THE STUDIES ON ANTIBACTERIAL AND MECHANICAL PROPERTIES OF GLASS IONOMER CEMENT MODIFIED WITH ANTIBACTERIAL AGENTS

A THESIS

BY

NATTA TAWEESAK

Presented in Partial Fulfillment of the Requirements for the

Master of Science degree in General Dentistry

at Srinakharinwirot University

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Although glass ionomer cements (GIC) release substantial amounts of fluoride, secondary caries has been shown to be the principal cause for clinical failure of GIC. Attention has been given to the process of reducing secondary caries by increasing the antibacteriogenicity. Titanium dioxide (TiO_2), silver-zeolite (SZ), extract from pericarp of mangosteen (Man) and chitosan (Chi) have shown to inhibit various bacterial strains. This study aims to evaluate antibacterial, mechanical properties and fluoride releasing of highly viscous GIC modified with antibacterial agents. Each antibacterial agents was incorporated into the powder component of Fuji IX^{TM} (GC Co., Japan) at 3%, 5% and 7% (w/w). Agar diffusion was used to test the inhibitory effect to the growth of *S. mutans*. Compressive strength and flexural strength were evaluated by using a universal testing machine. Surface microhardness was tested by using Vickers microhardnesss tester. Fluoride releasing was determined by a fluoride ion-specific electrode. Data was analyzed by 1-way ANOVA with post hoc test (α =0.05). GIC containing SZ insignificantly increased of inhibition zone with increasing concentration of SZ. While GIC containing TiO_2, Man and Chi showed no inhibition zone. Thus, the mechanical properties and fluoride release testing was done especially in SZ groups. The mechanical properties showed no significant difference between the three experimental groups compared with the control group. Cumulative amounts of fluoride release from each group at any given time were not significantly different. Highly viscous GIC containing 3% (w/w) SZ demonstrated an antibacterial activity toward *S. mutans*, together with similar mechanical properties and fluoride release to that of the control.

Keywords: Glass ionomer cements, silver-zeolite, agar diffusion test, mechanical properties, fluoride release

การศึกษาคุณสมบัติการต้านเชื้อแบคทีเรียและคุณสมบัติเชิงกลของกลาสไอโอโนเมอร์ซีเมนต์ที่ดัดแปลงด้วยสารต้านเชื้อแบคทีเรีย

บทคัดย่อ

ของ

ณัฐฐา ทวีศักดิ์

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
ตามหลักสูตรปริญญาวิทยาศาสตร์มหาบัณฑิต สาขาทันตกรรมทั่วไป
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คณะกรรมการควบคุม: ดร.ปิยะนารถ เอกวรพจน์

แม้ว่ากลาสไอโอโนเมอร์ซีเมนต์จะเป็นวัสดุบูรณะที่มีการปลดปล่อยฟลูออไรด์ได้สูง แต่ปัญหาฟันผุกลับซ้ำก็ยังเป็นปัญหาหลักของการบูรณะด้วยวัสดุชนิดนี้ การเพิ่มความสามารถในการต้านทานฟันผุของกลาสไอโอโนเมอร์ซีเมนต์เพื่อลดอุบัติการของการเกิดฟันผุกลับซ้ำจึงมีความสำคัญ ขณะที่ไทเทเนียมไดออกไซด์ ซิลเวอร์ซีโอไลท์ สารสกัดจากเปลือกมังคุด และไคโตซานสามารถยับยั้งเชื้อแบคทีเรียได้หลายชนิด การศึกษานี้จึงนำสารต้านเชื้อแบคทีเรีย มาประยุกต์ใช้ในวัสดุบูรณะประเภทกลาสไอโอโนเมอร์ซีเมนต์เพื่อเพิ่มคุณสมบัติการต้านทานต่อเชื้อแบคทีเรีย และศึกษาผลของการเติมสารต้านเชื้อแบคทีเรียต่อคุณสมบัติเชิงกล รวมถึงการปล่อยฟลูออไรด์ สารต้านเชื้อแบคทีเรียปริมาณ 3% 5% และ 7% โดยน้ำหนักถูกผสมรวมกับส่วนผงของกลาสไอโอโนเมอร์ซีเมนต์ จากนั้นนำซีเมนต์ที่ได้รับการดัดแปลงด้วยสารต้านเชื้อแบคทีเรีย มาทดสอบคุณสมบัติการต้านทานแบคทีเรียต่อเชื้อสเตรปโตคอคคัสมิวแทนด้วยวิธีอาการ์ดิฟฟิวชั่น ทำการทดสอบความแข็งแรงต่อแรงอัดและความแข็งแรงต่อแรงดัดโค้งด้วยเครื่องทดสอบแรงดึงอัด ทดสอบความแข็งผิวแบบจุลภาคด้วยเครื่องทดสอบความแข็งผิว การประเมินการปล่อยฟลูออไรด์ด้วยวิธีใช้ขั้วไฟฟ้าเฉพาะฟลูออไรด์ไอออน วิเคราะห์ข้อมูลด้วยสถิติความแปรปรวนจำแนกทางเดียว และการทดสอบความแตกต่างของค่าเฉลี่ยแบบจับคู่พหุคูณ ผลการศึกษาพบว่ากลาสไอโอโนเมอร์ซีเมนต์ที่ดัดแปลงด้วยซิลเวอร์ซีโอไลท์แสดงผลการยับยั้งเชื้อสเตรปโตคอคคัสมิวแทน โดยการเพิ่มปริมาณซิลเวอร์ซีโอไลท์ในส่วนประกอบมีผลในการเพิ่มการยับยั้งเชื้ออย่างไม่มีนัยสำคัญทางสถิติ ขณะที่กลาสไอโอโนเมอร์ซีเมนต์ที่ดัดแปลงด้วยไทเทเนียมไดออกไซด์ สารสกัดจากเปลือกมังคุด และไคโตซานไม่แสดงผลในการยับยั้งแบคทีเรีย จึงนำเฉพาะซิลเวอร์ซีโอไลท์มาทดสอบคุณสมบัติเชิงกลและการปล่อยฟลูออไรด์ ค่าคุณสมบัติเชิงกลระหว่างกลุ่มทดลองและกลุ่มควบคุมแตกต่างกันอย่างไม่มีนัยสำคัญทางสถิติ ปริมาณฟลูออไรด์สะสมที่ทุกช่วงเวลามีความแตกต่างอย่างไม่มีนัยสำคัญทางสถิติจึงสรุปได้ว่ากลาสไอโอโนเมอร์ซีเมนต์ที่มีส่วนประกอบของซิลเวอร์ซีโอไลท์ 3% โดยน้ำหนัก มีคุณสมบัติในการยับยั้งเชื้อสเตรปโตคอคคัสมิวแทนโดยมีคุณสมบัติเชิงกลและการปล่อยฟลูออไรด์ไม่แตกต่างจากกลุ่มควบคุม

คำสำคัญ: กลาสไอโอโนเมอร์ซีเมนต์ ซิลเวอร์ซีโอไลท์ การทดสอบแบบอาการ์ดิฟฟิวชั่น คุณสมบัติเชิงกลการปล่อยฟลูออไรด์

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Natta Taweesak

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GLOSSARY OF ABBREVIATIONS

ANOVA	Analysis of variance
APF	Acidulated Phosphate Fluoride
ART	Atraumatic restorative treatment
ATP	Adenosine triphosphate
BHI	Brain heart infusion
CFU	Colony forming unit
CHX	Chlorhexidine
CPP-ACP	Casein phospopeptide-amorphous calcium phosphate
DNA	Deoxyribonucleic acid
FTIR	Fourier transform infrared spectroscopy
GIC	Glass ionomer cement
HA	Hydroxyapatite
ISO	International Standards Organization
MPa	Mega-pascal
ppm	part per million (mg/l)
RMGI	Resin modified glass ionomer cement
S. mutans	*Streptococcus mutans*
SZ	Silver-zeolite
VHN	Vickers hardness

CHAPTER I

INTRODUCTION

1.1 Background and rationale

Direct restorative materials can be classified into tooth-colored and metallic filling materials. Amalgam is an alloy consisting of various metals, extensively use since a long time. At present, the growing demand for aesthetic tooth-colored restorations requires widely used materials such as resin composite, compomers, and glass ionomer cements. The resin composite is an aesthetic material that can be bonded micromechanically to partially demineralized enamel and dentin. A gap formation along the restoration margin may be caused from the polymerization shrinkage. This leads to microleakage and colonization of microorganism [1]. Moreover, there are also some concerns about the release of Bisphenol A from resin composites. It is an estrogenic substance related to increased growth rate, precocious puberty, decreased sperm count and infertility, increased risks of breast, and prostate cancers, behavioral effects, and altered immune functions [2]. Glass ionomer cements are an alternative restorative materials. Although low physical strength limits the use of glass ionomer cements, a material compatible with biological tissues of the tooth may be advantageous. Furthermore, glass ionomer cements bond chemically to tooth structure through an ion exchange mechanism. They have a coefficient of thermal expansion close to that of the natural tooth, and possess fluoride releasing properties. Thus, a glass ionomer cement could be fulfill requirement of ideal restoration materials.

Restorative materials may be developed to enhance their mechanical properties. At the same time, the antibacterial properties should be incorporated to reduce or prevent secondary caries [3]. Nowadays, there are several fluoride-containing dental restorative materials including glass ionomer cement (GIC), resin modified glass ionomer cement (RMGI), compomers, and composite. Fluoride-containing dental materials have the ability to release fluoride ion into the environment surrounding a tooth. In this way, they decrease demineralization and enhance the remineralization process on tooth structure. Moreover, fluoride interrupts the pellicle and plaque formation on the tooth surface [4,5]. Fluoride released from restorative materials may reduce the occurrence of secondary caries, as they are the major cause for restoration failure at the tooth/restoration interface [6,7]. Among

fluoridated materials, glass ionomer cement has been reported to have the ability to continuously release fluoride for a long period [4,7,9]. The survey from Mjör (2000) has shown that 57% for amalgam, 50% for glass ionomer cement, and 47% for resin composite in permanent teeth of 19 years and older were replaced due to secondary caries [10]. Although glass ionomer cements release high fluoride, secondary caries has been shown to be the principal cause for clinical failure of glass ionomer cement. Thus, several studies have searched for alternative agents with synergistic effects. They aimed to obtain an anticariogenic effect for glass ionomer cement.

In an approach to enhance the anticariogenic potential of a glass ionomer cement, a variety of antibacterial agents have been adding into the materials. The antibacterial agents have been used, including synthetic and natural agents. Silver- zeolite (SZ) and titanium dioxide are synthetic agents. SZ, known commercially as Zeomic, is an inorganic antimicrobial agent that can be used in a powder form. It consists of silver ions encased in a three-dimensional alumino-silicate mineral [1]. Due to prolonged antimicrobial activity and low toxicity, the SZ has been used in several applications such as catheter and orthopedic prostheses to obtain an antimicrobial surface [11]. Titanium dioxide is an inorganic filler for various application. It's a nontoxic and stable chemical substance which has good biocompatibility with living tissue. Furthermore, it has high photocatalytic effect including killing bacteria [12,13]. Natural substances are considered biocompatible and have low cytotoxicity. The extract from pericarp of mangosteen is discarded natural materials, which has been use in Thai folk medicine for treatment of skin infections, wounds, and diarrhea. Several studies indicated that the extract from pericarp of mangosteen is effective in inhibiting bacterial species [14,16]. Chitosan is acquired by alkaline deacetylation of chitin, which is the composition of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and, cell walls of some fungi. Chitosan can be favorable for biomedical applications because of its biocompatibility, biodegradability, and non-toxicity [17]. Moreover, chitosan is extensively used as an antimicrobial agent either alone or mixed together with other natural polymers [18].

It is unclear how the glass ionomer cement develops anticariogenic properties. Glass ionomer cement was modified with each antibacterial agent developed in this study to integrate the advantage characteristics of both materials and allow a better result. This study surveyed and found out the proper antibacterial agent which has inhibitory effect

toward *S. mutans*. Thereafter, the mechanical properties and fluoride release were also investigated.

1.2 Research objectives

1.2.1 To prepare modified glass ionomer cement with eced antibacterial properties.

1.2.2 To evaluate antibacterial, mechanical properties (compressive, flexural strength, and surface microhardness) and ability to release fluoride of the modified glass ionomer cement.

1.3 Outline of study

1.3.1 Fabrication of antibacterial agents containing glass ionomer cement

Each antibacterial agent such as SZ, titanium dioxide nanoparticle, extract from pericarp of mangosteen, and shrimp chitosan will be blended together with powder portion of highly viscous glass ionomer cement at various concentrations (3%, 5%, and 7% w/w).

1.3.2 Investigation and selection of proper antibacterial agents

Four antibacterial agents containing glass ionomer cement will be investigated antibacterial potential toward *Streptococcus mutans* by using agar diffusion test.

1.3.3 Studies on chemical and mechanical properties

After focusing only on selected antibacterial agent containing glass ionomer cement, chemical property such as fluoride release will be determined. Fluoride releasing will be analyzed with an ion-specific electrode. The amount of fluoride ion released from cement is reported in ppm (mgF/g).

The mechanical properties such as compressive, flexural strength and surface microhardness will be investigated by using a universal testing machine and microhardness tester respectively.

CHAPTER II

LITERATURE REVIEW

2.1 Introduction

Currently, there are various kinds of dental restorative materials commonly used in dental practice. For indirect restoration, a dental casting alloy and ceramic are mainly used. They both offer great physical properties although the fabrication involve high costs. Direct restorative materials such as amalgams, resin composites, and glass ionomer cements can be placed directly into a prepared cavity and are inexpensive. Amalgam and resin composite are synthetic dental materials. Amalgam is an alloy with a long history that continues to be used due to its strength. Nevertheless, the toxicity of mercury released from an aged amalgam is suspicious. Resin composites are polymer based aesthetic materials with favorable physical properties. However, bishenol-A (BPA) leached from bis-GMA-based resin composite may produce estrogenic effects inducing changes in estrogen-sensitive organs or cells [19]. Shifting from synthetic materials to dental biomaterial like glass ionomer cements are desirable as they are highly biocompatible with teeth. A glass ionomer cement reacts to oral environment by releasing and recharging fluoride ion when exposed to an exogenous sources of fluoride (figure 2.1) [20,21]. It is known that fluoride ion play the important role in control bacterial growth [4]. Therefore, fluoride ion released from a glass ionomer cement may act as antibacterial agents. At present, the antibacterial effects of fluoride ion in a glass ionomer cement sill need be determined.

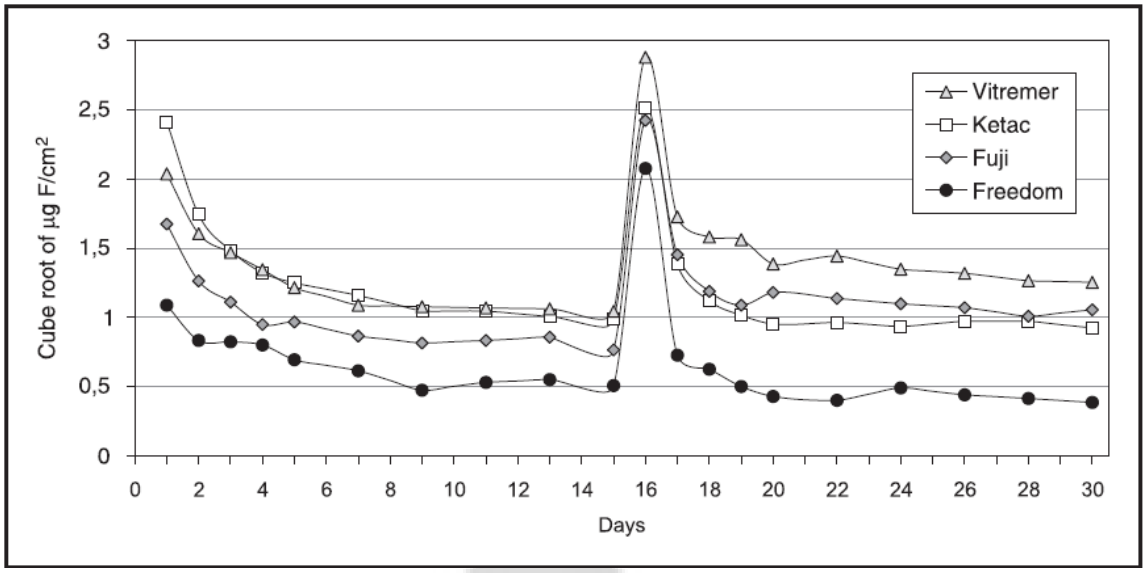

Figure 1 Fluoride release curves of various types of glass ionomer cements. Higher fluoride release was reported on the 1st day for Ketac-Fil followed by Vitremer, Fuji II LC, and Freedom, with a decrease in the initial 15 days. APF gel containing 1.23% fluoride ions was applied on the 16th day, the materials were able to take up fluoride and consequently release it.

Source: Pedrini D, Delbem ACB, França JGMD, Machado TDM. Fluoride release by restorative materials before and after a topical application of fluoride gel. Pesqui Odontol Bras. 2003;17(2):137-41.)

Dental caries demineralize the tooth structure. Remineralization process occurs when the mineral substance and collagen fibril lay contiguous to the carious lesion. However, in case of the caries lesion widely extended, it is necessary to seal off the cavity to prevent further demineralization [21]. Malone et. al. (1966) classified carious lesions into two categories: the affected and the infected dentin. In infected dentin, the surface open to oral environment and not only loss of mineral, but also infection. The affected dentin is demineralized but not infected. If the infected dentin is removed and is sealed with restorative materials, the softened layer is able to remineralize. This method is appropriate for treating root caries, but a restorative material must be able to provide prolonged sealing [20,21]. Glass ionomer cement has been shown to fulfill these requirements. It seals for a long term. In addition, it has a low coefficient of thermal expansion closer to that of the tooth and releases fluoride continuously [4,9,21].

Previous studies on the antibacterial properties of glass ionomer cement have not yet given conclusive results. Botelho et al. (2003) found that conventional glass ionomer cement (Fuji IX™) does not have antibacterial effects. Bacterial inhibition is related to the

duration after mixing the materials. Freshly mixed materials have more antibacterial properties than the set one [23]. Yap et al. (1999) also found that materials containing fluoride (fluoride-releasing composites, compomers, resin-modified glass ionomer cement, and glass ionomer cement) have no bacterial inhibition. None of the materials had zinc oxide, a strong antiseptic, and the materials were not set on bacterial plates [24]. The antibacterial properties of glass ionomer cement may however result from the low pH while materials are being set, or from fluoride contained in the materials themselves. Loyola-Rodriguez et al. (1994) and Friedl et al. (1997) reported that glass ionomer cement exhibits a correlation between fluoride released and bacterial inhibition. An amount of 115-165 ppm fluoride or more reveal the ability to inhibit oral bacteria in neutral condition. The inhibition was not associated to the low pH after the setting [25,26]. Fluoride release from glass ionomer cement suppresses bacterial growth by inhibiting an endolase enzyme. This enzyme changes 2-P-glycerate into P-enolpyruvate in the glycolysis pathway of carbohydrate metabolism [24]. Other studies however show that bacteria is inhibited due to the low initial pH of cement [24,27,28]. In experiments done using the liquid portion of a resin-modified glass ionomer cement, the material showed inhibition toward *S. mutans, S. sobrinus*, *Actinomyces viscosus,* and *L. salivarius*. The antibacterial properties of liquid part exhibited better result than mixed cement. The effect is not related to fluoride, but rather related to the low pH, other chemicals, or liquid had better agar diffusion capability [27].

The addition of antibacterial agents to dental materials is an important current trend towards a reduction in bacterial contamination and increase in anticariogenicity. There are several studies reporting the addition of various types of antibacterial agents such as soluble agents (chlorhexidine and benzalkonium chloride), polymerizable agents (quaternary ammonium and phosphonium), and inorganic fillers (silver and zinc oxide) in composition of dental bonding agents. The mixture of soluble antibacterial agents into resin and mixture of particles into fillers provide a way that resin composite and resin cement demonstrate antibacterial properties [29].

Previous studies have shown that the amount of fluoride released from some conventional glass ionomer cement is approximately 100 ppm to less than 10 ppm [30]. The anticariogenic effect of glass ionomer cement is still not enough to control bacterial growth, even though it releases fluoride after restoration of the tooth. Fluoride release does not provide a constant level and antibacterial properties of glass ionomer cement have

shown controversial results. Thus, the incorporation of antibacterial agents into glass ionomer cement offers a simple way to achieve antibacterial effects.

2.2 Background knowledge

The firstly dental materials science was introduced by G.V. black in 1900 with the amalgam [31]. Aesthetic dental composites were proposed in the mid-1960's as an option to silicate cements and unreinforced methyl methacrylate direct filling resin [20]. The idea of chemical adhesion with tooth structure resulted in the invention of polyacrylic acid-based cements, first the zinc polycarboxylate and subsequently, the glass-ionomer cements [32,33]. The glass ionomer cement was first presented to dental profession by Wilson and Kent in 1972 [34]. It is the restoration that bond to unmodified tooth. The cavity preparation is minimized, sealing enhanced and microleakage decreased [32]. It is believed that a restorative should mimic the tooth properties in all characteristic. The glass ionomer cements are one of the materials improved in this direction.

2.2.1 Composition and reactions of a glass ionomer cement

Glass ionomer or glass polyalkenoate cement is derived from dental silicate cement and zinc polycarboxylate cement. The main components of a glass ionomer cement are ion-leachable glass powder and a polyalkenoic acid. The ion-leachable glass is an aluminosilicate glass with high fluoride, formed by fusion of quartz, alumina, cryolite, fluorite, aluminum trifluoride, and aluminum phosphate in a silaminite crucible at 1100-1300º C. Then, the glass frit is cooled down to dull glow and quenched in water. Finally it is crushed into less than 45 μm particle size. The reaction of glass is influenced by the temperature. The powder is incompletely fused with massive fluorite inclusions at low temperature (1100-1200 ºC). Then, it becomes more disseminated, having smaller fluorite particles, more aluminum and less fluorine at higher temperatures (1300-1500 ºC). An earlier polyalkenoic acid contained 50% aqueous solution of polyacrylic acid but it was unstable on storage. The solution was unstable due to hydrogen bonding between the polyacid chains. To address this problem, itaconic acid, and some of the alkenoic acid were mixed together with the polyalkenoic acid as copolymer. Alkenoic acid is composed of maleic and fumaric acids. The copolymer reduces viscosity and prevent gelification. Moreover, this earlier glass ionomer cement still had a slow setting. The additional of tartaric acid helps to increase the rate of setting, the ultimate compressive, and tensile strengths. After that, the development

has been prepared of vacuum-dries polyakenoic acid which is mixed in powder form with glass powder. The liquid part only has water or tartaric acid which have a long shelf-life in dry condition [20,25].

The setting reaction of glass ionomer cement begins when hydrogen ion from polyacrylic acid attack on a glass surface [19]. Calcium and aluminum ions are released to form salt bridge between carboxyl groups of the polyacid and create a gel matrix around the glass particle. The matrix is attached to the tooth surface from multiple interactions of carboxyl groups of polyacid chain (figure 2.2) [20]. The forming of glass ionomer cement is a quite slow process. It is separated into 2 stages. In the initial stage of setting reaction (dissolution phase), calcium ions are released rapidly and form primarily calcium salt bridge between poly-acrylates chains. At this stage, glass ionomer cement can either absorb or release water. Cautions must be taken with the water balance of restoration during the first 24 hours period. During the later stage of the setting reaction (gelation phase), aluminum ion creates a cross-linking to stabilize the matrix structure [35].

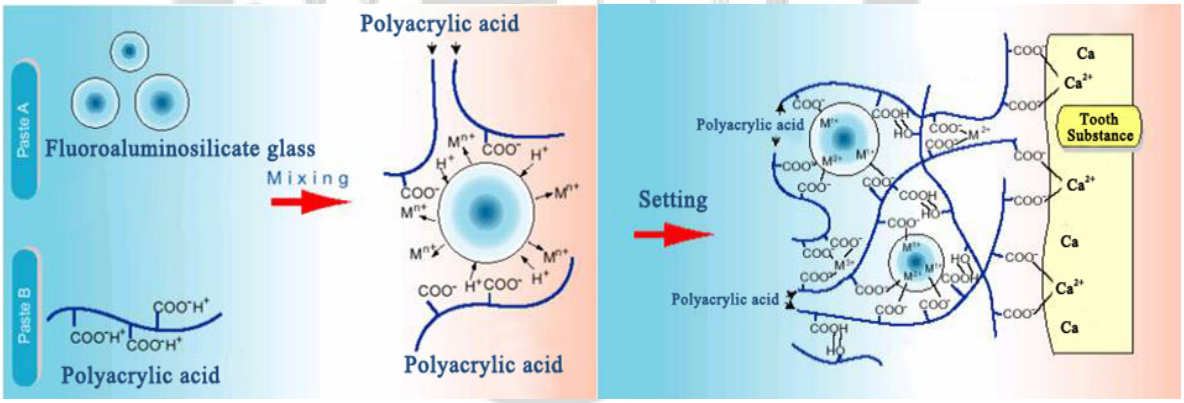

Figure 2 The reaction of powder portion (fluoroaluminosilicate glass) and liquid (polyalkenoic acid) of glass ionomer cement. The matrix is formed by the dissolution of the surface of the glass particles in the acid. The metallic polyalkenoate salt induce gelation continuously until the cement completed setting.

