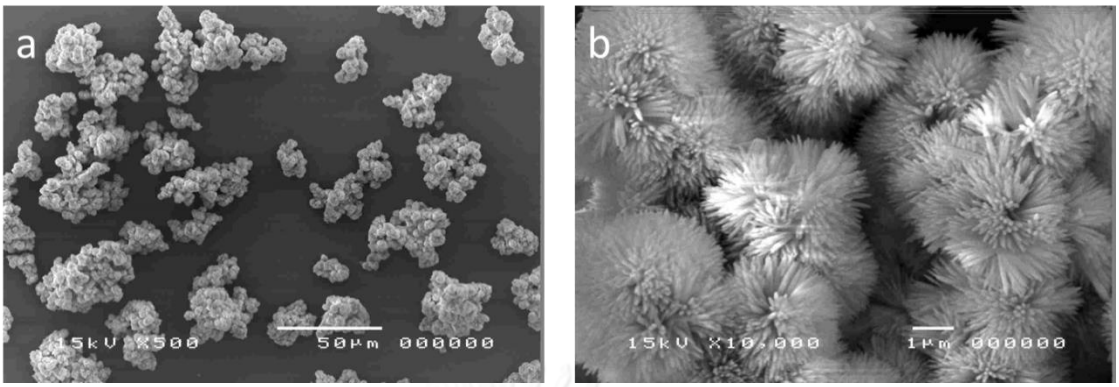

Figure 5 Representative SEM images of the FHA powder (a) at 500× magnification and (b) at 10,000× magnification

Potassium ion release

Amount of potassium ion released at different time points and accumulated concentrations of all varnishes were reported in Table 6.

The KCl varnish showed higher amount of potassium ion release compared with the KCl-FHA varnish at 10 min, 1 h and 3h. In contrast, the KCl-FHA varnish released higher amount of potassium ions than the KCl varnish at 6 h.

In terms of accumulated amount of potassium ion release, the KCl revealed higher amount than the KCl-FHA varnish over 6 h. The average cumulative potassium ion release of both varnishes over 6 h was illustrated in Figure 6.

The average of the total amount of potassium ion release from the KCl and KCl-FHA varnishes over 6 h were 172.5 and 129.2 ppm respectively (Table 6). The highest rates of ion release were at 10 min after immersion determined by the average slope (Figure 6).

The cumulative potassium ion release from KCl, KNO_3 and $K_3C_6H_5O_2$ varnishes over 6 h were illustrated in Figure 7. KCl varnish released higher amount of potassium ions than KNO_3 varnish and both KCl and KNO_3 varnish released higher amount of potassium ions than $K_3C_6H_5O_2$ varnish.

The cumulative potassium ion release from 0% KCl, 5% KCl and 10% KCl varnishes over 6 h were illustrated in Figure 8. The increase in amount of KCl resulted in a significant increase in potassium ion release rate. The formulation containing 10% of KCl released the highest amount of potassium ions over 6 h, and this formulation was chosen for all tests in this study.

Table 6 Amount of potassium ions released from different varnishes at different time points and accumulated concentrations (in brackets)

Group	Mean Concentration (ppm)			
	10 min	60 min	180 min	360 min
Plain	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
KCl	58.12 (58.12)	59.63 (117.7)	44.44 (162.2)	10.34 (172.5)
FHA	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
KCl-FHA	35.92 (35.92)	41.06 (76.99)	39.52 (116.5)	12.65 (129.2)

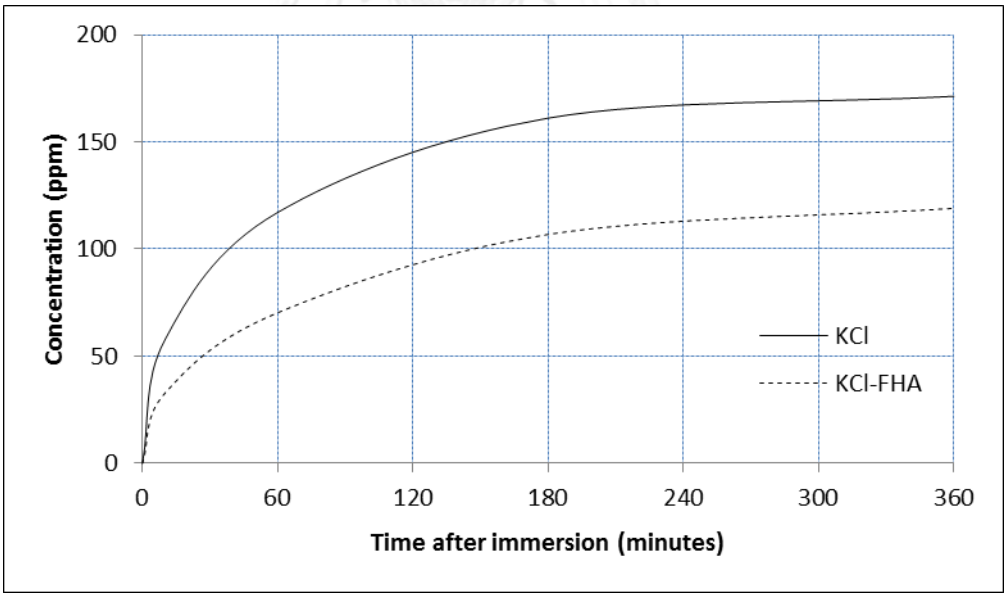

Figure 6 Average cumulative potassium ion release from KCl and KCl-FHA varnishes over 6 h (*n*=6)

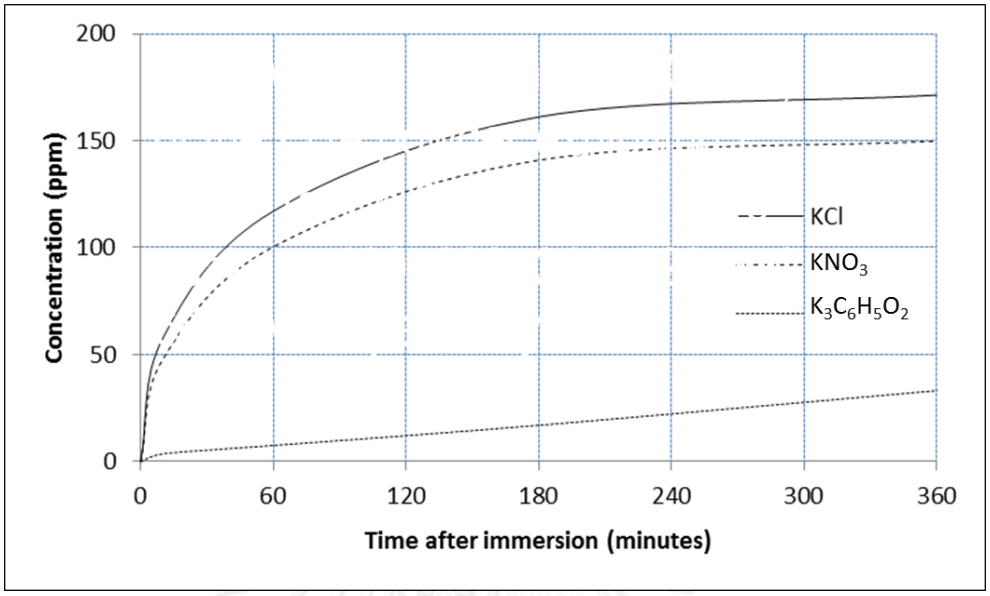

Figure 7 Average cumulative potassium ion release from KCl, KNO_3 and $K_3C_6H_5O_2$ varnishes over 6 h (*n*=6)

