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FLUORIDE RELEASE AND RECHARGE ABILITY OF RESIN-BASED SEALANT WITH BINARY ACTIVE FILLERS

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การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของสารตัวเติมชนิดต่างๆ ต่อสมบัติการปลดปล่อยฟลูออไรด์ การดูดกลับฟลูออไรด์ และสมบัติการทำให้กรดแลคติกเป็นกลาง ในเรซินเคลือบหลุมร่องฟัน สารตัวเติมซิลิกาที่มีรูพรุนขนาดกลางได้รับการสังเคราะห์โดยใช้กระบวนการโซลเจล โดยใช้เถ้าแกลบเป็นวัตถุดิบในการสังเคราะห์ สารตัวเติมชนิดต่างๆ ได้แก่ แคลเซียมอลูมิเนตซีเมนต์ ซิลิกาที่มีรูพรุนขนาดกลางที่ได้จากการสังเคราะห์ ผงกลาสไอโอโนเมอร์ซีเมนต์ ผงกลาสไอโอโนเมอร์ซีเมนต์ที่ผ่านการปรับสภาพพื้นผิวด้วยกรดแอซีติก และสารตัวเติมที่ได้รับการผสมสองชนิด ถูกเติมเข้าไปในเรซินเคลือบหลุมร่องฟัน ด้วยปริมาณร้อยละ 5 โดยมวล เรซินเคลือบหลุมร่องฟันที่ไม่มีสารตัวเติมเป็นกลุ่มควบคุม แช่ชิ้นตัวอย่างแต่ละชิ้นในน้ำปราศจากไอออนปริมาณ 3 มิลลิลิตร วัดปริมาณฟลูออไรด์ที่ปลดปล่อยออกมาทุก 3 วัน ตั้งแต่วันที่ 3 จนถึงวันที่ 27 ทั้งก่อนและหลังการดูดกลับฟลูออไรด์จากการแช่ในฟลูออไรด์เจล วัดค่าความเป็นกรดด่างของกรดแลคติกหลังการแช่ชิ้นตัวอย่าง วิเคราะห์ศักย์ซีตา วิเคราะห์การกระจายของขนาดสารตัวเติม วิเคราะห์หาค่าพื้นที่ผิวด้วยวิธีบรูนเนอร์ เอมเมตต์ และเทลเลอร์ (บีอีที) รวมทั้งศึกษาลักษณะพื้นผิวของสารตัวเติมโดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด พบว่าเรซินเคลือบหลุมร่องฟันที่มีสารตัวเติมสองชนิด ได้แก่ แคลเซียมอลูมิเนตซีเมนต์ และซิลิกาที่มีรูพรุนขนาดกลางที่ได้จากการสังเคราะห์ มีสมบัติการดูดกลับและปลดปล่อยฟลูออไรด์สูงสุด โดยอัตราส่วนของ แคลเซียมอลูมิเนตซีเมนต์ ซิลิกาที่มีรูพรุนขนาดกลางที่ได้จากการสังเคราะห์ ที่ 1 ต่อ 1 และ 2 ต่อ 1 ให้ผลสูงสุดไม่แตกต่างกัน และสามารถเพิ่มค่าความเป็นกรดด่างของกรดแลคติกได้ เรซินเคลือบหลุมร่องฟันที่มีสารตัวเติมสองชนิดนี้มีแนวโน้มที่สามารถเพิ่มการคืนแร่ธาตุ ป้องกันรอยผุกลับซ้ำ จากการปลดปล่อยฟลูออไรด์ และค่าความเป็นกรดด่างที่เหมาะสม

ภาควิชา	ทันตกรรมประดิษฐ์	ลายมือชื่อนิสิต	....
สาขาวิชา	ทันตกรรมประดิษฐ์	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
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KEYWORDS: CALCIUM ALUMINATE CEMENT / FLUORIDE RECHARGE / FLUORIDE RELEASE / MESOPOROUS SILICA / PIT AND FISSURE SEALANT

ATIKOM SURINTANASARN: FLUORIDE RELEASE AND RECHARGE ABILITY OF RESIN-BASED SEALANT WITH BINARY ACTIVE FILLERS. ADVISOR: ASST. PROF. NIYOM THAMRONGANANSKUL, Ph.D., CO-ADVISOR: KRISANA SIRALERTMUKUL, Ph.D., 152 pp.

This study was to evaluate the effects of different types of active filler in a resin-based pit and fissure sealant on fluoride release, recharge, and lactic acid neutralizing abilities. Mesoporous silica was synthesized from rice hull ash using sol-gel method. Resin-based sealant was incorporated with 5% w/w of the following fillers: calcium aluminate cement (CAC), synthesized mesoporous silica (SI), glass-ionomer powder (GIC), acetic acid-treated GIC (GICA), and combination of fillers. Resin-based sealant without filler added was the control. Ten specimens of each group were separately stored in 3 mL of deionized water and the fluoride concentration, before and after fluoride recharge with fluoride gel, were measured every 3 days (from day 3 to day 27). The lactic acid pH change after storage for 24 hours of specimens was measured. Zeta potential, particle size distribution, particle surface area (BET), and morphology of fillers were evaluated. The CAC and SI mixture (CAC+SI) group demonstrated the highest fluoride release after being recharged with fluoride gel. CAC:SI weight ratio of 1:1 and 2:1 showed higher fluoride recharge property than those of 1:2. The CAC+SI group also demonstrated increased lactic acid pH. These findings suggest that a resin-based sealant containing synthesized mesoporous silica and calcium aluminate cement may enhance remineralization due to fluoride recharge and higher pH.

Department:	Prosthodontics	Student's Signature	....
Field of Study:	Prosthodontics	Advisor's Signature	....
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CHAPTER 1

INTRODUCTION

Background and significance of problem

Dental caries is the most common oral disease which caused by the imbalance of oral homeostasis including host, bacteria, and environment result in dental hard tissue destruction (Yip and Smales, 2012). Occlusal surface is the most frequently attacked by this disease due to the morphology and structural irregularity (Carvalho et al., 1989). Thus, pit and fissure sealant is described as a material that is applied to pit and fissure of dental caries-susceptible teeth for occlusal caries prevention (Simonsen, 2002).

The most of sealants are based on Bis-GMA due to high retention rate (Chen and Liu, 2013; Haznedaroglu et al., 2016; Kumaran, 2013). It can bond to enamel surface using mechanical bonding between acid-treated rough enamel and low-viscosity resin materials. They have less filler particles to reduce viscosity; however, some sealants contain more filler to improve wear resistance. Nevertheless, microleakage and partial loss of material can cause secondary caries on enamel-resin interface (Hicks et al., 2000).

In addition to resin-based sealant, glass-ionomer materials are also used as pit and fissure sealant. This type of sealant can bond to enamel and dentin using physicochemical bond following acid conditioning. Even though wear resistance and long-term retention of glass-ionomer are still questionable, the important advantage of glass-ionomer over conventional resin sealant is the property of fluoride release (Hicks and Flaitz, 2005). Fluoride-releasing restorative materials tend to provide inhibitive effect on tooth demineralization and reduce secondary caries on tooth structure (Hicks and Flaitz, 2000; Hicks et al., 2000; Tantbirojn et al., 1997).

Fluoride-releasing restorative materials tend to provide inhibitive effect on tooth demineralization in adjacent area. The amount of mineral loss is significantly decreased compared with that of non-fluoride material from 0.2 mm to 7 mm distance from the margin of restoration (Tantbirojn et al., 1997). The placement of fluoride-releasing material also reduces susceptibility of adjacent enamel and inhibits demineralization along the margin. Hence, it is the material that could reduce prevalence of secondary caries on tooth structure (Hicks and Flaitz, 2000). Acid production of dental caries-related oral streptococci could also be inhibited by GIC at low pH (Nakajo et al., 2009). Moreover, clinical experience associated with GIC showed that fewer secondary caries is seen compared with those of resin composite filling (Forsten, 1993).

However, there is no clear correlation between in vitro and in vivo studies. It could be implied that artificial caries model could not precisely predict the clinical outcome (Papagiannoulis et al., 2002). Systematic review also shows that resin-based sealant has more effective long-term anti-caries effect and more cost-effective because of higher retention rate (Muller-Bolla et al., 2006; Simonsen and Neal, 2011).

For overcoming both limitations, resin-based sealant containing fluoride is introduced but long-term significant difference is not found due to the short fluoride releasing period. Soluble fluoride salt was added to unpolymerized resin. When sealant was applied to pit and fissure and polymerized, fluoride ion can be slowly dissolved in oral cavity. However, larger amounts of fluoride are released on first two days, and then the release tapers off. Released fluoride then decreases by approximately one-half for each of first three days. This condition is called “burst effect” (Cooley et al., 1990). Moreover, it is suggested that fluoride recharge ability is more important than fluoride release alone (Dionysopoulos et al., 2013; Hatibovic-Kofman et al., 1997; Preston et al., 2003). However, only glass-ionomer sealant which has high dislodged rate showed highest fluoride release and recharge while resin sealant did not show fluoride recharge (Han et al., 2002; Koga et al., 2004).

The capacity to buffer lactic acid solution of GIC, which is the mechanism that might be the complement property, was found in 1999. This mechanism might be clinically beneficial against secondary caries (Nicholson et al., 1999). Buffers have ability to change the pH of acid, moving to neutral solution (Nicholson et al., 2000). Although lactic acid, acetic acid, and propionic acid are the major acids in carious dentin (Hojo et al., 1991), lactic acid is dominant in active lesion (Hojo et al., 1994).

Thus, the property of lactic acid buffering has potential clinical importance (Nicholson et al., 2000). Normally, local plaque pH above 6 is classified in dental caries safe zone, pH between 5.5 and 6 is potentially cariogenic, and pH below 5.5 is cariogenic or danger zone (Xu et al., 2011). This trend shows that higher pH tends to reduce demineralization process and stimulate remineralization process. It results in reduced prevalence of dental cavity.

Glass-ionomer phase on glass particles plays an important role in fluoride recharge ability (Shimazu et al., 2011). Moreover, secondary cement-forming reactions of glass-ionomer cement are believed to contribute cement hardening (Wilson, 1996). When acetic acid reacts with standard glass, the stable cement can be prepared from a surface-modified standard glass (Wasson and Nicholson, 1993a). Treated glass-ionomer cement powder with acetic acid in resin-based material looks like to play an important role in enhancing ability of materials.

Calcium aluminate cements are derived from the class of hydraulic cement. Necessary mechanical properties are qualified according to the standard to use as direct restorative material with bioactive activities (Jefferies, 2014a; Loof et al., 2003). This material is marked as a bioceramic with claims as uniqueness and biocompatibility alternative to amalgam and resin composite (Sunnegårdh-Grönberg et al., 2003).

Mesoporous silica materials possess high well-ordered honeycomb-like pore structure and large surface area. The synthesis and modification are encouraged due to their wide range of applications, including catalysis and drug delivery (Zhuang et al., 2015). This structure with numerous empty channels is able to absorb and encapsulate relatively large amounts of molecules (Slowing et al., 2008). Several techniques are available to synthesize mesoporous silica such as chemical vapor deposition, combustion, micro-emulsion, sol-gel process, frame spray pyrolysis etc.; however, the most common and popular process to synthesize this particle is a sol-gel technique. This offers excellent control of reaction and low temperature synthesis (Singh et al., 2014). Metal alkoxide is the most used precursor for this purpose. Alkoxysilanes, such as tetraethoxysilane (TEOS), are widely used for the production of silica gel (Alothman, 2012). Moreover, rice hull ash which is the waste product from rice hull burning to generate energy is rich of silica. It can also be an economically practical raw material for silica-based production (Kalapathy et al., 2000a; b; Ma et al., 2012). However, the effect of both calcium aluminate cement and mesoporous silica using as filler in dental material on fluoride release and recharge capability has not been investigated.

Many studies have examined the fluoride release and recharge ability of conventional materials used as pit and fissure sealants (Bayrak et al., 2010; Koga et al., 2004). However, there has been no report on the effect of adding a material or a combination of materials to conventional pit and fissure sealants to provide fluoride release and fluoride recharge capabilities. The aim of this study was to evaluate the addition of materials that have the potential for fluoride release and fluoride recharge to resin-based pit and fissure sealant that has a high retention rate. This study compared the fluoride release and recharge abilities of a conventional resin-based sealant and resin-based sealants incorporated with (1) calcium aluminate cement (CAC), (2) synthesized mesoporous silica (SI), (3) unmodified glass-ionomer powder (GIC), and (4) acetic acid-modified glass-ionomer powder (GICA), and a combination of fillers for possible application of these materials as fillers in resin-based pit and fissure sealants. The ability of these materials to neutralize lactic acid, surface potential of fillers, particle size distribution of fillers, and morphological structure of fillers, were also examined.

Objectives

The objectives of this study are:

a. To find proper method to synthesize active fillers and use applied existing fillers which have potential to increase ability of pit and fissure sealant.
b. To evaluate morphological structure, filler surface potential, particle size distribution, and proportion of fillers used in this study.
c. To compare the properties between conventional pit and fissure sealant and active filler-containing pit and fissure sealant.

d. To evaluate the effect of active filler on fluoride releasing ability, fluoride recharge ability, acid neutralizing ability, and particle distribution of pit and fissure sealant.

Research Hypothesis

The null hypotheses are as follows:

a. There is no difference of fluoride release and recharge properties between conventional resin-based sealant and active filler-filled resin-based sealant.
b. There is no difference of neutralizing property between conventional resin-based sealant and active filler-filled resin-based sealant.

The alternative hypotheses are as follows:

a. There is a difference of fluoride release and recharge properties between conventional resin-based sealant and active filler-filled resin-based sealant.
b. There is a difference of neutralizing property between conventional resin-based sealant and active filler-filled resin-based sealant.

Assumption

This is a preliminary in vitro study, uncomplicated procedure are used in filler incorporation. Amount of filler loading is designed to control the viscosity of material because excessive loading of filler can cause difficulty in manipulation. Therefore, further in vitro study with advance instrument and clinical research are required to confirm the effects of these additives.

Research design

Experimental research

CHAPTER 2

LITERATURE REVIEW

Polymer composites

Polymer composites are the materials modified through the incorporation of additive yields. They are the mixtures of polymers with the additives. Thus, they consist of two or more components and phases. The mixtures are characterized by unique structures that are responsible for the properties. The objectives for using additives are (Xanthos, 2005):

- Property modification
- Property enhancement
- Cost reduction
- Improving of processing characteristics

Modified polymers can be classified as particulate, fiber-reinforced or structural composites. However, particulate fillers are the most common in dental material application (van Noort, 2007). Due to unfavorable geometrical features of filler additives, they could only moderately enhance the modulus of the polymer while the strength could not be gained. Generally, the major purpose is lowering the cost of materials by replacing the more expensive polymer. Nevertheless, the term of reinforcing filler was coined to describe discontinuous additives. Inorganic reinforcing fillers that stiffer than the matrix can cause an overall reduction of matrix strain and improve the mechanical properties of polymer. The reinforcing capacity depends on the aspect ratio, defined as the ratio of the length of filler to its height when the length is larger than the height. Thus, the spheres have minimal reinforcing capacity because the aspect ratio is unity comparing to platelets and fiber (Fig. 1). Moreover, the ratio of its surface area to its volume that needs to be high for effective reinforcement is also the useful parameter (Xanthos, 2005).

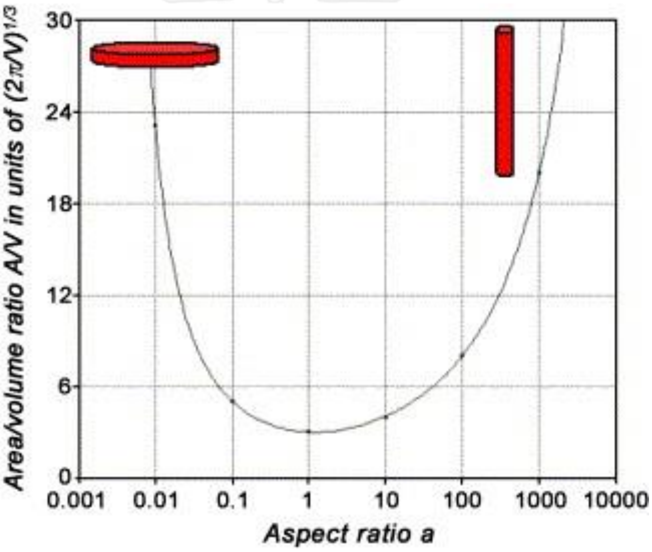


Fig. 1 – Plot of the function describing the ratio of surface area to volume (A/V) versus aspect ratio (Fischer, 2003).

The definition of fillers is very broad and contains a wide range of materials but usually defined in polymer science term as a variety of solid particulate materials. Pigments and elastomeric materials are not commonly included in this term. Fillers can be classified according to shapes, sizes, aspect ratio, forms, chemical structure, and inherent properties (Xanthos, 2005). However, the effects attained by adding fillers into polymeric matrix are also useful. Thus, all fillers are divided into two groups: active and inactive. Active fillers are the fillers that improve mechanical and physical properties while inactive fillers are introduced to attain definite color and decrease their cost (Lipatov, 1995). Moreover, they can also be classified according to their specific function. The examples of primary functions are as follows (Xanthos, 2005):

- Modification of mechanical properties
- Enhancement of fire retardant ability
- Modification of electrical and magnetic properties
- Modification of surface properties
- Enhancement of process ability

Principle mechanisms in polymers (Hohenberger, 2009)

Additives can affect many properties of a polymer such as density, shrinkage, expansion coefficient, permeability, mechanical properties, etc. The effectiveness of filler depends on type, shape, incorporation method, and surface treatment. Some basic principles of filler application are as followed:

- The filler must retain its structure and remain inert, insoluble with no volatiles, no catalytic activity, and low additive adsorption.
- The filler must be compatible to the matrix.
- The handling of process should not be difficult.
- The filler must be available in sufficient amounts in constant quality.

The formulator should focus on the main effect needed because fillers can affect nearly all properties. After this consideration, the formulation can be adjusted by changing filler loading, adding additives, varying the matrix, and treatment the surface of filler.

Most filler can increase the modulus and the tensile strength but decrease the elongation at break. However, bad dispersion, presence of agglomerates, weak filler-matrix bonding, phase changes of the matrix, and very low filler loading are the exceptions from this rule. The formulator should compare the volume fraction rather than the mass fraction because the volume is presented in composite.

Fillers also have strong impact in the composite viscosity. This property depends on filler loading, particle size distribution (PSD), the degree of dispersion and aspect ratio. For example, Spherical fillers tend to cause less viscosity than platy fillers. The viscosity normally increases after filler loading. However, this effect is nonlinear and dependent on shear rate and temperature. Furthermore, the presence of proper coupling agents can reduce the viscosity sharply.

Properties of fillers and guiding selection (Hohenberger, 2009)

Average particle sizes of fillers are range between 1 and 100 μm. The characteristic shape size is the aspect ratio, defined as the average ratio of filler diameter to filler thickness or its height. The relationship between particle shape and range of the aspect ratio is shown in Table 1.

Table 1– Relationship between particle shape and range of the aspect ratio

Particle shape	Range of the aspect ratio
Spherical	1-2
Cubic	1-4
Platy	2-50
Acicular	10-100
Fibrous	>100

The particle size distribution (PSD) of filler is a cumulative curve indicating the amount smaller than a given size by percent of total number, volume, or weight. It can be measured by sieving or sedimentation techniques. The sedimentation techniques are applicable according to Stoke's law, an equivalent spherical diameter (ESD). ESD is defined as the diameter of spherical particle settling with the same velocity as the actual particle. It is not a real diameter of particle being measured.