Source: http://mi.gceurope.com/gic/what.php)

Finally, the solidified glass ionomer cement acquires a structure compose of unreacted glass (glass core) surrounded by a siliceous hydrogel. This siliceous hydrogel comprises silica (calcium and aluminum ions taken out) reacts with hydronium ion in a

matrix of cross-linked polyalkenoic molecules (polysalt of aluminum carboxylate and calcium carboxylate) (figure 2.3) [35]. Light-cured varnish is recommend to use for seal newly placed restorations. The vanish used is not completely water-proof. The glass ionomer cement still may have water loss during the first two weeks [20].

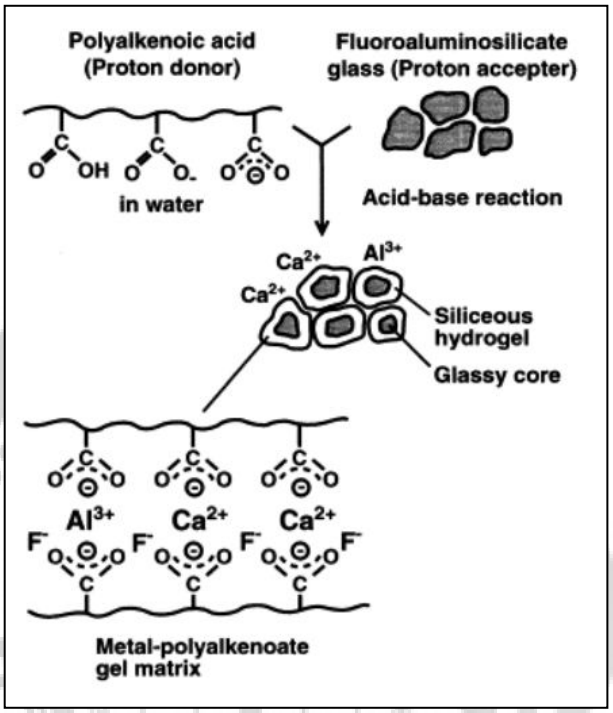

Figure 3 Glass ionomer cement's structure composes a glass core surrounded by a siliceous hydrogel in the matrix of cross-linked polyalkenoic molecules (polysalt of aluminum carboxylate and calcium carboxylate).

Source: Ikemuraa K, Tayb FR, Kouroa Y, Endoc T, Yoshiyamad M, Miyaia K et al. Optimizing filler content in an adhesive system containing pre-reacted glass-ionomer fillers. Dent Mater. 2003;19(2):137- 46)

Glass ionomer cement binds to the mineralized tooth structure through an ion exchange mechanism. The existence of polyalkenoic acid in freshly mixed cement induces the ion release from the glass particle (calcium and aluminum) and from the tooth structure (calcium and phosphate). The ions act as buffer acid, which affect the initial setting and create ion-enriched interface layer attached between teeth and restoration. The result would be a calcium phosphate and polyalkenoate crystalline structure as interface between enamel and cement. Moreover, many studies have found that there is a chemical adhesion

between glass ionomer cement and collagen fibers. When the cement is properly placed, the chemical adhesion may help to reduce microleakage between the restoration and the cavity wall. Thus, post-insertion sensitivity will not occur when using glass ionomer cement [35,37].

2.2.2 Type of glass ionomer cements

There are various types of glass ionomer cement. Several products are generated to satisfy the requirement for specific applications. They can be classified as follows:

2.2.2.1 Classification according to application [19,35,38]

2.2.2.1.1 Type I Luting cement

Glass ionomer cements are designed as cementing metal inlays, crowns, bridges, and orthodontic brackets (figure 2.4a). They have bonding ability, ease of manipulation, fluoride release, and low solubility in the oral environment. The fine grained glass powder with no more than 15 μm particle size provides flow properties and minimal film thickness. Required radio-opacity is provided by mixing strontium or lanthanum instead of calcium in the glass. If translucency is not requested, phase-separated glass, a cermet ionomer, or a glass combined with zinc oxide can be used instead. The examples products are Fuji I, Fuji Plus, and Ketac-Cem Radiopaque.

In the study of physical and mechanical tests, the resin cements exhibited higher flexural strength than other materials [39]. Conventional glass ionomer cements show bond strength less than resin cement [19]. Zinc phosphate and conventional glass ionomer cements revealed brittle fracture without plastic deformation. Different types of luting cement may be appropiate for specific applications. Glass ionomer cements may prevent marginal gap formation and crown dislodgment. Resin cements may be proper for adhesively bonded restorations with margins located on supra-gingival enamel, where strength and energy absorption is required [40].

2.2.2.1.2 Type II a: Aesthetic filling materials

In anterior restoration, where aesthetic must be taken into account, glass ionomer cement with enough translucency is required. It must be bear in mind that clear glasses having a high silica/alumina ratios forms cements that tend to harden slowly. The products are Fuji II LC (figure 2.4c) which has 11 shades (A1, A2, A3, A3.5, A4, B2, B3, B4, C2, C4, D2) and Ketac-fil Plus Aplicap which has 8 shades (A1, A2, A3, A3.5, A4, C2, C3, DG0) [41]. As filling material, glass-ionomer cement creates tooth color not as well

as composites. Composites offer diverse shades and opacities that generate a more identical of the color and opacity of enamel and dentin [42].

Type II b: Bis-reinforced filling materials

They are a derivatives of Type II which include cermet. Cermet is glass-metal powders sintered to high density that was introduced by McLean and Gasser in 1985. Nowadays, a commercial cermet is Ketac-Silver Aplicap as shown in figure 2.4d [38]. Even though it is not aesthetic, it is more resistant to abrasion.

2.2.2.1.3 Type III: Lining, base and fissure-sealing materials

Lining, base, and fissure sealant materials should be strong and radio-opaque. Some commercial products contain zinc oxide that tend to buffer the systems and make the cement radio-opaque. These materials may have loss of strength. The type III classification also includes non-aesthetic glass ionomer cements. These glass ionomer cements are useful for the double laminate or sandwich technique. Glass ionomer cements are used as a dentin and resin composites are used as enamel. The glass ionomer cements have a coefficient of thermal expansion close to that of the tooth resulting in good bonding to dentin and good biocompatibility. The resin composite, on the other hand, exhibits superior physical and aesthetic qualities. Therefore, the features of both can be integrated. The products are Fuji Lining LC, Ketac-Bond, Photac-Bond Aplicap, and Vitrebond (figure 2.4e). Set glass ionomer cement has a compressive strength comparable to zinc phosphate cement, a tensile strength more than zinc phosphate cement and a modulus of elasticity approximately half of zinc phosphate cement [43].

2.2.2.2 Classification according to development [44]

2.2.2.2.1 First generation

The first era of glass ionomer cements for filling were Aluminosilicate Poly-Acrylate (ASPA I; Dentsply). It was not active, slowly to set, moist sensitive and poorly translucent. ASPA II composed of tartaric acid. It had superior qualities and was the first glass ionomer cements with practical usage. Glass ionomer cements are used only in pedodontics and restoration of Class III and V. They are not recommended for permanent filling of occlusal surfaces.

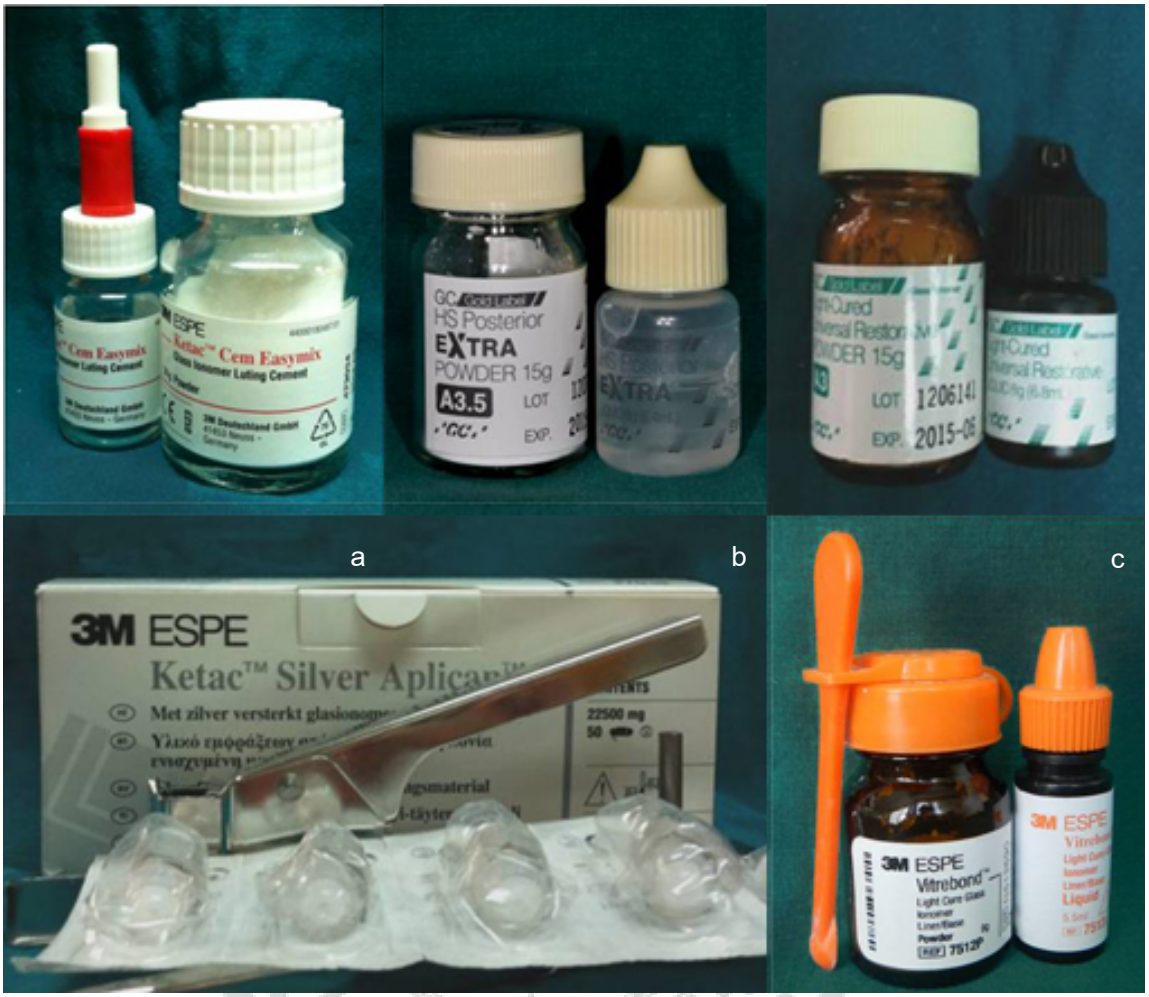

Figure 4 Various type of glass ionomer cements categorized according to application a) Type I Luting cement, b) and c) Type II Aesthetic filling materials, d) Type II Bis-reinforced filling materials, and e) Type III Base and lining.

2.2.2.2.2 Second generation

Polyacrylic acid has been blended with the powder in 1969 [45]. The liquid portion consists of water or an aqueous solution of tartaric acid. Thus, the benefit comprises an increase in shelf life by prevention gelation and a decrease in viscosity while mixing. Furthermore, the strength is improved because the molecular weight of the polyacid can be increased. These products are Chemfil and Ketac-Cem.

2.2.2.2.3 Reinforced cements

The second generation of glass ionomer cements had a tensile strength of approximately 7-14 MPa, which was not sufficient for high stress areas [46]. Some processes or agents were used for reinforce the cement as follows:

Dispersed phases such as alumina, titanium oxide and zirconium oxide were applied to improve the strength. Fiber-reinforced glasses: the alumina fibers or other fibers such as glass fibers, silica fibers, and carbon fibers were added to increase the flexural strength. Glasses reinforced with metals: the amalgam powder was mixed with powder of glass ionomer cement and was called “Miracle Mix”.

Cermet ionomer is made by sinter of metal and glass powder. Reinforced glass ionomer cements are the addition of metals to the filled portion. The powder is composed of fluoroaluminosilicate glass and a silver alloy. The product is cermet (ceramic or glass and metal). They have been used for core buildup and temporary posterior material because of proper strength, ease to manipulate, and radio-opaque properties.

Conventional glass ionomer cements with a high viscosity: The highly viscous glass ionomer cements are beneficial when atraumatic restorative treatment (ART) is required. These glass ionomer cements are an option for posterior preventive restorations. The samples are Fuji IX and Ketac Molar. They can be applied for intermediate restorations, replacing amalgam, and core buildup.

2.2.2.2.4 Resin modified glass ionomer cements

Resin modification of glass ionomer cement was created to generate favorable physical properties of resin composites and resin cements while maintaining the advantage of conventional glass ionomer cements. Resin modified glass ionomer cements were achieved by incorporating methacrylate into polyacrylic acid. The setting reaction begins with light activation, and is followed by an acid-base reaction. The samples of these cements are Fuji II LC, Vitremer, and Vitrebond.

Table 1 The examples of glass ionomer cement products [19]

Clinical application	Product	Classification	Manufacturer
Luting	Fuji I®	Conventional	GC Corp., Japan
	Fuji Plus®	Resin-modified	GC Corp., Japan
	Ketac-Cem Radiopaque®	Conventional	ESPE, USA
	Aquacem®	Conventional	Dentsply, Germany
	Advance®	Resin-modified	Dentsply, Germany
	Vitremer luting cement®	Resin-modified	3M, USA
	Hy-Bond Glas Ionomer CX®	Resin-modified	Shofu, Japan
Filling	Fuji II®	Conventional	GC Corp., Japan
	Fuji II LC®	Resin-modified	GC Corp., Japan
	Ketac-Fil Plus Aplicap®	Conventional	ESPE, USA
	Photac-Fil Quick Aplicap®	Resin-modified	ESPE, USA
	Chem-Fil II®	Conventional	Dentsply, Germany
	Glas Ionomer F®	Conventional	Shofu, Japan
	Vitremer®	Resin-modified	3M, USA
Filling (Highly vicous)	Fuji IX®	Conventional	GC Corp., Japan
	Fuji IX GP®	Conventional	GC Corp., Japan
	Ketac-Molar®	Conventional	ESPE, USA
	Ketac-Molar Aplicap®	Conventional	ESPE, USA
	Hi-Fi®	Conventional	Shofu, Japan
Metal reinforced	Miracle Mix®	Metal-reinforced	GC Corp., Japan
	Ketac-Silver Aplicap®	Metal-reinforced	ESPE, USA
	Hi-Dense®	Metal-reinforced	Shofu, Japan
	Aqua Silver®	Metal-reinforced	Voco, Germany

Table 1 (Cont.)

Clinical application	Product	Classification	Manufacturer
Base/liner	GC Lining cement®	Conventional	GC Corp., Japan
	Fuji Lining LC®	Resin-modified	GC Corp., Japan
	Ketac-Bond®	Conventional	ESPE, USA
	Photac-Bond Aplicap®	Resin-modified	ESPE, USA
	Vitrebond®	Resin-modified	3M, USA
	BaseLine®	Conventional	Denstply, Germany
	Hy-Bond Liner®	Conventional	Shofu, Japan
Fissure protection	Fuji III®	Conventional	GC Corp., Japan
	Fuji III LC®	Resin-modified	GC Corp., Japan
Bonding agent	Fuji Bond LC®	Resin-modified	GC Corp., Japan
Orthodontic bracket bonding	Fuji Ortho®	Resin-modified	GC Corp., Japan
	Fuji Ortho LC®	Resin-modified	GC Corp., Japan
Orthodontic band bonding	Multi-CureGlass-ionomer®	Resin-modified	3M Unitek, USA
Root canal sealing	Ketac-Endo Aplicap®	Conventional	ESPE, USA

2.3 Glass ionomer cement and development

2.3.1 The improvement on mechanical properties

Earlier, glass ionomer cement was not aesthetically appealing and was susceptible to water loss and water uptake in the oral environment. The drawback was the lack of sufficient physical strength which made it susceptible to fracture and exhibited low wear resistance. Thus, application of glass ionomer cements have been limited to the low masticatory stress areas [20]. There have been however attempts to improve its mechanical properties. In the past, amalgam alloy powder was added to glass ionomer cement powder under the product “Miracle mix” to gain radiopacity and to increase the strength. Later on, silver particles were sintered to glass to form a cermet (ceramic-metal) called Ketac Silver. Metal reinforced glass ionomer cements have mechanical properties similar to that of amalgam while still maintaining the favorable features of conventional glass ionomer cement. However, the reinforcing effects of metal addition to glass ionomer cement is still in

debate. Some studies have found that a reinforced glass ionomer cement has higher strength than conventional glass ionomer cement, others found the opposite. A lower strength would result from the lack of interfacial bonding to transfer stress from the matrix to the reinforcement [47].

The problem of moisture sensitivity, lack of command cure and low strength of conventional glass ionomer cement still go on. To solve these troubles, in the late 1980s to early 1990s the development of light cured glass ionomer cements emerged and were called “resin modified glass ionomer cement”. These are glass ionomer cements with the incorporation of a small quantity of a resin such as hydroxyethyl methacrylate (HEMA) or Bis – GMA in the liquid [46]. A highly viscous glass ionomer cement like Fuji IX GP, Chem-Flex, and Ketac-Molar become to replace metal-reinforced glass ionomer cement. The high powder:liquid ratio of these cement creates a "condesable" mass. It is usually used in atraumatic restorative treatment recommended by the World Health Organization. By developing the chemistry further than mixing with metal, makes highly viscous glass ionomer cement to have improved mechanical properties, wear resistance, and tooth-color. The properties of highly viscous glass ionomer cements are better than metal-reinforced glass ionomer cements. After that, a fast-set highly viscous glass ionomer cement was developed. It has 3 minutes faster than the 5-6 minutes setting time of the highly viscous glass ionomer cement. Fast setting reaction improves mechanical properties and wear resistance. It can sustain a faster masticatory load than highly viscous glass ionomer cement [47,48].

2.3.2 The improvement as a smart dental biomaterial

A biomaterial is “any material, natural or man-made, that comprises whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function [49].” Smart materials are “materials that have properties which may be altered in a controlled fashion by stimuli, such as stress, temperature, moisture, pH, electric or magnetic fields.” Glass ionomer cement is a smart dental biomaterial. Little or no change in dimension was displayed when heating or cooling between 20°C and 50°C in wet condition. Moreover, the potential of fluoride release has been the subject of smart behavior [50].

The incorporation of agents which induce tissue responses such as hydroxyapatite (HA), casein phospopeptide-amorphous calcium phosphate (CPP-ACP), bioactive glass particle, and nanoparticle will be advantageous**.** New conceptions consider more “smart” will be started.

2.3.2.1 Bioactive additive

2.3.2.1.1 Hydroxyapatite

Hydroxyapatite is a group of calcium phosphate, which is the main mineral composition of the enamel and dentin of the tooth. Hydroxyapatite is also the inorganic matrix of bone. Hydroxyapatite has great biological behavior. Glass ionomer cement interacts with hydroxyapatite through the carboxylate groups in the polyacid. The addition of hydroxyapatite into glass ionomer cement may not only enhance the biocompatibility of glass ionomer cement, but also improve the mechanical properties. Moreover, it promotes bond strength to the tooth structure because it has the same composition and structure to enamel and dentin [51].

Lucas (2003) performed a test by adding 8% hydroxypatite in the powder part of glass ionomer cement. It was found that fracture toughness of 8% hydroxyapatite-added glass ionomer cement has increased after mixing 15 minutes and 24 hours. The strength of cement increased within the first 15 minutes. It might be that dissolving hydroxypatite in the acidic solution, causes calcium out from the surface of hydroxyapatite. The mechanism is the same as that of glassionomer cement attached to enamel and dentin. The reaction occurs between apatite of the tooth and polyacrylic acid, creating polyacrylates ions, which makes strong ionic bonds [51]. The results are in agreement with the work of Arita et al. (2003) who also found that the incorporation of hydroxyapatite either in whisker or granule form can enhance the flexural strength and microstructural property of glass ionomer cement [53].

2.3.2.1.2 Bioactive glass

Bioactive glass, first introduced by Hench et al., is composed of silicon, sodium, calcium and phosphorous oxide. It was firstly used as biomaterial to substitute the lost osseous tissues. It generates a strong bond with bone by production of hydroxyapatite and builds a strong bond between the collagen and the hydroxyapatite. It is not reject by the body. In some studies, bioactive glass has been incorporated to glass ionomer cement to enhance its bioactivity and tooth regeneration capacity [44].

In 2003, Yli-Urpo et. al. demonstrated that glass ionomer cement disks containing 30wt% of bioactive glass (S53P4) inhibit the growth of *S. mutans*, but the bioactive glass made the glass ionomer cement brittle [54]. After that in 2005, it was noticed that mixing bioactive glass particles with the powder part of conventional glass ionomer cement caused the compressive strength decrease but calcium increase.This makes it appropriate for usage in areas which requires bioactivity but does not need high compressive strength such as root surface fillings and liners [55].

2.3.2.1.3 Casein phospopeptide-amorphous calcium phosphate (CPP-ACP)

CPP–ACP is a milk product which increases remineralization and prevents dental caries. CPP is a peptide which contains elements that can bind calcium. ACP is the precursor of hydroxyapatite. CPP supports the ACP to bind with the enamel. CPP has antibacterial property against *S. mutans*. CPP-ACP has been found to prevent demineralization and enhance remineralization. CPP-ACP itself can release calcium and phosphate ions [56,57].

Mazzaoui et al. (2003) studied the effect of CPP-ACP incorporation into glass ionomer cements. They found that the addition of 1.56% w/w of CPP-ACP into glass ionomer cement increased microtensile bond strength and compressive strength significantly. Furthermore, including CPP-ACP in glass ionomer cements also improves the calcium, phosphate and fluoride ion release [58]. While Zraikat et al. (2011) reported that 5% CPP-ACP reduces the strength of cement and prolongs setting time but is still in ISO limits. The 3% or 5% mixing of CPP-ACP reduces the release of fluoride of glass ionomer cement significantly, but it has higher release of calcium and inorganic phosphate and a significantly smaller area of demineralized enamel compared to control group. This makes the 3% mixing of CPP-ACP in glass ionomer cement increase the performance to prevent caries without affecting its mechanical properties [57].

2.3.2.1.4 Nanoparticles

The use of nanoparticles, defined as particles smaller than 0.1 μm, or 100 nm, is starting to play a role in dental research. Mashoverinia et al. (2008) have shown that mixing of hydroxyapatite and fluoroapatite nanobioceramics in conventional glass ionomer cement (glass powder/HA and glass powder/FA ratio of 20:1 by wt.) improves its mechanical properties and bond strength to dentin. The experimental glasses have wider ranges of particle size distribution because the small sizes of the nanoceramics added

into the glass powder of glass ionomer cement. The addition of small size may fill the space between the glass particles and may result in a strengthen glass ionomer cement [51]. However, the mixing of ytterbium fluoride and barium sulfate nanoparticle in glass ionomer cement reduces the compressive strength in a 24 hour period. The reduction showed an increase for barium sulfate incorporation, which even 1% in the powder caused a fall of more than 10% in strength from 160 to 142 MPa. There may be impediment of the barium sulfate with normal glass ionomer cement reaction. Moreover, the bivalent cations like Ca^{2+} and Ba^{2+} provide less strength than trivalent cations like Al^{3+} and Yb^{3+} [12,59].

2.3.2.2 Antibacterial agents

2.3.2.2.1 Antibiotics

Pinheiro et al. (2005) studied antibacterial activity of glass ionomer cement containing antibiotics on caries lesion. They stated that glass ionomer cement with 1% of metroniazol, 1% of ciprofloxacin and 1% of cefaclor significantly reduceviable bacteria in infected dentin in comparison to the conventional glass ionomer cement[60]. The results concur with Yesilyurt et. al. (2009), who showed that a glass ionomer cement containing three antibiotic mixtures (ciprofloxacin,metronidazole and minocycline) at 1.5, 3.0, and 4.5% w/w exhibit inhibition against *S. mutans* or *L. casei.* Nevertheless, the incorporation of antibiotics at 3% and 4.5% decreases compressive strength and bond strength to dentin [61].