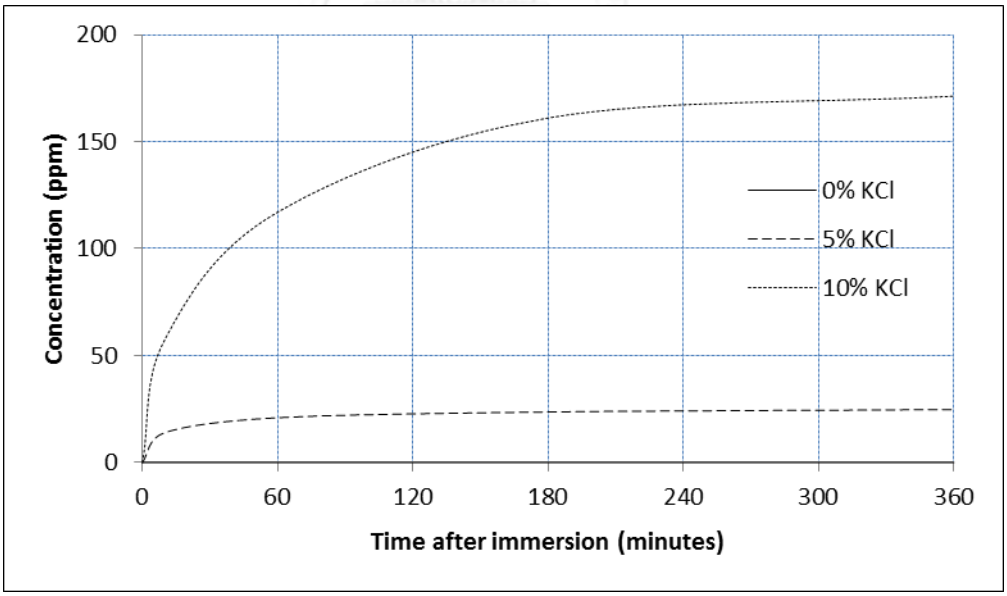

Figure 8 Average cumulative potassium ion release from 0% KCl, 5% KCl and 10% KCl varnishes over 6 h (*n*=6)

Calcium ion release

The average of the total amount of calcium ion release from all varnishes over 6 h was reported in Table 7. Accumulated concentrations were reported in Figure 9. The KCl-FHA varnish showed the highest amount of calcium ion release followed by FHA varnish over 6 h. Calcium ions released from KCl and plain varnishes were lower than KCl-FHA and FHA varnishes over 6 h.

Table 7 Amount of calcium ions released from different varnishes at different time points and accumulated concentrations (in brackets)

Group	Mean Concentration (ppm)			
	10 min	60 min	180 min	360 min
Plain	0.000 (0.000)	0.005 (0.005)	0.117 (0.122)	0.074 (0.196)
FHA	0.000 (0.000)	0.037 (0.037)	0.210 (0.247)	0.213 (0.461)
KCl	0.015 (0.015)	0.071 (0.085)	0.038 (0.124)	0.058 (0.182)
KCl-FHA	0.000 (0.000)	0.038 (0.038)	0.149 (0.186)	0.988 (1.18)

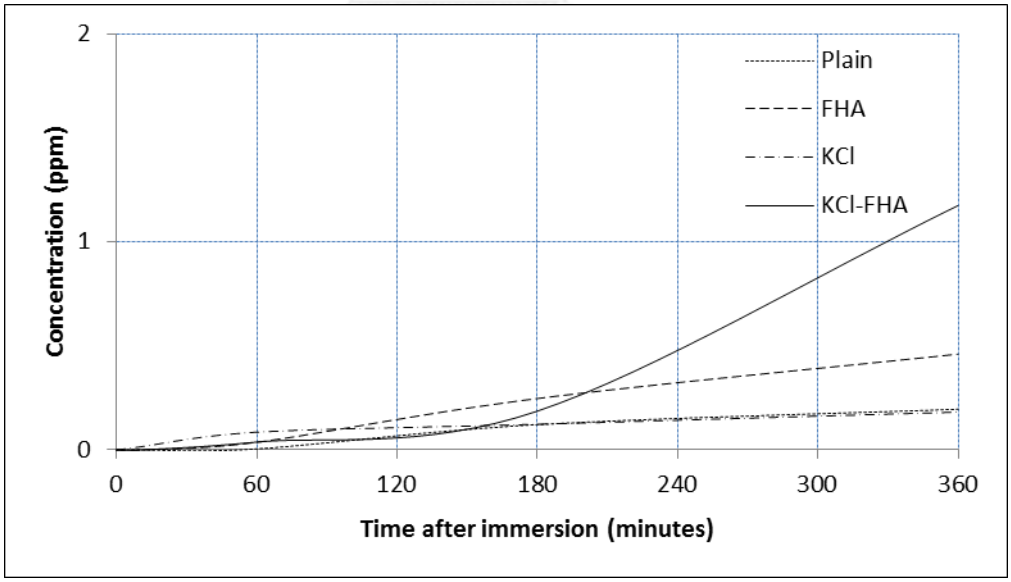

Figure 9 Average cumulative calcium ion release from different varnishes over 6 h (n=6).

Dentin permeability reduction

Figure 10 showed the average percent reduction in hydraulic conductance of the 5 varnish groups. The highest reduction was observed in the KCl-FHA varnish group at 54.02 percent, followed by the FHA varnish group at a slightly lower percentage reduction 48.13 percent. The difference between these groups was not significant ($p>0.05$). The hydraulic conductance reduction of the F varnish group (38.56 percent) was significantly lower than that of the KCl-FHA varnish and the FHA varnish groups ($p<0.05$) and higher, but not significantly, than the KCl varnish group (32.49 percent) ($p>0.05$). The plain varnish group had the lowest percent reduction at 23.17 percent, which was significantly different compared to the other groups ($p<0.05$).

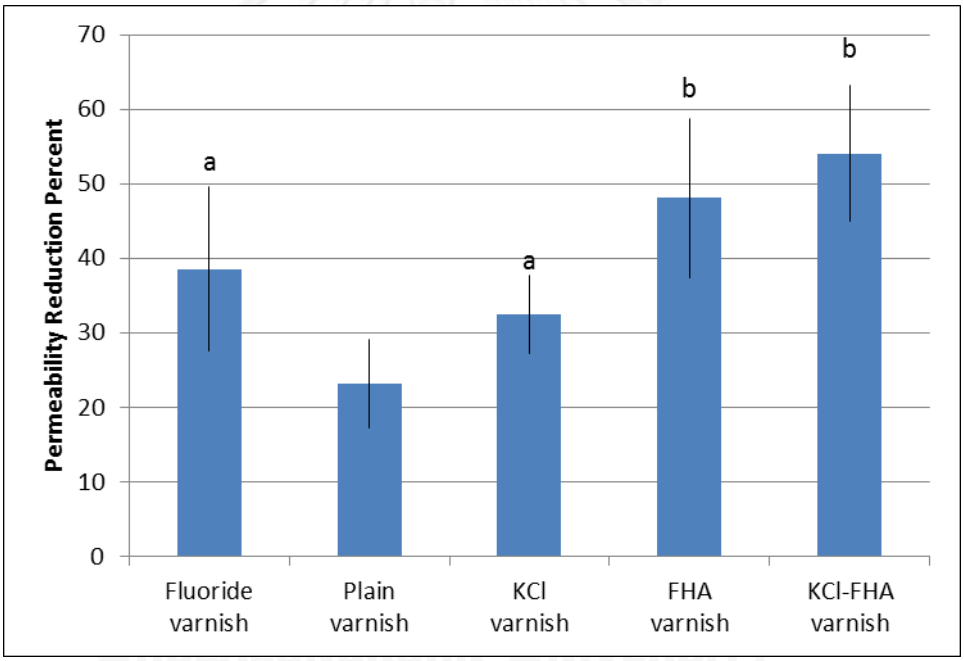

Figure 10 Average PR% from dentin permeability measurement after each specimen was painted with the assigned varnish and left for 5 min with respect to the maximum hydraulic conductance (n=15/group) (Same superscript letters signifies no significant difference ($p>0.05$).)