The scattering of light is a function of particle size. The principle of sizing by light scattering is measuring the angular variation of light that passed through the particles. Large particles scatter light at small angle and small particles scatter at large angle. The angular scattering intensity data are then analyzed to calculate the size of particles. However, the important disadvantage of light-scattering techniques is that the effects of particle shape are unable to be estimated easily. The calculated data are reported as a volume equivalent sphere diameter (Morrison and Ross, 2002).

Specific surface area is defined as the total surface per unit weight of the filler. It can be done by both liquid nitrogen adsorption (BET) and permeability of air (Blaine method). Brunauer-Emmett-Teller (BET) Surface Area Analysis is a specific surface area measurement by nitrogen multilayer adsorption measured as a function of relative pressure by a fully automated analyzer. This technique analyzes external area and pore area to determine the total specific surface area yielding important information in studying the surface porosity and particle size (Emmett, 1938). Generally, this technique shows more reliable results than Blaine technique.

The hardness of fillers is commonly provided in Mohs' scale but this scale is non-linear and not relate to physically defined hardness which is linked to actual wear and abrasion properties. However, Tröger value is well associated in the formula $H_M = \left(\frac{5}{4}\right)^2 (2^{M-1})$ when M is Mohs' hardness. The comparison of different hardness scales is shown in Table 2.

Table 2– Comparison of different hardness scales

Filler	Mohs' Hardness	Vickers' hardness	Tröger's Hardness	$H_M = \left(\frac{5}{4}\right)^2 (2^{M-1})$
Talc	1	-	1.08	1.56
Gypsum	2	-	2.36	3.12
Calcite	3	-	6.99	6.25
Fluorite	4	-	12.1	12.5
Apatite	5	200	25.7	25
Orthoclase	6	400	49.5	50
Quartz	7	600	100	100
Topaz	8	900	143	200
Corundum	9	1500	342	400
Diamond	10	2600	850	800

Filler which is surface-treated with proper coupling agent or some filler, such as carbon black, can bond to macromolecular chain in the matrix of polymer. Other fillers may be described as nonreactive fillers but these filler can still immobilize polymer chains which can increase glass transition temperature (T_g). Nonreactive fillers seem to have less effect than more reactive fillers (Seymour, 1991).

Dental composites

Resin-based composites are probably the most numerous materials used in dentistry. They have been applied in a huge variety of clinical application, ranging from restorative materials, dental cements and indirect restoration. The dental composites have three main components: resin matrix, fillers and coupling agent. Moreover, they also contain other components, including activator, initiator, inhibitor, pigment, Ultraviolet absorber and other additive (Anusavice et al., 2013).

Matrix

Matrix is initially a fluid monomer but it can be converted to a rigid polymer by polymerization. This ability allows this material to be used as restorative materials. The most resin matrix used in dental composite are based on bisphenol-a-glycidyl methacrylate (Bis-GMA) and urethane dimethacrylate (UDMA). However, they are highly viscous because of high molecular weights and are difficult to manipulate in clinical applications. Thus, low-viscosity monomers, such as methyl methacrylate (MMA), ethylene glycol dimethacrylate (EDMA) and triethylene glycol dimethacrylate (TEGDMA), are added to control and dilute the viscous components (Anusavice et al., 2013; van Noort, 2007).

Filler

The main objectives of filler used in dental composites are to strengthen them, to reduce polymerization shrinkage, and to control thermal expansion. The amounts of filler vary from 30-70% by volume or 50-85% by weight of a composite (Anusavice et al., 2013). Hardness, compressive strength and stiffness could increase when the level of filler increased (Li et al., 1985). Flexural strength also increased with increasing filler volume fraction while the polymerization shrinkage decreased (Ikejima et al., 2003). Viscosity of materials that directly affects clinical manipulation can also be adjusted with the filler loading, filler shape, and filler size. Moreover, the translucency is also important for acceptable esthetics. The refractive index of filler should closely match to that of the matrix for the similarity of translucency between restoration and tooth structure. A wide range of filler type, filler load, and filler morphology, that have been identified to influence the properties, are continuously being developed in modern materials because the manufacturers are improving physical and mechanical properties by adding filler volume without increasing their viscosity (Beun et al., 2007). Thus, distribution of smaller particles can achieve maximum filler loading. However, the high surface-to-volume ratio cause polar with monomer to increase viscosity. Therefore, the small particles were developed as loosely bound (clusters) particles which are not bound to each other and act to decrease physical properties. This system shows high translucency while maintaining properties (Mitra et al., 2003). The classification of filled resins by filler particle sizes is shown in Table 3.

Table 3 – Classification of reinforcing filler particles by size range (Anusavice et al., 2013)

Class of filler	**Particle size (μm)**
Macrofillers	10-100
Small/fine fillers	0.1-10
Midfillers	1-10
Minifillers	0.1-1
Microfillers	0.01-0.1
Nanofillers	0.005-0.1

Fine-particle composites have as high as or higher filler loadings than those of macrofilled composites. They are more polishable and provide higher strength but brittleness. Microfilled composites are then developed to reduce surface roughness and to increase translucency. However, surface area of the fillers is high and the smaller particles have large surface area-to-volume ratio. They require proper amount of monomer to wet their surface in order to prevent high-viscosity composites. Thus, agglomeration is necessary to increase the load of filler particles (Ferracane, 1995). However, filler particle clustering is the one of the harmful factors to the performance of particle reinforced composites. The clustering of particles into large agglomerates make inter-particle spacing very small and thus protective so that the monomer could not penetrate. The mechanical properties of agglomerates would be relatively low (Lim et al., 2002). On the contrary, the study of Beatty et al found that agglomerated-particle composites seem to have higher hardness and wear resistance (Beatty et al., 1998).

Recently, nanoparticles have been fabricated with complicated technique. The particles in nanofilled composited are mostly discrete and rarely effect on viscosity, comparing to microfilled composites (Anusavice et al., 2013). Thus, these types of composites have good optical properties and high polishability like those of microfilled composites. However, no manufacturers have advertised that the products contain only homogeneously dispersed nanoparticles. The first product of nanocomposites on the market is launched in 2003. Mitra et al. published their research toward the development of new dental nanocomposite, consisted of nanomeric particles (nanomers) and filler fraction of agglomerated nanoparticles. It was described as “nanocluster” in Filtek Supreme Universal Restorative (3M ESPE, St. Paul, MN, USA) (Fig. 2 and Fig. 3) (Mitra et al., 2003). Due to their small particle size, more filler can be added to the composite. Theoretically, nanofiller levels can be as much as 90-95% by weight. However, small particles and the increase in filler loading also increase the surface area of particles that can be limited due to wettability of fillers (Chen, 2010). Composites with nanocluster have high filler loading and better mechanical properties than common homogeneous nanocomposites but they are weaker than hybrid composite or microfilled composite because these particles are not chemically bound to each other (Anusavice et al., 2013).

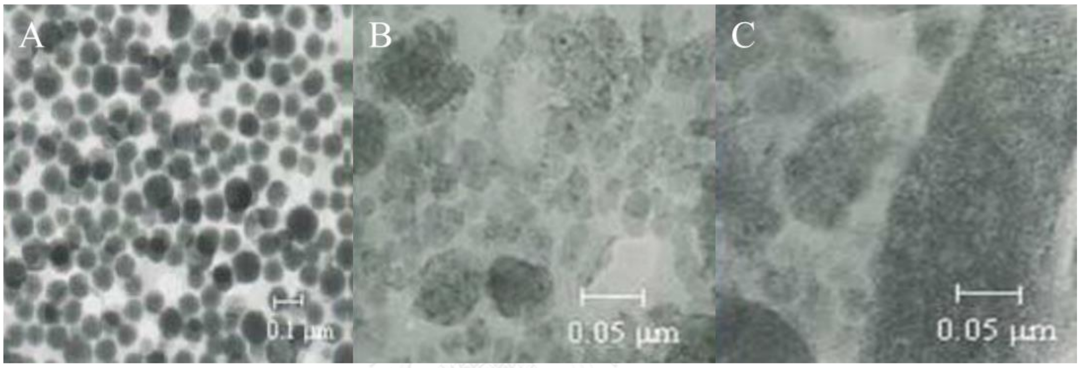


Fig. 2 – Transmission electron microscopic (TEM) images of composites

(Mitra et al., 2003)

A. Composite with nanometric particles

B. Composite with nanocluster particles

C. Composite with large-particle-size hybrid fillers

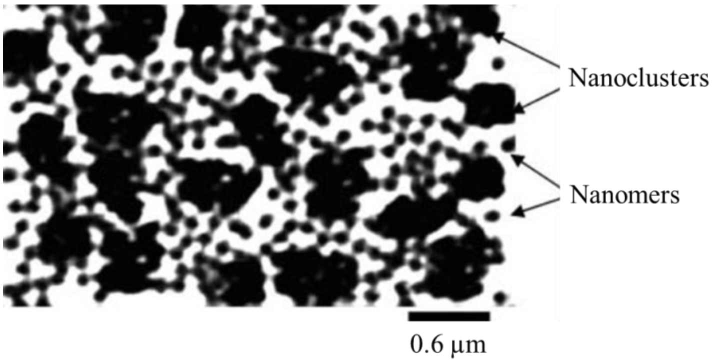


Fig. 3 – Nanomers and nanoclusters in nanocomposite (Chen, 2010)

Moreover, the other manufacturers also develop composites with this nanotechnology. The summary of 3 typical examples with different fillers and compositions of nanocomposites is shown in Table 4.

Table 4 – Comparison of typical examples of commercial nanocomposites (Chen, 2010)

Brand	**Filler types**	**Filler compositions**	**Manufacturer**
Filtek Supreme	Nanofilled	58-60 % v/v (78.5 % w/w), combination of aggregated zirconia-silica/silica cluster filler with primary particle size of 2-20 nm and 75 nm, and nonagglomerated/non-aggregated 20-nm and 75-nm silica fillers	3M ESPE, St. Paul, MN, USA

Premise	Nanohybrid	69 % v/v (84 % w/w), polymerized resin fillers (PPRF) (30-50 µm), barium glass (0.4 µm), silica nanoparticles (20 nm)	Kerr/Sybron, Orange, CA, USA
Ceram-X	Nanohybrid	57% v/v (76 % w/w), contains glass fillers (1.1–1.5 µm) with methacrylate-modified silicon-dioxide-containing nanofiller (10 nm)	Dentsply DeTrey, Konstanz, Germany

Filler modifiers and coupling agents

Coupling agents have been generally used in a wide range of applications due to their ability to bond polymers with different materials. They can enhance the adhesion between organic polymers and inorganic substrates. The bond of them has high initial strength as showed by failure of the composite by polymer rupture. The bond also exhibits excellent strength after environmental aging. The siliceous matter or metal can be in the form of fibers, fillers, or massive structures (Sterman and Marsden, 1966).

Surface modification of fillers with silane coupling agents can generate following several benefits (Weissenbach and Mack, 2005):

- Improved wettability

- Improved dimensional stability
- Improved filler dispersion
- Improved mechanical properties
- Controlled rheological properties

Silane coupling agents commonly have the general formula Y-$(CH_2)_3Si(X)_3$ and Y-$(CH_2)_2Si(CH_3)(X)_2$. X is the silicon functional group that is a hydrolysable group. It reacts with hydroxyl groups of the fillers to produce a bond. Y is the organofunctional group that tightly bounds to silicon by short carbon chain and links with the polymer (Weissenbach and Mack, 2005).

The function of silane coupling agent is to replace the adsorbed water and form a strong chemical bond between the oxide groups on the surface of glass and the polymer molecules. X groups present as an intermediate because they are hydrolyzed to form a silanols. These silanols are able to form hydrogen bonds with the hydroxyl groups on the glass surface. When coated glass is dried, the water is then removed and a condensation reaction occurs to form a covalent bond (Fig. 4). Then, the organofunctional group can react and form a strong bond to the resin. The silane coupling agents that is most commonly used in resin composite and other dental applications is gamma-MPTS or gamma-methacryloxypropyltrimethoxysilane (Fig. 5) (van Noort, 2007).

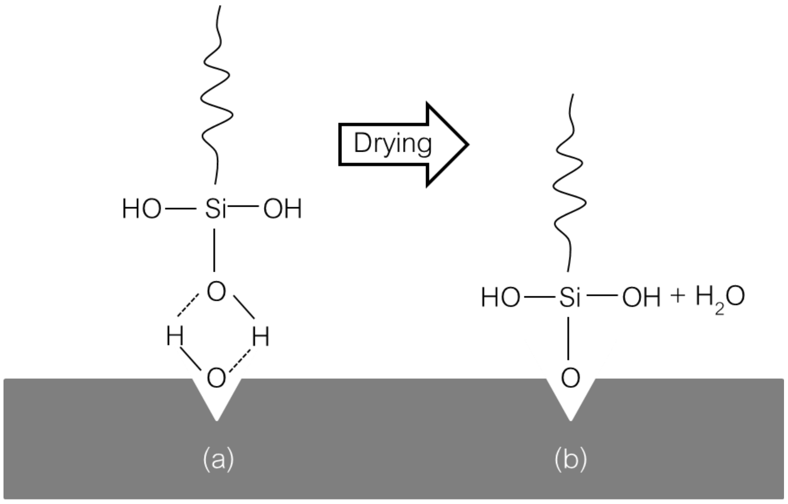
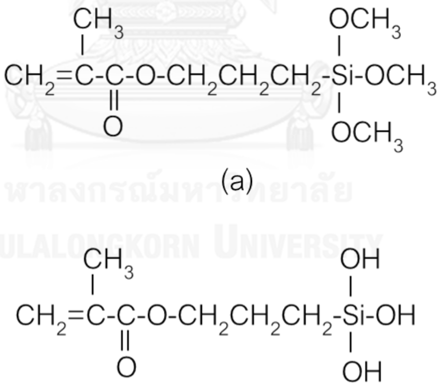


Fig. 4 – Chemical structure of (a) hydrogen bond formation between hydroxyl group and silane coupling agent and (b) covalent bond after the release of water



$$\mathrm{CH_2{=}\overset{\displaystyle CH_3}{\overset{|}{C}}-\underset{\underset{\displaystyle O}{\|}}{C}-O-CH_2CH_2CH_2-\overset{\displaystyle OCH_3}{\overset{|}{\underset{\underset{\displaystyle OCH_3}{|}}{Si}}}-OCH_3}$$

(a)

$$\mathrm{CH_2{=}\overset{\displaystyle CH_3}{\overset{|}{C}}-\underset{\underset{\displaystyle O}{\|}}{C}-O-CH_2CH_2CH_2-\overset{\displaystyle OH}{\overset{|}{\underset{\underset{\displaystyle OH}{|}}{Si}}}-OH}$$

(b)

Fig. 5 – Structure of gamma-methacryloxypropyltrimethoxysilane (gamma-MPTS) (a) before acid activation and (b) after acid activation

Pit and fissure sealant

The term pit and fissure sealant is described as a material that is applied to pit and fissure of dental caries-susceptible area of posterior teeth (Simonsen, 2002). It can bond to enamel surface using mechanical bonding between acid-treated rough enamel and low-viscosity resin materials (Hicks and Flaitz, 2005). The objective of sealant is to seal pit and fissure area against plaque impaction and oral bacteria causing dental caries, especially in a child patient and high-dental caries- risk patient (Norling, 2003). Cyanoacrylates-based was the first materials used experimentally but it was not marketed. Bis-GMA resin is generally used as commercial pit and fissure sealant nowadays because of bacterial degradation in the oral cavity over time of cyanoacrylates-based material (Hicks and Flaitz, 2005). Other dimethacrylates, such as urethane dimethacrylate, are alternative material used as resin in sealant material. Pit and fissure sealant can be classified by various properties, such as process of polymerization or amount of filler. The light-curing pit and fissure sealants have diketones and aromatic ketones, which are sensitive to blue region of visible light (Sanders et al., 2011). Light-curing sealant seems to have lower retention rate than that of chemical-curing but light-curing material are more popular among nowadays practitioners (Beun et al., 2012). The sealant is polymerized in oral cavity when it exposes to a curing light to change into cross-linked polymer (Powers and Wataha, 2017). Properties of light-curing resin-based pit and fissure sealant are listed in Table 5.

Table 5 – Properties of light-curing resin-based pit and fissure sealant (Powers and Wataha, 2017)

Setting time	Activated by light
Tensile strength	20-31 MPa
Compressive strength	92-150 MPa
Elastic modulus	2,100-5,200 MPa
Knoop hardness	20-25 kg/mm^2
Water sorption (7 days)	1.3-2.0 mg/cm^2
Water solubility (7 days)	0.2 mg/cm^2
Wear	22-23×10^{-4} mm^3/mm

The differences between restorative resin materials and pit fissure sealant include filler particles such as porcelain, quartz, and glass in restorative materials to improve mechanical strength. The most of sealants are unfilled Bis-GMA and have few filler particles to reduce viscosity and some sealants contain more filler to improve wear resistance (Hicks and Flaitz, 2005). The penetration of material which is believed as an important factor for retention of sealant might have been inversely proportional to the viscosity because unfilled resin should have penetrated deeper into the undercut of prepared enamel surface than that of filled resin material, such as filler-containing sealant or flowable resin composite. However, the addition of filler to pit and fissure sealant possibly seems to have minor effect on clinical result, including microleakage level. Filler-containing materials could not provide a higher retention rate and there are other factors that may cause the sealant loss (Autio-Gold, 2002; Beun et al., 2012; Park et al., 1993; Waggoner and Siegal, 1996).

The term “unfilled resin” in material of pit and fissure sealant is used for colorless or transparent material which is generally used before introduction of color sealant. In 1977, the first color sealant, Concise White Sealant, was launched in US market. It is the first self-curing sealant which has white color from the addition of titanium dioxide. For the dentist, it is easier to see the material during application and the time for follow-up is saver due to the color of material. For the parents or patients, the sealant is clearly visible to check any loss of sealant (Babu et al., 2014; Simonsen, 2002; Simonsen and Neal, 2011). Some clinician disagree using opaque sealant because it may obscure periodic examination underneath the material. However, application of pit and fissure sealant over incipient caries or stained pit and fissure does not indicate any cause of concern (Simonsen, 2002).

According to the study of sealant and the study comparing between filled and unfilled sealant, there are various classification of pit and fissure sealant based on filler content. When using the term of unfilled resin described above, filled sealant should be opaque and are available in numerous color shade. Unfilled sealant should be colorless or clear or tinted transparent material. However, some studies classify some opaque sealant as an unfilled sealant because of different method to categorize. The studies comparing between filled and unfilled resin are demonstrated in Table 6.