2.3.2.2.2 Chlorhexidine

Chlorhexidine is a synthetic cationic bis-guanide. The efficacy of chlorhexidine results from positive charged reacting with the negatively charged of phosphate groups on bacterial cell walls. This enhances the permeability of cell wall, which chlorhexidine molecule infiltrate into the bacteria. Chlorhexidine will be stable as a salt. The most common oral preparation is chlohexidine gluconate [62]. Chlorhexidine is beneficial in condition that oral hygiene is difficult, compromised or impossible. It has various application such as mouth rinse, spray, gel tooth paste and chewing gum. Chlorhexidine is an effective adjunctive chemotherapeutic agent for antiplaque and antigingivitis. Moreover, it appears to inhibit the activity of *S. mutans* and reduces bacteria remaining within the root canal [63].

Glass ionomer cement containing chlorhexidine was studied by Takahashi et al. (2006). They demonstrated that glass ionomer cement containing 1% chlorhexidine diacetate has an antibacterial effect with optimum physical and bonding properties. However the addition of 2% or more chlorhexidine diacetate significantly decreased compressive strength and bond strength of the glass ionomer cement [64]. In agreement with Palmer et. al. (2004), increasing the percentage of chlorhexidine acetate into glass ionomer cement decreases compressive strength in direct proportion and the working and setting time increased [65].

2.3.2.2.3 Titanium dioxide nanoparticles

Titanium dioxide nanoparticles is a substance with various capabilities. Its particle size is less than 50 nm. Photo-induced activities can be occurred by semiconductor band gap and can be effective only by simple energy stimulation such as light. The photocatalytic activities of titanium dioxide nanoparticle include killing bacteria and virus [66,67]. Titanium dioxide nanoparticles also have excellent mechanical properties, with elastic modulus value at approximately 230 GPa [12].

Several studies demonstrated improvements in physical, mechanical, and antibacterial properties of dental restorative material containing titanium dioxide nanoparticles. Microhardness and flexural strength of dental resin-based composites improve by incorporating titanium dioxide nanoparticles treated with the organosilane allyltriethoxysilane (ATES) [68]. Moreover, adding a small amount of acrylic acid modified titanium dioxide nanoparticles (AP25) to the resin increases the degree of vinyl conversion (DC) and mechanical properties rapidly. These properties increase significantly the functionality of dental adhesives [13]. While the additional of 3% titanium dioxide nanoparticles to glass ionomer cement increases both physical and antibacterial properties without changing the bonding strength with dentin or fluoride releasing of glass ionomer cement [12].

2.3.2.2.4 Chitosan

Chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose] is a group of partially and fully deacetylated chitin compounds. As chitosan is highly biocompatible to human tissue and nontoxic, it is used in several applications in the food, pharmaceutical, textile, agriculture, water treatment, and cosmetics industries [18].

The antimicrobial properties of chitosan have been studied. Luis et al. (2011) stated that nanoparticle complexes from chitosan have antimicrobial effect to biofilms of *S. mutans* [69]. Correspond to Costa et al. (2013) reported potential oral antimicrobial of chitosan. Therefore, chitosan is capable of interfering with *S. mutans* adhesion and primary biofilm formation [70]. Modified self-etching primer with chitosan contains an antibacterial activity against *Enterococcus faecalis* and did not perturb the bond strength to radicular dentin [71].

2.3.2.2.5 Extract from pericarp of mangosteen

Mangosteen, *Garcinia mangostana* is a tree widespread in Southeast Asian countries. The hull has been used in Thai folk medicine for treatment of skin infections, wounds, and diarrhea. There are some studies indicating that the extract from pericarp of mangosteen may inhibit bacteria. Extracts from mangosteen pericarp contain xanthone derivative compounds [14,16].

Suksamrarn et al. (2003) found that α, β-Mangostins, and garcinone B exhibit strong inhibitory effect against Mycobacterium spp [15]. Moreover, Treesuwan et al. (2012) examined the effect of extract from mangosteen pericarp containing polyphenolic compounds on inhibition of *S. mutans*. They found that the extract inhibit and disinfect *S. mutans* with minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of 0.25 mg/ML and 1 mg/ML, respectively. The mechanism of action may involve the inhibition of glucosyltransferases and insoluble glucan synthesis. Glucan is a polymer of D-glucose which functions like a cement holding bacteria cell together and fasten them to the tooth [14]. Moreover, gel containing mangosteen pericarp extract could improve the clinical effect such as probing pocket depth (PPD), bleeding on probing (BOP) and gingival index (GI) of periodontal treatment [72].

2.3.2.2.6 Silver-zeolite

Silver is common element used in medicine due to its attractive physiochemical properties and low cost [73]. Studies on the antibacterial effects of metal ions give the following sequence of efficacy: Ag^{+}, Cu^{2+}>Sn^{2+}>Zn^{2+}>Al^{3+}>Fe^{3+} [1]. Silver has the highest antibacterial effect among metal ions. It has long been used in medicine to prevent and treat diseases [73]. A diversity of silver compounds have been used as topically applied agents to treat burns and ocular infections [74]. Silver has direct or indirect effect on microbial cells. The antimicrobial action of silver has been proposed to occur by three

mechanisms. Firstly, positive charge of silver causes electrostatic attraction to the negative charge of bacterial cell membrane causing the cell membrane breaks. Moreover, low concentrations of silver induce proton leakage through bacterial membrane leading to cell death. Secondly, silver can react with the cell membrane or with enzyme proteins. Many oxidative enzymes are inhibited by silver resulting in the metabolites efflux, interference with DNA replication, inactivation of ATP production and inhibition of bacterial growth. Finally, silver is related to formation of free radicals that induced membrane damage [73,75].

Silver has been shown to be a biocompatible material. However, excess of silver is presented to precipitate in skin, liver, kidneys, spleen, corneas, gingival, mucous membranes, and nails. In dentistry, the drawback of silver compound is the black staining on cavity that silver containing material restored. There are a small number of studies reporting that gingiva near the teeth may increase of erythema or appear small white lesion in the mucosa. The lesion generally heal after 48 hours without treatment. These symptoms are mild and transient [75].

There are several types of inorganic carriers containing silver for example zeolite, phosphate, titanium dioxide, activated carbon, montmorillonite, water-soluble glass, and mesoporous silica [73]. These were developed to prolong the life of silver. Silver based materials have to release silver ion to a pathogenic environment to be effective [76]. The prolonged persistent isolation of silver into an environment allow a higher efficacy of silver containing materials. Furthermore, inorganic materials containing silver do not change the color of the product [73]. Zeolites are aluminum silicate crystalline structures with empty spaces of 3-10 angstroms in their composition [1,11]. Zeolite presents a strong affinity for silver ion and can electrostatically bind silver ion up to 40% (w/w) in its structure as figure 2.5 [74]. SZ continuously releases a small amount of silver ion into water, resulting in a long term antimicrobial property which is not deleterious to tissue cells [1,76].

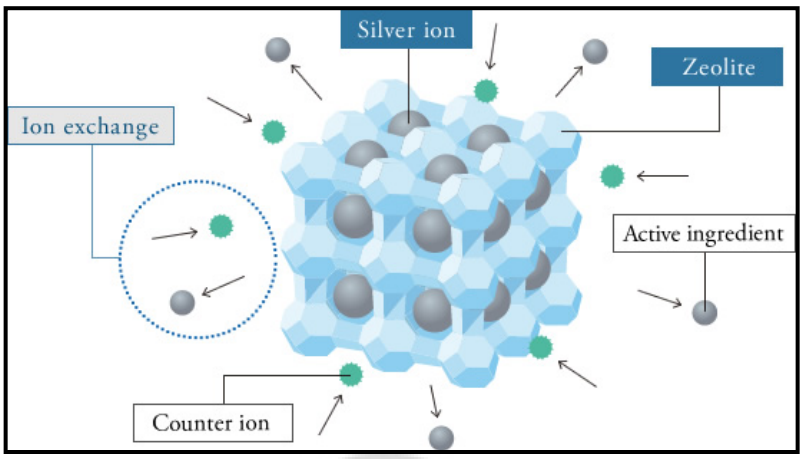

Figure 5 Structure of SZ. Silver in void space of zeolite which is aluminum silicate cystalline structure with empty spaces of 3-10 angstroms in their composition. Silver ion is released from SZ by an ionic exchange mechanism.

Source: http://www.zeomic.co.jp/en/product/zeomic/index.html)

In dentistry, dental caries is a common disease that cause tooth loss. An approach for caries control would be to develop a procedure to make the tooth surface uncolonizable by oral bacteria without affecting the microbial ecology. The antibacterial agent incorporation into restorative material may influence the prognosis of restoration. SZ has antimicrobial effects against cariogenic bacteria. Syafiuddin et al. (1993) reported that a new composite resin (Clearfill SC-II, Kuraray Co., Osaka, Japan) containing Zeomic (Zeomic-Zeolite with high content of Ag ion) has strong antibacterial effect against *S.mutans* Ingbritt. Although Zeomic reduces the mechanical properties (tensile and compressive strength) of composite, it is still effective for clinical use [1]. Furthermore, Lee et al. (2007) showed that a glass ionomer cement (Fuji I) containing 1wt% Zeomic could inhibit *S. mutans* and increased compressive strength. Film thickness and setting time were increased in direct proportion to the amount of Zeomic [77]. Tissue conditioners containing SZ have antimicrobial effects for four weeks on *Candida albicans* (*C. albicans*) aa nosocomial respiratory infection-causing bacteria [76]. Although the incorporation of a silver-zinc zeolite to acrylic resins resulted in antimicrobial activity against *C. albicans* and *S. mutans*, this addition affected the mechanical properties [11].

While antibacterial property of glass ionomer cement is controversial, incorporation of antibacterial agents may confer antibacterial properties to glass ionomer cement. However, this additive should not endanger the basic physical and mechanical properties of glass ionomer cement. The objective in this study was therefore to evaluate the antibacterial, mechanical properties and fluoride release of glass ionomer cement containing different percentages antibacterial agents.

2.4 Summary

Although glass ionomer cements are widely used as restorative materials, they have some disadvantages due to lack of adequate strength and toughness. Improvements have been made to enhance their properties to be smart dental biomaterials. While the antibacterial activity of glass ionomer cement is still unclear. The addition of a variety agents such as titanium dioxide nanoparticle, extract from pericarp of mangosteen, chitosan and SZ is interested to improve the antibacterial property while maintaining the other properties.

CHAPTER III

MATERIALS AND METHODS

3.1 Fabrication of antibacterial agents containing glass ionomer cement

Highly viscous glass ionomer cement (Fuji IX™, Lot number: 1205021, GC corp., Japan), titanium dioxide nanoparticle (P25 Degussa, Lot number: MKBL0267V, Sigma-Aldrich Co Ltd., Thailand), shrimp chitosan (Lot number: CS-250213/1-1, Marine Bio Resources Co.,Ltd, Thailand), and extract from pericarp of mangosteen were used as received. SZ (Zeomic® IM10D-L, Lot number: IML030) was supplied from Sinanen Zeomic Co., Ltd., Japan. The composition of materials are as shown in table 2, figure 6, and figure 7.

Table 2 Compositions of materials used in the experiment

Name and Manufacturer	Classification	Composition	Lot number
Fuji IX™, GC Corp.,Japan (GIC)	Highly viscous glass ionomer cement	Powder: 95% Aluminofluorosilicate glass 5% Polyacrylic acid powder Liquid: 50% distilled water 40% polyacrylic acid 10% polybasic carboxylic acid	1205021
Titanium dioxide, Sigma-Aldrich Co Ltd., Thailand (TiO_2)	Titanium Dioxide nanoparticle P25 Degussa	~21 nm particle size ≥99.5% trace metals basis	MKBL0267V

Table 2 (Cont.)

Name and Manufacturer	Classification	Composition	Lot number
Zeomic®, Sinanen Zeomic Co., Japan (SZ)	SZ	2.4% Silver, 97.6% Glass ($MX_2/nO,Al_2O_3,YSiO_2,ZH_2O$ M: ions such as silver and natrium X,Y,Z: Indicates the molar ratio of each element)	IML030
Extract from pericarp of mangosteen, Department of Chemistry, Faculty of Science, Srinakharinwirot University (Man)	Crude extract	Xanthones	
Shrimp Chitosan, Marine Bio Resources Co.,Ltd, Thailand (Chi)	Food grade	Particle size 100 mesh	CS-250213/1-1

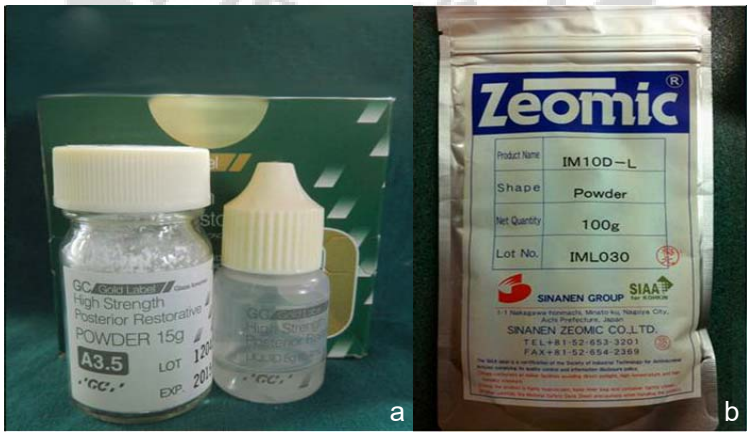

Figure 6 Materials used in this experiment. a) Highly viscous glass ionomer cement (Fuji IX™GP) provided as powder and liquid system. b) SZ (Zeomic®) as white powder

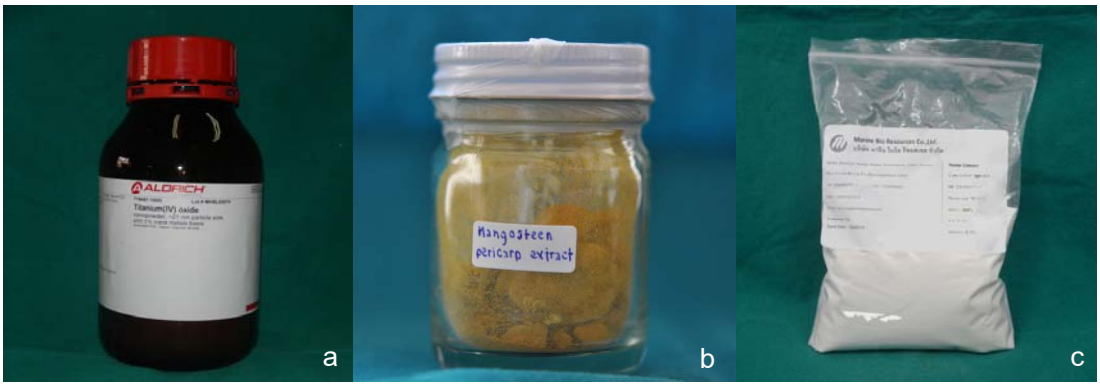

Figure 7 Antibacterial agents used in this experiment. a) Titanium dioxide (P25 Degussa) b) Extract from pericarp of mangosteen c) Shrimp chitosan

The powder of highly viscous glass ionomer cement was weighed by digital scales (Scaltec, Germany) and then was blended with antibacterial agents at 3%, 5%, and 7% (w/w) as table 2 in the bottle by using vortex mixer (Scientific Industries Inc., USA) 300 rpm for 15 min to obtain an adequate particle distribution (figure 8 and figure 9).

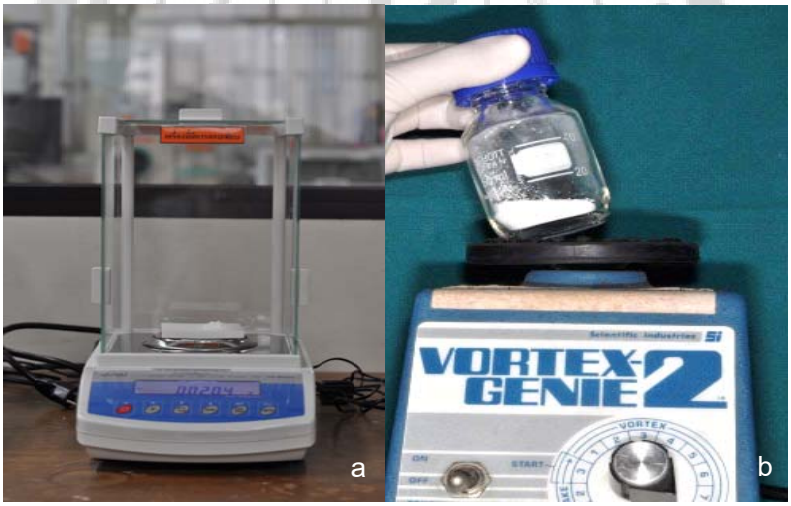

Figure 8 The equipment used in this experiment. a) Digital scales was used for weigh the substances. b) Blender (Vortex mixer) was using to vibrate material in the bottle.

Table 3 Compositions of antibacterial agents containing glass ionomer cement

Materials	GIC powder (wt%)	Antibacterial powder (wt%)	GIC liquid (wt%)	Powder/liquid ratio
Control	5	0	1.39	3.6:1
3%antibacterial powder	4.85	0.15	1.39	3.6:1
5%antibacterial powder	4.75	0.25	1.39	3.6:1
7% antibacterial powder	4.65	0.35	1.39	3.6:1

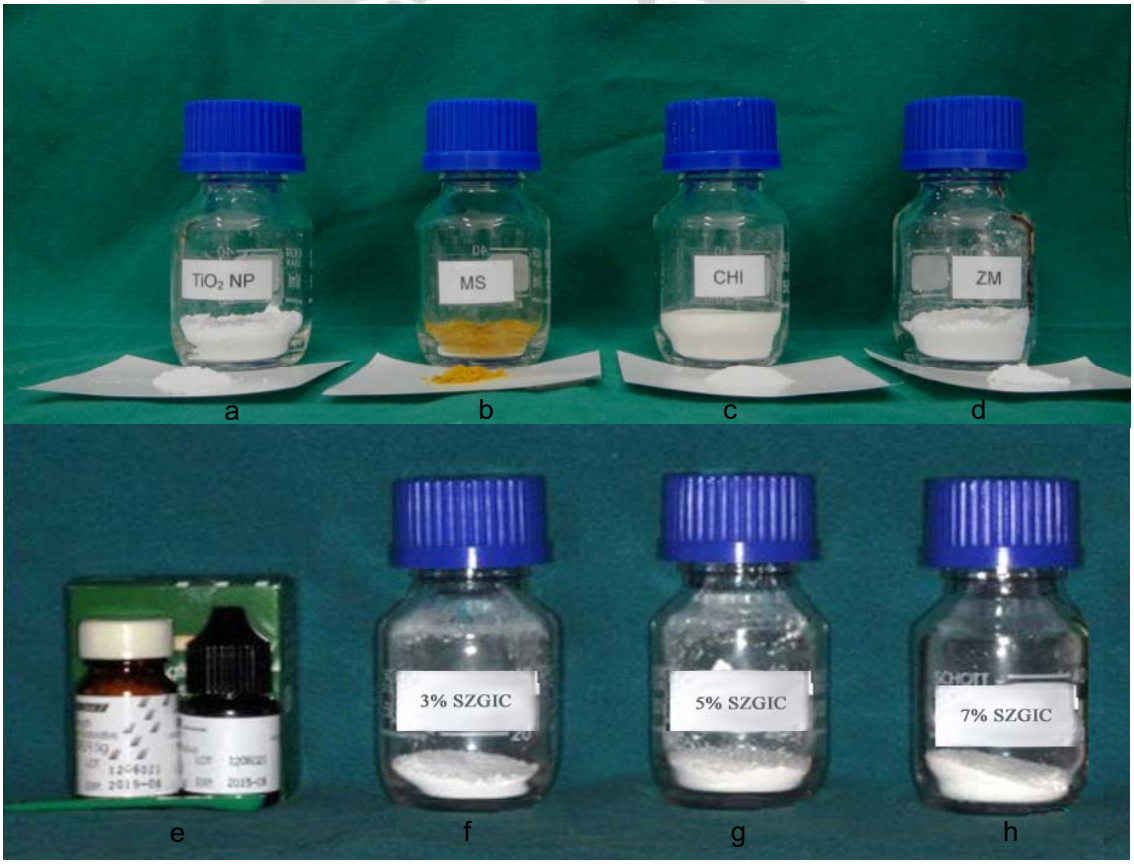

Figure 9 Materials used in this study a) Titanium dioxide b) Extract from pericarp of mangosteen c) chitosan c) SZ e) Conventional glass ionomer cement powder (Fuji IX^{TM}GP) f), g), h) Conventional glass ionomer cement powder mixed with SZ (Zeomic$^{®}$) at 3.0%, 5.0%, and 7.0%.

3.2 Antibacterial properties [64]

3.2.1 Specimen preparation

A powder/liquid ratio 3.6g/1g according with manufacturer's instructions of both glass ionomer cement and the one containing antibacterial agents was used in all of specimens. Disc-shaped specimens (5 mm in diameter and 2 mm thick) were prepared in silicone mold on the bottom glass plate as indicated in figure 3.5. The mold was filled to excess and any extruded cement was removed. The top glass plate was placed on the mold and was squeezed together to obtain smooth and flat surface. A 100% antibacterial agent was a powder of antibacterial agent mixed with sterile de-ionized water and filled in silicone mold. The specimens were left for 1 hour. Chlorhexidine gluconate 0.12% w/v (CHX: C-20, Osoth Inter Laboratories Co., Ltd) 1 µL was dropped on a paper disc as a positive control. Then, all of the specimens were placed on ten plates of Brain Heart Infusion (BHI) agar with *S. mutans*.

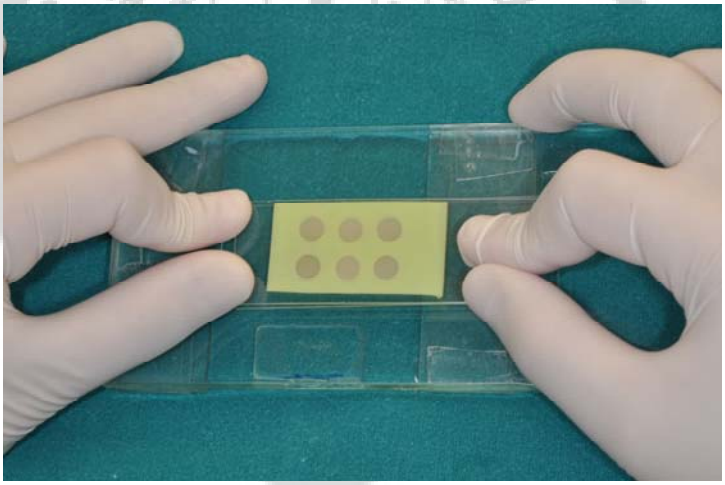

Figure 10 Disc-shaped specimens in silicone mold.

3.2.2 Bacterial suspension

The microorganism used was *Streptococcus mutans* (ATCC 25175). It was obtained from Department of Stomatology, Faculty of dentistry, Srinakharinwirot university, Bangkok, Thailand. Culture was begun from freeze-dried stock into 10 mL of sterilized Brain Heart Infusion broth (Difco Laboratories, Inc., USA) as figure 3.6a. The bacteria was allowed to grow at 37ºC for 24 hours in an incubator prior to being used. While the specimens were being set, concentration of *S. mutans* suspension was determined by UV-

spectrophotometer (Shimadzu Co., Ltd., Japan) at 600 nm (figure 3.6e). The suspension was adjusted to an optical density of approximately 0.1 as 0.5 Mcfarland which was correspond to 10^6-10^8 CFU/mL by opacity tube method. Then, the bacterial suspension was spread on Brain Heart Infusion agar plate and left at room temperature for 30 min (figure 11f).

Figure 11 Preparation of bacterial suspension and BHI a) Cultivation of *S. mutans* b) BHI powder c) Laminar flow hood d) Preparation BHI agar e) UV-spectrophotometer f) *S. mutans* spread on BHI agar.

3.2.3 Agar-diffusion tests

Antibacterial activity of experimental specimens against *S. mutans* was evaluated by using agar-diffusion tests. Each plate had six tested specimens: glass ionomer cement, 100% antibacterial agent, 3%, 5%, and 7% antibacterial agent containing glass ionomer cement, and chlorhexidine gluconate on a paper disc was used as a positive control (figure 12 and table 4). Specimens were placed onto 10 Brain Heart Infusion agar plates, inoculated with *S. mutans*. Then, the plates were kept in an incubator at 37 ºC for 48 hours. The inhibition zone around each specimen was evaluated by using a digital caliper (Mitutoyo Co., Japan) at two different points as indicated in figure 3.8 at 24 and 48 hours. The size of inhibition zones was calculated by deducting the specimen diameter and the average value was calculated (figure 13).