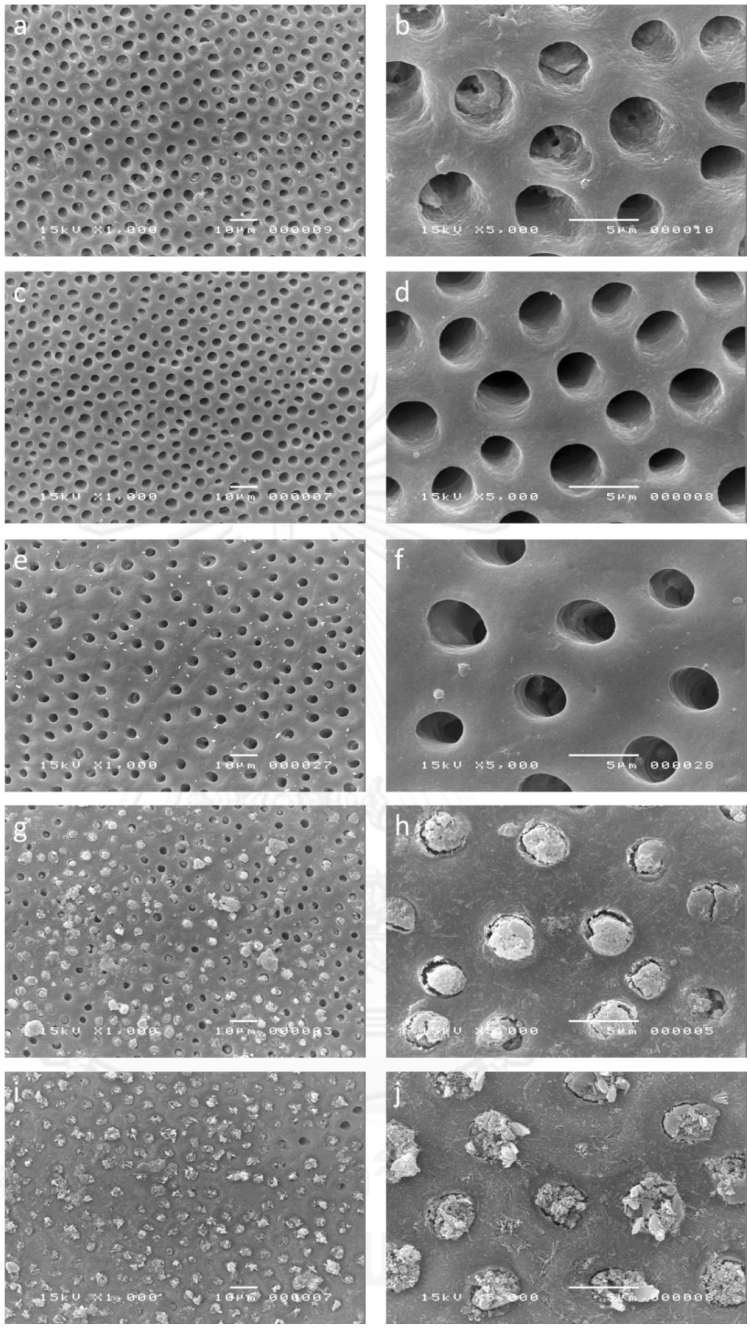

Figure 11 Representative SEM images of the dentin discs after treatment with the 5 types of varnishes and stored in artificial saliva for 12 h; (a, b) F varnish, (c, d) plain varnish, (e, f) KCl varnish, (g, h) FHA varnish, (i, j) KCl-FHA varnish, at 1,000× and 5,000× magnification respectively

Dentinal tubule occlusion

We used SEM to analyze the ability of the 5 vanishes to occlude the dentinal tubules of dentin discs (Figure 11). Representative SEM images demonstrated that the F (Figure 11a, b), plain (Figure 11c, d), and KCl (Figure 11e, f) varnishes did not occlude the dentinal tubules. The FHA varnish was able to occlude many of the dentinal tubules (Figure 11g, h). Most, but not all, of the dentinal tubules were observed to be occluded by the KCl-FHA varnish (Figure 11i, j).

We also observed the dentinal tubule occlusion of dentin discs treated with 5% FHA varnish. Only few dentinal tubules were occluded according to representative SEM images in Figure 12.

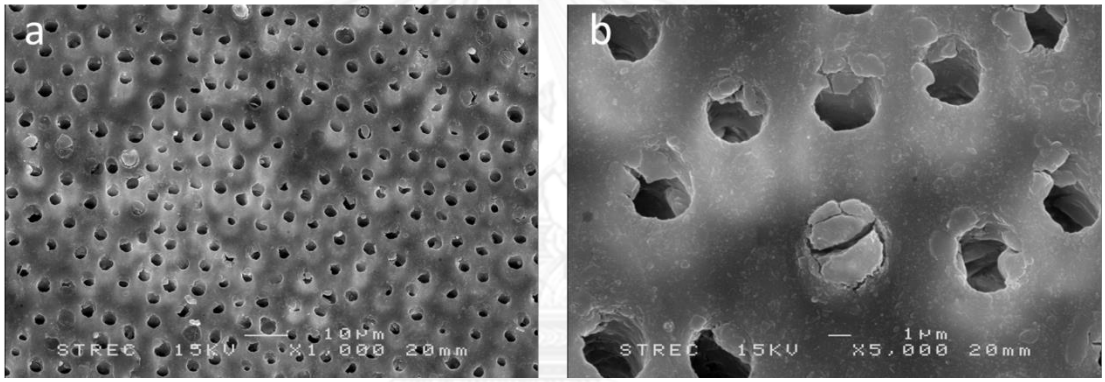

Figure 12 Representative SEM images of dentin discs after treatment with 5% FHA varnish and storage in artificial saliva for 12 h (a) at 1,000× magnification and (b) at 5,000× magnification

Direct contact test

To investigate the biocompatibility of the vanishes with gingival fibroblasts compared to that of commercial F varnish, we performed a direct contact assay. Phase contrast images indicated that after 24 h the gingival fibroblasts presented a typical fusiform morphology, with some round cells observed (Figure 13). While the cells did not attach directly to the varnish drops, they were present in close proximity to both the F varnish (Figure 13a) and the KCl-FHA (Figure 13i) varnish drops.

After 48 h, the living gingival fibroblasts had proliferated, but still did not contact the F (Figure 13b) or KCl-FHA (Figure 13j) varnish drops.