Table 6 – The study comparing between filled and unfilled sealant

Authors, year	**Sealant classified as filled sealant**	**Sealant classified as unfilled sealant**
Lajarin et al., 2000	Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)*	Helioseal (Ivoclar Vivadent AG, Schaan, Liechtenstein)
Koch et al., 1998	Helioseal F (Ivoclar Vivadent AG, Schaan, Liechtenstein)	Delton (Dentsply Professional, York, PA, USA)
Kumaran, 2013	Delton FS+ (Dentsply Professional, York, PA, USA) Helioseal F (Ivoclar Vivadent AG, Schaan, Liechtenstein)	Clinpro Sealant (3M ESPE, St. Paul, MN, USA)

Kusgöz et al., 2010	Grandio Seal (Voco, Cuxaven, Germany)	Clinpro Sealant (3M ESPE, St. Paul, MN, USA)
Ansari and Hashemi, 2008	Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)*	Prisma shield (Dentsply Professional, York, PA, USA)
Ashwin and Arathi, 2007	-	Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)*
Garcia-Godoy et al., 1996	Prisma shield (Dentsply Professional, York, PA, USA) FluroShield (Dentsply Caulk, Milford, DE, USA)	Helioseal (Ivoclar Vivadent AG, Schaan, Liechtenstein) Delton (Dentsply Professional, York, PA, USA) Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)*
Duangthip and Lussi, 2003	-	Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)*

Reddy et al., 2015	Helioseal F (Ivoclar Vivadent AG, Schaan, Liechtenstein)	Clinpro Sealant (3M ESPE, St. Paul, MN, USA)
Yazici et al., 2006	-	Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)*
Baca et al., 2007	Delton Plus (Dentsply Professional, York, PA)	Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)* Delton (Dentsply Professional, York, PA, USA)

*Concise sealant is classified in both filled and unfilled sealant.

It would be noted that Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA) are classified in both filled and unfilled sealant in different study. Filler content is a description of quantity of filler in composite material. Generally, the term “unfilled resin” is used for the resin without any filler in resin matrix. This group of material is usually used for unfilled sealant or bonding agent. The term “filled resin” commonly means the material which resin matrix is filled with filler approximately 30% w/w. Filler content descriptor is shown in Table 7 (Berg, 1998). Definition of both terms is also performed in Table 8 (Younger, 2012). Composition of available sealants which are classified as unfilled sealant described above from manufacturer's material safety data sheet is also shown in Table 9.

Table 7 – Filler content descriptor (Berg, 1998)

Category	**Filler content (approximate ranges)**
Unfilled resin	0% w/w
Unfilled bonding agent	0% w/w
Unfilled sealant	0% w/w
Filled sealant	15-50% w/w
Filled bonding agent	15-50% w/w
Flowable composite	50-70% w/w
Composite resin	70-85% w/w

Table 8 – Definition of unfilled/filled sealant (Younger, 2012)

Unfilled sealant	"Resin-based sealant material that does not contain filler particles; has a low viscosity that permits deeper penetration into the pits and fissures of a tooth"

Filled sealant	"Adhesive agent that contains, in addition to Bis-GMA, microparticle of glass, quartz, silica, and other fillers used in composite restoration; fillers make the sealant more resistant to abrasion"

Table 9 – Composition of sealant from manufacturer's material safety data sheet

Product	Composition (% w/w)
Helioseal (Ivoclar Vivadent AG, Schaan, Liechtenstein)	Bis-GMA (50-100%) TEGDMA (25-50%) Mequinol (0.3-1%)
Delton (Dentsply Professional, York, PA, USA)	Aromatic and aliphatic dimethacrylates (90-100%) Dichlorodimethyl Silane-, reaction products with silica (1-10%) Ethyl 4– dimethylaminobenzoate (1-5%) Titanium Dioxide (1-5%)
Clinpro Sealant (3M ESPE, St. Paul, MN, USA)	TEGDMA (40-50%) Bis-GMA (40-50%) Silane treated silica (5-10%) Tetrabutylammonium tetrafluoroborate (<5%)

	Diphenylliodonium hexafluorophosphate (<1%) Thiphenylantimony (<0.5%) EDMAB (<0.5%) Titanium Dioxide (<0.5%) Hydroquinone (<0.05%)
Concise Light Cure White Sealant (3M ESPE, St. Paul, MN, USA)	TEGDMA (45-55%) Bis-GMA (40-50%) Silane treated silica (5-10%) 4-(dimethylamino)-benzeneethanol (<0.5%)

Material safety data sheet (MSDS) is the essential tool when trying to find the cause of problem, such as occupational contact dermatitis, from material but the most of this sheet contains some undeclared material (Kanerva et al., 1997). In manufacturer's material safety data sheet of concise light cured white sealant, silane treated silica is found as filler in resin matrix (5-10% w/w). Moreover, opaque white color of material should be the effect of titanium dioxide filler since addition of 1% titanium dioxide to the clear resin which was introduced by 3M can change clear resin to be a colored resin. Other manufacturers have then followed this regime with various colors and tints (Simonsen, 1996). Thus, the authors who classified concise as unfilled resin probably classify based on quantity of filler which is less than common filled resin (15-50% w/w). The acceptable method to determine the filler particle weight content of dental material is the use of thermogravimetric analysis. Thermogravimetry is a technique which the mass of substance is observed as a function of time and temperature (Beun et al., 2008; 2012; Sabbagh et al., 2004). For example, Clinpro Sealant is classified as unfilled sealant (Reddy et al., 2015) while it is detected 6.5 % w/w by thermogravimetric analysis (Beun et al., 2012). However, filler measurement using this method has a minute difference from manufacturer's material safety data sheet. Nevertheless, Concise sealant is usually used as control in experimental study and as representative of resin-based sealant because it does not have any supplementary particle in its composition and it is known as basic pit and fissure sealant (Ashwin and Arathi, 2007; Ganesh and Shobha, 2007).

In addition to resin-based sealant, glass-ionomer materials are also used as pit and fissure sealant. This type of sealant can bond to enamel and dentin using physicochemical bond following acid conditioning. Even though wear resistance and long-term retention of glass-ionomer are still questionable, the important advantage of glass-ionomer over conventional resin sealant is the property of fluoride releasing (Hicks and Flaitz, 2005). However, resin-based sealant performs less microleakage which means better sealing ability (Ganesh and Shobha, 2007). Moreover, systematic review shows that resin-based sealant has more effective long-term anti-caries effect because of higher retention rate (Muller-Bolla et al., 2006).

Fluoride-releasing restorative materials tend to provide inhibitive effect on tooth demineralization in adjacent area. The amount of mineral loss is significantly decreased compared with that of non-fluoride material from 0.2 mm to 7 mm distance from the margin of restoration (Tantbirojn et al., 1997). The placement of fluoride-releasing material also reduces susceptibility of adjacent enamel and inhibits demineralization along the margin. Hence, it is the material that could reduce prevalence of secondary caries on tooth structure (Hicks and Flaitz, 2000). However, there is no clear correlation between in vitro and in vivo studies. It could be implied that artificial caries model could not precisely predict the clinical outcome (Papagiannoulis et al., 2002). Nevertheless, GIC could inhibit acid production of dental caries-related oral streptococci at low pH (Nakajo et al., 2009). Moreover, clinical experience associated with GIC showed that fewer secondary caries is seen compared with those of resin composite filling (Forsten, 1993).

A variable mixture of glass-ionomer and resin-based material are applied as pit and fissure sealant because of improved physical property, bonding ability, simple solution, and fluoride-releasing capability (Hicks and Flaitz, 2005). Properties, which the fluoride-containing resin-based sealant should have, are: (a) the constant rate of fluoride releasing for a prolonged period of time, (b) good mechanical properties and good enamel bonding, and (c) the sealant functions as a reservoir and a sealer of fluoride ion (Kadoma et al., 1983). There are 2 main methods to incorporate fluoride into pit and fissure sealant. First, soluble fluoride salt was added to unpolymerized resin. When sealant was applied to pit and fissure and polymerized, fluoride ion can be slowly dissolved in oral cavity. However, larger amounts of fluoride are released on first two days, and then the release tapers off. Released fluoride decreases by approximately one-half for each of first three days. This condition is called “burst effect” (Cooley et al., 1990). Second, because of the dissolution of fluoride salt, anion exchange system is used to solve this problem. In the latter method, an organic fluoride compound is chemically bond to the resin polymer. This method can avoid dissolution of fluoride salt that was incorporated into pit and fissure sealant (Ripa, 1993). Surface reaction-type pre-reacted glass-ionomer filler was introduced to incorporate in pit and fissure sealant. Glass-ionomer phase on glass particles plays an important role in fluoride recharge ability (Shimazu et al., 2011).

Mesoporous silica

The term “silica” is used for the compound silicon dioxide, SiO_2. Silica is used as reinforcing filler in thermoplastics, thermosets, and elastomers. Other applications of this filler include adhesives, sealant, coating, and electronic components. Most of silica filler is coated with coupling agents before use. Basic properties of precipitated silica are listed in Table 10 (Hohenberger, 2009). This type of material as filler in thermoplastic polymer can improve a range of properties including increased heat resistance, increased elasticity, increased hardness, and improved scratch resistance. These products which have very small particle size and high surface area are called silica gel. This physical property can promote their use in various applications include viscosity control agent, processing aid, selective adsorbent, and moisture removal agent (Lutz and Grossmann, 2001).

Table 10 – Properties of precipitated silica (97.5-99.4% SiO_2)

Temperature of decomposition	2000 °C
Acid solubility	None (except HF)
Water solubility	0.015 g/100 mL
Loss of ignition	3-18%
Density	2.0 g/cm^3
Hardness	1 *Mohs*
Thermal conductivity	0.015 W/m.K
Refractive index	1.48-1.65

Mesoporous silica is the solid material which has honeycomb-like porous structure with many empty channels that are able to absorb large amount of bioactive molecule (Slowing et al., 2008). This particle has become an attractive topic in biomedical application since it was used as drug delivery system in 2001 (Vallet-

Regi et al., 2001). It is potentially suitable for various medical substance controlled release applications because of high surface area, large pore volume, tunable pore size, and good thermal and chemical stability (Slowing et al., 2008).

Synthesis of mesoporous silica

The production of general fillers can normally be divided as top-down and bottom-up approaches (Fig. 6). The top-down approach involves etching or grinding bulk materials to smaller size and bottom-up approach involves building up from the atom or molecule. There are many techniques to produce or synthesize the particles and they can be classified into three categories (Singh et al., 2014):

1. Gaseous state preparation
    - 1.1 Gas condensation
    - 1.2 Vacuum deposition and vaporization
    - 1.3 Chemical vapor deposition
2. Solid state preparation
    - 2.1 Mechanical milling
3. Liquid state preparation
    - 3.1 Micro-emulsion method
    - 3.2 Sol-Gel process

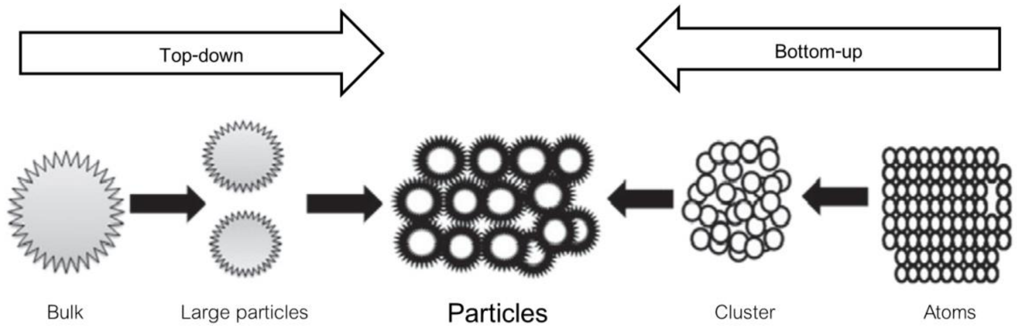


Fig. 6 – Approaches for particles preparation (Adapted from Singh et al., 2014)

Nowadays, it is generally accepts that conventional top-down approach cannot reduce the size of particles to nano-level. Therefore, the bottom-up approach is widely suggested to use as a promising method for small particles preparation. The trend of new researches seems to focus on particle preparation via this route because this method is more precise and faster than the other method. There are varieties of synthesized method in this route such as chemical vapor deposition, micro-emulsion method, or sol-gel process etc.; However, the most common and popular process to synthesize this particle is a sol-gel technique. This offers excellent control of reaction and it does not require high temperature synthesis (Singh et al., 2014).

High cost of precursors and long process duration are some disadvantages of this method. However, Low processing temperature of sol-gel synthesis can minimize the energy use and minimize evaporation loss of volatile component. For this reason, the purity of materials can be ensured by avoiding contamination during heat treatment and evaporation loss. Thus, a higher purity of synthesized particles would be obtained (Milea et al., 2011).

Sol-gel process

Sol-gel polymerization is a convenient way to synthesize silica gels because this method is able to produce homogeneous and high purity product at room temperature. For example, silica which is normally obtained from melt glass but the sol-gel method process is more effective for the production without high temperature condition (Alothman, 2012). Sol-gel process is the process for preparing inorganic polymers or ceramics from solution. It includes transformation from liquid precursors to a network structure (Danks et al., 2016). The sol-gel process is a number of processes which solution undergoes a sol-gel transition from "sol" to "gel" (Fig. 7). The term "sol" means the dispersion of colloidal particle that has a diameter ranged from 1-100 nm in liquid and the term "gel" means the substance which can be classified into 4 categories (Hench and West, 1990):

- Well-ordered lamellar structure
- Covalent polymeric networks

- Polymer network from physical aggregation
- Particular disordered structure

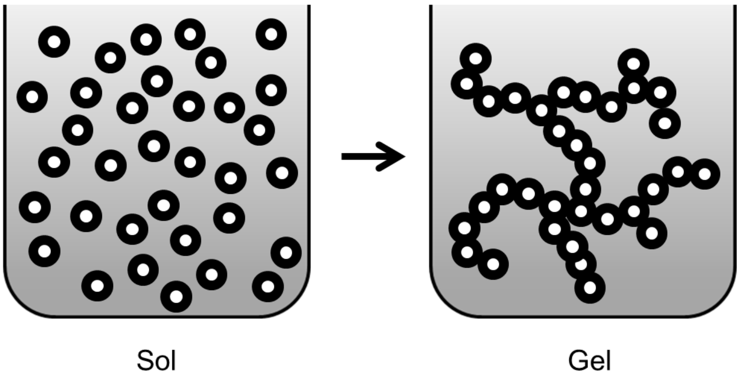


Fig. 7 – Sol-gel transition

There are wide varieties of synthetic parameters including precursor in sol-gel process which can provide the control of structural and chemical properties of product. It is also possible to design the composition, homogeneity, and pore structure of product (Ward and Ko, 1995). The gel can be synthesized from different ways. The same precursors with small difference in conditions can probably result in different structures and properties of product. Alkoxide is one of many precursors which can be used to synthesize silica. Metal element which is bound to reactive ligands is used as a precursor for colloid synthesis. In the present of water, Metal alkoxide is the most used precursor for this purpose. Alkoxysilanes, such as tetraethoxysilane (TEOS) and tetramethoxysilane (TMOS), are widely used for the production of silica gel (Alothman, 2012). The sol-gel process with an alkoxide as a precursor can be described in Fig. 8 and can be summarized in following steps (Danks et al., 2016):

- Synthesis of “sol” from hydrolysis and partial condensation of alkoxides
- Formation of “gel” via polycondensation
- Syneresis or aging when condensation process continues in gel network (This process results in expulsion of solvent and shrinkage)
- Gel drying

Normally, sol-gel process associates with hydrolysis and condensation reactions of alkoxide monomer in order to form a colloidal particle or sol and then consequently change to a network or gel. In hydrolysis step, silanol group is generated using a proper catalyst. The rate of reaction depends on pH, water-to-alkoxide ratio, and the solvent. Co-solvent for alkoxysilane is necessary in the process because it is not soluble in water. In the next step, the silanol group condenses with another silanol group or an alkoxide to form siloxane linkage with either an alcohol or a water molecule as by products. The siloxane particles will aggregate in the sol when the numbers of siloxane linkage increase. The dispersion of these particles in the solution will form small silicate cluster and the lead to the formation of gel network. The water and alcohol which is trapped in the network can be removed by heat treatment (Alothman, 2012).

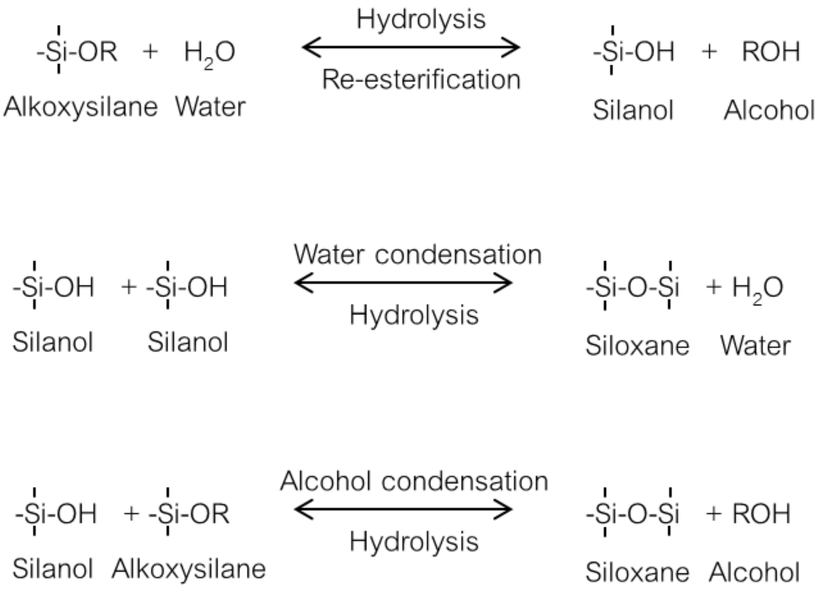


Fig. 8 – The sol-gel process with an alkoxide as a precursor

Calcium aluminate cement

Cement can be explained as adhesive substance which can unite fragments or masses of solid matter (Blezard, 2004). It also means a powder consisting alumina, silica lime, and other substances the can harden when it is mixed with water (University of Texas at Austin, 1991).

Hydraulic cement is the group of cement which sets and hardens under water and hence it often termed water-based cement. The example of hydraulic cement is Portland cement which is the common type of cement that is generally used as building material and construction industry material around the world. This material is commonly used in various dental applications, especially in restorative dentistry. This group of cement includes zinc phosphate cement, polycarboxylate cement, and glass-ionomer cement. Calcium aluminate cement is also derived from this class of cements (Jefferies, 2014b). The constituents of available calcium aluminate cement are shown in Table 11.

Table 11 – Composition (%) of available calcium aluminate cement in market

Product	A	B	C	D	E	F	G
CaO	28	28	28	27	18	18	18
Al_2O_3	71	71	71	72	81	81	81
Na_2O	≤0.3	≤0.3	≤0.3	≤0.3	≤0.6	≤0.8	≤0.8
SiO_2	≤0.3	≤0.3	≤0.3	≤0.3	≤0.3	≤0.3	≤0.3
Fe_2O_3	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2
MgO	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4

*from product data of Almatis

Calcium aluminate cement is the cement other than Portland cement in long-term and continuous production. In ternary diagram of CaO-SiO_2-Al_2O_3 system (Fig. 9), obvious difference of compositions is found between two types of cement. Since high alumina contained, this cement is probably termed aluminous cement or high-alumina cement (Scrivener, 2001). The term “high-alumina cement” is used to distinguish it from Portland cement which contains much less alumina. This cement is obtained by fusing or sintering a mixture which has suitable proportion of aluminous and calcareous materials and then grinded to obtain fine powder product. Calcareous material means the material containing or composing large portion of calcium carbonate (Blezard, 2004; University of Texas at Austin, 1991).

Setting reaction of calcium aluminate cement is shown in Fig. 10. After mixing this cement with water, acid-base reaction begins. The powder acts as the base and the water acts as the weak acid. Water starts dissolving the calcium aluminate and forms calcium ions, aluminum hydroxyl ions, and hydroxyl ions. At body temperature, new solid phases, Katoite and Gibbsite, are then precipitated when the solution saturates. Precipitations continue until a connected cluster of hydrates is built up (Jefferies, 2014a; Kraft, 2002).