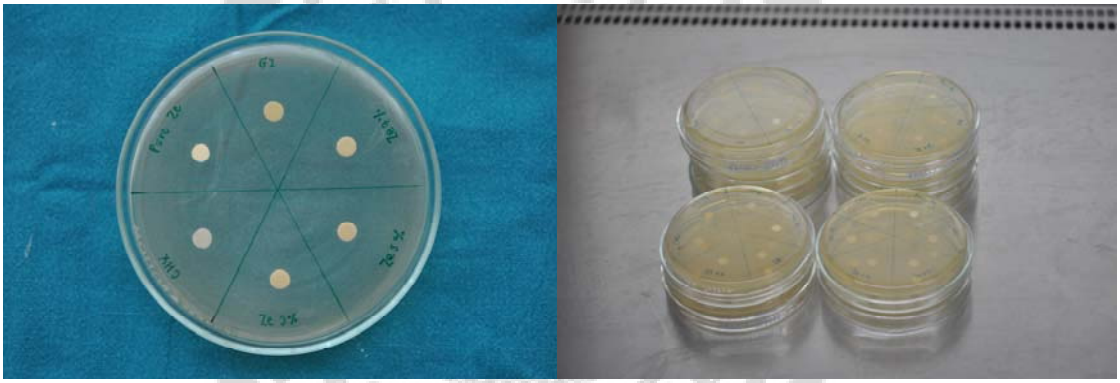

Figure 12 Dish-shaped specimens were placed on BHI agar plate with *S. mutans* inoculation.

Table 4 Specimens containing in each plate for agar diffusion test

Group	Number of Plates	Specimens in each place					
		Positive control	Experimental groups				
Titanium dioxide (TiO_2)	10	CHX	GIC	3%TiO_2	5%TiO_2	7%TiO_2	100%TiO_2
Silver-zeolite (SZ)	10	CHX	GIC	3%SZ	5%SZ	7%SZ	100%SZ
Extract from pericarp of mangosteen (Man)	10	CHX	GIC	3%Man	5%Man	7%Man	100%Man
Chitosan (Chi)	10	CHX	GIC	3%Chi	5% Chi	7% Chi	100% Chi

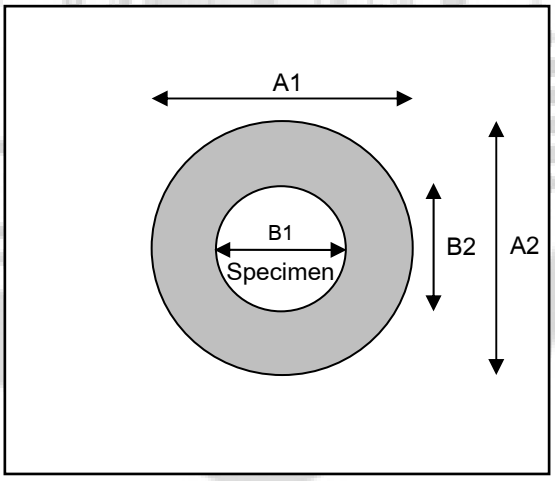

Figure 13 The size of inhibition zone was distance calculated by deducting diameter of specimen (A-B=X). Each specimen was evaluated at two different points for average value (X1+X2/2=X^2).

3.3 Physical properties

A powder/liquid ratio 3.6g/1g according with manufacturer's instructions of both glass ionomer cement and the one containing silver zeolite was used in all of specimens. The specimens were prepared on a silicone mold (6 mm diameter, 1.5 mm thick), placed between two glass plates. The whole assembly was transferred to the cabinet and kept at 37°C in 100% relative humidity for 1 hour. After 1 hour, the specimen was placed in a

plastic container containing with 4 ml of de-ionized water and then incubated at 37°C for 23 hours. The homogeneity and color of specimens were observed. Then, the specimens were immersed in de-ionized for 29 days. The homogeneity and color of specimens were compared with each other after mixing, 1 and 30 days.

3.4 Mechanical properties

All specimens were divided into 4 groups; group I: glass ionomer cement as control group, group II: 3%SZGIC, group III: 5%SZGIC and group IV: 7%SZGIC for compressive, flexural strength and surface microhardness test. Ten specimens were fabricated for each group.

3.4.1 Compressive strength

Compressive strength test was evaluated under ISO standard 9917-1 indicating specimen and test method [78].

3.4.1.1 Specimen preparation

Powder and liquid were mixed on a mixing pad with a plastic spatula as indicated in figure 3.9a (powder to liquid ratio is 3.6g/1.0g, mixing time 30 seconds from the start of mixing). Stainless steel split mold with dimensions of 4 mm diameter and 6 mm height was prepared and was coated with petroleum jelly (figure 3.9b). The split mold was carefully filled with mixed cement to avoid trapping air bubbles. The mold was filled to excess and placed on the bottom glass plate with some pressure. The extruded cement was removed. Then, the mold was pressed with the glass plate on the top (working time is 2 minutes from the start of mixing). The mold and plates were transferred to the screw clamp and tighten (figure 3.9c). The whole assembly was brought into the cabinet and kept at 37°C in 100% humidity for 1 hour. Then, the specimen was removed from the mold and checked for air-voids or shipped edges (figure 3.9d). Defective specimens were discarded. Specimens were immersed in water and stored in the incubator (Contherm Scientific Ltd., New Zealand) at 37°C for 23 hours (figure 3.10).

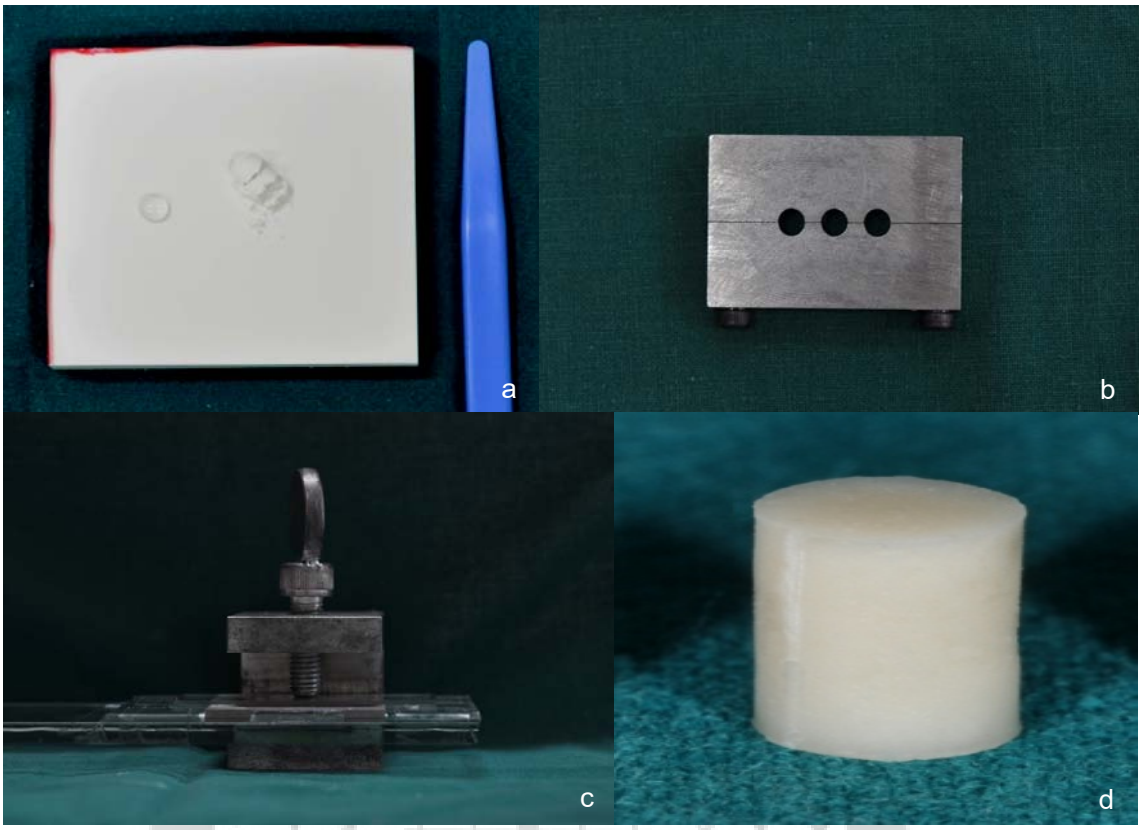

Figure 14 Specimen preparation for compressive strength test. a) Powder and liquid were prepared. b) Stainless steel split mold used for compressive strength test. c) Split mold and plate with screw clamp tightened. d) Specimen was removed from the mold.

Figure 15 Specimen preparation for compressive strength test. a) Before testing, specimens were immersed in de-ionized water. b) The specimens were maintained in an incubator at a 37 ˚C for 23 hours.

3.4.1.2 Compressive strength test

Before testing, specimens were measured to determine the dimensions by using a digital caliper (Mitutoyo Co., Japan) at 24 hours after the end of mixing. Then, each specimen was placed with the flat ends between the platens of the universal testing machine. Compressive strength was evaluated by using a universal testing machine (LLOYD Instrument Ltd., UK) by using 10 kN load cell with a crosshead speed of 0.75 mm/min (figure 3.11). The compressive load was applied along the long axis of specimen until fracture. The maximum load applied to fracture the specimen was recorded (figure 3.12). The stress at maximum load (compressive strength) was calculated by LRX console program.

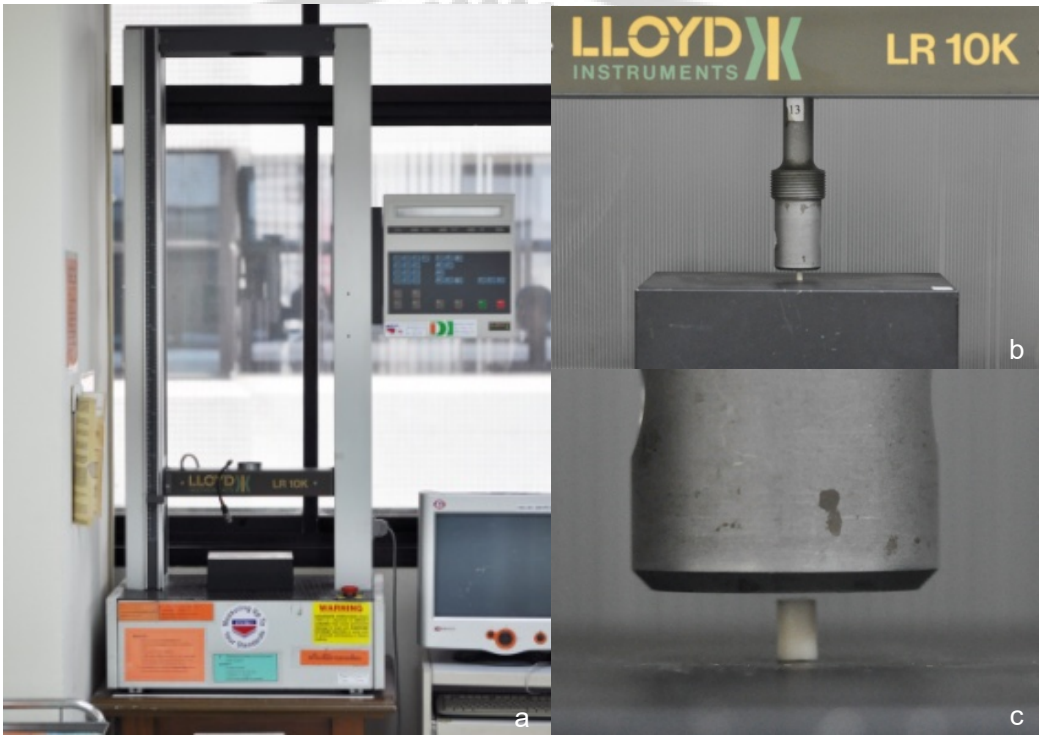

Figure 16 Compressive strength test. a) The universal testing machine (LLOYD Instrument Ltd., UK) for compressive strength testing. b), c) The specimen was placed between the platens of the universal testing machine.

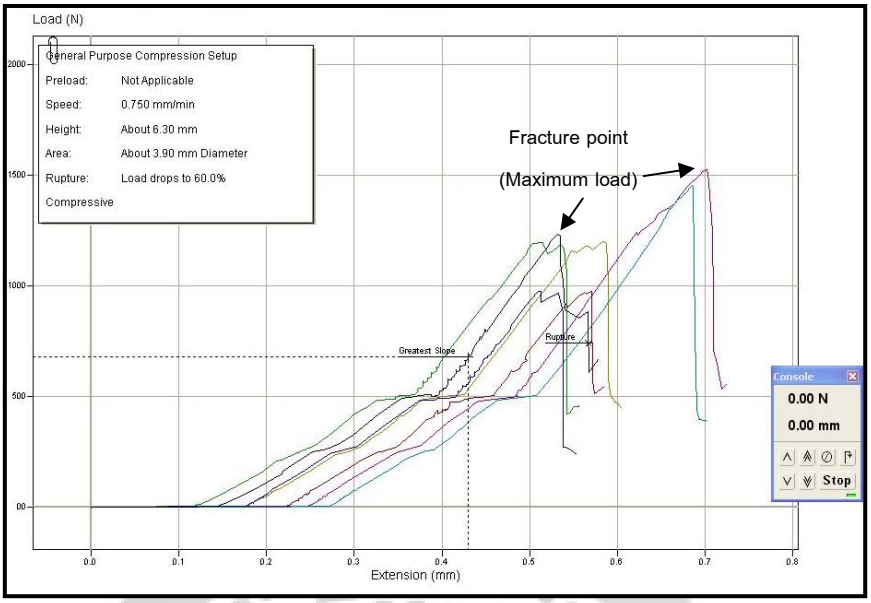

Figure 17 The graph of compressive strength test. The maximum load applied to fracture the specimen was recorded.

The maximum load and diameter of specimen was used to automatic calculate the compressive strength (C) in megapascals, using the equation:

$$C = 4p/\pi d^2$$

where p is the maximum load applied, in newtons and d is the measured diameter of the specimen, in millimeters.

3.4.2 Flexural strength

Flexural strength test was evaluated under ISO standard 9917-2 indicating specimen and test method [79].

3.4.2.1 Specimen preparation

Powder and liquid were mixed on a mixing pad with a plastic spatula as indicated in figure 18a (powder to liquid ratio was 3.6g/1.0g, mixing time 30 seconds from the start of the mixing). Bar-shaped stainless steel split mold with dimensions of 25 mm length, 2 mm width, 2 mm thick was prepared and was coated with petroleum jelly (figure 18b). The split mold was carefully filled with mixed cement to avoid trapping air bubbles. Mixed cement was overfilled into a split mold which placed on the bottom glass plate.

The extrude cement was removed. The top glass plate and clamp were compressed to cement (figure 18c). The assembly was left to set in a water bath at 37°C for 1 hour. After that, the specimen was removed from the mold and stored in distilled water at 37°C for 23 hours in incubator (figure 18e and figure 18f). Defective specimens were discarded.

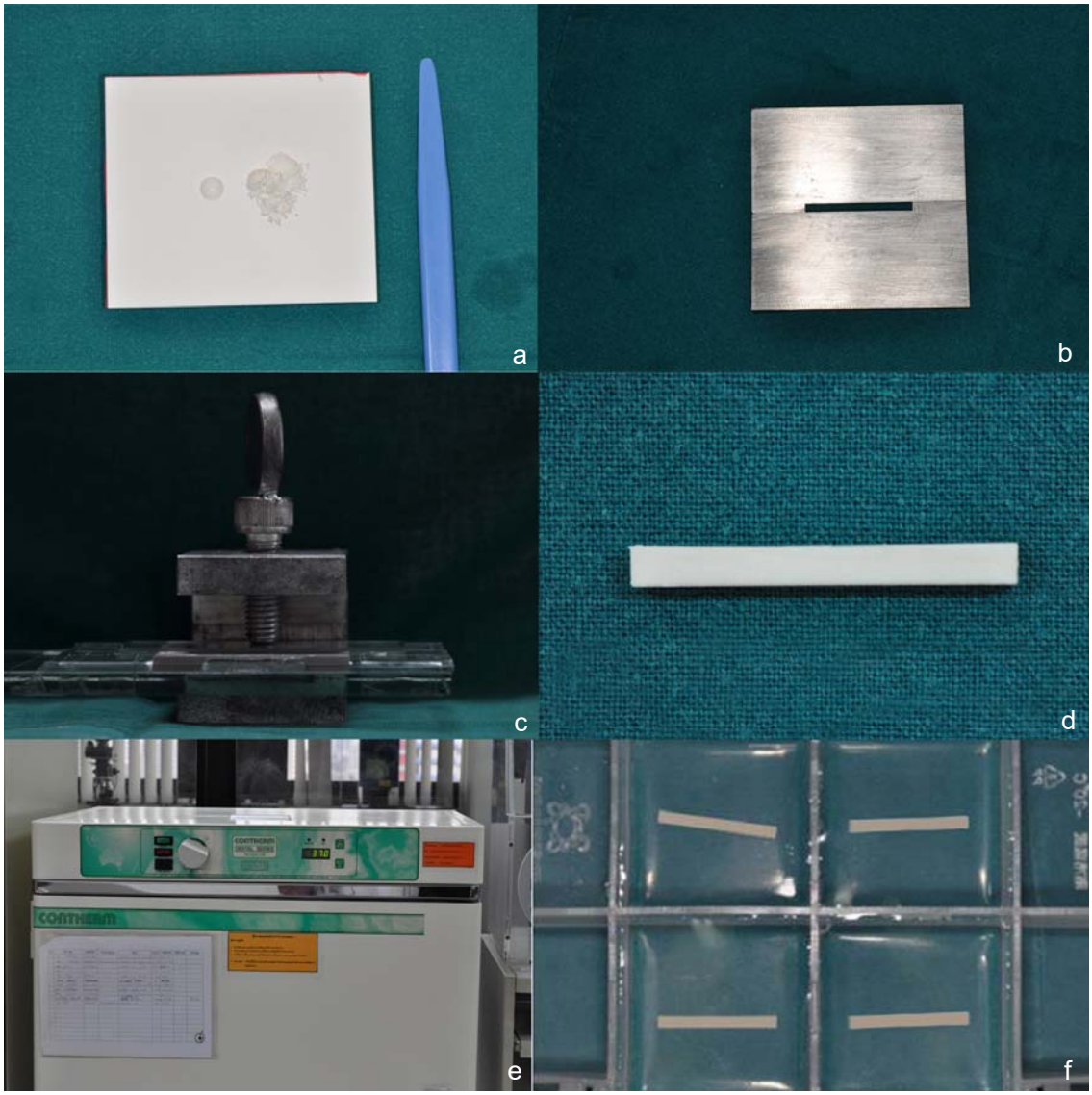

Figure 18 Specimen preparation for flexural strength test. a) Powder and liquid were prepared according to the manufacturer's instructions. b) A stainless steel split mold was used for flexural strength test. c) Split mould and plate were tightened with the screw clamp. d) The specimen was then removed from the mold. e) The incubator was maintained at a temperature of 37 ˚C. f) The specimen in de-ionized water.

3.4.2.2 Flexural strength test

Specimen dimensions were measured by using a digital caliper (Mitutoyo Co., Japan) after 23 hours storage in water. By using a universal testing machine as figure 19a (Shimadzu Co., Ltd., Japan), specimen was placed center on two rods which mounted parallel with 20 mm (figure 19b). The upper rod was fixed to the center of the specimen. Flexural strength was tested by the three-point bending mode by using 500 N load cell with a crosshead speed of 0.5mm/min. The stress at maximum load (flexural strength) was calculated by Trapezium2 program.

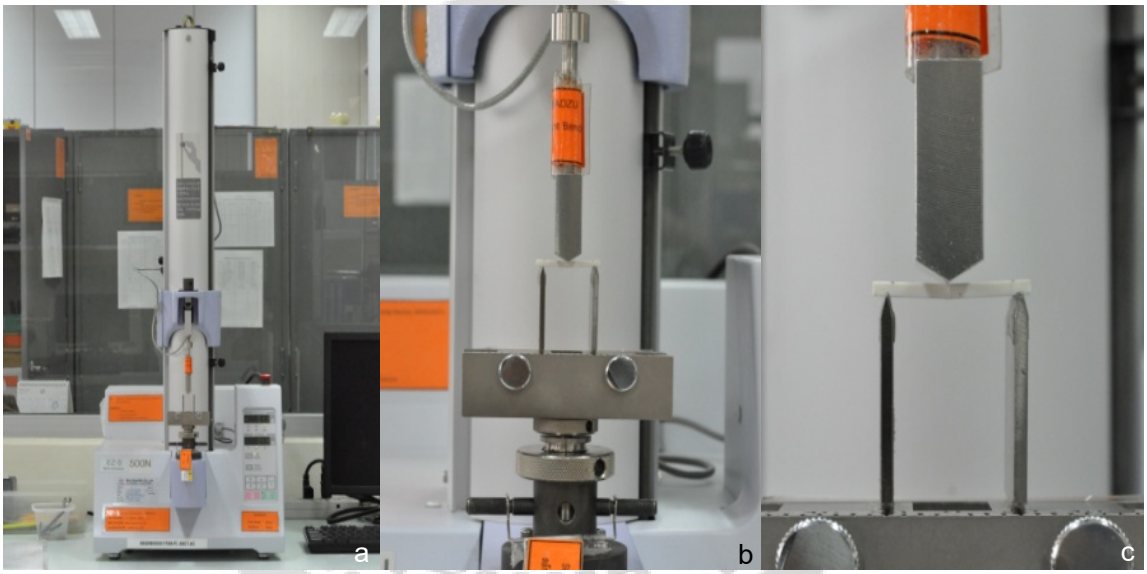

Figure 19 Flexural strength test. a) The universal testing machine (Shimadzu Co., Ltd., Japan) for three point bending test b), c) The specimen positioned in the center of test jig, perpendicular to the three rods. The two rods on the lower block were mounted parallel with 20 mm apart.

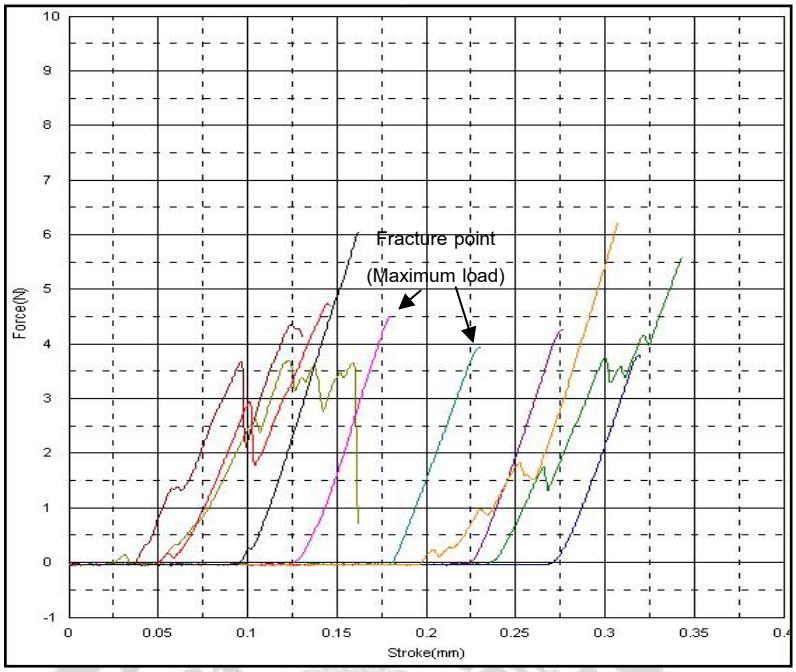

Figure 20 The graph of flexural strength test. The maximum load applied to fracture the specimen was recorded.

The maximum load was recorded when the specimen fractures (figure 20). The flexural strength (σ) was automatic calculated in megapascals, using the following equation:

$$\sigma = 3PI/2bd^2$$

where P is the maximum load applied, in newtons, I is the distance between the support, in millimeters (In this case, I = 20 millimeters), b is the width of specimen, in millimeters, and d is the height of specimen, in millimeters.

3.4.3 Surface microhardness [12, 80]

The surface microhardness test was performed according to Palma-Dibb et al. in 2002 and Elsaka el al. in 2011.

3.4.3.1 Specimen preparation

Powder and liquid were mixed on a mixing pad with a plastic spatula as shown in figure 21a (powder to liquid ratio is 3.6g/1.0g, mixing time 30 seconds from the start of mixing). Disc-shaped silicone mold with dimensions of 6 mm diameter, 3 mm thick

were prepared as shown in figure 3.16b. The silicone mold on the bottom glass plate was carefully filled with mixed cement to avoid trapping air bubbles. The extruded cement was removed. The glass plate was placed on the top of the mold to obtain a smooth, flatten surface. The assembly was left until it set at 37°C in 100% humidity for 1 hour. Then, the specimen was removed from the mold (figure 21c) and immersed in water at 37°C and stored in a incubator for 23 hours (figure 21 e and figure 21 f). Any defective specimen was discarded.

3.4.3.2 Surface microhardness test

The specimens were tested for surface microhardness by using a microhardness tester as figure 22a (Type D, Future tech corp., Japan) at 24 hours after the end of mixing. Each specimen was indented with a square based pyramidal diamond indenter 50g load and a dwell time of 10 seconds. Indentations were made on flat and no void surface. Five indentations per each specimen were spaced at least 1 mm from each other or the margin of the specimen (figure 22b). The dimension of the identations was measured at 2 diagonal length by microhardness tester (figure 22e). The vickers hardness number (VHN) for each indentation was automatic computed by microhardness tester. Five reading were recorded for each specimen and the mean was calculated.