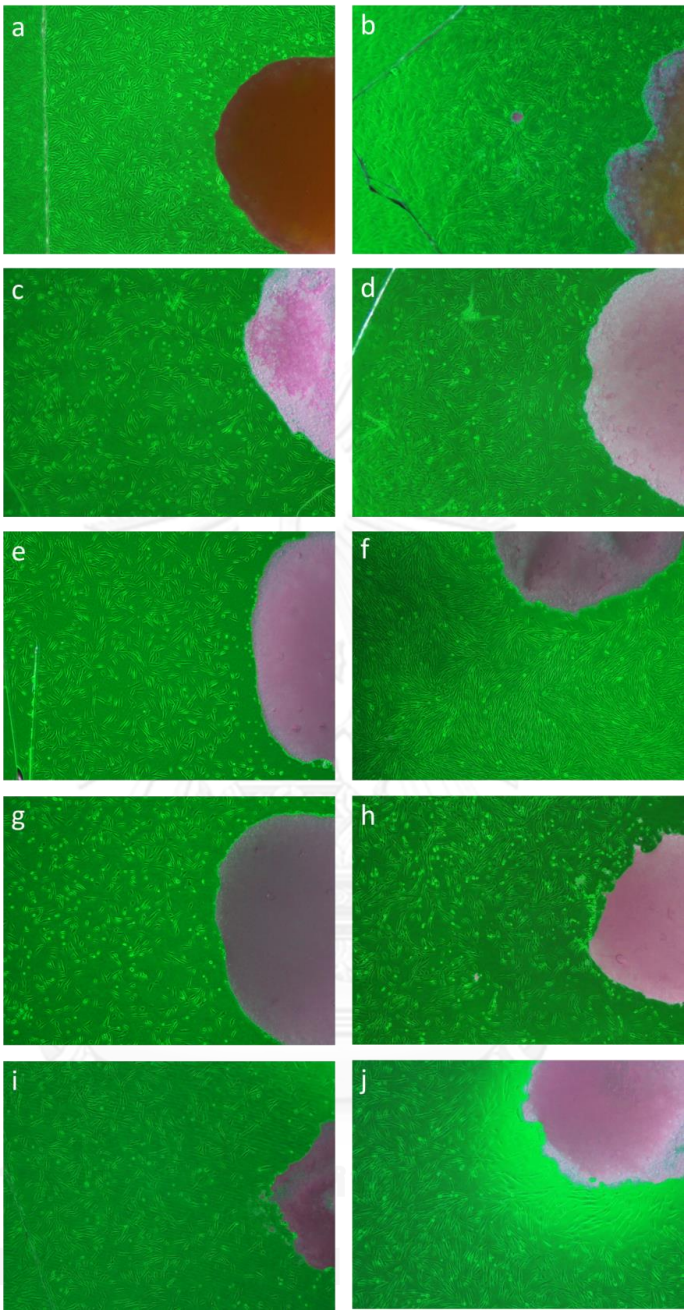

Figure 13 Phase contrast images from direct contact test of gingival fibroblasts interaction with 5 types of varnishes; (a, b) F varnish, (c, d) plain varnish, (e, f) KCl varnish, (g, h) FHA varnish, (i, j) KCl-FHA varnish, at 24 and 48 h respectively

MTT assay

The biocompatibility of the KCl-FHA varnish determined by cell viability was not significantly different from the F varnish ($p>0.05$). All varnishes showed slightly higher cell viability than blank control Figure 14. The F varnish showed the higher amount of cell viability than the KCl-FHA group. However, multiple comparisons revealed there was no statistically difference among all groups ($p>0.05$).

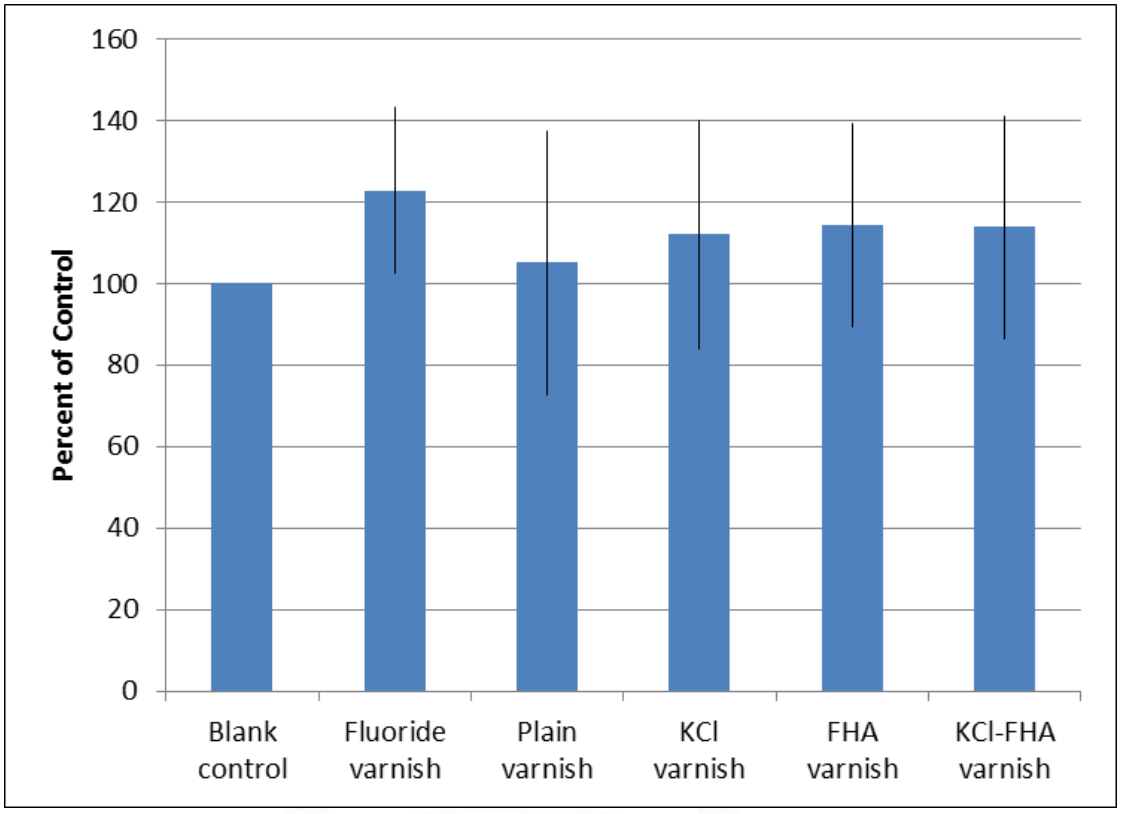

Figure 14 Average percentage of gingival fibroblast viability from MTT assay after 48 h incubation with different varnishes compared to blank control (There is no significant difference between any groups ($p>0.05$).)

The biocompatibility of varnishes to pulpal fibroblasts was quite similar to gingival fibroblasts. The F varnish showed the higher amount of cell viability than the KCl-FHA group but it was not different significantly ($p>0.05$). All varnishes except the plain varnish showed slightly higher cell viability than blank control (Figure 15). Multiple comparisons revealed there was a significant difference between both varnishes to blank control ($p<0.05$).