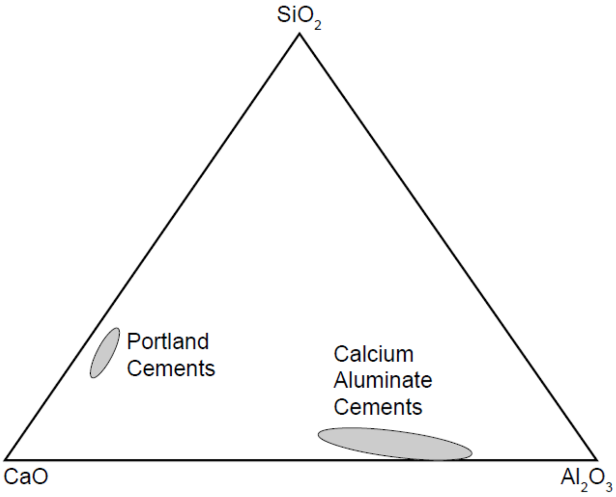
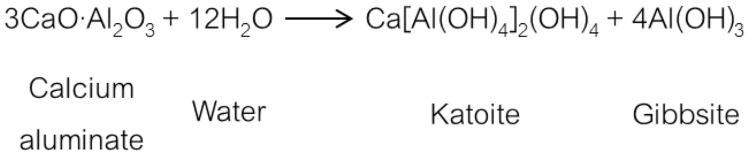


Fig. 9 – Ternary diagram of composition range of CaO-SiO_2-Al_2O_3 system of calcium aluminate cement compared to portland cement (Scrivener, 2001)



$$3CaO{\cdot}Al_2O_3 + 12H_2O \longrightarrow Ca[Al(OH)_4]_2(OH)_4 + 4Al(OH)_3$$

Fig. 10 – Chemical reaction of calcium aluminate cement at 37°C (Kraft, 2002)

Calcium aluminate cement as dental restorative material

There have been two calcium aluminate cement-based dental material products which are launched in Swedish market. First, dental ceramic composes of calcium aluminate cement and oxide (Doxadent, Doxa Dental AB, Uppsala, Sweden) which is purposed to restore class I, class II, and class V cavities as a permanent restorations (Fig. 11). Second, calcium aluminate-based luting agent (Ceramir, Doxa

Dental AB, Uppsala, Sweden) is intended to use as a permanent cement of dental prosthesis. However, calcium aluminate cement as restorative material would be focused in this review.

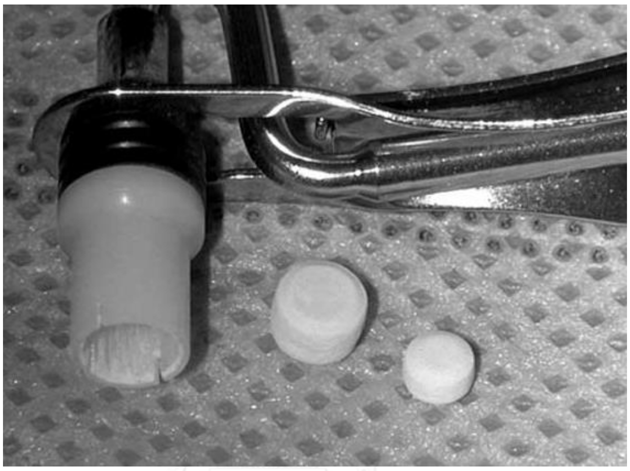


Fig. 11 – Calcium aluminate cement (Doxadent) tablets and carrier

(Geirsson et al., 2004)

Doxadent is marketed as a bioceramic material alternative to amalgam and resin composite with claim of biocompatibility. Although it can set directly in oral cavity with acid-base reaction without any heat treatment which can be found in other ceramic e.g. feldspathic porcelain, this product can probably be classified as ceramic materials due to its composition and setting reaction (Sunnegårdh-Grönberg et al., 2003). This restorative system has several advantages that allow this system to be used as biomaterial:

- The biocompatibility of this material (Jefferies, 2014a)
- Exhibited bioactivity (Loof et al., 2008)
- Rapid strength development (Scrivener and Capmas, 2004)
- Good sealing and tight bond between material and tooth (Engqvist et al., 2004)
- Proper retention due to slight expansion of material (Engqvist et al., 2004)
- High temperature resistance (Scrivener and Capmas, 2004)
- Resistance to chemically aggressive conditions (Scrivener and Capmas, 2004)
- Thermal expansion coefficient which is close to that of tooth substance (Kraft et al., 2004)

However, some studies raise concerns about its mechanical properties. Unacceptable failure rate of these restorations is reported due to their poor mechanical properties and difficulty of handling. High expansion of the cement can also increase the rate of cusp fracture when tooth is restored with this material (van Dijken and Sunnegårdh-Grönberg, 2003). Moreover, strength and modulus of this material are probably not proper to use as posterior restoration (Sunnegårdh-Grönberg et al., 2003). Higher porosity level of this material compared with that of other tooth color materials might be the reason of lower mechanical properties (Geirsson et al., 2004).

Buffer properties in dental materials

Glass-ionomer cements are generally used in clinical dentistry. They are mainly used for dental restoration and as a luting agent for fixed restorations (van Noort, 2007). The important advantage of glass-ionomer over conventional restorative materials is the property of fluoride releasing (Hicks and Flaitz, 2005). This property may be clinically beneficial against secondary dental caries and they can also be the buffer solution of lactic acid. The first study of neutralization property of glass-ionomer cement was published in 1987. In that study, a pH change was found when glass ionomers exposed to aqueous sodium fluoride solution (Billington et al., 1987). However, the capacity to buffer lactic acid solution of GIC, which is the mechanism that seems to be the complement property, was found in 1999. This mechanism which the pH of lactic acid solutions increase when glass-ionomer cements are stored because buffers have ability to change the pH of acid, moving to neutral solution might be clinically beneficial against secondary caries (Nicholson et al., 1999; Nicholson et al., 2000). Buffering is the term of the study of Czarnecka et al. applied to the ability of dental cements to increase the pH of lactic acid solutions to which they are exposed (Czarnecka et al., 2002).This effect is seemed to be important for long-term clinical result because of its property to protect restored teeth from the development of secondary caries (Nicholson et al., 2000; Patel et al., 2000). Dental caries can be arrested by relatively minor changes in pH. Thus, it is suggested that the presence the material that has lactic acid buffering property in oral cavity is likely to be beneficial. Moreover, the acid can also be sufficiently neutralized to increase the pH above the critical value (Nicholson et al., 1999).

Although lactic acid, acetic acid, and propionic acid are the major acids in carious dentin (Hojo et al., 1991), lactic acid is dominant in active lesion (Hojo et al., 1994). Thus, Lactic acid buffering is generally studied because the property of lactic acid buffering has potential clinical important (Nicholson et al., 2000). Normally, local plaque pH above 6 is classified in dental caries safe zone, pH between 5.5 and 6 is potentially cariogenic, and pH below 5.5 is cariogenic or danger zone (Xu et al., 2011). This trend shows that higher pH tends to reduce demineralization process and stimulate remineralization process. It results in reduced prevalence of dental cavity.

The lactic acid, which the pH is about 2 to 3, does not cause the adverse effect to cement specimens. Surface of cement is still smooth and has no other imperfections (Nicholson et al., 1999). The lactic acid concentration is various in different studies. Lowered pH is used because the clear change can be easily studied while high pH is used to mimic the pH of active dental caries. The volume of lactic acid is also various. Most of studies use larger amount while the study of Nicholson et al. use thin film and small volume of lactic acid to mimic clinical conditions (Nicholson et al., 2000). Table 12 shows experimental design in buffering test.

Table 12 – Experimental design in buffering test

Author, year	Type of materials tested	Diameter of specimens	Thickness of specimens	Result
Nicholson et al., 2000	glass-ionomer cements	13 mm	0.8 mm	Thin film (20 µL) and small volume (1.5 mL) of lactic acid (0.0002 mol/L) have a change in pH when applied in lactic acid. More rapid change is found in thin film lactic acid.
Nicholson et al., 1999	Glass-ionomer cement, zinc phosphate, and zinc polycarboxylate cement	6 mm	12 mm	The pH of lactic acid (0.02 mol/L) is increased over a period of 7 days.
Czarnecka et al., 2002	glass-ionomer cements	6 mm	12 mm	The pH of water and lactic acid (0.02 mol/L) is increased for storage periods of 1 week up to 6 weeks. In water, pH change does not vary significantly with time. In lactic acid, the change in first week is greater.

Wang et al., 2011	S-PRG filler-containing pit and fissure sealants	13 mm	1 mm	Most of S-PRG filler-containing pit and fissure sealants can increase pH of lactic acid (pH 4.0)

Zeta potential

Zeta potential is a scientific term of the potential at liquid-solid interface. It is one of methods to determine surface potential such as the surface of shear. It is useful to study in suspension and emulsion (Kirby and Hasselbrink, 2004). The symbol ζ is generally used to donate this potential. Thus, ζ potential can be simply called to explain (Kohler, 1993). The value of zeta potential can be calculated from electrokinetic experiments. The velocity of particle relative to the surrounding solution that is stimulated by the model of electrical double layers is measured and it can be converted to zeta potential value.

Ions of opposite charge to the charge of particle will be attached to the surface of particle. Higher concentration of counter ions is found where they are close to the surface of particle. The liquid layer around the particle can be divided to two layers: the inner layer (stern layer) and outer layer (diffuse layer). In stern layer, ions are firmly bound to the surface while they are weaker in diffuse layer. Within the diffuse layer, the ions are stable and the boundary is formed. The ions in this boundary will move when the particle moves. This boundary is called slipping plane and the zeta potential is the potential that exists at this plane (Fig. 12).

The movement of particles happens when an electric field is applied. Particles are attracted to the electrode that has opposing charge to the charge of particles. The particle will move with constant velocity when equilibrium is reached. However, there are many factors that affect the velocity such as viscosity of medium, dielectric constant of medium, strength of electric field, and zeta potential. It means the zeta potential is not the only factor that affects the velocity. Thus, zeta potential has to be calculated using Henry equation before analyzing.

The particles that have large negative or large positive zeta potential value tend to repel each other. Moreover, there is low chance to flocculate. The value between -30 and +30 mV is generally known as unstable suspension because small negative or positive value means there is low force of particle to repel each other. It might be suggested that the particles seem to come together and flocculate (Malvern Instruments, 2004).

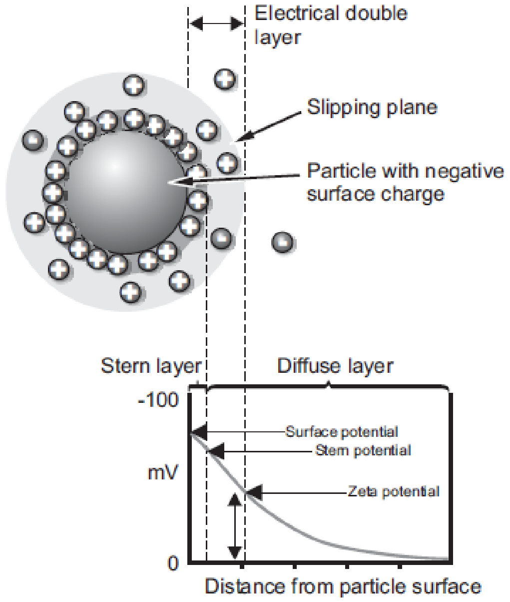


Fig. 12 – The liquid layer surrounding the particle (Malvern Instruments, 2004)

Particle sizing

The particle size strongly affects important properties of composites such as rheological properties and optical properties. There are various techniques to measure the size of particle: single-particle detection, light scattering, sizing by flow, acoustic, or surface area measurement. However, light-scattering technique is one of strong techniques of particle size measurement. Particle-size distributions (PSD) of suspended particles in solution can be measured in two modes: Mie scattering and quasi-elastic light scattering. Mie scattering technique is to measure the intensity of scattered light as a function of scattering angle while quasi-elastic light scattering technique is to measure scattered light at fixed angle (time dependence). In Mie scattering technique, single detector on goniometer or multiple detectors can be used to measure the intensity of light at many angles. However, Mie measurements are based on spherical shape or nearly spherical shape (Morrison and Ross, 2002).

This technique is based on the principle that the particle in suspension will scatter the light at different angle related to the size. The diffraction angle appears to be inversely proportional to the size of particles. For example, the small particles can scatter at higher angle than those of large particles. The laser diffraction can be described by Mie theory. However, this assumption requires spherical shape of particles (Rawle, 1997).

Colloidal stability (Raj and Cannon, 2002; Shi, 2002; Zaman, 2002)

The particles in dispersion medium usually shows Brownian movement and the stability of colloids can be determined by the interaction between the particles. There are two basic forces in this principle: attractive force and repulsive force. The particles tend to come together, adhere together, and flocculate when attraction dominates. On the contrary, the particles tend to repel each other and the system is stable when repulsion dominates. However, van der Waals force is a basic primary attractive force. Colloidal stability can normally be achieved by repulsive potentials, opposing to van der Waals attractive potential. The repulsive force should be at least as strong as the attractive force to stabilize sufficiently. Colloidal stability is normally achieved by polymeric and/or electrostatic stabilization.

Electrostatic potential is the stabilization from a surface charge and potential that fall off away from the surface of particles. The surface charge can be caused by different mechanisms to form a charged layer such as absorbed ions on surface, ionization, or direct charge exchange with the fluid. To maintain neutrality, counter-ions around the fluid must balance the surface charge. They form the double-layer that is an ionic cloud around the particle. Mutual repulsion surrounding particles play an important role in electrostatic stabilization of colloid (Fig. 13).

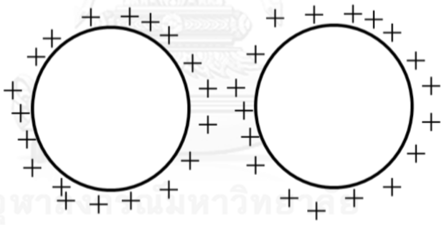


Fig. 13 – Schematics of electrostatic stabilization

Polymeric stabilization is the mechanism that affected by polymer molecules. There are two different mechanisms in this type of stabilization: steric stabilization and depletion stabilization. Steric stabilization is achieved by macromolecules anchored to the particles (Fig. 14). The particles are separated by overlapping polymer chains. Depletion stabilization of particles is achieved by macromolecules that are free in the solution (Fig. 15).

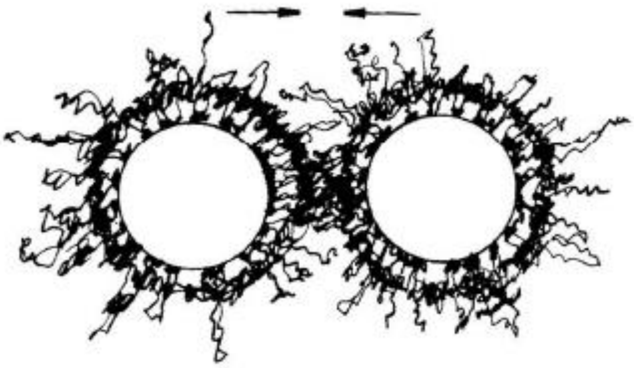


Fig. 14 – Schematics of steric stabilization

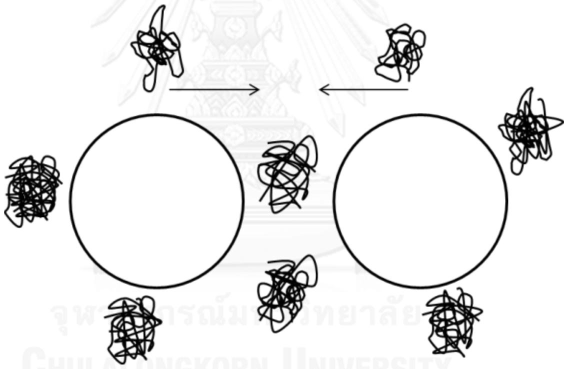


Fig. 15 – Schematics of depletion stabilization

Electrosteric stabilization is a combination of electrostatic and steric stabilization. The source of electrostatic charge is from net charge on the particle surface and charge associated with polymeric macromolecules attached to the particle surface (Fig. 16).

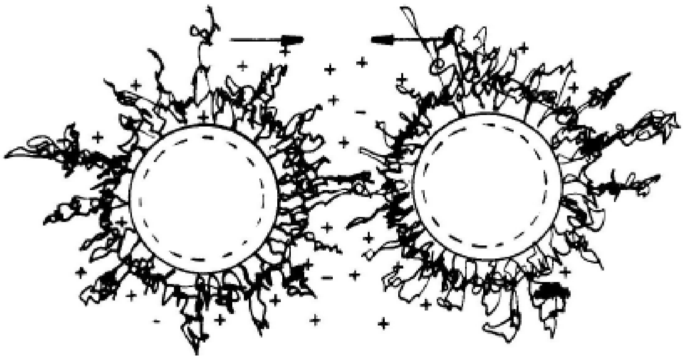


Fig. 16 – Schematics of electrosteric stabilization

CHAPTER 3

MATERIALS AND METHODS

Part 1: Mesoporous silica synthesis

The rice hull ash used for the experiments was collected from a local industry in Ayutthaya, Thailand. The rice hull ash had been burned during the process of rice manufacturing. The obtained rice hull ash was burned at 600ºC for 3 hours to prevent incomplete combustion. White rice hull ash from complete combustion was mainly free from carbon or it contained a small amount of carbon (Fig. 17), while incomplete combustion resulted in black or grey rich hull ash (Fig. 18).

Fig. 17 – Rice hull ash after complete combustion

Fig. 18 – Rice hull ash after incomplete combustion

Mesoporous silica was extracted from rice hull ash using method adapted from that of Kalapathy et al (Kalapathy et al., 2000b). Rice hull ash was washed with hydrochloric acid (HCl) to remove other minerals prior to silica extraction process. Ten grams of rice hull ash was suspended in 60 mL of distilled water. The pH was adjusted using 1 N of HCl until the pH decreased to 7. This suspension was stirred on the stirrer for 120 minutes and then filtered through Whatman grade 42 ashless filter paper under vacuum. The residue was then washed with 100 mL of distilled water through filter paper. This residue was used for the mesoporous silica extraction.

The washed rice hull ash residue was suspended in 60 mL of 1 N sodium hydroxide (NaOH) and boiled with stirring at 100°C for 1 hour to dissolve silica and produce sodium silicate solution (Fig. 19). The dispersion was filtered through

Whatman grade 42 ashless filter paper and then washed with 100 mL of boiling water (Fig. 20 and Fig. 21).