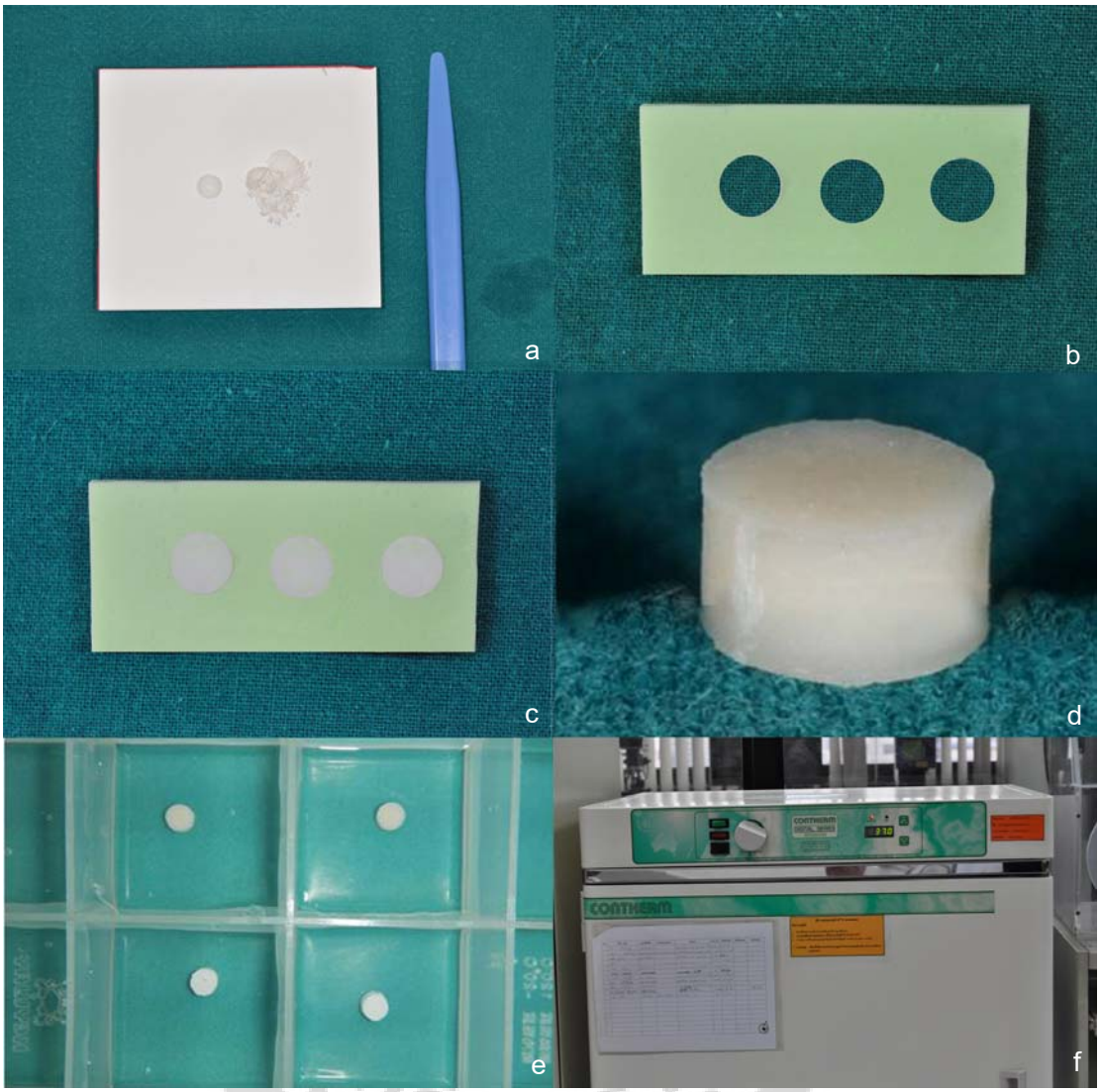

Figure 21 Specimen preparation for surface microhardness test. a) Powder and liquid were prepared according to manufacturer's instructions. b) The silicone mold was used for surface microhardness test. c) Specimens in silicone mold. d) Specimen after removed from the mold e) The specimen in de-ionized water. f) The incubator maintained at a temperature of 37 ˚C.

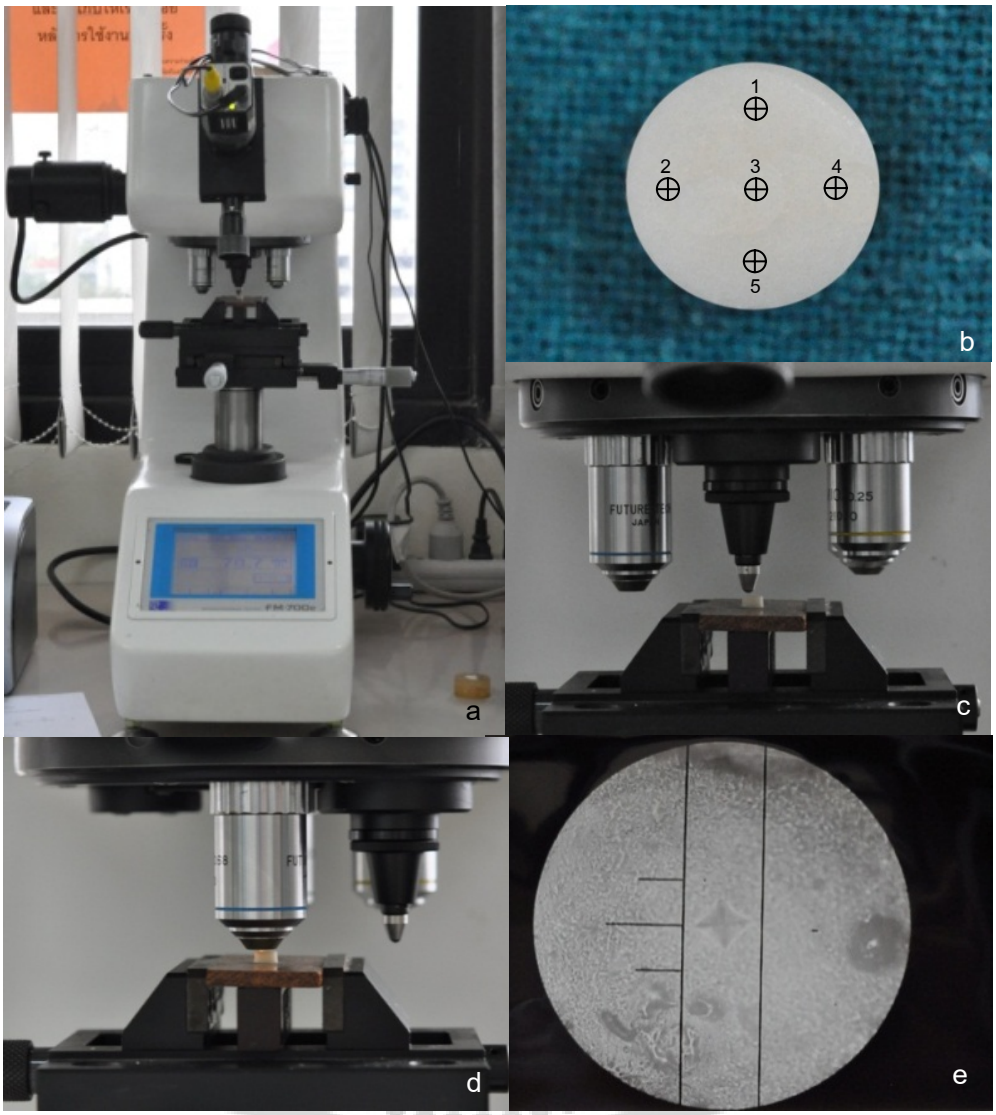

Figure 22 Surface microhardness test. a) The microhardness tester (Type D, Future tech corp., Japan) for surface microhardness test. b) The position on the specimen for surface microhardness test. c), d) The specimen on the microhardness tester. e) The indentation on the specimen.

The vickers hardness value, VHN was automatic calculated by using the equation:

$$VHN = 1.8544 \times L/d^2$$

where L is applied load, in kilogram, and d is the mean indentations diagonal length, in millimeters.

3.5 Fluoride release measurements [34]

Fluoride release measurements were made according to Attar and Onen in 2002. Forty disc-shaped specimens were divided into 4 groups; group I: glass ionomer cement as control group, group II: 3%SZGIC, group III: 5%SZGIC and group IV: 7%SZGIC.

3.5.1 Specimen preparation

A powder/liquid ratio 3.6g/1g of both glass ionomer cement and the one containing SZ was used in all of specimens according to manufacturer' instructions. The specimens were prepared on a silicone mold (6 mm diameter, 1.5 mm thick), placed between two glass plates. The whole assembly was transferred to the cabinet and kept at 37°C in 100% relative humidity for 1 hour.

3.5.2 Fluoride release

After 1 hour, each specimen was placed in a plastic container with 4 ml of deionized water and then incubated at 37°C. All specimens were washed with 1 mL deionized water over the original container after incubation of 23 hours. The rinsed water was kept to determined fluoride ion. A 0.5 mL of buffer solution (TISAB III) was added to 5 mL of the collected and washed specimen deionized water. Fluoride release was determined by a calibrated fluoride ion-specific electrode (Fisher Scientific Ltd., USA) as shown in figure 3.18. For the next measurement, each specimen was placed in a new plastic container with 4 ml of deionized water and stored at 37°C. The amount of fluoride was measured at 1, 2, 3, 4, 5, 15, and 30 days. Data was recorded in parts per million (ppm).

Figure 23 Disc-shaped specimens in deionized water. De-ionized water 5 mL and buffer solution (TISAB III) 0.5 mL were measured for the fluoride release.

3.6 Statistical analysis

The mean values of antibacterial test were analyzed by using one-way analysis of variance (ANOVA). Dunnett T3 multiple comparison tests were used to assess significant differences between the values, with 95% confidence level.

The data of compressive strength, surface microhardness, and cumulative fluoride release were analyzed by using one-way analysis of variance (ANOVA). Significant level was set at $p<0.05$.

The flexural strength of each group was analyzed by using one-way analysis of variance (ANOVA) and Tukey HSD multiple comparison tests were used to evaluate significant differences between the values, with 95% confidence level.

CHAPTER IV

RESULTS

4.1 Antibacterial properties

The mean distance of inhibition zone was calculated from 10 specimens per group. The data in the control and experimental groups of all concentrations is presented in table 5, figure 24 and figure 25.

Table 5 Mean (standard deviation) of the inhibition zone in millimeters. All data showed inhibition zone at 24 hours; no increased in the inhibition zone was detected at 48 hours.

Group	TiO_2	Mangosteen	Chitosan	SZ
CHX	19.04(0.79)	12.24(0.49)	13.81(0.56)	16.59(1.27)
100%Antibac	0.00(0.00)	2.04(0.18)	0.00(0.00)	6.54(0.64)
GIC	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
3%Antibac	0.00(0.00)	0.00(0.00)	0.00(0.00)	2.60(0.54)
5%Antibac	0.00(0.00)	0.00(0.00)	0.00(0.00)	2.66(0.54)
7%Antibac	0.00(0.00)	0.00(0.00)	0.00(0.00)	2.90(0.54)

For titanium dioxide, extract from pericarp of mangosteen and chitosan, there were no inhibition zone of antibacterial agent containing glass ionomer cement (figure 24). Chlorhexidine as positive control was shown the highest inhibition zone. The 100% extract from pericarp of mangosteen was only one in experimental group that exhibited inhibition zone as 2.04 mm.

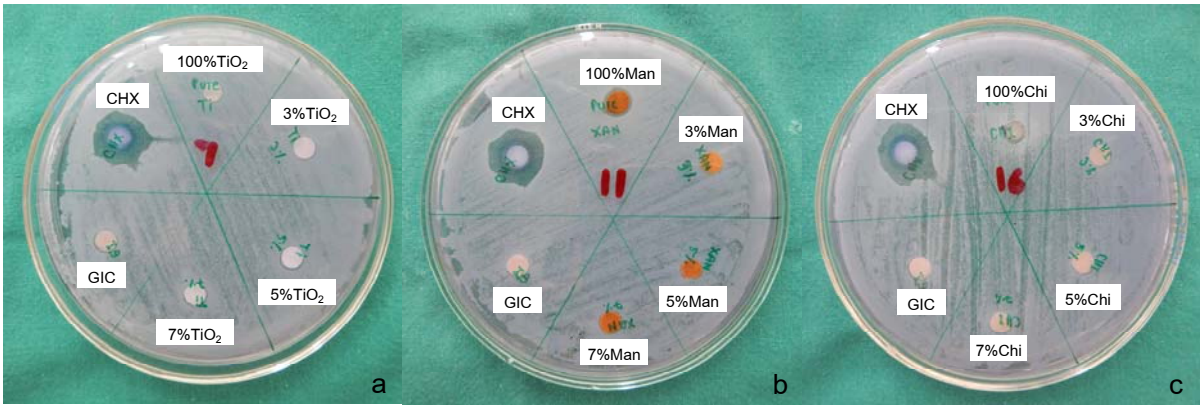

Figure 24 Agar diffusion test of a) titanium dioxide, b) extract from pericarp of mangosteen and c) chitosan group.

For the SZ agent, the inhibition zone of chlohexidine as positive control group was the highest (16.59 mm), whereas no inhibition zone against *S. mutans* was produced in glass ionomer cement. The inhibition zone of SZ containing glass ionomer cement ranged from 2.60 to 2.90 mm (figure 4.2). The 3%SZGIC, 5%SZGIC, and 7%SZGIC insignificantly increased the size of the inhibition zone according to increasing concentration of SZ incorporated into the glass ionomer cement ($p>0.05$). However, there was a significant decrease in inhibition zone for 3%SZGIC, 5%SZGIC and 7%SZGIC compared to 100% SZ and chlohexidine ($p<0.05$). All specimens showed no significant difference of inhibition zone between 24 and 48 hours.

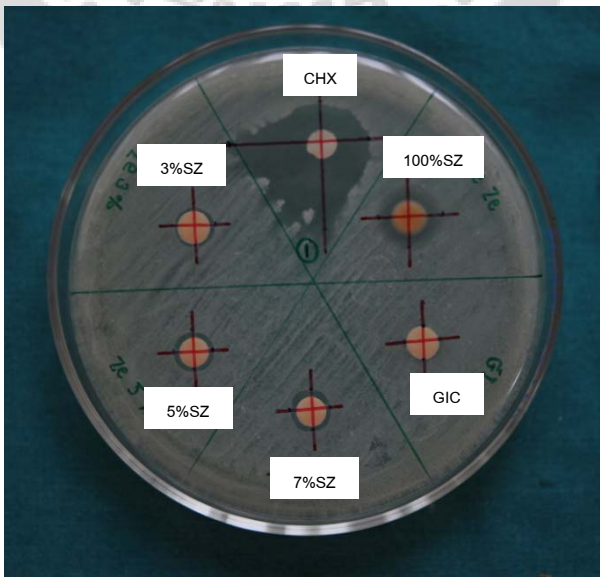

Figure 25 Agar diffusion test showing inhibition zone of glass ionomer cement, 3%SZGIC, 5%SZGIC, 7%SZGIC, 100%SZ and chlorhexidine.

4.2 Physical properties

After mixing the powder and liquid of glass ionomer cement modified with SZ, the specimen demonstrated homogeneous mixture as unmodified. In addition, after immersing specimens in de-ionized water for 1 and 30 days, the color of the modified groups at any percents was not different from that of the control group as shown in figure 26.

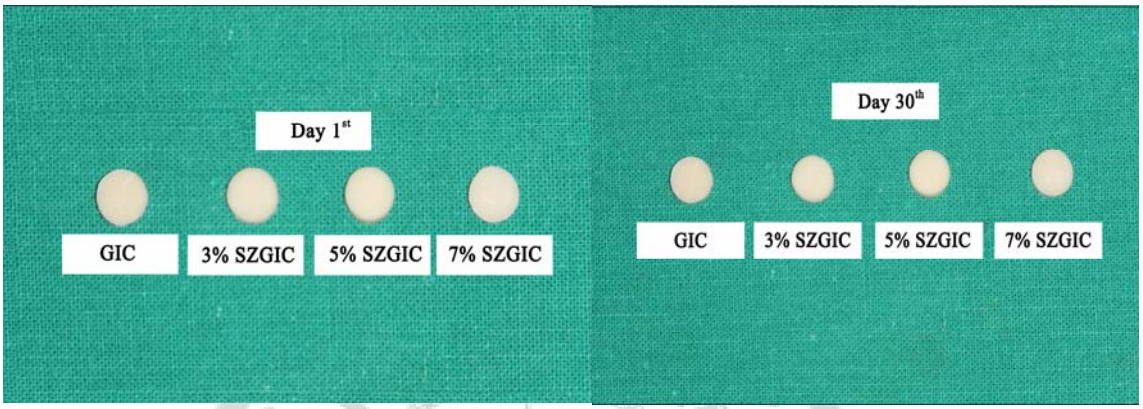

Figure 26 Glass ionomer cement, 3%, 5%, and 7% SZGIC after immersed in deionized water for 1 and 30 days. There was no change in color between control and experimental group.

4.3 Mechanical properties

4.3.1 Compressive strength test

The results of compressive strength test are summarized in table 6. Data is shown as mean (SD) of ten specimens per group. The average compressive strength ranged from 100.22 to 115.10 MPa. The 3%SZGIC was the highest while 5%SZGIC was the lowest of compressive strength. Statistical analysis of average compressive strength showed no significant difference between the three experimental groups compared with that of the control group ($p>0.05$). There was no significant increase in the compressive strength from 106.09 MPa in GIC to 115.10 MPa in 3%SZGIC groups ($p>0.05$). However, 5%SZGIC (w/w) group decreased compressive strength compared with the control group ($p>0.05$) (table 6 and figure 27).

Table 6 Mean (standard deviation) of the compressive strength of glass ionomer cement and the one containing SZ at different percents.

Material	n	Compressive strength (MPa)
GI	10	106.09 (15.80)[a]
3%SZGIC	10	115.10 (8.61)[a]
5%SZGIC	10	100.22 (15.85)[a]
7%SZGIC	10	112.20 (7.42)[a]

Mean compressive strength for each group with identical superscript letter (column) are not significantly different ($p>0.05$), while the mean with the different letters are significantly different ($p<0.05$)

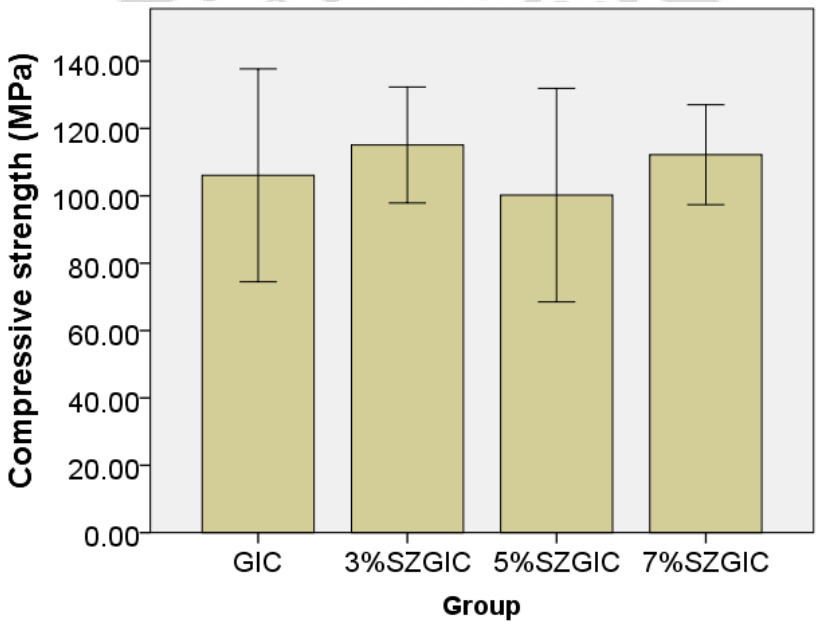

Figure 27 The graph shows a compressive strength of glass ionomer cement and the one containing SZ at different percents.

4.2.2 Flexural strength test

The results of flexural strength test are summarized in table 7. Data was shown as mean (SD) of ten specimens per group. The flexural strength ranged from 10.67 to 14.66 MPa. The group of 3%SZGIC was the highest while 7%SZGIC was the lowest of the flexural strengths. Average flexural strength of 3%SZGIC showed an increase compared with that of the control group but the difference was not significant ($p>0.05$). There was no significant difference between 3%SZGIC and 5%SZGIC compared with the control group ($p>0.05$). However, the incorporation of 7% SZ into glass ionomer cement significant decreased the flexural strength compared with the 3% SZGIC ($p<0.05$) (table 7 and figure 28).

Table 7 Mean (standard deviation) of the flexural strength test of glass ionomer cement and the one containing SZ at different percents.

Material	n	Flexural strength (MPa)
GI	10	11.66 (2.45)^{ab}
3%SZGIC	10	14.66 (2.60)^{a}
5%SZGIC	10	11.76 (2.87)^{ab}
7%SZGIC	10	10.67 (2.06)^{b}

Mean flexural strength for each group with identical superscript letter (column) are not significantly different ($p>0.05$), while the mean with different letters are significantly different ($p<0.05$).

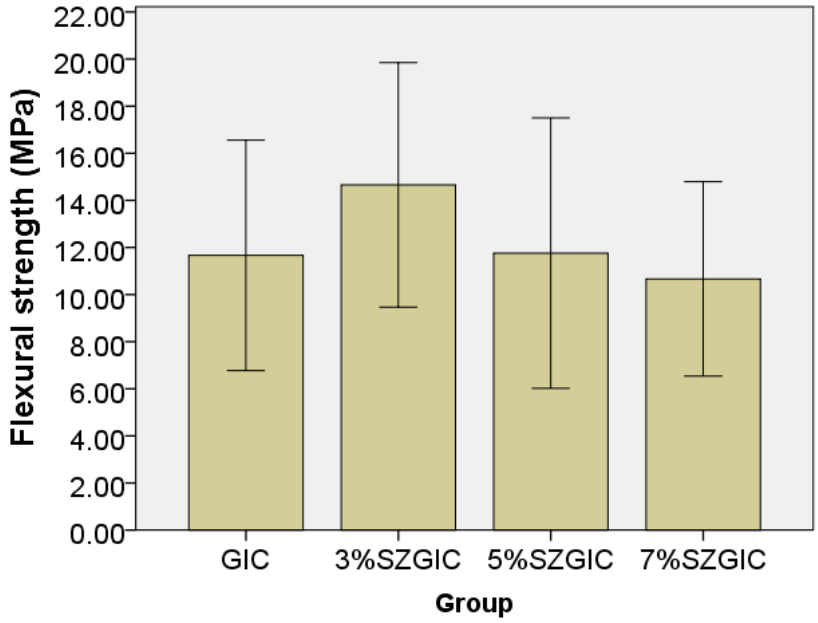

Figure 28 The graph shows a flexural strength of glass ionomer cement and the one containing SZ at different percents.

4.2.3 Surface microhardness test

The results of surface microhardness test are summarized in table 8. Data is presented as mean (SD) of ten specimens per group. The microhardness ranged from 68.34 to 69.63 VHN. The 5%SZGIC gave the highest while glass ionomer cement as control group gave the lowest of surface microhardness. Data for microhardness showed no significant increase for 7%SZGIC, 3%SZGIC and 5%SZGIC groups, respectively, when compared to the control group ($p>0.05$). The incorporation of 3%SZGIC, 5%SZGIC and 7%SZGIC into glass ionomer cement showed no significant difference on the surface microhardness compared with the control group($p>0.05$) (table 8 and figure 29).

Table 8 Mean (standard deviation) of the surface microhardness test of glass ionomer cement and the one containing SZ at different percents.

Material	n	Microhardness strength (VHN)
GI	10	68.34 (2.56)[a]
3%SZGIC	10	69.12 (2.47)[a]
5%SZGIC	10	69.63 (4.00)[a]
7%SZGIC	10	68.96 (4.16)[a]

Mean surface microhardness for each group with identical superscript letter (column) are not significantly different (P>0.05), while the mean with the different letters are significantly different (p<0.05).