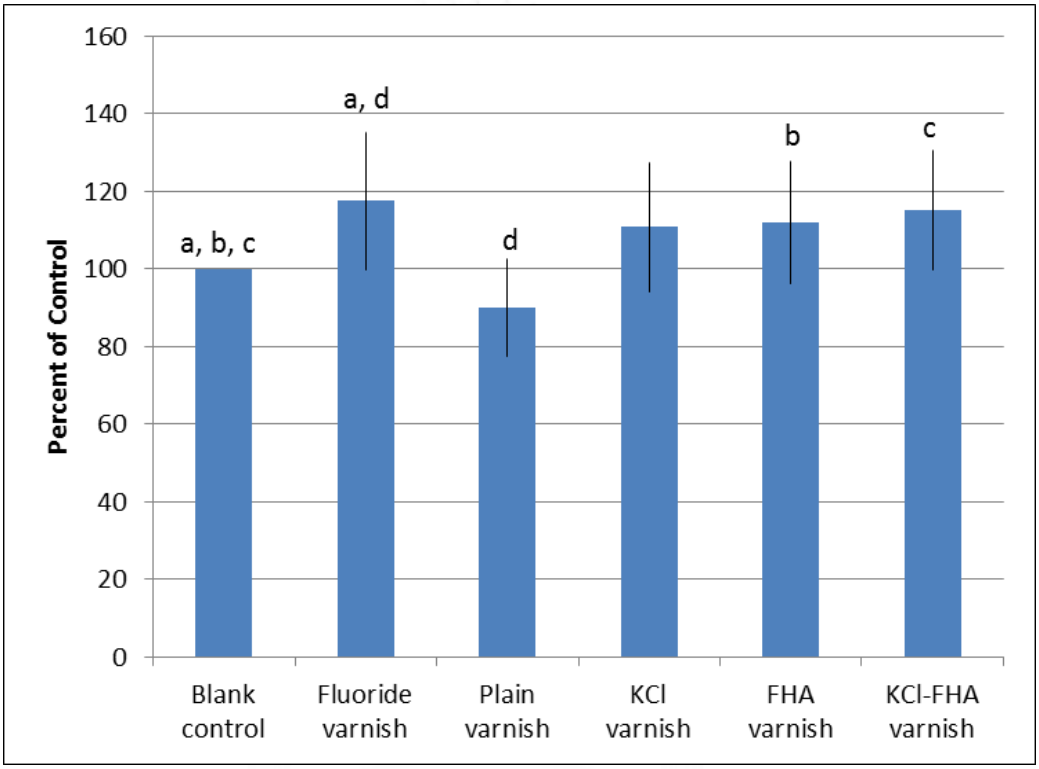

Figure 15 Average percentage of pulpal fibroblast viability from MTT assay after 48 h incubation with different varnishes compared to blank control (Same superscript letters signifies the significant differences ($p<0.05$).)

CHAPTER V
Discussion

In the present study we synthesized a novel rosin based varnish containing KCl and FHA for use in treating DH. We assayed the physical characteristics and cytotoxicity of the novel varnish and found that the KCl-FHA varnish could release potassium ions and reduce the hydraulic conductance of dentin discs. In addition, we determined that the KCl-FHA varnish exhibited toxicity similar to that of commercial fluoride varnish.

Varnish formulation

The objective of using varnish in this study is due to its sustained release property. Moreover, it is easy to apply so patients can use it by themselves. Even though gel is widely used in dentistry, especially fluoride gel which used for fluoride ions delivery to tooth surface, it has some limitations. One of them is its limited contact time to tooth surface. Thus, it should contain much higher concentration of potassium ions and be controlled by the Food and Drug Administration regulations regarding the toxicity of high potassium ion concentration. Consequently, it would be limited for professional use only. Because of these reasons, varnish was chosen to formulate a novel material in this study.

In summary, four varnishes were formulated to test their physical properties and biological responses. The plain varnish represented a varnish with no active ingredient and a negative control for the entire study. The 10% KCl varnish was assigned as the “KCl varnish” which represented a varnish with KCl as the active ingredient for nerve desensitization purpose and a negative control for the dentin permeability measurement and the observation of dentinal tubule occlusion. The 10% FHA varnish was assigned as the “FHA varnish” which represented a varnish with FHA as the active ingredient for dentinal tubule occlusion purpose and a negative control for the measurement of potassium ion release. The KCl-FHA varnish represented a varnish with two active ingredients, KCl and FHA, for dual mechanisms for DH management.

Potassium ion release

KCl, KNO_3 and $K_3C_6H_5O_2$ are the effective desensitizing agents that mostly used in DH management[31]. Consequently, they were selected for the measurement of potassium ion release in this study. These compounds were prepared and incorporated to varnishes with 10% (w/w) concentration. The aim was to compare the amount of potassium ion release.

Based on a study on sustained-release of clotrimazole from clotrimazole varnish[60], our study measured potassium ion release from four different immersion periods after varnish application. The 10-min time point represents immediate potassium ion release which results in immediate desensitizing effect of the varnish. The 6-h time point represents expected duration of which varnish attached on tooth surface before removal by toothbrushing. The 1-h and 3-h time points represent intermediate points of time between 10-min and 6-h time points.

Incorporation of KCl, KNO_3 and $K_3C_6H_5O_2$ into rosin-based varnish can result in the release of potassium ions when immersed in deionized water. The ion releasing rate was inversely proportional to the time. Most of potassium ions were released from the varnishes in the first 3 h. Among 3 different potassium compounds, KCl showed the highest amount of potassium ion release followed closely by the KNO_3 varnish. The varnish containing $K_3C_6H_5O_2$ released dramatically lower amount of potassium ions.

In terms of DH management, the KCl and KNO_3 varnishes showed their potentials to provide large amount of potassium ions which will increase the concentration of potassium ions in saliva or dentinal fluid after applying on tooth surface with dentin exposure. However, the KCl varnish showed higher amount of potassium ion release than the KNO_3 varnish. On the other hand, the $K_3C_6H_5O_2$ varnish released such little amount of potassium ions over 6 h. These may result from the molecular weight differences. KCl has the highest proportion of potassium with respect to its molecular weight when compared with KNO_3 and $K_3C_6H_5O_2$. Thus, KCl was chosen for formulating the varnishes in this study. However, a clinical study by Ajcharanukul *et al.*[32] indicated that effect of the KCl was due to the potassium ions and not to the chloride ions. Hence, the chloride ion release measurement did not conduct in this study.

In addition, 10% (w/w) incorporation of potassium compounds were based on the effective amount of the compound which reported its efficacy for DH relief[36]. However, 0% and 5% (w/w) of KCl also prepared and incorporated to varnishes in

order to investigate the appropriate concentration. Figure 8 illustrated the average cumulative potassium ion release from the 0% KCl, 5% KCl and 10% KCl varnishes. It revealed that the higher percentage of KCl incorporation in the varnish resulted in the higher amount of potassium ion release. The potassium ion release characteristic was similar to the release of clotrimazole from clotrimazole varnish in previous study[60]. The 10% KCl varnish showed approximately 6 times of the released ions to the 5% KCl varnish. Thus, 10% of KCl was chosen for formulating the varnishes in this study.

Results from the measurement of potassium ion release suggested that both the KCl and KCl-FHA varnishes showed their potentials to release potassium ions over 6 h. The KCl varnish released higher amount of potassium ions than the KCl-FHA varnish at 10 min, 1 h and 3h. In contrast, the KCl-FHA varnish released higher amount of potassium ions than the KCl varnish at 6 h. This may result from the temporary binding of potassium ions and FHA which caused the slower release of potassium ions from the KCl-FHA varnish. This would be an advantage for DH management in terms of the sustained effect.

However, *in vivo* and clinical studies are required to investigate the degree of nerve desensitization induced by the varnish containing KCl to identify the appropriate percentage of KCl to add into the varnish. If 10% KCl is insufficient, a higher percentage of KCl incorporation should be considered and tested. The amount of potassium ions required to be released to induce nerve desensitization after each varnish application on the dentin surface should also be investigated.

Calcium ions released from all varnishes were by far lower than potassium ions. The varnish containing FHA showed higher amount of calcium ions. These may be accounted for the dissolution of calcium from the apatite.