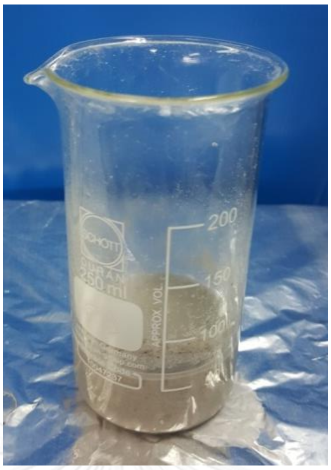


Fig. 19 – The washed rice hull ash residue was suspended in 60 mL of 1 N NaOH and boiled with stirring at 100°C

Fig. 20 – The dispersion was filtered through Whatman grade 42 ashless filter paper and then washed with 100 mL of boiling water

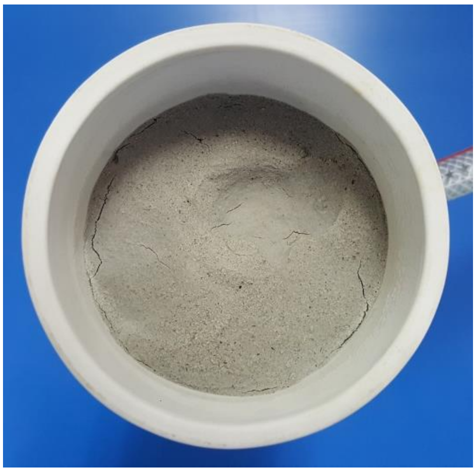


Fig. 21 – The filtered dispersion after washing with boiling water

After 30 minutes, filtrated solution was titrated with 1 N HCl to pH 7 (Fig. 22). The pH was verified with pH-indicator strips pH 0 - 14 Universal indicator (Merck Millipore, Darmstadt, Germany) (Fig. 23). The gel was slowly precipitated in neutral solution and it was allowed to age for 18 hours (Fig. 24 and Fig. 25). After aging, 100 mL of distilled water was added to the gel and the mixture was stirred for 15 minutes to crush the gel into slurry. The slurry was then divided into two equal parts and was centrifuged at 2,500 rpm for 15 minutes (Fig. 26). The supernatant was discarded and the washing step was repeated.

Fig. 22 – Titration of solution

Fig. 23 – The pH-indicator strips pH 0 - 14 Universal indicator

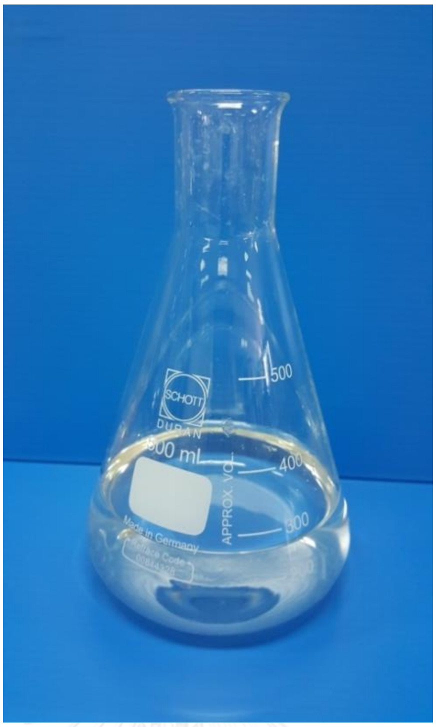


Fig. 24 – The solution before aging

Fig. 25 – The gel was precipitated after aging

Fig. 26 – The slurry was divided into two equal parts

After centrifuging process was repeated, the gel was collected (Fig. 27) and dried at 80°C for 12 hours. The dried gel was ground and washed with distilled water through filter paper before dried at 80°C and ground. The mesoporous silica particle was sieved (<45 μm) prior to use.

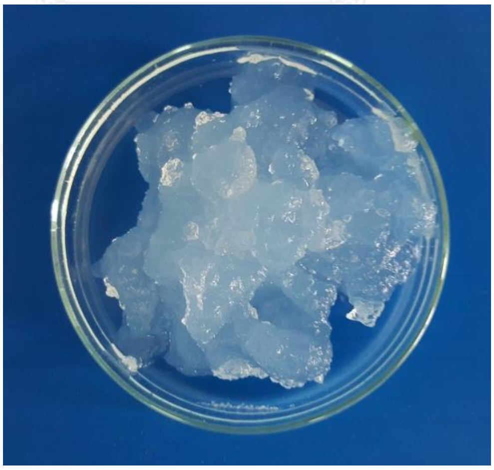


Fig. 27 – The collected gel

Part 2: Additional filler preparation

The groups of filler which would be used as filler in resin-based pit and fissure sealant are listed in Table 13.

Table 13 – Materials used in this study and their preparation

Sample	Filler	Filler preparation
Control group	-	-
SI	Mesoporous silica synthesized from rice hull ash	Described in part 1
CAC	Calcium aluminate cement	Calcium aluminate powder (CA-25R, Almatis BV, Rotterdam, The Netherlands) was mixed with distilled water (Water-cement ratio is 0.6) and allowed to set for 90 minutes. After final setting, cement was dried at 100 °C for 2 hours, milled for 2 hours, and then sieved (<45 μm) before use.
GIC	The powder of conventional glass-ionomer cement	The powder of conventional glass-ionomer cement (GC Fuji IX Gold label, GC Corporation, Tokyo, Japan) without

		any surface treatment was sieved (<45 μm) before use.
GICA	The powder of conventional glass-ionomer cement	The powder of conventional glass-ionomer cement (GC Fuji IX Gold label, GC Corporation, Tokyo, Japan) was suspended in polyethylene bottle containing 4% acetic acid solution for 24 hours. The selection of this concentration of acetic acid was made because it was used in the ISO standard solubility test (International Standards Organization, 2015). Acetic acid is believed that it is corrosive enough to form the soluble complex on the surface of dental ceramic (Milleding et al., 1999). The dispersion of glass-ionomer cement powder in acetic acid solution was filtered through Whatman grade 42 ashless filter paper under vacuum and the then washed thoroughly using distilled water. After the

		powder was filtered and dried in desiccator for 24 hours, it was milled and sieved (<45 µm) prior to use.
Combination of filler (X+Y)	Mixture of 2 groups of filler (equal % w/w)	Use 2 groups of filler which had highest fluoride recharge ability in pilot study

Varying the weight of each filler to find optimal weight ratio

Combination of filler (X+Y)	Mixture of 2 groups of filler (weight ratio 1:1)	Use 2 groups of filler which had highest fluoride recharge ability in pilot study
Combination of filler (X+Y)	Mixture of 2 groups of filler (weight ratio 1:2)	Use 2 groups of filler which had highest fluoride recharge ability in pilot study
Combination of filler (X+Y)	Mixture of 2 groups of filler (weight ratio 2:1)	Use 2 groups of filler which had highest fluoride recharge ability in pilot study

Part 3: Specimen preparation (for fluoride release, recharge, and neutralizing ability testing)

Six-mL-bottle of resin-based pit and fissure sealant (Concise Light Cured White Sealant, 3M ESPE, St. Paul, MN, USA) was used in this study. The 5% w/w of filler was added to the sealant and mixed for 60 seconds. The procedure was performed in an amber plastic chamber to prevent visible light that could affect the polymerization of light-curing material. This weight proportion of filler to pit and fissure sealant was chosen to achieve the suitable viscosity of the experimental sealant. The viscosity should not obviously increase because of the difficulty when handling the material during sealant placement. In control group, the sealant alone without filler adding was placed in the chamber and stirred using disposable brush tip for 60 seconds.

Twenty specimens of each group were prepared in a plastic mold (10 mm diameter and 1 mm deep) (Bayrak et al., 2010). A transparent polyester film (0.15 mm thick) was placed on a 1-mm thick clear glass slide and the mold was then placed over the film. The pit and fissure sealant were prepared as described above, and were slowly placed into the mold, slightly overfilled it. A second polyester film was placed on the mold followed by second glass slide and pressed with a constant 1-kg weight in order to extrude and allow the excess cement to be drained, to obtain smooth surface of specimen, and to prevent oxygen inhibited layer. A round weight tip was used to allow exposure of the specimen to the curing light.

An LED light curing unit with 10-mm-diameter light guide (Elipar Freelight 2, 3M ESPE, St Paul, MN, USA) which was previously measured the light intensity using a radiometer (Demetron Research Corp., Danbury, CT, USA) that could produce above 1,000 mW/cm^2 was used for light activation. Light activation of specimens was performed for 20 seconds per section. At the beginning, irradiation was performed around the weight for seven overlapping sections and then the weight was removed before central section of specimen was irradiated. The opposing side of specimen was irradiated in the same way as was done in first side. Therefore, the specimen was received eight overlapping sections of light activation on each side. After light curing, specimen was gently removed from plastic mold and immediately wet polished on each side with 600-grit silicon carbide paper for 10 seconds (Shimazu et al., 2011).

Part 4: Fluoride release and recharge ability measurement

Ten specimens for each group were individually stored in 3 mL deionized distilled water in a plastic container at 37°C. Then, the fluoride concentration of the solution was measured after 72 hours and then every 3 days using fluoride selective electrode (Orion 9609BNWP, Thermo Scientific, Beverly, MA, USA) coupled to electrochemistry benchtop meter (Orion Versastar, Thermo Scientific, Beverly, MA, USA). One mL of solution was pipetted into a new plastic measuring container and 0.1 mL of Total Ionic Strength Adjustment Buffer (TISAB III, Thermo Fisher Scientific, Chelmsford, MA, USA) was added before measurement. The meter was calibrated so that the fluoride readings were based on a calibration curve derived using 1, 0.1 and 0.01 ppm fluoride standard solutions (Certipur, Merck, Darmstadt, Germany).

After each measurement, each specimen was rinsed with deionized water, cleaned in 50 mL of deionized water using an ultrasonic bath for 1 minute, and then stored in 3 mL of fresh deionized water in a new plastic container. The experimental procedure of fluoride release and recharge from day 3–27 is shown in Table 14 and Fig. 28.

To determine the fluoride recharge ability of the specimens, each specimen was individually soaked in 2 mL of acidulated phosphate fluoride (APF) gel (60 Second Taste Gel, Pascal, Bellevue, WA, USA) in a plastic tube for 4 minutes on day 9 and day 18 after taking fluoride measurements. The APF gel contained 2.72% w/v sodium fluoride, generating 1.23% w/w fluoride ions. After soaking in the fluoride gel, each specimen was rinsed with deionized water and cleaned in 50 mL of deionized water using an ultrasonic bath for 1 minute and then stored in 3 mL of fresh deionized water in a new plastic container.

Table 14 – Regime of experimental procedure of fluoride release and recharge from day 3 to day 27

Measurement day	Storage in fresh deionized water	Fluoride recharge after measurement
3	Yes	No
6	Yes	No
9	Yes	Yes
12	Yes	No
15	Yes	No
18	Yes	Yes
21	Yes	No
24	Yes	No
27	Yes	No

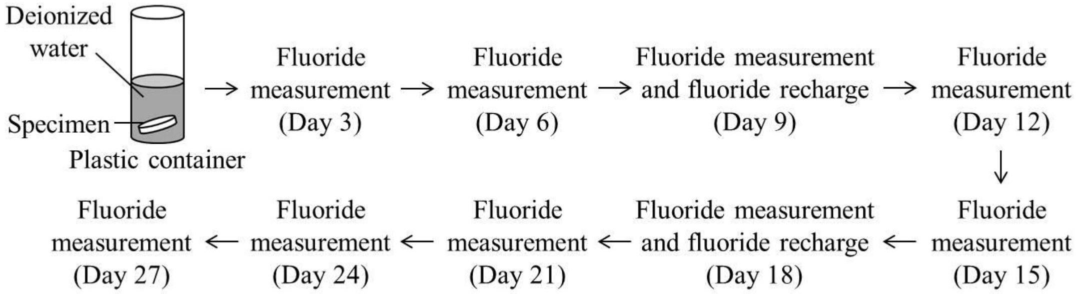


Fig. 28 – The experimental procedure of fluoride release and recharge

Part 5: Neutralizing ability measurement

Ten specimens for each group, prepared as described above, were stored in individual containers in 2 mL of deionized water at 37°C for 24 hours. Lactic acid at a concentration of 0.0002 mol/L was freshly prepared (Nicholson et al., 2000). Each specimen was stored in a plastic container with 2 mL of the lactic acid solution for 24 hours. Two mL of the lactic acid solution alone was placed in 10 containers as the control group. After 24 hours, the pH of the test solutions and controls were measured using a pH meter (Orion 420A, Orion Research Inc., Boston, MA, USA) that was calibrated at pH 4.00 and pH 7.00 immediately before use.

Part 6: Zeta potential measurement

The zeta potential is electrical charge of surface of particle. This data can provide information about the stability of particles in suspension. The particles that have large negative or large positive zeta potential value tend to repel each other. Moreover, there is low chance to flocculate. The value between -30 and +30 mV is generally known as unstable suspension because small negative or positive value means there is low force of particle to repel each other. It might be suggested that the particles seem to come together and flocculate (Malvern Instruments, 2004). However, the relative zeta potential can only be used to compare the colloidal stability of homogeneous particles in same media (Sadat-Shojai et al., 2010). In this study, the surface potential of mesoporous silica synthesized from rice hull ash, calcium aluminate cement powder, glass-ionomer cement powder, and acetic acid-treated glass-ionomer cement powder was determined. A Zetasizer Nano ZS90 and Mastersizer 2000 (Malvern instruments, Worcestershire, UK) was used to measure the zeta potential of the samples by electrophoresis. Samples were dispersed in suitable media, distilled water, and then placed in disposable measurement chambers. The electrophoresis was measured and then converted to zeta potential data.

Part 7: Particle size distribution measurement

The particle size distribution of mesoporous silica synthesized from rice hull ash, calcium aluminate cement powder, glass-ionomer cement powder, and acetic acid-treated glass-ionomer cement powder was determined using laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK).

Sample was dispersed in suitable media, distilled water, and then placed in disposable measurement chambers before measurement.

Part 8: Scanning electron microscopy (SEM)

The morphology of mesoporous silica synthesized from rice hull ash, calcium aluminate cement powder, glass-ionomer cement powder, and acetic acid-treated glass-ionomer cement powder was observed using an ultra-high resolution Schottky field emission scanning electron microscope (JSM-7610F, JEOL, Tokyo, Japan). The samples were platinum coated by a rotary pumped sputter coater (Quorum Q150R S, Quorum Technologies, East Sussex, UK) before SEM observations (50,000X).

Part 9: particles surface area measurement

The specific-surface-area of mesoporous silica synthesized from rice hull ash, calcium aluminate cement powder, glass-ionomer cement powder, and acetic acid-treated glass-ionomer cement powder was measured using multipoint BET particle surface area analyzer (Autosorb-1, Quantachrome Instruments, Boynton Beach, FL, USA).

Part 10: Statistical analysis

The differences of fluoride release and pH between the groups on each experimental day were statistically analyzed using one-way ANOVA or the independent t-test (SPSS version 22) at a significance level of 0.05. Data was also evaluated using the Bonferroni post hoc test when equal variances were assumed or the Tamhane's T2 post hoc test when equal variances were not assumed. The differences in fluoride release between experimental days were evaluated using the paired t-test.

CHAPTER 4

RESEARCH RESULT

According to preliminary pilot study, highest fluoride recharge ability was found in resin-based pit and fissure sealant with calcium aluminate cement filler and resin-based pit and fissure sealant with synthesized mesoporous silica filler. Thus, combination of filler explained in chapter 3 was the mixture of calcium aluminate cement and synthesized mesoporous silica (X+Y=CAC+SI). The specimens were fabricated with varying filler proportions. Resin-based sealant was incorporated with 5% w/w of the following fillers: a CAC and SI mixture with CAC/SI weight ratio of 1: 1 (CAC1: SI1), CAC/SI weight ratio of 1: 2 (CAC1: SI2), and CAC/SI weight ratio of 2: 1 (CAC2: SI1).

The amount of fluoride released from each material is shown in Table 15 and Fig. 29. At day 3 and 6, initial fluoride release was only found in the GIC and GICA groups with the released fluoride in these groups returning to baseline level by day 9. In these groups, a significant decrease in fluoride release was found at day 6 compared with day 3. GICA released significantly higher fluoride level at day 6 compared with the GIC group, however, a significant difference was not found at day 3 between these groups. After recharge at day 9 and 18, increased fluoride release was found at day 12 and 21 and the fluoride level decreased to baseline by day 15 and 24, in both groups. At day 12 and 21, the GICA group released significantly more fluoride compared with that of the GIC group.

The control group did not demonstrate significant differences in fluoride release before or after recharge throughout this study. The initial fluoride release in the CAC, SI, and CAC+SI groups was similar to baseline, however, fluoride release was found after these groups were recharged at day 9 and 18. After being recharged, the CAC+SI group showed the highest fluoride release at day 12, 15, 21, and 24. Although the mean fluoride release in the CAC+SI group at day 24 was 0.0106 ppm, it was reported as a trace level of fluoride release because it was out of the measurement range of the standard curve. However, nine days after each recharge at day 9 and 18, the fluoride level decreased to baseline in the CAC+SI group by day 18 and day 27, respectively.

Table 15 – Amount of fluoride released from specimens containing filler and control (ppm)

Group	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
Control	ND	ND	ND	ND	ND
CAC	ND	ND	ND	0.1064^{c} (0.0096)	0.0224^{a*} (0.0022)
SI	ND	ND	ND	0.1151^{c} (0.0049)	ND
CAC+SI	ND	ND	ND	0.3073^{d} (0.0227)	0.0219^{a*} (0.0025)
GIC	0.1095^{a} (0.0064)	0.0478^{a*} (0.0037)	ND	0.0374^{a} (0.0029)	ND
GICA	0.1062^{a} (0.0049)	0.0554^{b*} (0.0043)	ND	0.0794^{b} (0.0039)	ND

Group	Day 18	Day 21	Day 24	Day 27
Control	ND	ND	ND	ND
CAC	ND	0.0818^{c} (0.0121)	ND	ND
SI	ND	0.0949^{c} (0.0031)	ND	ND
CAC+SI	ND	0.1848^{d} (0.0089)	TR	ND
GIC	ND	0.0317^{a} (0.0029)	ND	ND
GICA	ND	0.0505^{b} (0.0028)	ND	ND

Values with the same letters are not significantly different (p>0.05) on the same experimental day (a = lowest values).

ND: Not detectable (less than 0.01 ppm)

TR: Trace (more than or equal to 0.01 ppm but less than 0.02 ppm)

*: significantly different from previous experimental day (p<0.05)

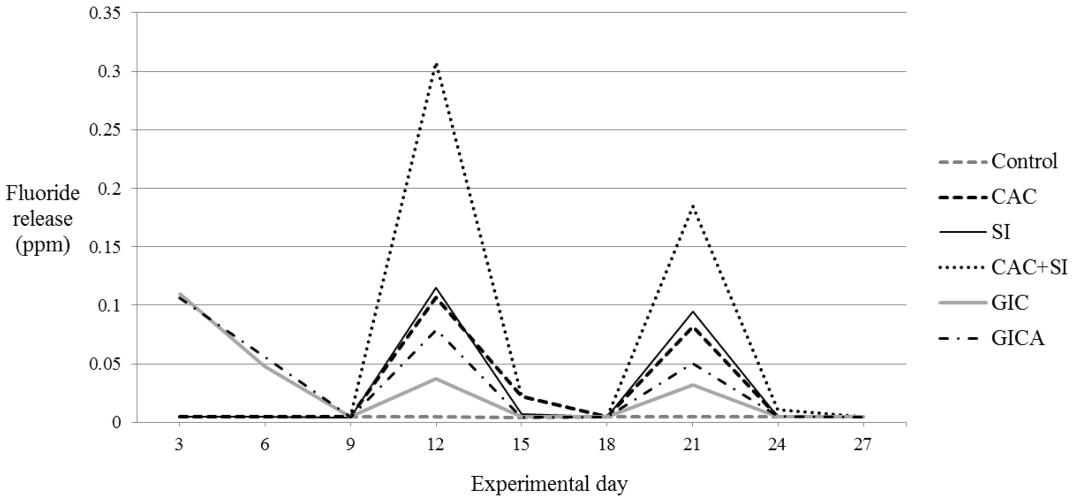


Fig. 29 – Amount of fluoride released from specimens containing filler and control

Fluoride release and recharge of specimens varying filler proportion of calcium aluminate cement and mesoporous silica fillers was demonstrated in Table 16 and Fig. 30. The initial fluoride was similar to baseline, however, fluoride release was found after these groups were recharged at day 9 and 18. After being recharged, CAC1: SI1 and CAC2: SI1 groups showed the highest fluoride release at day 12, 15, 21, and 24. Significant difference between two groups was not found in all experimental days which the fluoride level was more than baseline. The pattern of fluoride release and recharge is same as that of CAC+SI group. In CAC1: SI2 group, fluoride release also increased after recharge but the level was less than that of CAC1: SI1 and CAC2: SI1 group at day 12, 15, 21, and 24.