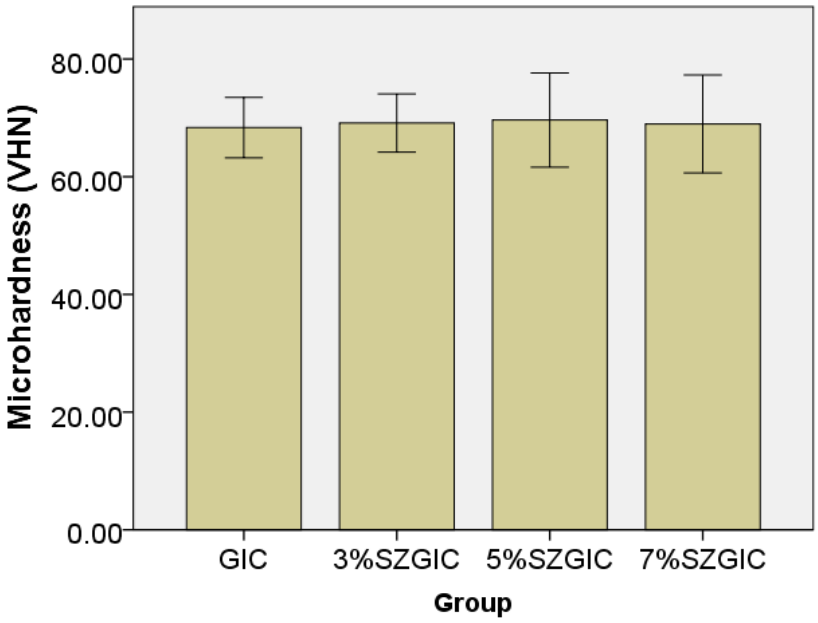

Figure 29 The graph shows a surface microhardness of glass ionomer cement and the one containing SZ at different percents.

4.4 Fluoride release

The amounts of fluoride release (ppm) in each group are presented in table 9. Data is shown as mean (SD) of ten specimens per group on day 1, 2, 3, 4, 5, 15 and 30 and cumulative amounts of fluoride release on day 5, 15 and 30. The glass ionomer cement as control group gave the highest while 5%SZGIC shown the lowest of cumulative amounts of fluoride release 30 days. Although there was a significant difference in the fluoride release between the group at days 4, 5, 15 and 30 (p<0.05), cumulative amounts of fluoride released from each group at any given time were no significant difference (p>0.05).

Table 9 Mean (standard deviation) of the fluoride release(ppm) from glass ionomer cement and the one containing SZ at different percents.

Group	1 Day	2 Days	3 Days	4 Days	5 Days	15 Days	30 Days	Cumulative fluoride release		
								5 Days	15 Days	30 Days
GI-control	8.50 (1.43)	0.78 (0.17)	0.47 (0.09)	0.74 (0.23)	0.30 (0.12)	0.21 (0.10)	0.05 (0.03)	10.79 (1.71)	10.99 (1.79)	11.05 (1.81)
3%SZGIC	7.90 (0.99)	0.68 (0.15)	0.39 (0.09)	0.44 (0.11)	0.18 (0.08)	0.06 (0.03)	0.006 (0.002)	9.59 (1.01)	9.65 (1.00)	9.66 (1.00)
5%SZGIC	7.90 (2.18)	0.64 (0.13)	0.38 (0.08)	0.39 (0.09)	0.25 (0.13)	0.06 (0.02)	0.005 (0.002)	9.56 (2.28)	9.62 (2.30)	9.62 (2.30)
7%SZGIC	7.80 (1.14)	0.75 (0.13)	0.46 (0.12)	0.47 (0.13)	0.33 (0.13)	0.07 (0.02)	0.008 (0.002)	9.81 (1.24)	9.88 (1.24)	9.89 (1.24)

The patterns of fluoride ion release from the 3%SZGIC, 5%SZGIC and 7%SZGIC were identical with that of control group. The greatest amount of fluoride was released from all groups on the first day approximately 7-9 ppm. This was followed by a sharp drop on the second day to 0-1 ppm then gradually diminished but continuous release of fluoride (figure 30 and figure 31).

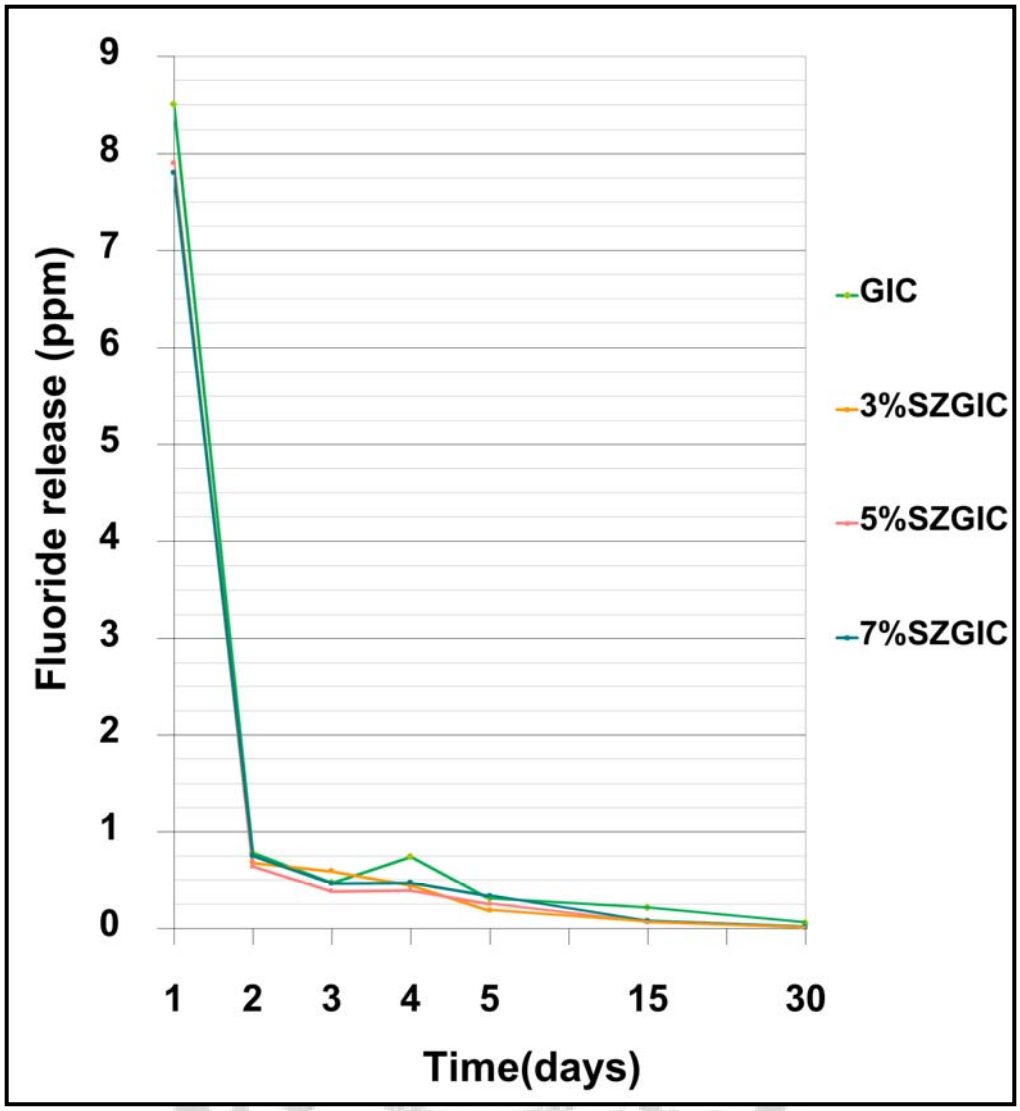

Figure 30 The graph shows fluoride release (ppm) from glass ionomer cement and the one containing SZ at different percents.

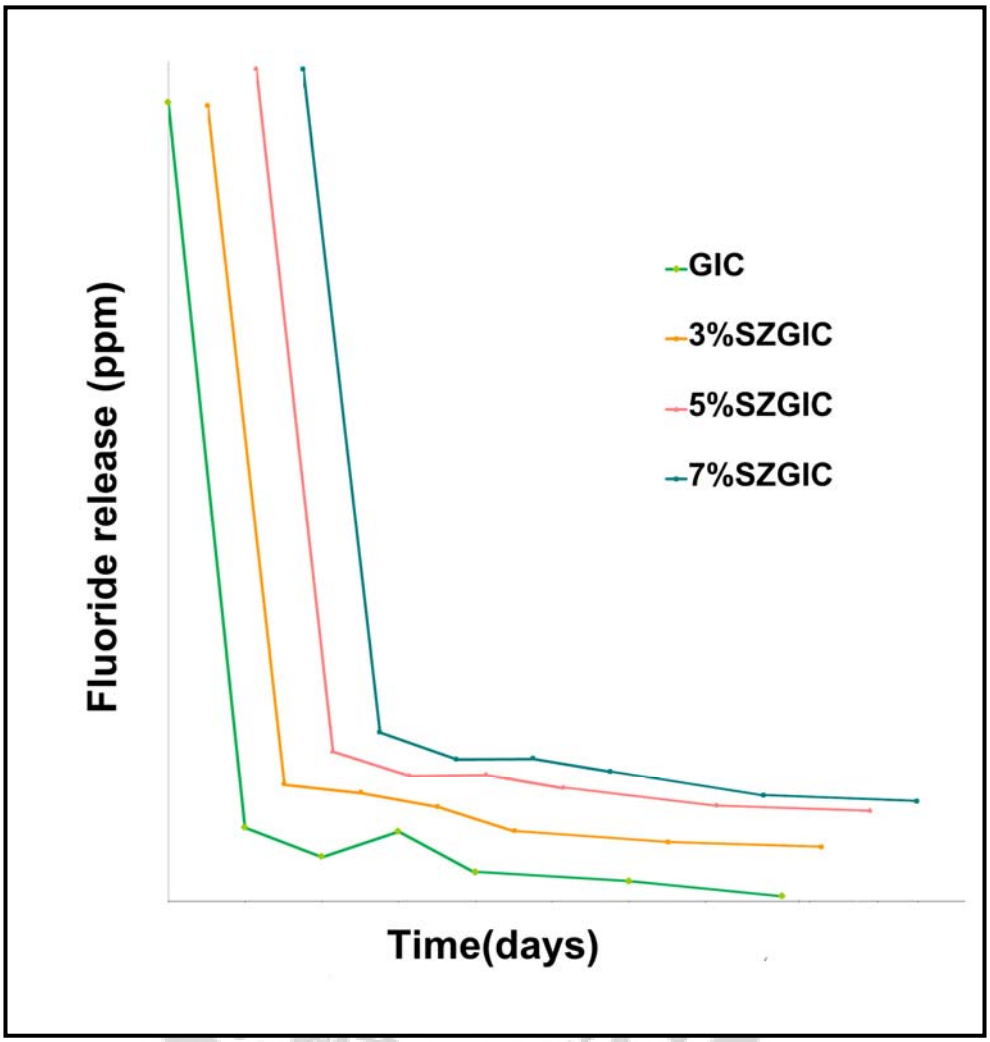

Figure 31 The graph shows the pattern of fluoride release in each group.

CHAPTER V
DISCUSSION

5.1 Antibacterial properties

Titanium dioxide, extract from pericarp of mangosteen and chitosan containing glass ionomer cement have no antibacterial effect against *S. mutans* in an agar diffusion test. From the literature review, it seems that only titanium dioxide has been added into glass ionomer cement and the study has demonstrated antibacterial action [12]. This disagrees with the current study. The difference is the use of direct contact test whereas, in our study, agar diffusion test was used. In addition, titanium dioxide is a photo-induced activities substance but in this study, agar diffusion test was done under a dark incubator. Both 100% chitosan and chitosan containing glass ionomer cement limit inhibition on *S. mutans.* The antibacterial properties of chitosan is pH dependent. Chitosan exhibits more inhibitory activity at lower pH and the inhibitory effect is diminished with increasing pH. While the pH of brain heart infusion agar is 7.2-7.6, the lack of inhibition zone may be due to decrease antibacterial activity of chitosan under neutral condition [18]. The absence of an inhibition effect may be also associated with the elution from the material and the binding, or perhaps synergism or antagonism between the antibacterial agents and glass ionomer cement [23].

The finding from this study is that the conventional glass ionomer cement alone did not show an inhibition zone. The reasons are firstly, the conventional glass ionomer cement from this study release fluoride approximately at 9.28 ppm during 48 hours which is closed to the results obtained by Mazzaoui et al [30]. The amounts of fluoride may be less effective to present antibacterial activity [64]. The effective one is 140±25 ppm [25]. Secondly, This experiment did not use freshly mixed glass ionomer cement. The conventional glass ionomer cement was not set on the bacterial plates, so low pH at level approximately 2-3 at gelation phase of glass ionomer cement while setting could not exhibit antibacterial activity [28]. Finally, this glass ionomer cement did not contain zinc oxide, which is a strong antiseptic and may inhibit metabolism or cause cell lysis of bacteria [24, 81].

This study demonstrates that SZ modified glass ionomer cement, 100% SZ, 100% extract from the pericarp of mangosteen and chlorhexidine as positive control have antibacterial activity against *S. mutans.* The 100% extract from pericarp of mangosteen has

shown antibacterial activity in agreement with Treesuwan et al. (2012) which reported an antibacterial effect of polyphenol from the pericarp of mangosteen extracts towards *S. mutans* [14]. However, extract from pericarp of mangosteen containing glass ionomer cement showed no antibacterial activity against *S. mutans*. This may be associated with inadequate mixing ratio of extract from pericarp of mangosteen. Otherwise, Fuji IX consists of high amounts of glasses, which turn into highly viscous form suddenly after mixing. The effect in viscosity may limit the release of extract from pericarp of mangosteen [64].

Silver has the highest antibacterial effect among the metal ions and has long been used in medicine to prevent and treat diseases. Zeolites presents a strong affinity for silver ion, acting as host for silver ion. SZ continuously releases a small amount of silver ion accumulating on the surface. Silver ion is released from SZ by an ionic exchange mechanism. The level of silver ion depends on the cations in the environment. SZ can sustain a long term release of silver ion with low ionic strength [74]. In this study, glass ionomer cements containing SZ have antibacterial effect against *S.mutans*. The exposure of SZ containing glass ionomer cement to a brain heart infusion agar that contains water may induce leaching of silver ion [55]. Silver ions have been proposed to have three antibacterial mechanisms: destruction of cell membrane, interference with DNA replication and formation of free radicals that induced membrane damage [73, 75]. Antibacterial effects were enhanced by increasing concentrations of SZ according to Lee et al. (2007) [77].

Silver ion presents not hazard to tissue [74]. However, there is a concern about the long term toxicity of silver. The cations may precipitate in oral epithelial cell and induce argyria [76]. In this study, the inhibition zone of glass ionomer cement containing SZ is at a nearby distance which may suppress bacteria around the border restoration and mostly sustain within the surface of material. Moreover, the quite low concentration (3%-7% SZGIC) and Vickers hardness is comparable to control. This may not be enough to affect the surrounding tissue. The agar diffusion test is a satisfactory method to primarily differentiate antibacterial property [27]. The test is inexpensive and can be achieved quickly and simply with a large amount of specimens [28]. The advantage of this test is that the inhibition of microbial growth could be direct identified [24]. However, There are some limitations. This procedure does not discriminate between bacteriostatic and bacteriocidal activities of materials, nor does it obtain any viability of test bacteria [24, 27]. Furthermore, the test does not reveal the clinical condition which many species of bacteria grow in

complicated biofilms [23]. Thus, further studies such as broth dilution method and toxicity related to the oral tissue are necessary to carry out to obtain further information.

5.2 Physical and mechanical Properties

The additions of SZ into glass ionomer cement present an aesthetically, satisfactory result. The color of the modified cement does not differ from that of the control group. These SZ containing cements are unlike mixture or sinter of silver particles to the glass (metal reinforced glass ionomer cement), which their color ranges from light to dark gray [47]. The silver powder is partially bonded to the glass [38], but the reinforced effect of silver addition to glass ionomer cement does not confirmed to be stronger than the conventional type because lacking of interfacial bonding. It is important for transfer of stress from the matrix to the reinforcement [47]. In this study, we found that incorporating 3% SZ insignificantly improved the mechanical properties of glass ionomer cement. SZ is composed of silver ions encased in a three-dimensional mineral of zeolite. The structure of zeolite is crystalline alumino-silicate, which is homologous to the powder of glass ionomer cement and may explain why the addition of 3% SZ does not jeopardise the mechanical properties of this glass ionomer cement.

Compressive, flexural strengths and microhardness of 3%SZGIC, 5%SZGIC and 7%SZGIC were comparable to that of conventional glass ionomer cement. The compressive strength of the control (Fuji IX^{TM}) was less than a previous result [82]. This could perhaps be due to stresses when specimens are removed from the stainless steel mold [57]. Surprisingly, 3%SZGIC has shown the highest compressive and flexural strengths. This may indicate that silver ion might not disrupt the homogeneity and crosslink of the final set cement. Although the compressive strength of 5%SZGIC was the lowest (100.22 MPa) and showed no significant difference compared with the highest (3%SZGIC: 115.10 MPa), further studies should be conducted with larger number of specimens. However, glass ionomer cement containing 7% SZ significantly decreased the flexural strength compared with 3%SZGIC. There may be insufficient polyacrylic acid to bind with the increased amount of SZ, cationic properties of silver may hamper the normal glass ionomer cement reaction or SZ probably represent as filler which had not been adhered into the matrix of glass ionomer cement [12, 55]. Furthermore, the alteration in the powder:liquid ratio by adding SZ may affect mechanical properties of glass ionomer cement [57, 83]. The powder:liquid ratio of glass ionomer cement used was lower than manufacturer's recommendation, diminishing

the mechanical properties [84]. Therefore a slight modification by adding SZ to the powder of glass ionomer cement may not jeopardize the basic mechanical properties of glass ionomer cement.

5.3 Fluoride release

The patterns of fluoride release for glass ionomer cement containing SZ were comparable to that of the control. The burst of fluoride release occurred during the first 24 hours, followed by a rapid drop on the next day then gradually decrease and continuous fluoride release. The rapid fall is compatible with previous reports on the highest solubility and the high early erosion of glass ionomer cement [24, 36]. The impaired amount of fluoride release of SZ containing glass ionomer as compared with control group may be due to the formation of silver fluoride, thus retaining the fluoride ions bound to the cement. However, this effect was insignificant [85]. This study may indicate that the incorporation of SZ does not impede fluoride release.

This is a preliminary study to investigate the antibacterial, mechanical properties and fluoride release of glass ionomer cement containing SZ. Data is limited and could not explain the exact structure of the modified glass ionomer cement. Spectroscopic techniques such as Fourier transformation infrared spectroscopy (FTIR) and Raman used to evaluate the exact chemical interaction between SZ and glass ionomer cement need to be performed [86]. Thus, further studies should be carried out to evaluate the structure of modified materials to clarify the antibacterial mechanism and mechanical properties and investigating their biocompatibility like cell culture, long term effect and clinical performance for the potential application in dentistry.

CHAPTER VI

CONCLUSIONS

6.1 SZ containing glass ionomer cement had homogeneous mixture and color compared to unmodified glass ionomer cement.

6.2 SZ containing glass ionomer cement, 100% SZ and the extract from the pericarp of mangosteen exhibit an antibacterial activity against *S. mutans*. The 3%SZGIC, 5%SZGIC and 7%SZGIC increased the inhibition zone according to increasing concentration of SZ incorporated into the glass ionomer cement.

6.3 Chemical and mechanical properties

6.3.1 Glass ionomer cement containing 3%, 5%, and 7% SZ exhibited no significant difference mechanical properties compared to the unmodified glass ionomer cement.

However, a decrease flexural strength was found for glass ionomer cement containing 7% SZ compared to 3% SZ.

6.3.2 The patterns of fluoride release from glass ionomer cement containing 3%, 5%, and 7% SZ are similar to unmodified glass ionomer cement. Although the cumulative amounts after 30 days for glass ionomer cement containing 3%, 5%, and 7% SZ were less than unmodified glass ionomer cement, there were no significant difference.

6.3.3 In this study, the incorporation of 3% (w/w) SZ into glass ionomer cement is an optimal concentration to provide the antibacterial property for glass ionomer cement and does not affect to the mechanical properties and fluoride releasing ability of glass ionomer cement.

6.4 The characterization, long term pharmacological testing and clinical effects of SZ containing glass ionomer cement should be investigated in future studies.

REFERENCES

REFERENCES

1. Syafiuddin T, Igarashi T, Shimomura H, Hisamitsu H, Goto N. Bacteriological and Mechanical Evaluation of Antibacterial Filler-Containing Composite Resins. J Showa Univ Dent Soc. 1993;13:443-9.
2. Kang YG, Kim JY, Kim J, Won PJ, Nam JH. Release of bisphenol A from resin composite used to bond orthodontic lingual retainers. Am J Orthod Dentofacial Orthop. 2011;140:779-89.
3. Syafiuddin T, Hisamitsu H, Toko T, Igarashi T, Goto N, Fujishima A, et al. In vitro inhibition of caries around a resin composite restoration containing antibacterial filler. Biomaterials. 1997;18:1051-7.
4. Wiegand A, Buchalla W, Attin T. Review on fluoride-releasing restorative materials--Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. Dent Mater. 2007;23:343-62.
5. Forss H, Seppä L. Prevention of enamel demineralization adjacent to glass ionomer filling materials. Scand J Dent Res. 1990;98:173-8.
6. Torii Y, Itota T, Okamoto M, Nakabo S, Nagamine M, Inoue K. Inhibition of artificial secondary caries in root by fluoride-releasing restorative materials. Oper Dent. 2001;26:36-43.
7. Pedrini D, Delbem AC, de França JG, Machado Tde M. Fluoride release by restorative materials before and after a topical application of fluoride gel. Pesqui Odontol Bras. 2003;17:137-41.
8. Creanor SL, Carruthers LMC, Saunders WP, Strang R, Foye RH. Fluoride Uptake and Release Characteristics of Glass Ionomer Cements. Caries Res. 1994;28:322-8.
9. Tam LE, Chan GP-L, Yim D. In vitro caries inhibiton effects by conventional and resin-modified glass-ionomer restorations. Oper Dent. 1997;22:4-14.
10. Mjor IA, Moorhead JE, Dahl JE. Reasons for replacement of restorations in permanent teeth in general dental practice. Int Dent J. 2000;50:361-6.
11. Casemiro LA, Gomes Martins CH, Pires-de-Souza Fde C, Panzeri H. Antimicrobial and mechanical properties of acrylic resins with incorporated silver-zinc zeolite - part I. Gerodontology. 2008;25:1-8.

12. Elsaka SE, Hamouda IM, Swain MV. Titanium dioxide nanoparticles addition to a conventional glass-ionomer restorative: Influence on physical and antibacterial properties. J Dent. 2011;39:589-98.
13. Sun J, Forster AM, Johnson PM, Eidelman N, Quinn G, Schumacher G, et al. Improving performance of dental resins by ading titanium dioxide nanoparticles. Dent Mater J. 2011;27:972-82.
14. Treesuwan P, Juntavee A, Rattanathongkom A, Peerapattana J, Nualkaew N, Chatrchaiwiwatana S. Inhibitory effects of polyphenol from mangosteen extracts on Streptococcus mutans in vitro. The 4th Annual Northeast Pharmacy Research Conference of 2012 “Pharmacy Profession in Harmony”; February 11 – 12, 2012; Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand2012.
15. Suksamrarn S, Suwannapoch N, Phakhodee W, Thanuhiranlert J, Ratananukul P, Chimnoi N, et al. Antimycobacterial Activity of Prenylated Xanthones from the Fruits of Garcinia mangostana. Chem Pharm Bull. 2003;51:857-9.
16. Torrungruang K, Vichienroj P, Chutimaworapan S. Antibacterial activity of mangosteen pericarp extract against cariogenic Streptococcus mutans. CU Dent J. 2007;30:1-10.
17. Petri DFS, Donega J, Benassi AM, Bocangel JAJS. Preliminary study on chitosan modified glass ionomer restoratives. Dent Mat. 2007;23:1004-10.
18. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: A state of the art review. Int J of Food Microbiol. 2010;144:51-63.
19. Saito S, Tosaki S, Hirota K. Characteristics of Glass-Ionomer Cements. In: Davidson CL, Mjör IA, editors. Advance in Glass-ionomer cements. Germany: Quintessence Publishing; 1999. p. 15-49.
20. Bowen RL, Marjenhoff WA. Dental Composites/Glass Ionomer: The Materials. Adv Dent Res. 1992;6:44-9.
21. Mount GJ. Glass ionomer cements and future research. Am J Dent. 1994;7:286-91.
22. Malone M, Bell C, Massler M. Physio-chemical characteristics of active and arrested carious lesion of dentin. J Dent Res. 1966;45:16-26.
23. Botelho MG. Inhibitory Effects on Selected Oral Bacteria of Antibacterial Agents Incorporated in a Glass Ionomer Cement. Caries Res. 2003;37:108-14.
24. Yap AUJ, Khor E, Foo SH. Fluoride release and antibacterial properties of new-generation tooth colored restoratives. Oper Dent. 1999;24:297-305.
25. Loyola-Rodriguez JP, Garcia-Godoy F, Lindquist R. Growth inhibition of glass ionomer

cements on mutans streptococci. Pediatr Dent J. 1994;16:346-49.