Dentin permeability reduction and dentinal tubule occlusion

The XPS surface chemical analysis revealed the basic elements found in hydroxyapatite and also showed the presence of fluorine (Figure 3 and Table 5). The FTIR spectra demonstrated the typical spectra of FHA (Figure 4). The SEM images of the apatite from our study (Figure 5b) showed the typical morphology of FHA powder, and resembled the FHA synthesized by Okazaki *et al*.[66]. All of the above confirmed that the synthesized apatite was FHA.

Regarding to the amount of FHA, 0%, 5%, 10% and 15% of FHA were prepared and incorporated into the varnishes. However, the 15% FHA varnish was not able to mix homogeneously with the other ingredients due to the large amount of FHA powder. Hence, it was considered not to formulate for any test in this study. Obvious dentinal tubule occlusion was found from the 10% FHA varnish specimens. The 5% FHA varnish showed partial occlusion of dentinal tubules (Figure 12) while the 0% FHA varnish showed no occlusion. Thus 10% of FHA was chosen for formulating the varnishes in this study.

Though SEM observation can demonstrate the degree of dentinal tubule occlusion, it tends to be only a qualitative measurement. Representative SEM images may be selected under examiner's preference. Consequently, we also conducted the dentin permeability test as a quantitative measurement. There were several automated apparatus designed to measure the fluid flow or the dentin permeability[65, 67-73]. The measurement system and apparatus used in this study were modified to fit with the application of varnish on dentin disc surface.

Adding the FHA into rosin-based varnish showed the highest reduction of dentin permeability among all groups and showed a significant reduction compared to the F varnish. This may infer that the superior reduction of dentin permeability of varnishes containing FHA was the result from dentinal tubule occlusion by the apatite.

For the other groups, the effects may come from the occlusion by rosin or other compositions, such as KCl or NaF as some authors reported previously[4, 57, 74]. According to NaF, although mechanism of action of fluoride varnish is not yet clear, it was believed that the occlusion of dentinal tubules was the result from CaF_2 crystal formation, by chemical reaction between fluoride ions from fluoride varnish and calcium ions from dentin[22] or it may be as a result of occlusion of the tubules by the resin[21]. Generally, CaF_2 crystal is very small in size, approximately 0.05 μm. Hence, a single application may not completely occlude the tubules. Moreover, CaF_2 crystal is a soluble substance, thereby, it can be dissolved into the saliva according to the Gibbs free energy[41, 55, 56]. The SEM images also showed the patent dentinal tubules in the F varnish group after treatment and immersion in artificial saliva for 12 h. However, there is no study reported regarding KCl effect to the dentin permeability reduction. Potassium and chloride solutions were tested and reported with no effect to hydraulic conductance[65]. Hence, the reduction should result from

the direct occlusion of KCl particles to dentinal tubule orifices. Further study should be conducted to test this hypothesis.

In contrast, FHA is not soluble confirmed with the SEM images after treatment and immersion in artificial saliva. They also revealed the dentinal tubule occlusion only in the varnish containing FHA groups. These precipitates might be the FHA itself from the FHA containing varnishes or the new insoluble compounds which formed by the elements from artificial saliva interacted with the FHA and/or the tooth. Thus the chemical analysis of the precipitates should be conducted in future study.

One of the ideal properties of desensitizing agents is to have a long-lasting effect. The effectiveness of the tubular occluding agents will depend on their resistance to removal. *In vitro* results demonstrate that a number of agents can occlude tubules, but this does not necessarily correlate with the *in vivo* situation when there must be resistant to the oral challenges of day-to-day activity[21].

Although the present study demonstrates the ability of the FHA containing varnishes to produce precipitates that occluded dentinal tubules after immersion in artificial saliva over 12 h, it only represents the situation without food or beverage consumption. Consequently, an acid resistance test is suggested in future studies to determine the long-lasting effect of this biomaterial.

Combination of KCl and FHA

The combination of incorporating KCl and FHA did not decrease the property to reduce dentin permeability as the KCl-FHA varnish showed no significant difference to the FHA varnish in terms of dentin permeability reduction percent. The KCl-FHA varnish tended to reduce dentin permeability greater than the FHA varnish. With this regard, KCl may be the cause of this escalation since the KCl varnish showed significantly higher dentin permeability reduction than the plain varnish. Thus, from this study the KCl and FHA revealed no antagonistic effect and tended to have the additive effect when formulated to the varnish in terms of the dentinal tubule occlusion.

Two treatment modalities are used in the DH treatment: alteration of fluid flow in the tubules and modification or blocking of the pulpal nerve response[21]. In accordance with our results, the KCl-FHA varnish appeared to be the promising dental biomaterial for DH management purpose. This is because it was able to release potassium ions and occlude tubules.

Biological responses

In terms of biological response measurement, two assays were chosen for different purposes. Firstly, the direct contact test was designed to test the safety of this novel material when applied on tooth surface. The varnish may be accidentally contacted with adjacent gingiva. Secondly, due to the material property, the MTT assay was designed to test safety profile of the ion release. Apart from the gingival fibroblast, the pulpal fibroblast was included in this test since the ions released from the varnish may be able to diffuse along the dentinal tubules and reach the pulp cavity.

The KCl-FHA varnish showed its biocompatibility with both gingival and pulpal fibroblasts with respect to the F varnish. With direct contact test, the tested varnishes showed good performance only with gingival fibroblasts as the varnishes were designed to paint on dentin surface with no pulp exposure. With regards to gingival cells, they were able to attach nearby the varnish drops in the same manner as they reacted with the F varnish. Less than 20% of the cells were round or showed changes in morphology in both groups. Moreover, cells showed their ability to proliferate in the next 24 h. These were considered as no cytotoxic effects in the KCl-FHA varnish and the F varnish based on ISO 10993-5 biological evaluation of medical devices.

MTT assay also presented the cytotoxicity results in the same manner. All varnishes exhibited the number of living gingival cells with no significant difference to the blanks. Furthermore, all varnishes except the plain varnish manifested more viable pulpal fibroblasts than the blanks significantly. This might be a result of the released calcium, chloride or potassium ions from the varnishes. Because each of the OD was compared with respect to the blank control, the blanks were compared with themselves. Therefore, there was no standard deviation in the blank control groups.

From the direct contact test and MTT assay results, they suggested that the KCl-FHA varnish was biocompatible with gingival and pulpal fibroblasts with respect to the F varnish and appeared to be safe for applying on dentin surfaces with no pulp exposure.

Results from this study suggested that the novel varnish might be developed for an in-office product for professional use or a home use product for DH

management. For example, dentists may apply the varnish on any exposed dentin surface with minimal loss of tooth structure where restoration is not required. Moreover, patients may apply it on their sensitive areas routinely for the long lasting effect under supervision of dentists. However, it needs further researches to confirm the clinical efficacy and safety of this biomaterial.

CHAPTER VI

Conclusion

This study introduced the novel biomaterial for DH treatment, the varnish containing 10% (w/w) KCl and 10% (w/w) FHA, with two approaches; release potassium ions for nerve desensitization and dentinal tubule occlusion by the FHA. The *in vitro* tests of the varnish properties and biological responses exhibited the potential of this promising biomaterial for DH management.