Table 16 – Amount of fluoride released from specimens containing calcium aluminate cement and mesoporous silica when weight ratio was varied (ppm)

Group (CAC:SI)	Day 3	Day 6	Day 9	Day 12	Day 15
Control	ND	ND	ND	ND	ND
CAC1: SI1	ND	ND	ND	0.3015^{b} (0.0312)	0.0228^{b*} (0.0036)
CAC2: SI1	ND	ND	ND	0.2856^{b} (0.0121)	0.0214^{b*} (0.0029)
CAC1: SI2	ND	ND	ND	0.1818^{a} (0.0203)	0.0121^{a*} (0.0015)

Group	Day 18	Day 21	Day 24	Day 27
Control	ND	ND	ND	ND
CAC1: SI1	ND	0.1866^{b} (0.0054)	TR	ND
CAC2: SI1	ND	0.1920^{b} (0.0071)	TR	ND
CAC1: SI2	ND	0.1188^{a} (0.0130)	ND	ND

Values with the same letters are not significantly different ($p>0.05$) on the same experimental day (a = lowest values).

ND: Not detectable (less than 0.01 ppm)

TR: Trace (more than or equal to 0.01 ppm but less than 0.02 ppm)

*: significantly different from previous experimental day ($p<0.05$)

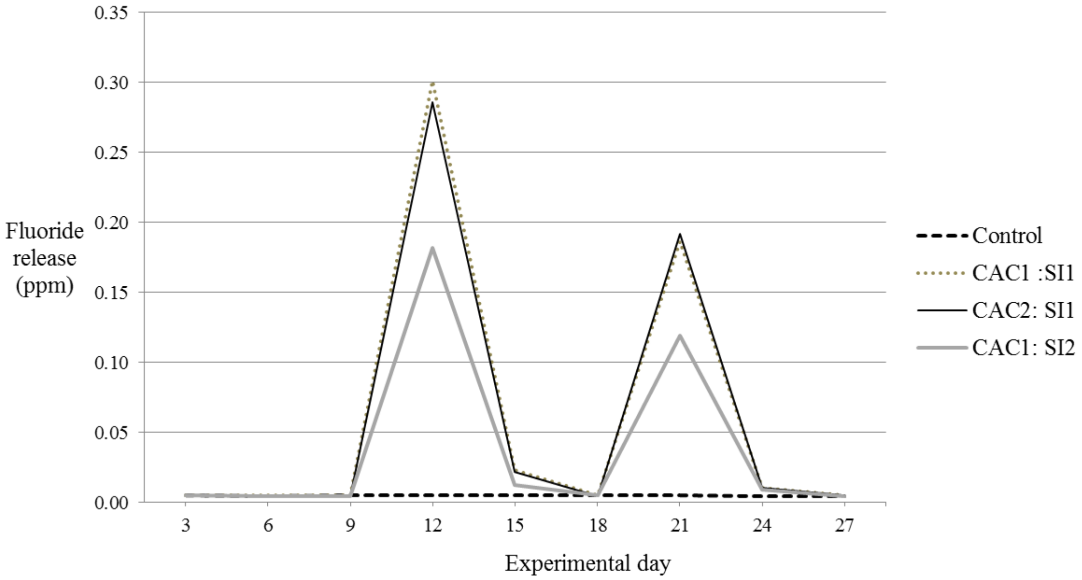


Fig. 30 – Amount of fluoride released from specimens containing calcium aluminate cement and mesoporous silica when weight ratio was varied

The results of pH change of lactic acid solution are shown in Table 17 and Fig. 31. The pH was measured before soaking specimen in lactic acid solution and significant difference was not found between groups. After 24 hours, pH of lactic acid solution without specimen soaking did not statistically change. The result of resin-based sealant without active filler added was in the same way.

The pH value of lactic solution increased in all groups with filler incorporation. The highest increase of pH value was found in CAC, CAC+SI, and GICA groups.

Table 17 – Mean lactic acid solution pH change after 24 hours

Group	Initial pH	pH after 24 hours	pH difference
No specimen	3.77^{a} (0.03)	3.78^{a} (0.03)	0.01^{a} (0.02)
Control	3.77^{a} (0.03)	3.77^{a} (0.03)	0.00^{a} (0.03)
CAC	3.77^{a} (0.02)	4.48^{d} (0.11)	0.71^{d} (0.11)
SI	3.77^{a} (0.02)	4.21^{b} (0.03)	0.45^{b} (0.03)
CAC+SI	3.77^{a} (0.03)	$4.39^{c,d}$ (0.06)	0.61^{d} (0.06)
GIC	3.77^{a} (0.02)	$4.36^{c,d}$ (0.04)	$0.59^{c,d}$ (0.05)
GICA	3.78^{a} (0.03)	4.30^{c} (0.05)	0.52^{c} (0.05)

Mean (SD)

Values with the same letters in the same column are not significantly different (*p*>0.05) (a = lowest values).

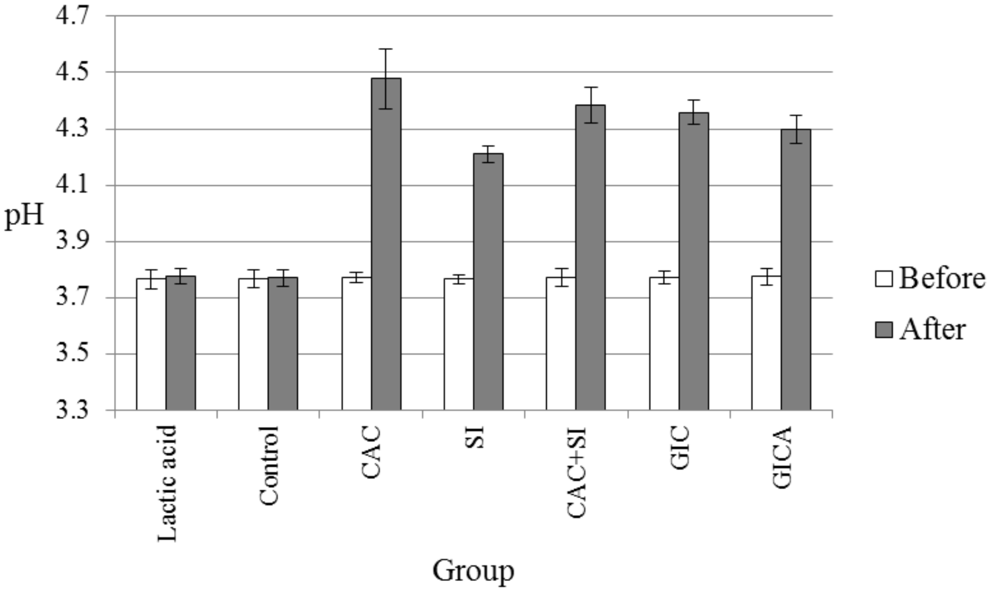


Fig. 31 – Value and standard deviation of pH of lactic acid solution containing specimen for 24 hours

The zeta potentials which are shown in Table 18 and Fig. 32 indicate negative surface charge in the solution. The most negative surface charge was found in untreated glass-ionomer cement powder group (GIC). This result indicated that the dispersion of GIC was probably the most stable filler particle in suspension. However, this value was less negative than dividing line between stable and unstable suspension. Electrostatic stabilization might not play an important role in colloidal stability of these fillers. Particle size of fillers is demonstrated in Table 19.

Table 18 – Zeta potential of filler particles

Filler particles	Zeta potential (mV)
CAC	-14.7 ± 0.3
SI	-14.0 ± 1.4
GIC	-28.2 ± 7.0
GICA	-20.3 ± 3.9

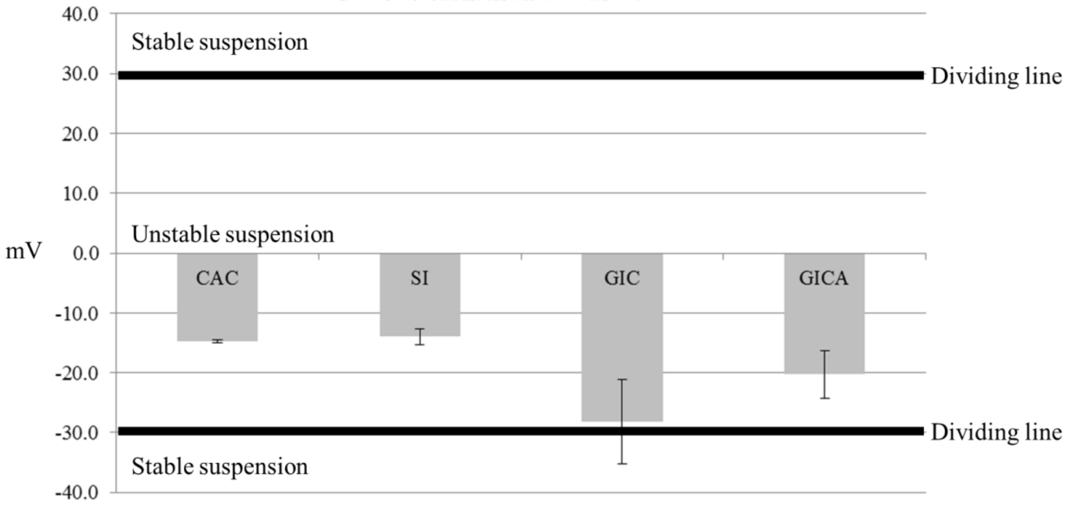


Fig. 32 – Zeta potential of filler particles and dividing line between stable and unstable suspensions

Table 19 – Median diameter of the particle size distribution

Filler particles	Median diameter (µm)
CAC	7.95
SI	6.97
GIC	6.09
GICA	12.93

The filler particle surface morphology was evaluated using SEM. The irregular surface of the SI filler appeared as a clumping of fine cubic-shaped particles with a particle size of about 100 nm into coral-like agglomerates (Fig. 33). The CAC filler demonstrated a gel-like structure with micro-tunnels on the surface (Fig. 34). The GIC filler demonstrated irregular-shaped particles with a smooth surface (Fig. 35A), however, the particle surface appeared rough after acetic acid treatment (GICA) (Fig. 35B).

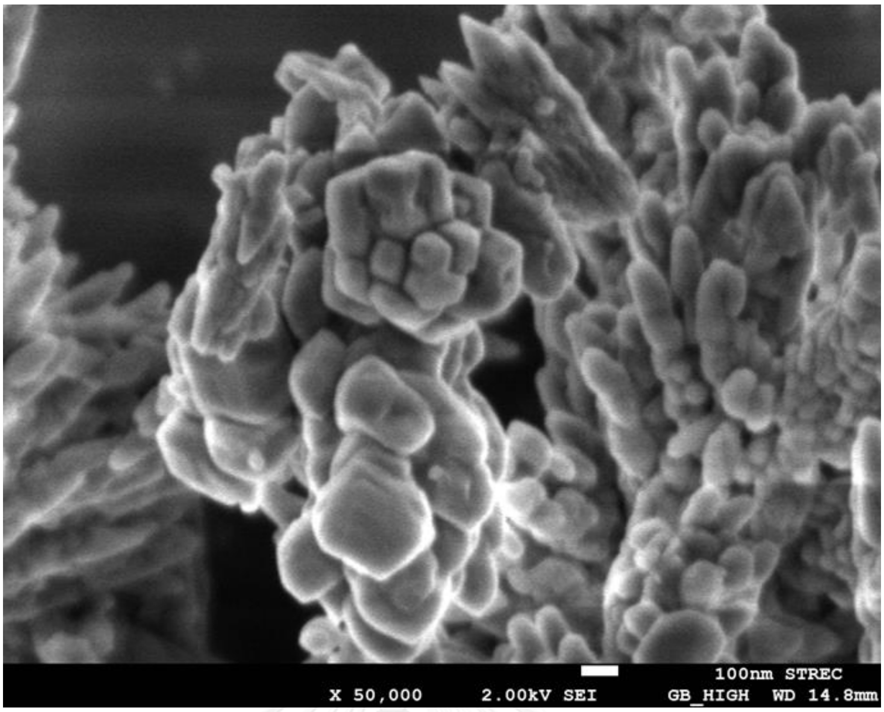


Fig. 33 – SEM images of the SI filler.

High-magnification view of the SI filler showed the irregular surface of the filler appeared as a clumping of fine cubic-shaped particles with a particle size of about 100-200 nm into coral-like agglomerates. Surface roughness and micro-groove between cubic-shaped particles could be seen on the surface of filler (Fig. 33).

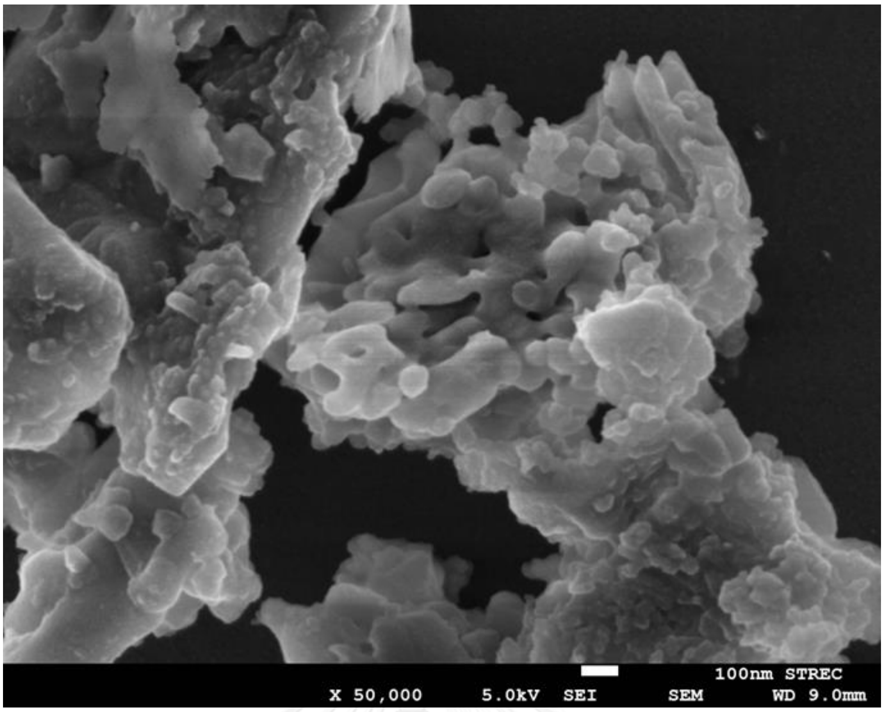


Fig. 34 – SEM images of the CAC filler.

High-magnification view of the CAC filler showed a gel-like structure consisted with plate-shaped and bulk-shaped crystals. Interconnected micro-channels or micro-tunnels could be seen between the crystalline structures (Fig. 34).

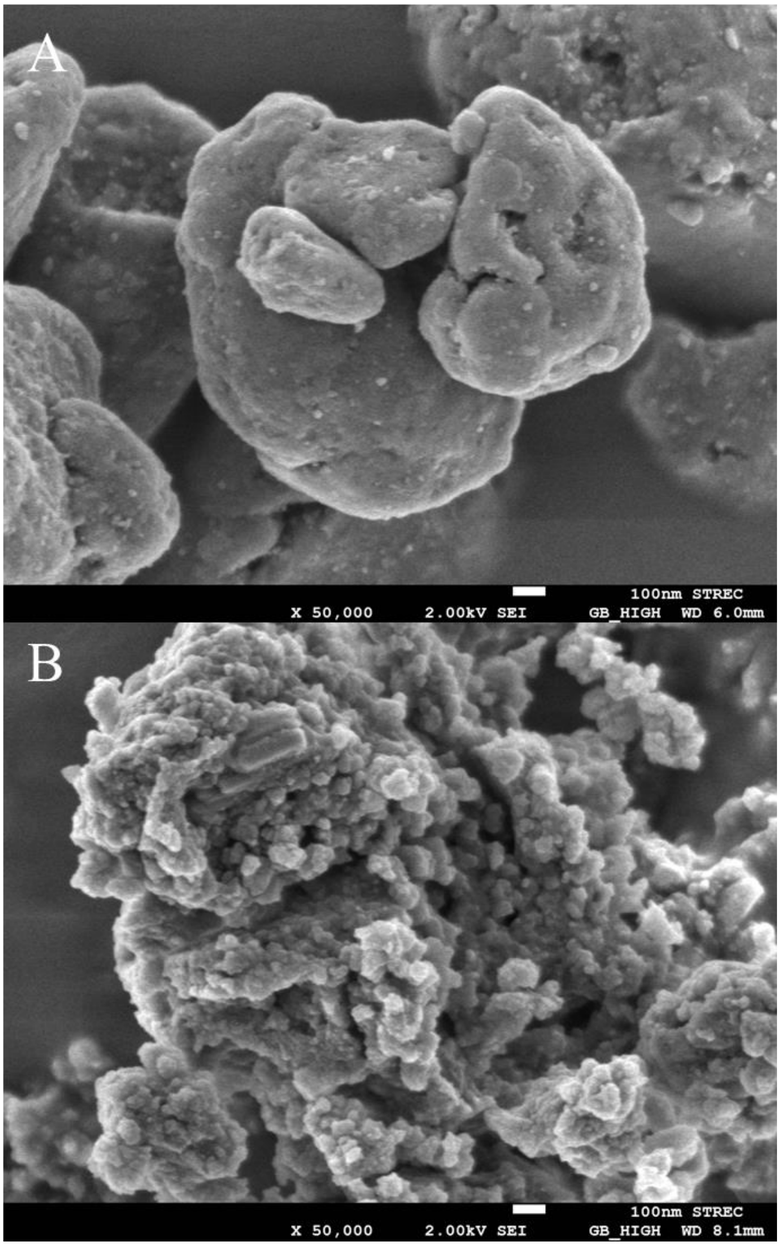


Fig. 35 – SEM images of the GIC (A) and GICA (B) filler.

High-magnification view of the GIC filler demonstrated irregular-shaped particles with a smooth surface (Fig. 35A), however, the particle surface appeared rough after acetic acid treatment (GICA) (Fig. 35B).

The BET surface areas are shown in Table 20. The results showed that the specific surface areas of glass-ionomer cement powder (GIC) were increase from 1.67 to 23 m^2/g after surface treatment with acetic acid. A higher specific surface area of this particle may be attributed to the surface roughness which was also seen in scanning electron microscope image (Fig. 35). The highest specific surface area was found in calcium aluminate cement filler (CAC), which may be due to interconnected micro-channels or micro-tunnels on the surface of this filler. They were also can be seen in SEM (Fig. 34).