26. Friedl K-H, Schmalz G, Hiller K-A, Shams M. Resin-modified glass ionomer cements: fluoride release and influence on *Streptococcus mutans* growth. Eur J Oral Sci. 1997;105:81-5.
27. Meiers JC, Miller GA. Antibacterial activity of dentin bonding systems, resin modified glass ionomers and polyacid-modified composite resins. Oper Dent. 1996;21:257-64.
28. Vermeersch G, Leloup G, Delmee M, Vreven J. Antibacterial activity of glass-ionomer cements, compomers and resin composites: relationship between acidity and material setting phase. J Oral Rehabil. 2005;32:368-74.
29. Chen L, Shen H, Suh BI. Antibactirial dental restorative material: A state-of-the-art review. Am J Dent. 2012;25:337-46.
30. Mazzaoui SA, Burrow MF, Tyas MJ. Fluoride release from glass ionomer cements and resin composites coated with a dentin adhesive. Dent Mater. 2000;16:166-71.
31. Bayne SC. Dental Biomaterials: Where Are We and Where Are We Going? J Dent Educ. 2005;69:571-85.
32. Wilson AD. Glass-Ionomer Cement--Origins, Development and Future. Clin Mater. 1991;7:275-82.
33. Smith DC. Development of glass-ionomer cement systems. Biomaterials. 1998;19:467-78.
34. Attar N, Onen A. Fluoride release and uptake characteristics of aesthetic restorative materials. J Oral Rehabil. 2002;29:791-8.
35. Walls AWG. Glass polyalkenoate (glass-ionomer) cements: a review. J Dent. 1986;14:231-46.
36. Hewlett ER, Mount GJ. Glass Ionomers in Contemporary Restorative Dentistry--A Clinical Update. J Calif Dent Assoc. 2003;31:483-92.
37. Mount GJ. Adhesion of Glass-ionomer cement in the clinical environment. Oper Dent. 1991;16:141-8.
38. Wilson AD, McLean JW. Glass Ionomer Cement. Composition. West Germany: Quintessence Publishing; 1988. p. 21-42.
39. Attar N, Tam LE, McComb D. Mechanical and physical properties of contemporary dental luting agents. J Prosthet Dent. 2003;89:127-34.
40. Li ZC, White SN. Mechanical properties of dental luting cements. J Prosthet Dent. 1999;81:597-609.

41. Verdonschot EH, Oortwijn JC, Roeters FJ. Aesthetic properties of three Type II glass polyalkenoate (ionomer) cements. J Dent. 1991;19:357-61.
42. Villarroel M, Fahl N, Sousa AMD, Oliveira OBD. Direct Esthetic Restorations Based on Translucency and Opacity of Composite Resins. J Esthet Restor Dent. 2011;23:73-88.
43. Weiner R. Liners and bases in general dentistry. Aus Dent J. 2011;56:11-22.
44. Khoroushi M, Keshani F. A review of glass ionomers: From conventional glass-ionomer to bioactive glass-ionomer. Dent Res J. 2013;10:411-20.
45. McLean JW, Wilson AD, Prosser HJ. Development and use of water-hardening glass-ionomer luting cements. J Prosthet Dent. 1984;52:175-81.
46. Nagaraja Upadhya P, Kishore G. Glass Ionomer Cement – The Different Generations. Trends Biomater Artif Organs. 2005;18 158-65.
47. Yap AUJ, Cheang YSPP. Physico-mechanical properties of a fast-set highly viscous GIC restoration. J Oral Rehabil. 2003;30:1-8.
48. Carvalho T-S, Amerongen W-Ev, Gee Ad, Bonecker M, Sampaio F-C. Shear bond strengths of three glass ionomer cements to enamel and dentine. Med Oral Patol Oral Cir Bucal. 2011;16:406-10.
49. Tathe A, Ghodke M, Nikalje AP. A brief review: biomaterials and their application. Int J of Pha And Pha Sci. 2010;2:19-23.
50. McCabe JF, Yan Z, Naimi OT, Mahmoud G, Rolland SL. Smart materials in dentistry - future prospects. Dent Mater J. 2009;28:38-43.
51. Moshaverinia A, Ansari S, Moshaverinia M, Roohpour N, Darr JA, Rehman I. Effects of incorporation of hydroxyapatite and fluoroapatite nanobioceramics into conventional glass ionomer cements (GIC). Acta Biomat. 2008;4:432-40.
52. Lucas ME, Arita K, Nishino M. Toughness, bonding and fluoride-release properties of hydroxyapatite-added glass ionomer cement. Biomaterials. 2003;24:3787-94.
53. Arita K, Lucas ME, Nishino M. The Effect of Adding Hydroxyapatite on the Flexural Strength of Glass Ionomer cement. Dent Mater J. 2003;22:126-36.
54. Yli-Urpo H, Narhi T, Soderling E. Antimicrobial effects of glass ionomer cements containing bioactive glass (S53P4) on oral micro-organisms in vitro. Acta Odontol Scand. 2003;61:241-6.
55. Yli-Urpo H, Lassila LVJ, Närhi T, Vallittu PK. Compressive strength and surface characterization of glass ionomer cements modified by particles of bioactive glass.

Dent Mater J. 2005;21:201-9.

56. Farooq I, Moheet IA, Imran Z, Farooq U. A review of novel dental caries preventive material: Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complex. King Saud Univ J of Dental Sci. 2013;4:47-51.
57. Zraikat HA, Palamara JEA, Messer HH, Burrow MF. The incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass ionomer cement. Dent Mater. 2011;27:235-43.
58. Mazzaoui SA, Burrow MF, Tyas MJ, Dashper SG, Eakins D, Reynolds EC. Incorporation of Casein Phosphopeptide-Amorphous Calcium Phosphate into a Glass-ionomer Cement. J Dent Res. 2003;82:914-8.
59. Prentice LH, Tyas MJ, Burrow MF. The effect of ytterbium fluoride and barium sulphate nanoparticles on the reactivity and strength of a glass-ionomer cement. Dent Mater. 2006;22:746-51.
60. Pinheiro SL, Simionato MRL, Imparato JCP, Oda M. Antibacterial activity of glass-ionomer cement containing antibiotics on caries lesion microorganisms. Oper Dent. 2005;18:261-6.
61. Yesilyurt C, Er K, Tasdemir T, Buruk K, Celik D. Antibacterial Activity and Physical Properties of Glass-ionomer Cements Containing Antibiotics. Oper Dent. 2009;34:18-23.
62. Mohammadi Z, Abbott PV. The properties and application of chlorhexidine in endodontics. Int Endod J. 2009;42:288-302.
63. Ankola AV, Hebbal M, Mocherla M. A review of efficacy of various modes of chlorhexidine delivery. J Oral Biosci. 2008;50:239-42.
64. Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical Properties of glass-ionomer cements containing chlorhexidine for the ART approach. Dent Mater. 2006;22:647-52.
65. Palmer G, Jones FH, Billington RW, Pearson GJ. Chlorhexidine release from an experimental glass ionomer cement. Biomaterials. 2004;25:5423-31.
66. Zhang R, Gao L. Effect of peptization on phasetransformation of TiO_2 nanoparticles. Material Research Bulletin. 2001;36:1957-65.
67. Mills A, LeHunte S. An overview of semiconductor photocatalysis. J Photochem Photobio A. 1997;108:1-35.

68. Xia Y, Zhang F, Xei H, Gu N. Nanoparticle-reinforced resin-based dental composite. J Dent. 2008;36:450-55.

69. Chávez de Paz LE, Resin A, Howard KA, Sutherland DS, Wejse PL. Antimicrobial Effect of Chitosan Nanoparticles on Streptococcus mutans Biofilms. Appl Environ Microbiol. 2011;77:3892-5.

70. Costa EM, Silva S, Tavaria FK, Pintado MM. Study of the effects of chitosan upon Streptococcus mutans adherence and biofilm formation. Anaerobe. 2013;5:1-5.

71. Elsaka S, Elnaghy A. Effect of addition of chitosan to self-etching primer: antibacterial activity and push-out bond strength to radicular dentin. J Biomed Res. 2012;26:288-94.

72. Rassameemasmaung S, Sirikulsathean A, Amornchat C, Maungmingsook P, Rojanapanthu P, Gritsanaphan W. Topical application of Garcinia mangostana L. pericarp gel as an adjunct to periodontal treatment. Complement Ther Med. 2008;16:262-7.

73. Saengmee-anupharb S, Srikhirin T, Thaweboon B, Thaweboon S, Amornsakchai T, Dechkunakorn S, et al. Antimicrobial effects of silver zeolite, silver zirconium phosphate silicate and silver zirconium phosphate against oral microorganisms. Asian Pac J Trop Biomed. 2013;3:47-52.

74. Kawahara K, Tsuruda K, Morishita M, Uchida M. Antibacterial effect of silver-zeolite on oral bacteria under anaerobic conditions. Dent Mater. 2000;16:452-5.

75. Peng JJ-Y, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: A review. J Dent. 2012;40:531-41.

76. Matsuura T, Abe Y, Sato Y, Okamoto K, Ueshige M, Akagawa Y. Prolonged antimicrobial effect of tissue conditioners containing silver-zeolite. J Dent. 1997;25:373-7.

77. Lee J-H, Lee S-B, Kim K-N, Kim K-M, Lee Y-K. Antibacterial effect of silver-zeolites in glass-ionomer cements. Key Eng Mat. 2007;330-332:831-4.

78. ISO. ISO 9917-1: dentistry-water-based cements--part 1: powder/liquid acid-base cements. Geneva, Switzerland: International Organization for Standardization; 2007.

79. ISO. ISO 9917-2: dentistry-water-based cements--part 2: resin-modified cements. Geneva, Switzerland: International Organization for Standardization; 2010.

80. Palma-Dibb RG, Palma AE, Matson E, Chinelatti MA, Ramos RP. Microhardness of Esthetic Restorative Materials at Different Depths. Mat Res. 2002;6:85-90.

81. Boeckh C, Schumacher E, Podbielski A, Haller B. Antibacterial activity of restorative dental biomaterials in vitro. Caries Res. 2002;36:101-7.

82. Peez R, Frank S. The physical-mechanical performance of the new Ketac Molar Easymix compared to commercially available glass ionomer restoratives. J Dent. 2006;34:582-7.

83. Crisp S, Lewis BG, Wilson AD. Characterization of glass-ionomer cements 2. Effect of the powder : liquid ratio on the physical properties. J Dent. 1976;4:287-90.

84. Rosenstiel S, Land M, Crispin B. Dental luting agents: a review of the current literature. J Prosthet Dent. 1998;80:280-301.

85. Mallakh BFE, Sarkar NK. Fluoride release from glass-ionomer cements in de-ionized water and artificial saliva. Dent Mater. 1990;6:118-22.

86. Young AM. FTIR investigation of polymerisation and polyacid neutralisation kinetics in resin-modified glass-ionomer dental cements. Biomaterials. 2002;23:3289-95.

APPENDIX

APPENDIX A

7.1 Antibacterial properties

Table 10 The data from agar diffusion test of glass ionomer cement and titanium dioxide containing glass ionomer cement reported as diameter of inhibition zone at 2 different points. All specimens showed no significant difference of inhibition zone between 24 and 48 hours.

Plate		Diameter (mm)					
		GIC	3%TiO_2	5% TiO_2	7% TiO_2	100% TiO_2	CHX
1	X1	0.00	0.00	0.00	0.00	0.00	12.36
	X2	0.00	0.00	0.00	0.00	0.00	10.92
2	X1	0.00	0.00	0.00	0.00	0.00	15.78
	X2	0.00	0.00	0.00	0.00	0.00	14.42
3	X1	0.00	0.00	0.00	0.00	0.00	15.87
	X2	0.00	0.00	0.00	0.00	0.00	12.57
4	X1	0.00	0.00	0.00	0.00	0.00	19.56
	X2	0.00	0.00	0.00	0.00	0.00	20.60
5	X1	0.00	0.00	0.00	0.00	0.00	13.64
	X2	0.00	0.00	0.00	0.00	0.00	11.92
6	X1	0.00	0.00	0.00	0.00	0.00	12.17
	X2	0.00	0.00	0.00	0.00	0.00	11.63
7	X1	0.00	0.00	0.00	0.00	0.00	13.47
	X2	0.00	0.00	0.00	0.00	0.00	15.89
8	X1	0.00	0.00	0.00	0.00	0.00	16.27
	X2	0.00	0.00	0.00	0.00	0.00	13.77
9	X1	0.00	0.00	0.00	0.00	0.00	11.93
	X2	0.00	0.00	0.00	0.00	0.00	12.39
10	X1	0.00	0.00	0.00	0.00	0.00	13.64
	X2	0.00	0.00	0.00	0.00	0.00	11.92
Mean±SD		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	19.04±0.79

Table 11 The data from agar diffusion test of glass ionomer cement and extract from pericarp of mangosteen containing glass ionomer cement reported as diameter of inhibition zone at 2 different points. All specimens showed no significant difference of inhibition zone between 24 and 48 hours.

Plate		Diameter (mm)					
		GIC	3%Xan	5%Xan	7%Xan	100%Xan	CHX
1	X1	0.00	0.00	0.00	0.00	2.71	13.82
	X2	0.00	0.00	0.00	0.00	2.33	11.98
2	X1	0.00	0.00	0.00	0.00	2.34	12.15
	X2	0.00	0.00	0.00	0.00	2.82	9.53
3	X1	0.00	0.00	0.00	0.00	1.98	12.68
	X2	0.00	0.00	0.00	0.00	2.34	10.48
4	X1	0.00	0.00	0.00	0.00	1.84	12.92
	X2	0.00	0.00	0.00	0.00	0.88	13.76
5	X1	0.00	0.00	0.00	0.00	0.96	13.57
	X2	0.00	0.00	0.00	0.00	1.48	10.27
6	X1	0.00	0.00	0.00	0.00	2.18	11.21
	X2	0.00	0.00	0.00	0.00	2.94	8.55
7	X1	0.00	0.00	0.00	0.00	1.24	14.16
	X2	0.00	0.00	0.00	0.00	1.92	8.20
8	X1	0.00	0.00	0.00	0.00	1.65	13.87
	X2	0.00	0.00	0.00	0.00	1.83	9.85
9	X1	0.00	0.00	0.00	0.00	2.46	14.19
	X2	0.00	0.00	0.00	0.00	3.18	13.13
10	X1	0.00	0.00	0.00	0.00	2.07	16.38
	X2	0.00	0.00	0.00	0.00	1.65	14.10
Mean±SD		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.04±0.18	12.24±0.49

Table 12 The data from agar diffusion test of glass ionomer cement and chitosan containing glass ionomer cement reported as diameter of inhibition zone at 2 different points. All specimens showed no significant difference of inhibition zone between 24 and 48 hours.

Plate		Diameter (mm)					
		GIC	3%Chi	5% Chi	7% Chi	100% Chi	CHX
1	X1	0.00	0.00	0.00	0.00	0.00	13.17
	X2	0.00	0.00	0.00	0.00	0.00	12.55
2	X1	0.00	0.00	0.00	0.00	0.00	16.72
	X2	0.00	0.00	0.00	0.00	0.00	14.28
3	X1	0.00	0.00	0.00	0.00	0.00	11.82
	X2	0.00	0.00	0.00	0.00	0.00	11.66
4	X1	0.00	0.00	0.00	0.00	0.00	14.72
	X2	0.00	0.00	0.00	0.00	0.00	16.52
5	X1	0.00	0.00	0.00	0.00	0.00	17.38
	X2	0.00	0.00	0.00	0.00	0.00	15.26
6	X1	0.00	0.00	0.00	0.00	0.00	13.85
	X2	0.00	0.00	0.00	0.00	0.00	17.43
7	X1	0.00	0.00	0.00	0.00	0.00	12.15
	X2	0.00	0.00	0.00	0.00	0.00	11.69
8	X1	0.00	0.00	0.00	0.00	0.00	11.87
	X2	0.00	0.00	0.00	0.00	0.00	13.37
9	X1	0.00	0.00	0.00	0.00	0.00	14.11
	X2	0.00	0.00	0.00	0.00	0.00	10.97
10	X1	0.00	0.00	0.00	0.00	0.00	12.68
	X2	0.00	0.00	0.00	0.00	0.00	14.00
Mean±SD		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	13.81±0.56

Table 13 The data from agar diffusion test of glass ionomer cement and SZ containing glass ionomer cement reported as diameter of inhibition zone at 2 different points. All specimens showed no significant difference of inhibition zone between 24 and 48 hours.

Plate		Diameter (mm)					
		GIC	3%SZGIC	5%SZGIC	7%SZGIC	100%SZ	CHX
1	X1	0.00	3.10	2.43	2.74	7.76	21.46
	X2	0.00	3.46	2.43	2.68	7.30	20.05
2	X1	0.00	3.14	3.47	3.72	6.26	16.79
	X2	0.00	2.86	3.09	3.71	7.50	14.24
3	X1	0.00	3.12	3.40	2.63	6.51	15.49
	X2	0.00	3.13	3.17	3.50	5.99	16.05
4	X1	0.00	2.33	2.31	1.72	6.80	14.71
	X2	0.00	2.53	1.79	2.36	6.54	17.27
5	X1	0.00	3.70	3.31	4.00	7.25	22.30
	X2	0.00	2.89	3.08	3.38	7.03	14.92
6	X1	0.00	1.89	2.27	3.40	5.84	18.36
	X2	0.00	2.17	2.72	2.80	5.68	15.14
7	X1	0.00	1.90	2.08	2.36	5.31	16.86
	X2	0.00	2.13	1.92	2.30	5.61	16.01
8	X1	0.00	2.13	2.31	2.46	6.22	16.50
	X2	0.00	2.09	2.84	2.46	7.64	17.69
9	X1	0.00	2.11	2.20	2.75	6.18	14.35
	X2	0.00	2.03	1.91	3.26	5.95	15.34
10	X1	0.00	2.57	3.39	2.82	6.60	20.99
	X2	0.00	2.69	3.00	2.93	6.84	11.29
Mean±SD		0.00±0.00	2.60±0.54	2.66±0.54	2.90±0.54	6.54±0.64	16.59±1.27

7.2 Mechanical properties

7.2.1 Compressive strength

Table 14 The data from compressive strength test of glass ionomer cement by using the universal testing machine (LLOYD Instrument Ltd., UK).

Number of specimen	Diameter (mm)	Height (mm)	Maximum force at failure (N)	Compressive strength (MPa)
1	4.00	6.00	1128.00	89.80
2	3.95	6.20	1378.00	112.00
3	3.93	6.21	1385.00	114.00
4	3.91	6.20	1496.00	125.00
5	3.88	6.28	1264.00	107.00
6	3.93	6.33	1590.00	131.00
7	3.96	6.40	1088.00	88.30
8	3.89	6.28	1072.00	90.50
9	4.01	6.33	1431.00	114.00
10	3.98	6.33	1111.00	89.30
Mean±SD				106.09±15.80

Table 15 The data from compressive strength test of glass ionomer cement containing 3% SZ by using the universal testing machine (LLOYD Instrument Ltd., UK).

Number of specimen	Diameter (mm)	Height (mm)	Maximum force at failure (N)	Compressive strength (MPa)
1	3.85	6.26	1503.00	129.00
2	3.98	6.27	1298.00	105.00
3	3.97	6.27	1304.00	106.00
4	3.99	6.30	1595.00	128.00
5	3.99	6.36	1449.00	116.00
6	3.85	6.29	1264.00	109.00
7	3.96	6.33	1447.00	118.00
8	4.05	6.33	1408.00	110.00
9	3.96	6.32	1479.00	120.00
10	3.95	6.46	1342.00	110.00
Mean±SD				115.10±8.61

Table 16 The data from compressive strength test of glass ionomer cement containing 5% SZ by using the universal testing machine (LLOYD Instrument Ltd., UK).

Number of specimen	Diameter (mm)	Height (mm)	Maximum force at failure (N)	Compressive strength (MPa)
1	4.01	6.27	1210.00	95.80
2	4.03	6.28	1196.00	93.80
3	3.99	6.31	1542.00	123.00
4	3.99	6.34	974.20	77.70
5	3.99	6.44	1199.00	95.60
6	3.95	6.37	1201.00	98.20
7	3.91	6.37	976.20	81.30
8	3.96	6.30	1528.00	124.00
9	4.01	6.28	1456.00	115.00
10	4.01	6.40	1236.00	97.80
Mean±SD				100.22±15.85

Table 17 The data from compressive strength test of glass ionomer cement containing 7% SZ by using the universal testing machine (LLOYD Instrument Ltd., UK).

Number of specimen	Diameter (mm)	Height (mm)	Maximum force at failure (N)	Compressive strength (MPa)
1	3.96	6.33	1346.00	109.00
2	3.98	6.29	1392.00	112.00
3	4.04	6.36	1495.00	117.00
4	4.03	6.30	1572.00	123.00
5	4.06	6.35	1623.00	123.00
6	3.93	6.44	1356.00	112.00
7	4.00	6.34	1450.20	115.00
8	3.99	6.46	1288.00	103.00
9	4.06	6.46	1360.00	105.00
10	4.00	6.41	1294.00	103.00
Mean±SD				112.20±7.42

7.2.2 Flexural strength

Table 18 The data from flexural strength test of glass ionomer cement by using three-point bending mode of a universal testing machine (Shimadzu Co., Ltd., Japan).

Number of specimen	Width (mm)	Height (mm)	Maximum force at failure (N)	Flexural strength (MPa)
1	2.52	2.17	6.35	16.05
2	2.50	2.32	6.57	14.64
3	2.56	2.23	3.91	9.20
4	2.52	2.33	5.46	11.97
5	2.45	2.37	5.62	12.25
6	2.47	2.38	5.56	11.93
7	2.45	2.38	5.92	12.79
8	2.49	2.29	3.91	8.99
9	2.53	2.32	4.49	9.90
10	2.49	2.35	4.08	8.90
Mean±SD				11.66±2.45

Table 19 The data from flexural strength test of glass ionomer cement containing 3% SZ by using three-point bending mode of a universal testing machine (Shimadzu Co., Ltd., Japan).

Number of specimen	Width (mm)	Height (mm)	Maximum force at failure (N)	Flexural strength (MPa)
1	2.41	2.23	3.82	9.57
2	2.54	2.27	6.71	15.37
3	2.54	2.22	6.69	16.03
4	2.61	2.23	5.62	12.99
5	2.51	2.18	6.30	15.84
6	2.52	2.28	5.96	13.64
7	2.50	2.41	6.43	13.28
8	2.51	2.30	6.03	13.61
9	2.48	2.23	7.51	18.26
10	2.47	2.24	7.44	18.00
Mean±SD				14.66±2.60

Table 20 The data from flexural strength test of glass ionomer cement containing 5% SZ by using three-point bending mode of a universal testing machine (Shimadzu Co., Ltd., Japan).

Number of specimen	Width (mm)	Height (mm)	Maximum force at failure (N)	Flexural strength (MPa)
1	2.40	2.15	5.69	15.39
2	2.55	2.10	5.98	15.96
3	2.42	2.28	4.84	11.53
4	2.46	2.30	5.15	11.87
5	2.49	2.18	4.71	11.95
6	2.61	2.41	3.80	7.53
7	2.57	2.30	6.11	13.48
8	2.51	2.22	3.38	8.20
9	2.49	2.29	5.59	12.84
10	2.50	2.41	4.28	8.83
Mean±SD				11.76±2.87

Table 21 The data from flexural strength test of glass ionomer cement containing 7% SZ by using three-point bending mode of a universal testing machine (Shimadzu Co., Ltd., Japan).

Number of specimen	Width (mm)	Height (mm)	Maximum force at failure (N)	Flexural strength (MPa)
1	2.52	2.33	3.96	8.69
2	2.46	2.42	4.37	9.11
3	2.44	2.24	4.28	10.49
4	2.42	2.32	5.59	12.86
5	2.54	2.22	3.82	9.14
6	2.48	2.24	3.71	8.93
7	2.51	2.35	4.51	9.77
8	2.54	2.36	4.76	10.08
9	2.49	2.27	6.22	14.53
10	2.61	2.31	6.06	13.05
Mean±SD				10.67±2.06

7.2.3 Surface microhardness

Table 22 The data from surface microhardness test of glass ionomer cement (5 indentations per specimen) by using microhardness tester (Type D, Future tech corp., Japan).

Number of specimen	Indentations					Average (VHN)
	1	2	3	4	5	
1	73.00	58.90	68.70	73.30	67.30	68.24
2	68.40	69.40	70.30	70.50	64.90	68.70
3	71.30	73.00	78.00	62.10	68.60	70.60
4	62.60	69.90	61.70	74.70	69.20	67.62
5	70.90	69.80	61.30	68.50	66.90	67.48
6	76.40	71.70	70.10	75.20	75.30	73.74
7	51.90	65.80	70.40	67.60	67.30	64.60
8	68.10	66.90	70.10	66.70	65.10	67.38
9	73.10	70.20	69.00	66.80	68.10	69.44
10	73.40	66.10	62.10	63.60	62.90	65.62
Mean±SD						68.34±2.56

Table 23 The data from surface microhardness test of glass ionomer cement containing 3% SZ (5 indentations per specimen) by using microhardness tester (Type D, Future tech corp., Japan).