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APPENDIX

Statistical analysis

Dentin permeability reduction

Test of Homogeneity of Variances

PR

Levene Statistic	df1	df2	Sig.
2.211	4	70	.077

ANOVA

PR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9023.066	4	2255.767	29.602	.000
Within Groups	5334.247	70	76.204		
Total	14357.314	74			

Multiple Comparisons

PR

Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Plain	KCl	9.3200084*	3.1875493	.047	.080151	18.559865
	FHA	24.9557603*	3.1875493	.000	15.715903	34.195617
	KCl-FHA	30.8451925*	3.1875493	.000	21.605336	40.085049
	F varnish	15.3828614*	3.1875493	.000	6.143004	24.622718
KCl	Plain	-9.3200084*	3.1875493	.047	-18.559865	-.080151
	FHA	15.6357520*	3.1875493	.000	6.395895	24.875609
	KCl-FHA	21.5251842*	3.1875493	.000	12.285327	30.765041
	F varnish	6.0628531	3.1875493	.613	-3.177004	15.302710
FHA	Plain	-24.9557603*	3.1875493	.000	-34.195617	-15.715903
	KCl	-15.6357520*	3.1875493	.000	-24.875609	-6.395895
	KCl-FHA	5.8894322	3.1875493	.689	-3.350425	15.129289
	F varnish	-9.5728989*	3.1875493	.037	-18.812756	-.333042
KCl-FHA	Plain	-30.8451925*	3.1875493	.000	-40.085049	-21.605336
	KCl	-21.5251842*	3.1875493	.000	-30.765041	-12.285327
	FHA	-5.8894322	3.1875493	.689	-15.129289	3.350425
	F varnish	-15.4623311*	3.1875493	.000	-24.702188	-6.222474
F varnish	Plain	-15.3828614*	3.1875493	.000	-24.622718	-6.143004
	KCl	-6.0628531	3.1875493	.613	-15.302710	3.177004
	FHA	9.5728989*	3.1875493	.037	.333042	18.812756
	KCl-FHA	15.4623311*	3.1875493	.000	6.222474	24.702188

*. The mean difference is significant at the 0.05 level.

Cell viability: Gingival fibroblast

Test of Homogeneity of Variances

OD

Levene Statistic	df1	df2	Sig.
1.897	5	43	.115

ANOVA

OD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.232	5	.046	.644	.667
Within Groups	3.096	43	.072		
Total	3.328	48			

Multiple Comparisons

OD

Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Plain	KCl	-.0693266	.1352186	1.000	-.489599	.350946
	FHA	-.0924781	.1352186	1.000	-.512751	.327794
	KCl-FHA	-.0879378	.1352186	1.000	-.508210	.332335
	F varnish	-.1779320	.1352186	1.000	-.598204	.242340
	Blank control	.0494121	.1492773	1.000	-.414556	.513380
KCl	Plain	.0693266	.1352186	1.000	-.350946	.489599
	FHA	-.0231515	.1264855	1.000	-.416280	.369977
	KCl-FHA	-.0186112	.1264855	1.000	-.411740	.374518
	F varnish	-.1086054	.1264855	1.000	-.501734	.284524
	Blank control	.1187387	.1414150	1.000	-.320793	.558270
FHA	Plain	.0924781	.1352186	1.000	-.327794	.512751
	KCl	.0231515	.1264855	1.000	-.369977	.416280
	KCl-FHA	.0045403	.1264855	1.000	-.388589	.397669
	F varnish	-.0854539	.1264855	1.000	-.478583	.307675
	Blank control	.1418902	.1414150	1.000	-.297641	.581422
KCl-FHA	Plain	.0879378	.1352186	1.000	-.332335	.508210
	KCl	.0186112	.1264855	1.000	-.374518	.411740
	FHA	-.0045403	.1264855	1.000	-.397669	.388589
	F varnish	-.0899942	.1264855	1.000	-.483123	.303135
	Blank control	.1373499	.1414150	1.000	-.302182	.576881
F varnish	Plain	.1779320	.1352186	1.000	-.242340	.598204
	KCl	.1086054	.1264855	1.000	-.284524	.501734
	FHA	.0854539	.1264855	1.000	-.307675	.478583
	KCl-FHA	.0899942	.1264855	1.000	-.303135	.483123
	Blank control	.2273441	.1414150	1.000	-.212187	.666876
Blank control	Plain	-.0494121	.1492773	1.000	-.513380	.414556
	KCl	-.1187387	.1414150	1.000	-.558270	.320793
	FHA	-.1418902	.1414150	1.000	-.581422	.297641
	KCl-FHA	-.1373499	.1414150	1.000	-.576881	.302182
	F varnish	-.2273441	.1414150	1.000	-.666876	.212187

Cell viability: Pulpal fibroblasts

Test of Homogeneity of Variances

OD

Levene Statistic	df1	df2	Sig.
.331	5	48	.892

ANOVA

OD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.852	5	.170	5.383	.001
Within Groups	1.520	48	.032		
Total	2.372	53			

Multiple Comparisons

OD

Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Plain	KCl	-.2089647	.0838833	.244	-.468100	.050171
	FHA	-.2210205	.0838833	.169	-.480156	.038115
	KCl-FHA	-.2525289	.0838833	.062	-.511664	.006606
	F varnish	-.2759812*	.0838833	.028	-.535117	-.016846
	Blank control	.0431947	.0838833	1.000	-.215941	.302330
KCl	Plain	.2089647	.0838833	.244	-.050171	.468100
	FHA	-.0120558	.0838833	1.000	-.271191	.247079
	KCl-FHA	-.0435642	.0838833	1.000	-.302700	.215571
	F varnish	-.0670165	.0838833	1.000	-.326152	.192119
	Blank control	.2521594	.0838833	.063	-.006976	.511295
FHA	Plain	.2210205	.0838833	.169	-.038115	.480156
	KCl	.0120558	.0838833	1.000	-.247079	.271191
	KCl-FHA	-.0315084	.0838833	1.000	-.290644	.227627
	F varnish	-.0549607	.0838833	1.000	-.314096	.204175
	Blank control	.2642152*	.0838833	.042	.005080	.523351
KCl-FHA	Plain	.2525289	.0838833	.062	-.006606	.511664
	KCl	.0435642	.0838833	1.000	-.215571	.302700
	FHA	.0315084	.0838833	1.000	-.227627	.290644
	F varnish	-.0234523	.0838833	1.000	-.282588	.235683
	Blank control	.2957237*	.0838833	.014	.036588	.554859
F varnish	Plain	.2759812*	.0838833	.028	.016846	.535117
	KCl	.0670165	.0838833	1.000	-.192119	.326152
	FHA	.0549607	.0838833	1.000	-.204175	.314096
	KCl-FHA	.0234523	.0838833	1.000	-.235683	.282588
	Blank control	.3191759*	.0838833	.006	.060041	.578311
Blank control	Plain	-.0431947	.0838833	1.000	-.302330	.215941
	KCl	-.2521594	.0838833	.063	-.511295	.006976
	FHA	-.2642152*	.0838833	.042	-.523351	-.005080
	KCl-FHA	-.2957237*	.0838833	.014	-.554859	-.036588
	F varnish	-.3191759*	.0838833	.006	-.578311	-.060041

*. The mean difference is significant at the 0.05 level.