Table 20 – Specific surface area measurement with BET test materials

Filler particles	BET surface area (m^2/g)
CAC	41.13
SI	6.65
GIC	1.67
GICA	23.00

CHAPTER 5

DISCUSSION AND CONCLUSION

Secondary caries is a common problem after tooth pits and fissures were sealed with a resin-based sealant. Numerous studies have investigated the prevention of secondary caries development beneath pit and fissure sealants (Li et al., 2011; Shimazu et al., 2011; Yang et al., 2013). Remineralization of demineralized tooth structure and acid neutralization are strategies to inhibit secondary caries (Yang et al., 2013). Generally, studies of dental caries indicate that fluoride-containing sealants reduce the amount of tooth demineralization adjacent to the material (Hicks et al., 2000; Jensen et al., 1990). Another strategy to prevent secondary caries is to develop acid neutralizing materials. A material with pH stabilizing properties neutralizes the acidic pH of the oral environment and maintain it at levels where demineralization will be inhibited (Chacko and Lakshminarayanan, 2001).

Fluoride-containing resin sealants release a slight amount of fluoride compared with that of a conventional sealant (Kuşgöz et al., 2010). There are two main methods to incorporate fluoride into a pit and fissure sealant. First, soluble fluoride salts are added to unpolymerized resin. When the sealant is applied and polymerized, fluoride ions slowly dissolve in the oral cavity. High amounts of fluoride are released during the first two days, and the release subsequently tapers off. The amount of released fluoride then decreases by approximately one-half in three days. This release pattern is called the burst effect (Cooley et al., 1990). Second, an anion exchange system, to avoid the dissolution of the fluoride salt from the pit and fissure sealant, is used to solve this problem. In this method, an organic fluoride compound is chemically bonded to the resin polymer (Ripa, 1993). Concise sealant was used in our study as a representative of resin-based sealants because it is commonly used as the control material in sealant studies (Ganesh and Shobha, 2007; Kantovitz et al., 2006). Conventional resin-based sealant normally does not demonstrate fluoride recharge as demonstrated by our control group results (Han et al., 2002; Koga et al., 2004). However, some studies found that resin-based materials can recharge low amounts of fluoride (Bayrak et al., 2010; Takahashi et al., 1993; Young et al., 1996). Fluoride in the surrounding water after recharge is probably due to the release of surface-retained fluoride, rather than fluoride being incorporated into the sealant (Young et al., 1996). The different findings from our study and other studies may result from different methods of rinsing and cleaning the material in water after fluoride recharge.

Fluoride in the blank control group (the solution of deionized water and TISAB) was also measured. The mean fluoride value of this group was similar to that of the resin-based sealant in deionized water group. Our results are in agreement with previous studies showing that resin-based materials do not have fluoride recharge ability (Han et al., 2002; Koga et al., 2004). Because the fluoride calibration curve used in our study had its lowest calibration point at 0.01 ppm, fluoride levels between 0.00 and 0.01 could not be evaluated. Thus, the data in this range was classified as not detectable (ND) and the data that was less than 0.02 ppm but greater than ND was reported as trace (TR). The ND and TR data were not statistically analysed because they were out of the measurement range of the selective electrode.

Fluoride release without charging was only found in the GIC and GICA groups. These materials contain intrinsic fluoride, because fluoride is added to reduce the materials' melting point during the manufacturing process (Billington et al., 2001). The initial high level of fluoride release agrees with previous studies and is due to the burst effect from the fluoro-alumino silicate glass filler in the GIC group and from the glass particles as they are dissolved by dilute acetic during surface treatment (Attar and Turgut, 2003; Bayrak et al., 2010; De Moor et al., 1996). In the present study, fluoride from the burst effect was found in the GIC and GICA groups at the first 2 measurement days that decreased with time to a baseline level by day 9. However, the resin sealant with other fillers did not show an initial fluoride burst effect because there were not any fluoride-containing components in these materials that would be the source of fluoride or intrinsic fluoride in these fillers (3M ESPE, 2016).

The fluoride recharge ability of a sealant is also important and this ability was found in all groups with incorporated filler. Fluoride recharge was detected in all groups, except for the control group, with the CAC+SI group demonstrating greater release compared with the other groups after fluoride recharge. Then, the fluoride release rate of these materials decreased and stabilized. However, the CAC+SI group had a significantly higher rate of fluoride release when measured 6 days later compared with the other groups. In the glass-ionomer filler (GIC and GICA) groups, the GICA group demonstrated a higher fluoride release compared with that of the GIC group after the first and second recharge. In a previous study, glass-ionomer plus acetic acid had a higher cement compressive strength after storage because of the growth of a hydrated silicate phase or silica gel that is believed to occur in the secondary phase of setting (Wasson and Nicholson, 1993b; Wilson, 1996). Silica gel formed by dilute acid is the only difference between the GIC and GICA fillers. Acetic acid was used in the present study as a surface treatment agent because a previous study showed that acid treatment created silica gel (Wasson and Nicholson, 1993b). The surface roughness seen in the GICA particle SEM images may be due to the formation of this thin silica gel layer on the surface of the glass core. This layer could serve as a reservoir for fluoride ions after fluoride recharge because seed-like inclusions were found in the silica gel layers after the depletion of ions from the surface of the glass core (Dhondt et al., 2001; Tay et al., 2001). The use of a 4% acetic acid solution as a corrosive medium was based on the method of ISO 6872 standard (International Standards Organization, 2015). Acetic acid is believed to be corrosive enough to form a soluble complex on the surface of dental ceramic (Milleding et al., 1999).

Fluoride-releasing sealants can recharge their fluoride content when exposed to fluoride such as fluoride gel, fluoride mouthwash, and toothpaste in the oral environment (Xu and Burgess, 2003). A higher fluoride recharge capability was normally found in materials with high fluoride release and our results finding higher fluoride recharge in the glass-ionomer groups is supported by those of previous studies (Dionysopoulos et al., 2013; Dionysopoulos et al., 2003; Kavaloglu Cildir and Sandalli, 2007; Xu and Burgess, 2003). Notably, sealant groups that did not initially demonstrate fluoride release could recharge a high level of fluoride. The CAC+SI group specimens did not initially release fluoride; however, they released more fluoride compared with the conventional and acid-treated glass-ionomer cements after recharge.

Mesoporous silica has received attention due to wide applications in catalysis, optical devices, sensors, separations, and drug delivery (Alothman, 2012; Katiyar et al., 2006; Vallet-Regi et al., 2001). Moreover, it can be used as a host material to confine desired molecules due to extremely high surface area combined with a large and uniform pore size (Alothman, 2012). Calcium aluminate powder reacts with water in a complex hydration process. After setting, calcium aluminate cement is more porous compared with amalgam and resin composite. The highest porosity level is found in calcium aluminate cement, followed by glass-ionomer cement

(Geirsson et al., 2004). The porosity of calcium aluminate cement and the rough surface of silica, as illustrated in our SEM micrographs, could have a major influence on their fluoride recharge properties. Higher porosity allows greater fluoride diffusion into the material (Xu and Burgess, 2003). Restorative materials with a high fluoride release, such as glass-ionomer cement and compomer, generally have low mechanical properties (El-Kalla and Garcia-Godoy, 1999; Xu and Burgess, 2003). However, a resin-based material containing active filler in this study seems to be mechanically stronger than these materials with lower resin content.

In our study, mesoporous silica and calcium aluminate cement each increased the fluoride recharge ability of the sealant due to their porous and rough surface and the combination of both fillers dramatically increased this ability. Calcium aluminate has potential to defluoridate industrial wastewater. The uptake of fluoride ions by calcium aluminate is likely due to a combination of both physisorption and chemisorption (Sakhare et al., 2012). However, the fluoride released from calcium aluminate is only due to physisorption because chemisorbed fluoride cannot be released. The combination of mesoporous silica and calcium aluminate cement may generate filler with different size of pores and surface roughness that might act as fluoride ion reservoirs for each other.

After the weight ratio is varied, the highest fluoride recharge ability is found in the groups which the proportion of calcium aluminate and mesoporous silica is 1:1 or 2:1. These results imply that combination of calcium aluminate and mesoporous silica filler which the weight of calcium aluminate is more than or equal to that of mesoporous silica has higher recharge property than the combination of filler which has more weight of mesoporous silica. Calcium aluminate seems to have more influence than mesoporous silica due to high surface area which is confirmed by BET (Brunauer, Emmett and Teller) specific surface area measurement. High surface area of calcium aluminate that is 41.13 m^2/g is in agreement to the surface morphology seen in high-magnification of SEM image.

The most negative surface charge was found in untreated glass-ionomer cement powder group (GIC). This result indicates that the dispersion of GIC is probably the most stable filler particle in suspension. However, this value is less negative than dividing line between stable and unstable suspension. Thus, electrostatic stabilization might not play an important role in colloidal stability of these fillers but the structure of Bis-GMA in resin-based pit and fissure sealant could contain a chain that allows for depletion stabilization of these fillers in the resin which is achieved by macromolecules that are free in the solution.

After specimens were stored in lactic acid solution for 24 hours, the groups with incorporated filler demonstrated a lactic acid solution pH change ranging from 0.45 to 0.71. These results indicate that all the fillers evaluated could neutralize a lactic acid solution and increase the pH of the solution after storage. Lactic acid can erode glass-ionomer, however, this disadvantage was accompanied by an increase in the pH of the acid solution (Matsuya et al., 1984; Nicholson et al., 2000). The results of our study confirmed the findings of previous studies showing that glass-ionomer material increased the pH of a lactic solution (Nicholson et al., 1999; Nicholson et al., 2000). In agreement with previous studies, calcium aluminate cement and silica filler also increase the pH of an acid solution (Kaga et al., 2014; Loof et al., 2008). Higher pH tends to reduce demineralization and stimulate remineralization, resulting in a reduced prevalence of dental caries (Nicholson et al., 1999; Nicholson et al., 2000; Xu et al., 2011).

The CAC group showed the highest neutralizing ability followed by the CAC+SI group that also demonstrated the highest fluoride recharge ability. Hence, the combination of mesoporous silica and calcium aluminate cement is promising as a new type of filler without fluoride incorporation as a dental restorative material for preventing dental caries.

The current study is the first report to demonstrate that a new formulation of resin-based pit and fissure sealant containing a combination of synthesized mesoporous silica and calcium aluminate cement is a promising material that can recharge with fluoride and neutralize lactic acid. This material could protect tooth structure by enhancing tooth remineralization and preventing secondary caries formation.

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APPENDIX

One-Sample Kolmogorov-Smirnov Test

Group			Day_3	Day_6	Day_9	Day_12	Day_15
Control	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004700	.004480	.004520	.004680	.004320
		Std. Deviation	.0006307	.0006877	.0006828	.0004894	.0004709
	Most Extreme Differences	Absolute	.137	.154	.174	.157	.180
		Positive	.137	.139	.141	.157	.180
		Negative	-.117	-.154	-.174	-.119	-.148
	Test Statistic		.137	.154	.174	.157	.180
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
CAC	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004630	.004710	.004580	.106410	.022440
		Std. Deviation	.0010155	.0008412	.0007361	.0096498	.0021521
	Most Extreme Differences	Absolute	.178	.172	.132	.217	.089
		Positive	.164	.152	.132	.217	.086
		Negative	-.178	-.172	-.103	-.143	-.089
	Test Statistic		.178	.172	.132	.217	.089
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.199[c]	.200[c,d]
SI	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004480	.004770	.004320	.115120	.006990
		Std. Deviation	.0007330	.0009141	.0006763	.0072547	.0008279

	Most Extreme Differences	Absolute	.211	.159	.139	.121	.200
		Positive	.139	.159	.139	.113	.159
		Negative	-.211	-.141	-.126	-.121	-.200
	Test Statistic		.211	.159	.139	.121	.200
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
CAC+SI	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004650	.004530	.004840	.307250	.021930
		Std. Deviation	.0008370	.0010296	.0008579	.0227261	.0024904
	Most Extreme Differences	Absolute	.138	.134	.172	.122	.200
		Positive	.138	.134	.172	.085	.200
		Negative	-.138	-.082	-.146	-.122	-.120
	Test Statistic		.138	.134	.172	.122	.200
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
GIC	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.109450	.047810	.004520	.037370	.004500
		Std. Deviation	.0064295	.0036665	.0008867	.0028511	.0008124
	Most Extreme Differences	Absolute	.129	.166	.182	.133	.144
		Positive	.129	.166	.182	.133	.144
		Negative	-.105	-.116	-.103	-.116	-.109
	Test Statistic		.129	.166	.182	.133	.144
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
GICA	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.106210	.055410	.004670	.079440	.004430
		Std. Deviation	.0049352	.0042904	.0008314	.0038865	.0004739

	Most Extreme Differences	Absolute	.151	.129	.173	.191	.159
		Positive	.114	.129	.146	.144	.079
		Negative	-.151	-.112	-.173	-.191	-.159
	Test Statistic		.151	.129	.173	.191	.159
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

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

One-Sample Kolmogorov-Smirnov Test

Group			Day_18	Day_21	Day_24	Day_27
Control	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004640	.004740	.004500	.004730
		Std. Deviation	.0007792	.0008897	.0007958	.0005736
	Most Extreme Differences	Absolute	.179	.163	.143	.149
		Positive	.120	.163	.117	.119
		Negative	-.179	-.100	-.143	-.149
	Test Statistic		.179	.163	.143	.149
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
CAC	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004750	.081780	.004790	.004680
		Std. Deviation	.0008182	.0120531	.0007866	.0009807

	Most Extreme Differences	Absolute	.113	.208	.210	.136
		Positive	.113	.208	.210	.112
		Negative	-.091	-.116	-.105	-.136
	Test Statistic		.113	.208	.210	.136
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
SI	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004680	.094850	.004780	.004370
		Std. Deviation	.0007131	.0147550	.0007285	.0007454
	Most Extreme Differences	Absolute	.247	.146	.230	.236
		Positive	.179	.123	.230	.236
		Negative	-.247	-.146	-.156	-.161
	Test Statistic		.247	.146	.230	.236
	Asymp. Sig. (2-tailed)		.084[c]	.200[c,d]	.142[c]	.122[c]
CAC+SI	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004610	.184840	.010610	.004700
		Std. Deviation	.0008048	.0088906	.0014487	.0008055
	Most Extreme Differences	Absolute	.246	.230	.252	.167
		Positive	.207	.230	.169	.167
		Negative	-.246	-.170	-.252	-.151
	Test Statistic		.246	.230	.252	.167
	Asymp. Sig. (2-tailed)		.089[c]	.142[c]	.071[c]	.200[c,d]
GIC	N		10	10	10	10

	Normal Parameters[a,b]	Mean	.004490	.031650	.004890	.004710
		Std. Deviation	.0006806	.0028972	.0010765	.0008252
	Most Extreme Differences	Absolute	.176	.233	.152	.146
		Positive	.165	.131	.152	.146
		Negative	-.176	-.233	-.115	-.098
	Test Statistic		.176	.233	.152	.146
	Asymp. Sig. (2-tailed)		.200[c,d]	.132[c]	.200[c,d]	.200[c,d]
GICA	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004280	.050490	.005020	.004480
		Std. Deviation	.0006070	.0028466	.0008149	.0009426
	Most Extreme Differences	Absolute	.213	.129	.261	.134
		Positive	.144	.129	.261	.134
		Negative	-.213	-.129	-.123	-.109
	Test Statistic		.213	.129	.261	.134
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.052[c]	.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

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

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Day_3	5	10	.109450	.0064295	.0020332
	6	10	.106210	.0049352	.0015607

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
Day_3	Equal variances assumed	.448	.512	1.264	18	.222	.0032400	.0025631	-.0021449	.0086249
	Equal variances not assumed			1.264	16.873	.223	.0032400	.0025631	-.0021708	.0086508

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Day_6	5	10	.047810	.0036665	.0011594
	6	10	.055410	.0042904	.0013567

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
Day_6 Equal variances assumed	.185	.672	-4.258	18	.000	-.0076000	.0017847	-.0113495	-.0038505
Equal variances not assumed			-4.258	17.573	.000	-.0076000	.0017847	-.0113560	-.0038440

Test of Homogeneity of Variances

Day_12

Levene Statistic	df1	df2	Sig.
7.268	4	45	.000

Robust Tests of Equality of Means

Day_12

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	790.131	4	15.185	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: Day_12

Tamhane

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CAC	SI	-.0087100	.0038177	.306	-.021002	.003582
	CAC+SI	-.2008400*	.0078077	.000	-.227444	-.174236
	GIC	.0690400*	.0031819	.000	.057845	.080235
	GICA	.0269700*	.0032897	.000	.015701	.038239
SI	CAC	.0087100	.0038177	.306	-.003582	.021002
	CAC+SI	-.1921300*	.0075439	.000	-.218516	-.165744
	GIC	.0777500*	.0024649	.000	.069286	.086214
	GICA	.0356800*	.0026026	.000	.027029	.044331

CAC+SI	CAC	.2008400*	.0078077	.000	.174236	.227444
	SI	.1921300*	.0075439	.000	.165744	.218516
	GIC	.2698800*	.0072430	.000	.243504	.296256
	GICA	.2278100*	.0072910	.000	.201453	.254167
GIC	CAC	-.0690400*	.0031819	.000	-.080235	-.057845
	SI	-.0777500*	.0024649	.000	-.086214	-.069286
	CAC+SI	-.2698800*	.0072430	.000	-.296256	-.243504
	GICA	-.0420700*	.0015243	.000	-.046987	-.037153
GICA	CAC	-.0269700*	.0032897	.000	-.038239	-.015701
	SI	-.0356800*	.0026026	.000	-.044331	-.027029
	CAC+SI	-.2278100*	.0072910	.000	-.254167	-.201453
	GIC	.0420700*	.0015243	.000	.037153	.046987

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

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Day_15	2	10	.022440	.0021521	.0006806
	4	10	.021930	.0024904	.0007875

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
Day_15	Equal variances assumed	.034	.856	.490	18	.630	.0005100	.0010409	-.0016768	.0026968
	Equal variances not assumed			.490	17.629	.630	.0005100	.0010409	-.0016801	.0027001

Test of Homogeneity of Variances

Day_21

Levene Statistic	df1	df2	Sig.
5.849	4	45	.001

Robust Tests of Equality of Means

Day_21

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	382.838	4	25.268	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: Day_21

Tamhane

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CAC	SI	-.0130700	.0060248	.364	-.032370	.006230
	CAC+SI	-.1030600*	.0047362	.000	-.118331	-.087789
	GIC	.0501300*	.0039201	.000	.036158	.064102
	GICA	.0312900*	.0039164	.000	.017318	.045262
SI	CAC	.0130700	.0060248	.364	-.006230	.032370
	CAC+SI	-.0899900*	.0054475	.000	-.107875	-.072105
	GIC	.0632000*	.0047550	.000	.046093	.080307
	GICA	.0443600*	.0047520	.000	.027252	.061468
CAC+SI	CAC	.1030600*	.0047362	.000	.087789	.118331
	SI	.0899900*	.0054475	.000	.072105	.107875
	GIC	.1531900*	.0029570	.000	.142865	.163515
	GICA	.1343500*	.0029520	.000	.124027	.144673

GIC	CAC	-.0501300*	.0039201	.000	-.064102	-.036158
	SI	-.0632000*	.0047550	.000	-.080307	-.046093
	CAC+SI	-.1531900*	.0029570	.000	-.163515	-.142865
	GICA	-.0188400*	.0012844	.000	-.022932	-.014748
GICA	CAC	-.0312900*	.0039164	.000	-.045262	-.017318
	SI	-.0443600*	.0047520	.000	-.061468	-.027252
	CAC+SI	-.1343500*	.0029520	.000	-.144673	-.124027
	GIC	.0188400*	.0012844	.000	.014748	.022932