Number of specimen	Indentations					Average (VHN)
	1	2	3	4	5	
1	72.70	71.50	69.50	68.20	69.00	70.18
2	66.40	62.00	73.00	69.70	68.20	67.86
3	80.80	65.30	65.50	70.50	70.30	70.48
4	72.70	65.70	71.90	76.80	63.60	70.14
5	73.50	66.30	70.90	72.50	72.10	71.06
6	57.00	70.70	70.60	71.40	70.90	68.12
7	61.10	68.90	73.00	62.20	61.70	65.38
8	75.70	75.70	74.00	73.00	69.90	73.66
9	71.50	67.50	70.30	68.40	63.90	68.32
10	61.70	64.80	66.30	66.80	70.60	66.04
Mean±SD						69.12±2.47

Table 24 The data from surface microhardness test of glass ionomer cement containing 5% SZ (5 indentations per specimen) by using microhardnesss tester (Type D, Future tech corp., Japan).

Number of specimen	Indentations					Average (VHN)
	1	2	3	4	5	
1	66.10	62.80	57.00	77.60	75.40	67.78
2	68.70	64.20	67.50	72.50	79.60	70.50
3	68.90	73.50	74.00	69.40	77.20	72.60
4	75.40	78.50	77.10	75.20	79.60	77.16
5	74.60	67.70	78.20	67.50	76.30	72.86
6	65.10	63.30	66.60	70.80	57.70	64.70
7	74.50	72.70	68.50	63.20	66.90	69.16
8	74.70	69.10	68.50	74.70	64.10	70.22
9	66.30	69.20	62.20	67.50	72.90	67.62
10	62.70	62.80	66.90	56.50	69.60	63.70
Mean±SD						69.63±4.00

Table 25 The data from surface microhardness test of glass ionomer cement containing 7% SZ (5 indentations per specimen) by using microhardnesss tester (Type D, Future tech corp., Japan).

Number of specimen	Indentations					Average (VHN)
	1	2	3	4	5	
1	75.00	71.20	60.80	70.60	77.30	70.98
2	81.90	79.60	60.90	75.40	60.40	71.64
3	71.30	67.30	63.30	60.60	67.40	65.98
4	72.60	69.70	77.40	61.70	67.10	69.70
5	75.20	68.70	56.50	72.30	69.60	68.46
6	82.30	75.20	79.90	72.90	80.10	78.08
7	64.80	56.50	56.50	66.40	67.10	62.26
8	65.70	73.60	68.30	62.80	65.00	67.08
9	70.20	70.10	74.10	60.50	62.90	67.56
10	69.80	70.20	67.50	68.30	63.30	67.82
Mean±SD						68.96±4.16

7.3 Fluoride release

Table 26 The data from fluoride release test of glass ionomer cement by using calibrated fluoride ion-specific electrode (Fisher Scientific Ltd., USA).

Number of specimen	Fluoride release test (ppm)						
	Day1	Day2	Day3	Day4	Day5	Day15	Day30
1	10.000	0.700	0.400	1.000	0.400	0.400	0.100
2	9.000	1.000	0.500	1.000	0.500	0.300	0.100
3	6.000	0.800	0.300	0.500	0.200	0.100	0.030
4	10.000	1.000	0.500	0.600	0.400	0.300	0.070
5	7.000	0.600	0.500	1.000	0.300	0.200	0.040
6	10.000	0.600	0.600	0.700	0.300	0.200	0.050
7	8.000	0.800	0.600	0.500	0.200	0.100	0.020
8	9.000	0.800	0.500	0.900	0.300	0.200	0.040
9	9.000	0.800	0.400	0.800	0.300	0.200	0.030
10	7.000	0.700	0.400	0.400	0.100	0.070	0.020
Mean±SD	8.50±1.43	0.78±0.17	0.47±0.09	0.74±0.23	0.30±0.12	0.21±0.10	0.05±0.03

Table 27 The data from fluoride release test of glass ionomer cement containing 3% SZ by using calibrated fluoride ion-specific electrode (Fisher Scientific Ltd., USA).

Number of specimen	Fluoride release test (ppm)						
	Day1	Day2	Day3	Day4	Day5	Day15	Day30
1	10.000	0.500	0.300	0.500	0.300	0.040	0.010
2	8.000	0.800	0.400	0.600	0.200	0.030	0.008
3	7.000	0.600	0.500	0.400	0.200	0.100	0.006
4	7.000	0.500	0.300	0.600	0.100	0.040	0.007
5	8.000	0.600	0.300	0.500	0.100	0.040	0.005
6	8.000	0.700	0.400	0.400	0.200	0.100	0.010
7	9.000	0.800	0.500	0.300	0.100	0.050	0.005
8	8.000	1.000	0.400	0.300	0.100	0.060	0.004
9	7.000	0.500	0.300	0.400	0.200	0.080	0.007
10	7.000	0.800	0.500	0.400	0.300	0.070	0.005
Mean±SD	7.90±0.99	0.68±0.15	0.39±0.09	0.44±0.11	0.18±0.08	0.06±0.03	0.006±0.002

Table 28 The data from fluoride release test of glass ionomer cement containing 5% SZ by using calibrated fluoride ion-specific electrode (Fisher Scientific Ltd., USA).

Number of specimen	Fluoride release test (ppm)						
	Day1	Day2	Day3	Day4	Day5	Day15	Day30
1	8.000	0.800	0.400	0.400	0.100	0.060	0.006
2	10.000	0.700	0.400	0.300	0.200	0.090	0.007
3	10.000	0.500	0.300	0.300	0.500	0.040	0.004
4	10.000	0.700	0.400	0.500	0.400	0.060	0.006
5	10.000	0.700	0.300	0.500	0.200	0.070	0.007
6	9.000	0.800	0.500	0.300	0.200	0.100	0.008
7	6.000	0.500	0.300	0.500	0.300	0.030	0.003
8	6.000	0.500	0.400	0.400	0.300	0.040	0.003
9	5.000	0.800	0.300	0.300	0.200	0.070	0.004
10	5.000	0.400	0.500	0.400	0.080	0.030	0.003
Mean±SD	7.90±2.18	0.64±0.13	0.38±0.08	0.39±0.09	0.25±0.13	0.06±0.02	0.005±0.002

Table 29 The data from fluoride release test of glass ionomer cement containing 7% SZ by using calibrated fluoride ion-specific electrode (Fisher Scientific Ltd., USA).

Number of specimen	Fluoride release test (ppm)						
	Day1	Day2	Day3	Day4	Day5	Day15	Day30
1	7.000	0.600	0.400	0.500	0.200	0.050	0.007
2	6.000	0.700	0.300	0.600	0.500	0.070	0.005
3	7.000	0.800	0.300	0.400	0.200	0.100	0.006
4	8.000	0.800	0.400	0.700	0.300	0.080	0.008
5	7.000	0.600	0.400	0.400	0.200	0.090	0.009
6	10.000	0.600	0.500	0.500	0.400	0.100	0.010
7	8.000	0.900	0.600	0.300	0.400	0.060	0.010
8	9.000	0.900	0.600	0.300	0.200	0.050	0.009
9	8.000	0.900	0.500	0.500	0.400	0.060	0.010
10	8.000	0.700	0.600	0.500	0.500	0.070	0.007
Mean±SD	7.80±1.14	0.75±0.13	0.46±0.12	0.47±0.13	0.33±0.13	0.07±0.02	0.008±0.002

APPENDIX B

8.1 Antibacterial properties

Table 30 The data from agar diffusion test was analyzed by Kolmogorov-Smirnov to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level was more than 0.05.

Tests of Normality[a]

Group		Kolmogorov-Smirnov[b]		
		Statistic	df	Sig.
Antibac	3%	0.219	10	0.192
	5%	0.242	10	0.100
	7%	0.157	10	0.200*
	SZ	0.179	10	0.200*
	CHX	0.151	10	0.200*

Table 31 The data from agar diffusion test was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level was less than 0.05.

Test of Homogeneity of Variances

Antibac

Levene Statistic	df1	df2	Sig.
6.533	5	54	0.000

Table 32 The data from agar diffusion test was analyzed by One-way ANOVA to compare means among different 6 groups. The data was statistically significant difference at the confidence level of 95%.

ANOVA

Antibac

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1771.393	5	354.279	731.973	0.000
Within Groups	26.136	54	0.484		
Total	1797.530	59			

Table 33 The data from agar diffusion test was analyzed by Dunnett T3 to perform all pairwise comparison between 2 different groups. The data was statistically significant difference when the significant level was less than 0.05.

Multiple Comparisons

Dependent Variable: Antibac

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett T3	GI	3%	-2.60000*	0.17014	0.000	-3.2415	-1.9585
		5%	-2.65900*	0.17053	0.000	-3.3019	-2.0161
		7%	-2.90100*	0.17231	0.000	-3.5507	-2.2513
		SZ	-6.54100*	0.20377	0.000	-7.3093	-5.7727
		CHX	-16.59300*	0.40145	0.000	-18.1066	-15.0794
	3%	GI	2.60000*	0.17014	0.000	1.9585	3.2415
		5%	-0.05900	0.24089	1.000	-0.8597	0.7417
		7%	-0.30100	0.24215	0.956	-1.1059	0.5039
		SZ	-3.94100*	0.26546	0.000	-4.8269	-3.0551
		CHX	-13.99300*	0.43602	0.000	-15.5335	-12.4525

	5%	GI	2.65900*	0.17053	0.000	2.0161	3.3019
		3%	0.05900	0.24089	1.000	-0.7417	0.8597
		7%	-0.24200	0.24242	0.993	-1.0478	0.5638
		SZ	-3.88200*	0.26571	0.000	-4.7686	-2.9954
		CHX	-13.93400*	0.43617	0.000	-15.4747	-12.3933
	7%	GI	2.90100*	0.17231	0.000	2.2513	3.5507
		3%	0.30100	0.24215	0.956	-0.5039	1.1059
		5%	0.24200	0.24242	0.993	-0.5638	1.0478
		SZ	-3.64000*	0.26685	0.000	-4.5301	-2.7499
		CHX	-13.69200*	0.43687	0.000	-15.2337	-12.1503
	SZ	GI	6.54100*	0.20377	0.000	5.7727	7.3093
		3%	3.94100*	0.26546	0.000	3.0551	4.8269
		5%	3.88200*	0.26571	0.000	2.9954	4.7686
		7%	3.64000*	0.26685	0.000	2.7499	4.5301
		CHX	-10.05200*	0.45021	0.000	-11.6157	-8.4883
	CHX	GI	16.59300*	0.40145	0.000	15.0794	18.1066
		3%	13.99300*	0.43602	0.000	12.4525	15.5335
		5%	13.93400*	0.43617	0.000	12.3933	15.4747
		7%	13.69200*	0.43687	0.000	12.1503	15.2337
		SZ	10.05200*	0.45021	0.000	8.4883	11.6157

8.2 Mechanical properties

8.2.1 Compressive strength

Table 34 The data from compressive strength test was analyzed by Kolmogorov-Smirnov to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Compressive	GI	0.238	10	0.114
	3%	0.223	10	0.172
	5%	0.251	10	0.075
	7%	0.134	10	0.200*

Table 35 The data from compressive strength test was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Compressive

Levene Statistic	df1	df2	Sig.
3.182	3	36	0.035

Table 36 The data from compressive strength test was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

Compressive

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1315.785	3	438.595	2.786	0.055
Within Groups	5668.305	36	157.453		
Total	6984.090	39			

8.2.2 Flexural strength

Table 37 The data from flexural strength test was analyzed by Kolmogorov-Smirnov to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Flexural	GI	0.164	10	0.200*
	3%	0.160	10	0.200*
	5%	0.168	10	0.200*
	7%	0.234	10	0.129

Table 38 The data from flexural strength test was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were not different when the significant level more than 0.05.

Test of Homogeneity of Variances

Flexural

Levene Statistic	df1	df2	Sig.
.223	3	36	0.880

Table 39 The data from flexural strength test was analyzed by One-way ANOVA to compare means among different 4 groups. The data was statistically significant difference at the confidence level of 95%.

ANOVA

Flexural

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	88.869	3	29.623	4.699	0.007
Within Groups	226.942	36	6.304		
Total	315.812	39			

Table 40 The data from flexural strength test was analyzed by Tukey HSD to perform all pairwise comparison between 2 different groups. The data was statistically significant difference when the significant level was less than 0.05.

Multiple Comparisons

Dependent Variable

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	GI	3%	-2.99700	1.12285	0.053	-6.0211	0.0271
		5%	-0.09600	1.12285	1.000	-3.1201	2.9281
		7%	0.99700	1.12285	0.811	-2.0271	4.0211
	3%	GI	2.99700	1.12285	0.053	-0.0271	6.0211
		5%	2.90100	1.12285	0.064	-0.1231	5.9251
		7%	3.99400*	1.12285	0.006	0.9699	7.0181
	5%	GI	0.09600	1.12285	1.000	-2.9281	3.1201
		3%	-2.90100	1.12285	0.064	-5.9251	0.1231
		7%	1.09300	1.12285	0.765	-1.9311	4.1171
	7%	GI	-0.99700	1.12285	0.811	-4.0211	2.0271
		3%	-3.99400*	1.12285	0.006	-7.0181	-0.9699
		5%	-1.09300	1.12285	0.765	-4.1171	1.9311

8.2.3 Surface microhardness

Table 41 The data from surface microhardness test was analyzed by Kolmogorov-Smirnov to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Summicro	GI	0.154	10	0.200*
	3%	0.159	10	0.200*
	5%	0.114	10	0.200*
	7%	0.159	10	0.200*

Table 42 The data from surface microhardness test was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data had no difference when the significant level more than 0.05.

Test of Homogeneity of Variances

Summicro

Levene Statistic	df1	df2	Sig.
0.858	3	36	0.472

Table 43 The data from surface microhardness test was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

Summicro

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.465	3	2.822	0.245	0.864
Within Groups	414.285	36	11.508		
Total	422.750	39			

8.3 Fluoride release

Table 44 The data from fluoride release test at day 1st was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[b]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Fluoride1	GI	0.236	10	0.120
	3%	0.260	10	0.054
	5%	0.232	10	0.136
	7%	0.230	10	0.143

Table 45 The data from fluoride release test at day 1st was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Fluoride1

Levene Statistic	df1	df2	Sig.
5.706	3	36	0.003

Table 46 The data from fluoride release test at day 1st was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

Fluoride1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.075	3	1.025	0.451	0.718
Within Groups	81.900	36	2.275		
Total	84.975	39			

Table 47 The data from fluoride release test at day 2nd was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Fluoride2	GI	0.243	10	0.096
	3%	0.182	10	0.200*
	5%	0.255	10	0.065
	7%	0.181	10	0.200*

Table 48 The data from fluoride release test at day 2nd was tested by Levene statistic to test of homogeneity of variances between groups for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Fluoride2

Levene Statistic	df1	df2	Sig.
0.589	3	36	0.626

Table 49 The data from fluoride release test at day 2nd was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

Fluoride2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.123	3	0.041	1.886	0.149
Within Groups	0.781	36	0.022		
Total	0.904	39			

Table 50 The data from fluoride release test at day 3rd was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Fluoride3	GI	0.224	10	0.168
	3%	0.248	10	0.082
	5%	0.245	10	0.091
	7%	0.195	10	0.200*

Table 51 The data from fluoride release test at day 3rd was tested by Levene statistic to test of homogeneity of variances between groups for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Fluoride3

Levene Statistic	df1	df2	Sig.
1.088	3	36	0.367

Table 52 The data from fluoride release test at day 3rd was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

Fluoride3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.065	3	0.022	2.364	0.087
Within Groups	0.330	36	0.009		
Total	0.395	39			

Table 53 The data from fluoride release test at day 4th was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Fluoride4	GI	0.169	10	0.200*
	3%	0.245	10	0.090
	5%	0.248	10	0.082
	7%	0.205	10	0.200*

Table 54 The data from fluoride release test at day 4th was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Fluoride4

Levene Statistic	df1	df2	Sig.
6.905	3	36	0.001

Table 55 The data from fluoride release test at day 4th was analyzed by One-way ANOVA to compare means among different 4 groups. The data was statistically significant difference at the confidence level of 95%.

ANOVA

Fluoride4

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.738	3	0.246	11.098	0.000
Within Groups	0.798	36	0.022		
Total	1.536	39			

Table 56 The data from fluoride release test at day 4th was analyzed by Dunnett T3 to perform all pairwise comparison between 2 different groups. The data was statistically significant difference when the significant level was less than 0.05.

Multiple Comparisons

Dependent Variable: Fluoride4

Dunnett T3

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GI	3%	0.300000*	0.080829	0.015	0.05214	0.54786
	5%	0.350000*	0.078387	0.005	0.10573	0.59427
	7%	0.270000*	0.083333	0.033	0.01772	0.52228
3%	GI	-0.300000*	0.080829	0.015	-0.54786	-0.05214
	5%	0.050000	0.043843	0.821	-0.07900	0.17900
	7%	-0.030000	0.052175	0.992	-0.18321	0.12321
5%	GI	-0.350000*	0.078387	0.005	-0.59427	-0.10573
	3%	-0.050000	0.043843	0.821	-0.17900	0.07900
	7%	-0.080000	0.048305	0.490	-0.22332	0.06332
7%	GI	-0.270000*	0.083333	0.033	-0.52228	-0.01772
	3%	0.030000	0.052175	0.992	-0.12321	0.18321
	5%	0.080000	0.048305	0.490	-0.06332	0.22332

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

Table 57 The data from fluoride release test at day 5th was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Fluoride5	GI	0.200	10	0.200*
	3%	0.245	10	0.091
	5%	0.244	10	0.093
	7%	0.251	10	0.075

Table 58 The data from fluoride release test at day 5th was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Fluoride5

Levene Statistic	df1	df2	Sig.
1.126	3	36	0.352

Table 59 The data from fluoride release test at day 5th was analyzed by One-way ANOVA to compare means among different 4 groups. The data was statistically significant difference at the confidence level of 95%.

ANOVA

Fluoride5

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.130	3	0.043	3.321	0.030
Within Groups	0.468	36	0.013		
Total	0.598	39			

Table 60 The data from fluoride release test at day 5th was analyzed by Tukey HSD to perform all pairwise comparison between 2 different groups. The data was statistically significant difference when the significant level was less than 0.05.

Multiple Comparisons

Dependent Variable: Fluoride5

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GI	3%	0.120000	0.051010	0.105	-0.01738	0.25738
	5%	0.052000	0.051010	0.739	-0.08538	0.18938
	7%	-0.030000	0.051010	0.935	-0.16738	0.10738
3%	GI	-0.120000	0.051010	0.105	-0.25738	0.01738
	5%	-0.068000	0.051010	0.548	-0.20538	0.06938
	7%	-0.150000*	0.051010	0.028	-0.28738	-0.01262
5%	GI	-0.052000	0.051010	0.739	-0.18938	0.08538
	3%	0.068000	0.051010	0.548	-0.06938	0.20538
	7%	-0.082000	0.051010	0.387	-0.21938	0.05538
7%	GI	0.030000	0.051010	0.935	-0.10738	0.16738
	3%	0.150000*	0.051010	0.028	0.01262	0.28738
	5%	0.082000	0.051010	0.387	-0.05538	0.21938

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

Table 61 The data from fluoride release test at day 15th was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Fluoride15	GI	0.227	10	0.155
	3%	0.194	10	0.200*
	5%	0.183	10	0.200*
	7%	0.163	10	0.200*

Table 62 The data from fluoride release test at day 15th was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Fluoride15

Levene Statistic	df1	df2	Sig.
6.921	3	36	0.001

Table 63 The data from fluoride release test at day 15th was analyzed by One-way ANOVA to compare means among different 4 groups. The data was statistically significant difference at the confidence level of 95%.

ANOVA

Fluoride15

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.154	3	0.051	16.657	0.000
Within Groups	0.111	36	0.003		
Total	0.265	39			

Table 64 The data from fluoride release test at day 15th was analyzed by Dunnett T3 to perform all pairwise comparison between 2 different groups. The data was statistically significant difference when the significant level was less than 0.05.

Multiple Comparisons

Dependent Variable: Fluoride15

Dunnett T3

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GI	3%	0.146000*	0.033714	0.008	0.03834	0.25366
	5%	0.148000*	0.033615	0.007	0.04042	0.25558
	7%	0.134000*	0.033270	0.014	0.02666	0.24134
3%	GI	-0.146000*	0.033714	0.008	-0.25366	-0.03834
	5%	0.002000	0.011146	1.000	-0.03066	0.03466
	7%	-0.012000	0.010055	0.790	-0.04173	0.01773
5%	GI	-0.148000*	0.033615	0.007	-0.25558	-0.04042
	3%	-0.002000	0.011146	1.000	-0.03466	0.03066
	7%	-0.014000	0.009718	0.632	-0.04265	0.01465
7%	GI	-0.134000*	0.033270	0.014	-0.24134	-0.02666
	3%	0.012000	0.010055	0.790	-0.01773	0.04173
	5%	0.014000	0.009718	0.632	-0.01465	0.04265

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

Table 65 The data from fluoride release test at day 30th was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
Fluoride30	GI	0.230	10	0.144
	3%	0.190	10	0.200*
	5%	0.217	10	0.198
	7%	0.192	10	0.200*

Table 66 The data from fluoride release test at day 30th was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

Fluoride30

Levene Statistic	df1	df2	Sig.
18.292	3	36	0.000

Table 67 The data from fluoride release test at day 30th was analyzed by One-way ANOVA to compare means among different 4 groups. The data was statistically significant difference at the confidence level of 95%.

ANOVA

Fluoride30

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.014	3	0.005	20.453	0.000
Within Groups	0.008	36	0.000		
Total	0.022	39			

Table 68 The data from fluoride release test at day 30th was analyzed by Dunnett T3 to perform all pairwise comparison between 2 different groups. The data was statistically significant difference when the significant level was less than 0.05.

Multiple Comparisons

Dependent Variable: Fluoride30

Dunnett T3

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GI	3%	0.043300*	0.009569	0.008	0.01206	0.07454
	5%	0.044900*	0.009564	0.006	0.01367	0.07613
	7%	0.041900*	0.009562	0.009	0.01067	0.07313
3%	GI	-0.043300*	0.009569	0.008	-0.07454	-0.01206
	5%	0.001600	0.000901	0.413	-0.00104	0.00424
	7%	-0.001400	0.000876	0.525	-0.00397	0.00117
5%	GI	-0.044900*	0.009564	0.006	-0.07613	-0.01367
	3%	-0.001600	0.000901	0.413	-0.00424	0.00104
	7%	-0.003000*	0.000829	0.011	-0.00543	-0.00057
7%	GI	-0.041900*	0.009562	0.009	-0.07313	-0.01067
	3%	0.001400	0.000876	0.525	-0.00117	0.00397
	5%	0.003000*	0.000829	0.011	0.00057	0.00543

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

Table 69 The data from cumulative fluoride release 5 days was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
c5	GI	0.217	10	0.198
	3%	0.143	10	0.200*
	5%	0.214	10	0.200*
	7%	0.223	10	0.171

Table 70 The data from cumulative fluoride release 5 days was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

c5

Levene Statistic	df1	df2	Sig.
5.788	3	36	0.002

Table 71 The data from cumulative fluoride release 5 days was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

c5

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.078	3	3.359	1.256	0.304
Within Groups	96.278	36	2.674		
Total	106.356	39			

Table 72 The data from cumulative fluoride release 15 days was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
c15	GI	0.211	10	0.200*
	3%	0.153	10	0.200*
	5%	0.212	10	0.200*
	7%	0.219	10	0.189

Table 73 The data from cumulative fluoride release 15 days was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

c15

Levene Statistic	df1	df2	Sig.
5.954	3	36	0.002

Table 74 The data from cumulative fluoride release 15 days was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

c15

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.707	3	4.236	1.536	0.222
Within Groups	99.274	36	2.758		
Total	111.981	39			

Table 75 The data from cumulative fluoride release 30 days was analyzed by Kolmogorov-Smirnov in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level more than 0.05.

Tests of Normality[c]

Group		Kolmogorov-Smirnov[a]		
		Statistic	df	Sig.
c30	GI	0.205	10	0.200*
	3%	0.152	10	0.200*
	5%	0.212	10	0.200*
	7%	0.220	10	0.187

Table 76 The data from cumulative fluoride release 30 days was tested by Levene statistic to test of homogeneity of variances between group for further statistical analysis. The variances of data were different when the significant level less than 0.05.

Test of Homogeneity of Variances

c30

Levene Statistic	df1	df2	Sig.
5.952	3	36	0.002

Table 77 The data from cumulative fluoride release 30 days was analyzed by One-way ANOVA to compare means among different 4 groups. The data was not statistically significant difference at the confidence level of 95%.

ANOVA

c30

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.562	3	4.521	1.626	0.200
Within Groups	100.081	36	2.780		
Total	113.643	39			

CURRICULUM VITAE

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