Cell viability: Gingival fibroblast ("KCl-FHA" vs "F varnish")

Case Processing Summary

	Group	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
OD	Plain	7	100.0%	0	.0%	7	100.0%
	KCl	9	100.0%	0	.0%	9	100.0%
	FHA	9	100.0%	0	.0%	9	100.0%
	KCl-FHA	9	100.0%	0	.0%	9	100.0%
	F varnish	9	100.0%	0	.0%	9	100.0%
	Control	9	100.0%	0	.0%	9	100.0%

Descriptives[a]

	Group			Statistic	Std. Error
OD	Plain	Mean		1.049686	.1316701
		95% Confidence Interval for Mean	Lower Bound	.727501	
			Upper Bound	1.371871	
		5% Trimmed Mean		1.049341	
		Median		.918008	
		Variance		.121	
		Std. Deviation		.3483663	
		Minimum		.6667	
		Maximum		1.4389	
		Range		.7723	
		Interquartile Range		.7539	
		Skewness		.165	.794
		Kurtosis		-2.394	1.587
	KCl	Mean		1.119013	.0988715
		95% Confidence Interval for Mean	Lower Bound	.891015	
			Upper Bound	1.347011	
		5% Trimmed Mean		1.127759	
		Median		1.138924	
		Variance		.088	
		Std. Deviation		.2966145	
		Minimum		.5722	

	Maximum		1.5084	
	Range		.9362	
	Interquartile Range		.4851	
	Skewness		-.569	.717
	Kurtosis		-.020	1.400
FHA	Mean		1.142164	.0881218
	95% Confidence Interval for Mean	Lower Bound	.938955	
		Upper Bound	1.345374	
	5% Trimmed Mean		1.137360	
	Median		1.076733	
	Variance		.070	
	Std. Deviation		.2643654	
	Minimum		.8781	
	Maximum		1.4927	
	Range		.6146	
	Interquartile Range		.5578	
	Skewness		.591	.717
	Kurtosis		-1.753	1.400
KCl-FHA	Mean		1.137624	.0963719
	95% Confidence Interval for Mean	Lower Bound	.915390	
		Upper Bound	1.359858	
	5% Trimmed Mean		1.145642	
	Median		1.119431	
	Variance		.084	
	Std. Deviation		.2891156	
	Minimum		.6819	
	Maximum		1.4490	
	Range		.7671	
	Interquartile Range		.5218	
	Skewness		-.658	.717
	Kurtosis		-.647	1.400
F varnish	Mean		1.227618	.0717365
	95% Confidence Interval for Mean	Lower Bound	1.062194	
		Upper Bound	1.393043	
	5% Trimmed Mean		1.236829	
	Median		1.191832	

	Variance	.046	
	Std. Deviation	.2152094	
	Minimum	.7923	
	Maximum	1.4972	
	Range	.7049	
	Interquartile Range	.2926	
	Skewness	-.712	.717
	Kurtosis	1.119	1.400

a. OD is constant when Group = Control. It has been omitted.

Tests of Normality[b]

	Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
OD	Plain	.233	7	.200*	.835	7	.089
	KCl	.177	9	.200*	.956	9	.750
	FHA	.234	9	.167	.806	9	.024
	KCl-FHA	.234	9	.170	.864	9	.107
	F varnish	.245	9	.126	.875	9	.139

a. Lilliefors Significance Correction

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

b. OD is constant when Group = Control. It has been omitted.

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
OD	KCl-FHA	9	1.137624	.2891156	.0963719
	F varnish	9	1.227618	.2152094	.0717365

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
OD	Equal variances assumed	.630	.439	-.749	16	.465	-.0899942	.1201402	-.3446800	.1646916
	Equal variances not assumed			-.749	14.783	.466	-.0899942	.1201402	-.3463947	.1664064

Cell viability: Pulpal fibroblast ("KCl-FHA" vs "F varnish")

Case Processing Summary

	Group	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
OD	Plain	9	100.0%	0	.0%	9	100.0%
	KCl	9	100.0%	0	.0%	9	100.0%
	FHA	9	100.0%	0	.0%	9	100.0%
	KCl-FHA	9	100.0%	0	.0%	9	100.0%
	F varnish	9	100.0%	0	.0%	9	100.0%

Descriptives

	Group			Statistic	Std. Error
OD	Plain	Mean		.898248	.0467073
		95% Confidence Interval for Mean	Lower Bound	.790540	
			Upper Bound	1.005955	
		5% Trimmed Mean		.898083	
		Median		.880969	
		Variance		.020	
		Std. Deviation		.1401219	
		Minimum		.6726	
		Maximum		1.1269	
		Range		.4543	
		Interquartile Range		.1954	
		Skewness		.201	.717
		Kurtosis		-.098	1.400
	KCl	Mean		1.107212	.0620742
		95% Confidence Interval for Mean	Lower Bound	.964069	
			Upper Bound	1.250356	
		5% Trimmed Mean		1.104623	
		Median		1.066999	
		Variance		.035	
		Std. Deviation		.1862225	

	Minimum		.8254	
	Maximum		1.4357	
	Range		.6103	
	Interquartile Range		.2526	
	Skewness		.567	.717
	Kurtosis		.226	1.400
FHA	Mean		1.119268	.0583023
	95% Confidence Interval for Mean	Lower Bound	.984823	
		Upper Bound	1.253713	
	5% Trimmed Mean		1.120790	
	Median		1.103349	
	Variance		.031	
	Std. Deviation		.1749069	
	Minimum		.8529	
	Maximum		1.3582	
	Range		.5053	
	Interquartile Range		.2936	
	Skewness		-.043	.717
	Kurtosis		-1.419	1.400
KCl-FHA	Mean		1.150776	.0578505
	95% Confidence Interval for Mean	Lower Bound	1.017373	
		Upper Bound	1.284180	
	5% Trimmed Mean		1.144208	
	Median		1.163221	
	Variance		.030	
	Std. Deviation		.1735516	
	Minimum		.9419	
	Maximum		1.4779	
	Range		.5360	
	Interquartile Range		.2696	
	Skewness		.620	.717
	Kurtosis		.220	1.400
F varnish	Mean		1.174229	.0657101
	95% Confidence Interval for Mean	Lower Bound	1.022701	
		Upper Bound	1.325756	
	5% Trimmed Mean		1.176808	

	Median	1.180327	
	Variance	.039	
	Std. Deviation	.1971302	
	Minimum	.8362	
	Maximum	1.4658	
	Range	.6296	
	Interquartile Range	.3106	
	Skewness	-.289	.717
	Kurtosis	-.456	1.400

Tests of Normality

	Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
OD	Plain	.169	9	.200*	.971	9	.906
	KCl	.199	9	.200*	.948	9	.663
	FHA	.192	9	.200*	.939	9	.569
	KCl-FHA	.194	9	.200*	.936	9	.545
	F varnish	.132	9	.200*	.987	9	.991

a. Lilliefors Significance Correction

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

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
OD	KCl-FHA	9	1.150776	.1735516	.0578505
	F varnish	9	1.174229	.1971302	.0657101

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
OD	Equal variances assumed	.317	.581	-.268	16	.792	-.0234523	.0875471	-.2090439	.1621393
	Equal variances not assumed			-.268	15.747	.792	-.0234523	.0875471	-.2092863	.1623817

VITA

Mr Yossakit Lochaiwatana was born on 25th March, 1985 in Bangkok, Thailand. In 2009, he received his Doctor of Dental Surgery (DDS) degree with 1st class honors from Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. Immediately after his graduation, he continued his study by enrolling the Doctor of Philosophy Program in Dental Biomaterials Science, Graduate School, Chulalongkorn University.

During his 6-year undergraduate studying at Faculty of Dentistry, Chulalongkorn University, he served in several positions, such as president of Dental Student Club of Chulalongkorn University, director of Chulalongkorn University Community Health Care Camp, committee of Student Government of Chulalongkorn University and Dental Student Union of Thailand. He also participated in the Medical and Dental Students Exchange Program which affiliated with Tokyo Medical and Dental University, Tokyo, Japan.

In December 2009, he started working with Johnson & Johnson Consumer (Thailand) Ltd as a Dental Science Liaison until now. Consequently, he involved a lot in oral care products and prevention of oral diseases, such as dental caries, periodontal diseases, oral biofilm and dentin hypersensitivity.