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

Paired Samples Statistics

Group			Mean	N	Std. Deviation	Std. Error Mean
GIC	Pair 1	Day_3	.109450	10	.0064295	.0020332
		Day_6	.047810	10	.0036665	.0011594
GICA	Pair 1	Day_3	.106210	10	.0049352	.0015607
		Day_6	.055410	10	.0042904	.0013567

Paired Samples Test

Group	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
GIC Pair 1 Day_3 - Day_6	.0616400	.0092964	.0029398	.0549898	.0682902	20.968	9	.000
GICA Pair 1 Day_3 - Day_6	.0508000	.0082066	.0025952	.0449293	.0566707	19.575	9	.000

Paired Samples Statistics

Group			Mean	N	Std. Deviation	Std. Error Mean
CAC	Pair 1	Day_12	.106410	10	.0096498	.0030515
		Day_15	.022440	10	.0021521	.0006806
CAC+SI	Pair 1	Day_12	.307250	10	.0227261	.0071866
		Day_15	.021930	10	.0024904	.0007875

Paired Samples Test

Group			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
CAC	Pair 1	Day_12 - Day_15	.0839700	.0100324	.0031725	.0767933	.0911467	26.468	9	.000
CAC+SI	Pair 1	Day_12 - Day_15	.2853200	.0224279	.0070923	.2692760	.3013640	40.229	9	.000

One-Sample Kolmogorov-Smirnov Test

Group			Day_3	Day_6	Day_9	Day_12	Day_15
Control	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004740	.004330	.004740	.004810	.004650
		Std. Deviation	.0006022	.0006447	.0006415	.0006027	.0006980
	Most Extreme Differences	Absolute	.174	.238	.186	.207	.215
		Positive	.133	.238	.186	.207	.215
		Negative	-.174	-.143	-.163	-.119	-.171
	Test Statistic		.174	.238	.186	.207	.215
	Asymp. Sig. (2-tailed)		.200[c,d]	.115[c]	.200[c,d]	.200[c,d]	.200[c,d]

CAC1_SI1	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004700	.004720	.004740	.301490	.022830
		Std. Deviation	.0008420	.0005029	.0004949	.0312325	.0036043
	Most Extreme Differences	Absolute	.162	.163	.168	.202	.237
		Positive	.162	.124	.100	.132	.237
		Negative	-.129	-.163	-.168	-.202	-.124
	Test Statistic		.162	.163	.168	.202	.237
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]	.117[c]
CAC2_SI1	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004900	.004780	.004490	.285610	.021410
		Std. Deviation	.0009866	.0006339	.0006100	.0120857	.0029433
	Most Extreme Differences	Absolute	.145	.125	.165	.224	.160
		Positive	.145	.125	.165	.224	.160
		Negative	-.128	-.102	-.078	-.109	-.147
	Test Statistic		.145	.125	.165	.224	.160
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.169[c]	.200[c,d]
CAC1_SI2	N		10	10	10	10	10
	Normal Parameters[a,b]	Mean	.004750	.004340	.004410	.181760	.012140
		Std. Deviation	.0006416	.0007442	.0007370	.0203415	.0014592
	Most Extreme Differences	Absolute	.148	.215	.163	.192	.182
		Positive	.131	.215	.163	.140	.156
		Negative	-.148	-.140	-.110	-.192	-.182
	Test Statistic		.148	.215	.163	.192	.182
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

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

One-Sample Kolmogorov-Smirnov Test

Group			Day_18	Day_21	Day_24	Day_27
Control	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004690	.004830	.004360	.004460
		Std. Deviation	.0003843	.0009889	.0004695	.0007336
	Most Extreme Differences	Absolute	.125	.182	.166	.188
		Positive	.092	.151	.166	.188
		Negative	-.125	-.182	-.116	-.150
	Test Statistic		.125	.182	.166	.188
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
CAC1_SI1	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004580	.186570	.010280	.004550
		Std. Deviation	.0007871	.0053595	.0010973	.0007678
	Most Extreme Differences	Absolute	.210	.136	.138	.174
		Positive	.148	.105	.138	.172
		Negative	-.210	-.136	-.135	-.174
	Test Statistic		.210	.136	.138	.174
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]

CAC2_SI1	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004420	.192000	.010270	.004690
		Std. Deviation	.0009355	.0071098	.0014205	.0008595
	Most Extreme Differences	Absolute	.179	.206	.130	.155
		Positive	.179	.206	.130	.149
		Negative	-.116	-.169	-.119	-.155
	Test Statistic		.179	.206	.130	.155
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]	.200[c,d]
CAC1_SI2	N		10	10	10	10
	Normal Parameters[a,b]	Mean	.004550	.118790	.008610	.004160
		Std. Deviation	.0007906	.0129559	.0017704	.0005835
	Most Extreme Differences	Absolute	.176	.221	.147	.159
		Positive	.175	.140	.096	.130
		Negative	-.176	-.221	-.147	-.159
	Test Statistic		.176	.221	.147	.159
	Asymp. Sig. (2-tailed)		.200[c,d]	.183[c]	.200[c,d]	.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

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

Test of Homogeneity of Variances

Day_12

Levene Statistic	df1	df2	Sig.
3.210	2	27	.056

ANOVA

Day_12

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.085	2	.042	82.629	.000
Within Groups	.014	27	.001		
Total	.098	29			

Multiple Comparisons

Dependent Variable: Day_12

Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CAC1_SI1	CAC2_SI1	.0158800	.0101170	.384	-.009943	.041703
	CAC1_SI2	.1197300*	.0101170	.000	.093907	.145553
CAC2_SI1	CAC1_SI1	-.0158800	.0101170	.384	-.041703	.009943
	CAC1_SI2	.1038500*	.0101170	.000	.078027	.129673
CAC1_SI2	CAC1_SI1	-.1197300*	.0101170	.000	-.145553	-.093907
	CAC2_SI1	-.1038500*	.0101170	.000	-.129673	-.078027

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

Test of Homogeneity of Variances

Day_15

Levene Statistic	df1	df2	Sig.
1.749	2	27	.193

ANOVA

Day_15

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	2	.000	42.513	.000
Within Groups	.000	27	.000		
Total	.001	29			

Multiple Comparisons

Dependent Variable: Day_15

Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CAC1_SI1	CAC2_SI1	.0014200	.0012592	.808	-.001794	.004634
	CAC1_SI2	.0106900*	.0012592	.000	.007476	.013904
CAC2_SI1	CAC1_SI1	-.0014200	.0012592	.808	-.004634	.001794
	CAC1_SI2	.0092700*	.0012592	.000	.006056	.012484
CAC1_SI2	CAC1_SI1	-.0106900*	.0012592	.000	-.013904	-.007476
	CAC2_SI1	-.0092700*	.0012592	.000	-.012484	-.006056

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

Test of Homogeneity of Variances

Day_21

Levene Statistic	df1	df2	Sig.
2.671	2	27	.087

ANOVA

Day_21

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.033	2	.017	201.987	.000
Within Groups	.002	27	.000		
Total	.036	29			

Multiple Comparisons

Dependent Variable: Day_21

Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CAC1_SI1	CAC2_SI1	-.0054300	.0040590	.576	-.015790	.004930
	CAC1_SI2	.0677800*	.0040590	.000	.057420	.078140
CAC2_SI1	CAC1_SI1	.0054300	.0040590	.576	-.004930	.015790
	CAC1_SI2	.0732100*	.0040590	.000	.062850	.083570
CAC1_SI2	CAC1_SI1	-.0677800*	.0040590	.000	-.078140	-.057420
	CAC2_SI1	-.0732100*	.0040590	.000	-.083570	-.062850

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

Paired Samples Statistics

Group			Mean	N	Std. Deviation	Std. Error Mean
CAC1_SI1	Pair 1	Day_12	.301490	10	.0312325	.0098766
		Day_15	.022830	10	.0036043	.0011398
CAC2_SI1	Pair 1	Day_12	.285610	10	.0120857	.0038218
		Day_15	.021410	10	.0029433	.0009308
CAC1_SI2	Pair 1	Day_12	.181760	10	.0203415	.0064325
		Day_15	.012140	10	.0014592	.0004614

Paired Samples Test

Group	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
CAC1_SI1Pair Day_12 1 - Day_15	.2786600	.0325611	.0102967	.2553672	.3019528	27.063	9	.000
CAC2_SI1Pair Day_12 1 - Day_15	.2642000	.0105010	.0033207	.2566880	.2717120	79.561	9	.000
CAC1_SI2Pair Day_12 1 - Day_15	.1696200	.0202778	.0064124	.1551141	.1841259	26.452	9	.000

One-Sample Kolmogorov-Smirnov Test

Group			Before	After	Difference
Lactic_acid	N		10	10	10
	Normal Parameters[a,b]	Mean	3.7650	3.7760	.0090
		Std. Deviation	.03342	.02675	.02331
	Most Extreme Differences	Absolute	.173	.200	.250
		Positive	.153	.102	.183
		Negative	-.173	-.200	-.250
	Test Statistic		.173	.200	.250

	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.077[c]
Control	N		10	10	10
	Normal Parameters[a,b]	Mean	3.7660	3.7700	.0040
		Std. Deviation	.03134	.02981	.03373
	Most Extreme Differences	Absolute	.197	.131	.171
		Positive	.197	.131	.120
		Negative	-.172	-.131	-.171
	Test Statistic		.197	.131	.171
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]
CAC	N		10	10	10
	Normal Parameters[a,b]	Mean	3.7710	4.4780	.7070
		Std. Deviation	.01792	.10602	.10541
	Most Extreme Differences	Absolute	.178	.125	.192
		Positive	.122	.107	.116
		Negative	-.178	-.125	-.192
	Test Statistic		.178	.125	.192
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]
SI	N		10	10	10
	Normal Parameters[a,b]	Mean	3.7660	4.2110	.4450
		Std. Deviation	.01647	.02923	.03408
	Most Extreme Differences	Absolute	.202	.214	.158
		Positive	.142	.214	.130
		Negative	-.202	-.153	-.158
	Test Statistic		.202	.214	.158
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]

CAC+SI	N		10	10	10
	Normal Parameters[a,b]	Mean	3.7710	4.3850	.6140
		Std. Deviation	.03213	.06294	.05700
	Most Extreme Differences	Absolute	.166	.154	.136
		Positive	.112	.154	.128
		Negative	-.166	-.111	-.136
	Test Statistic		.166	.154	.136
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]
GIC	N		10	10	10
	Normal Parameters[a,b]	Mean	3.7720	4.3590	.5870
		Std. Deviation	.02201	.04433	.04762
	Most Extreme Differences	Absolute	.193	.157	.156
		Positive	.141	.125	.144
		Negative	-.193	-.157	-.156
	Test Statistic		.193	.157	.156
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]
GICA	N		10	10	10
	Normal Parameters[a,b]	Mean	3.7750	4.2990	.5240
		Std. Deviation	.03028	.04886	.05275
	Most Extreme Differences	Absolute	.166	.173	.160
		Positive	.134	.173	.155
		Negative	-.166	-.137	-.160
	Test Statistic		.166	.173	.160
	Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

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

Paired Samples Statistics

Group			Mean	N	Std. Deviation	Std. Error Mean
Lactic_acid	Pair 1	Before	3.7650	10	.03342	.01057
		After	3.7760	10	.02675	.00846
Control	Pair 1	Before	3.7660	10	.03134	.00991
		After	3.7700	10	.02981	.00943
CAC	Pair 1	Before	3.7710	10	.01792	.00567
		After	4.4780	10	.10602	.03353
SI	Pair 1	Before	3.7660	10	.01647	.00521
		After	4.2110	10	.02923	.00924
CAC+SI	Pair 1	Before	3.7710	10	.03213	.01016
		After	4.3850	10	.06294	.01990
GIC	Pair 1	Before	3.7720	10	.02201	.00696
		After	4.3590	10	.04433	.01402
GICA	Pair 1	Before	3.7750	10	.03028	.00957
		After	4.2990	10	.04886	.01545

Paired Samples Test

Group			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Lactic_acid	Pair 1	Before - After	-.01100	.02331	.00737	-.02767	.00567	-1.492	9	.170
Control	Pair 1	Before - After	-.00400	.03373	.01067	-.02813	.02013	-.375	9	.716
CAC	Pair 1	Before - After	-.70700	.10541	.03333	-.78241	-.63159	-21.209	9	.000
SI	Pair 1	Before - After	-.44500	.03408	.01078	-.46938	-.42062	-41.297	9	.000
CAC+SI	Pair 1	Before - After	-.61400	.05700	.01802	-.65477	-.57323	-34.064	9	.000
GIC	Pair 1	Before - After	-.58700	.04762	.01506	-.62107	-.55293	-38.980	9	.000
GICA	Pair 1	Before - After	-.52400	.05275	.01668	-.56173	-.48627	-31.415	9	.000

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Before	1.559	6	63	.174
After	4.419	6	63	.001
Difference	4.489	6	63	.001

ANOVA

Before

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	6	.000	.195	.977
Within Groups	.046	63	.001		
Total	.047	69			

Robust Tests of Equality of Means

		Statistic[a]	df1	df2	Sig.
After	Brown-Forsythe	269.899	6	28.389	.000
Difference	Brown-Forsythe	262.634	6	29.741	.000

a. Asymptotically F distributed.

Multiple Comparisons

Tamhane

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
After	Lactic_acid	Control	.00600	.01267	1.000	-.0387	.0507
		CAC	-.70200*	.03458	.000	-.8404	-.5636
		SI	-.43500*	.01253	.000	-.4792	-.3908
		CAC+SI	-.60900*	.02163	.000	-.6914	-.5266
		GIC	-.58300*	.01637	.000	-.6427	-.5233
		GICA	-.52300*	.01762	.000	-.5880	-.4580
	Control	Lactic_acid	-.00600	.01267	1.000	-.0507	.0387
		CAC	-.70800*	.03483	.000	-.8463	-.5697
		SI	-.44100*	.01320	.000	-.4875	-.3945
		CAC+SI	-.61500*	.02202	.000	-.6978	-.5322
		GIC	-.58900*	.01690	.000	-.6499	-.5281
		GICA	-.52900*	.01810	.000	-.5949	-.4631
	CAC	Lactic_acid	.70200*	.03458	.000	.5636	.8404
		Control	.70800*	.03483	.000	.5697	.8463
		SI	.26700*	.03478	.000	.1287	.4053
		CAC+SI	.09300	.03899	.484	-.0494	.2354
		GIC	.11900	.03634	.130	-.0197	.2577
		GICA	.17900*	.03692	.007	.0398	.3182

	SI	Lactic_acid	.43500*	.01253	.000	.3908	.4792
		Control	.44100*	.01320	.000	.3945	.4875
		CAC	-.26700*	.03478	.000	-.4053	-.1287
		CAC+SI	-.17400*	.02194	.000	-.2567	-.0913
		GIC	-.14800*	.01679	.000	-.2086	-.0874
		GICA	-.08800*	.01801	.004	-.1537	-.0223
	CAC+SI	Lactic_acid	.60900*	.02163	.000	.5266	.6914
		Control	.61500*	.02202	.000	.5322	.6978
		CAC	-.09300	.03899	.484	-.2354	.0494
		SI	.17400*	.02194	.000	.0913	.2567
		GIC	.02600	.02434	.999	-.0613	.1133
		GICA	.08600	.02520	.067	-.0036	.1756
	GIC	Lactic_acid	.58300*	.01637	.000	.5233	.6427
		Control	.58900*	.01690	.000	.5281	.6499
		CAC	-.11900	.03634	.130	-.2577	.0197
		SI	.14800*	.01679	.000	.0874	.2086
		CAC+SI	-.02600	.02434	.999	-.1133	.0613
		GICA	.06000	.02086	.192	-.0136	.1336
	GICA	Lactic_acid	.52300*	.01762	.000	.4580	.5880
		Control	.52900*	.01810	.000	.4631	.5949
		CAC	-.17900*	.03692	.007	-.3182	-.0398
		SI	.08800*	.01801	.004	.0223	.1537
		CAC+SI	-.08600	.02520	.067	-.1756	.0036
		GIC	-.06000	.02086	.192	-.1336	.0136

Difference	Lactic_acid	Control	.00500	.01297	1.000	-.0416	.0516
		CAC	-.69800*	.03414	.000	-.8358	-.5602
		SI	-.43600*	.01306	.000	-.4829	-.3891
		CAC+SI	-.60500*	.01947	.000	-.6795	-.5305
		GIC	-.57800*	.01677	.000	-.6407	-.5153
		GICA	-.51500*	.01824	.000	-.5841	-.4459
	Control	Lactic_acid	-.00500	.01297	1.000	-.0516	.0416
		CAC	-.70300*	.03500	.000	-.8404	-.5656
		SI	-.44100*	.01516	.000	-.4944	-.3876
		CAC+SI	-.61000*	.02094	.000	-.6865	-.5335
		GIC	-.58300*	.01845	.000	-.6491	-.5169
		GICA	-.52000*	.01980	.000	-.5917	-.4483
	CAC	Lactic_acid	.69800*	.03414	.000	.5602	.8358
		Control	.70300*	.03500	.000	.5656	.8404
		SI	.26200*	.03503	.000	.1246	.3994
		CAC+SI	.09300	.03790	.449	-.0470	.2330
		GIC	.12000	.03658	.123	-.0183	.2583
		GICA	.18300*	.03728	.006	.0439	.3221
	SI	Lactic_acid	.43600*	.01306	.000	.3891	.4829
		Control	.44100*	.01516	.000	.3876	.4944
		CAC	-.26200*	.03503	.000	-.3994	-.1246
		CAC+SI	-.16900*	.02100	.000	-.2457	-.0923
		GIC	-.14200*	.01852	.000	-.2083	-.0757
		GICA	-.07900*	.01986	.024	-.1508	-.0072

	CAC+SI	Lactic_acid	.60500*	.01947	.000	.5305	.6795
		Control	.61000*	.02094	.000	.5335	.6865
		CAC	-.09300	.03790	.449	-.2330	.0470
		SI	.16900*	.02100	.000	.0923	.2457
		GIC	.02700	.02349	.998	-.0561	.1101
		GICA	.09000*	.02456	.037	.0034	.1766
	GIC	Lactic_acid	.57800*	.01677	.000	.5153	.6407
		Control	.58300*	.01845	.000	.5169	.6491
		CAC	-.12000	.03658	.123	-.2583	.0183
		SI	.14200*	.01852	.000	.0757	.2083
		CAC+SI	-.02700	.02349	.998	-.1101	.0561
		GICA	.06300	.02247	.221	-.0163	.1423
	GICA	Lactic_acid	.51500*	.01824	.000	.4459	.5841
		Control	.52000*	.01980	.000	.4483	.5917
		CAC	-.18300*	.03728	.006	-.3221	-.0439
		SI	.07900*	.01986	.024	.0072	.1508
		CAC+SI	-.09000*	.02456	.037	-.1766	-.0034
		GIC	-.06300	.02247	.221	-.1423	.0163

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

VITA

Atikom Surintanasarn, a son of Mr. Pravit Surintanasarn and Ms. Kandarat Benjapolsirijit, was born on October 12, 1984 in Bangkok, Thailand. He graduated his elementary education from Saint Dominic School in 1996 and high school education from Triamudomsuksa Pattanakarn School in 2002. He received degree of Doctor of Dental Surgery (D.D.S.) from Chulalongkorn University in 2008. After graduation, He worked as a general dentist at Proprathapchang Hospital, Ministry of Public Health and became a head of dental department and a member of hospital executive board in October, 2009. He also received degree of Master of Science in Prosthodontics from Chulalongkorn University in 2011.