Acid neutralizing, mechanical and physical properties of pit and fissure sealants containing melt-derived 45S5 bioactive glass

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ABSTRACT

Objectives. The aim of this study was to examine the effects of 45S5 bioactive glass (BAG) on the acid neutralizing, mechanical and physical properties of pit and fissure sealants.

Methods. 45S5BAG (<25 μm) was mixed with the silanized glass (180 ± 30 nm) and added into a resin matrix [Bis-GMA/TEGDMA 50/50 (wt%) containing 1% of DMAEMA/CQ 2:1 (wt%)] with varying filler proportions; 0% 45S5BAG + 50% glass (BAG0); 12.5% 45S5BAG + 37.5% glass (BAG12.5); 25% 45S5BAG + 25% glass (BAG25); 37.5% 45S5BAG + 12.5% glass (BAG37.5); and 50% 45S5BAG + 0% glass (BAG50). To evaluate the acid neutralizing properties, specimens were immersed in lactic acid solution (pH 4.0). Then, the change in pH and the time required to raise the pH from 4.0 to 5.5 were measured. In addition, flexural strength, water sorption and solubility were analyzed.

Results. The acid neutralizing properties of each group exhibited increasing pH values as more 45S5BAG was added, and the time required to raise the pH from 4.0 to 5.5 became shorter as the proportion of 45S5BAG increased (P < 0.05). Additionally, the flexural strength decreased according to the increasing proportions of 45S5BAG added (P < 0.05). Water sorption showed an increasing trend with increasing proportions of 45S5 BAG added (P < 0.05). However, the solubility results were similar among the groups (P > 0.05), except for BAG50.

Significance. The novel pit and fissure sealants neutralized the acid solution (pH 4.0) and exhibited appropriate mechanical and physical properties. Therefore, these compounds are suitable candidates for caries-inhibiting dental materials.

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1. Introduction

Tooth caries or dental caries, a bacterial infection in the mouth, is the most common dental disease. Miller’s chemo-parasitic theory is the most widely accepted mechanism for dental caries, which describes the formation of dental caries in the following steps: 1) various resident bacteria in the mouth metabolize acid from fermentable carbohydrate; 2) the pH decreases as acid is generated by bacteria, causing demineralization of the tooth structure; and 3) demineralization is sustained and caries are formed [1]. For these reasons,
cariogenic bacteria play an important role in the formation of dental caries [2]. Therefore, to inhibit this problem, preventive treatment should be considered for high-risk patients.

Occlusal surfaces are the sites most frequently attacked by dental caries because of their structural irregularity and morphological complexity [3]. These sites are considered to be ideal for the retention of bacteria and food remnants [4].

To inhibit caries on the occlusal surface, pit and fissure sealants have been used as the most effective preventive materials [5]. These materials are placed in the pits and fissures of occlusal surfaces to arrest caries progression by providing a physical barrier that inhibits the accumulation of microorganisms and food remnants [6]. The application of the pit and fissure sealant to patients with a high risk of dental caries has been proven to be cost-effective for public oral health services [7].

Nevertheless, secondary caries may form around the sealed pits and fissures on the material-tooth interfaces, due to either the partial loss of materials or the microleakage and gaps induced by polymerization shrinkage [8]. Fluoride released from pit and fissure sealants may prevent caries formation by enhancing remineralization and inhibiting microbial metabolism [9]. However, when considering the long-term effects, it has been shown that there is no significant difference in results between the pit and fissure sealants that contain fluoride and those that do not contain fluoride, due to the short fluoride releasing period [10]. A previous study used glass-ionomer materials as a pit and fissure sealant, which released high levels of fluoride while chemically bonded to the tooth structure. However, it exhibited high viscosity, which disabled it from penetrating deeply into narrow fissures and showed poor retention rates, which caused secondary caries [6].

To inhibit secondary caries formation, some dental composite resins have a cariostatic ability to neutralize acidic saliva and prevent decalcification of restorations around the area [11]. This neutralizing effect is due to the release of OHi- ions that originated from the alkaline glass filler embedded in the resin [12].

45S5 Bioglass®, a bioactive implant material discovered in 1971, has excellent biocompatibility and has been widely used as a bone filling material in the clinic due to its capacity for bone regeneration [13]. 45S5 Bioglass® is currently used in dentistry for air polishing or caries removal procedures, which simultaneously causes the remineralization of the dental hard tissue, including enamel and dentin [14]. Additionally, this glass particle possesses antibacterial properties [15] that are attributed to the high aqueous pH value, caused by the release of alkali ions [16].

There have been many studies that have examined either the remineralization effect or the antibacterial ability of the bioactive glass. However, the effects of bioactive glass’ acid neutralizing ability have not been investigated. Therefore, the aim of this study was to investigate the neutralizing ability of 45S5 bioactive glass (BAG) on acid that causes dental caries, for the possible application of these materials as dental pit and fissure sealants. This was accomplished by incorporating varying quantities of 45S5 BAG filler into the resin matrix in order to make novel pit and fissure sealants and examining its effects on the acid neutralizing ability, flexural strength, water sorption and solubility. The null hypothesis of this study was that pit and fissure sealants containing 45S5 BAG filler would not result in significant differences in the acid neutralizing, mechanical and physical properties compared to those of pit and fissure sealants without the 45S5 BAG filler.

2. Materials and methods

2.1. Preparation of 45S5 BAG powder

High purity silicon dioxide (SiO2, Junsei Chemical Co., Tokyo, Japan), sodium carbonate (Na2CO3, Duksan Pure Chemicals Co., Ansan-city, Korea), calcium carbonate (CaCO3, Samchun Pure Chemicals Co., Pyeongtaek city, Korea), and phosphorus pentoxide (P2O5, Sigma–Aldrich, Steinheim, Germany) powders were weighted and mixed to obtain an identical composition to 45S5 Bioglass® (45.0 SiO2, 24.5 CaO, 24.5 Na2O, 6.0 P2O5 in wt.%).

The powder mixture was melted in a platinum crucible for 4 h at 1400 °C. The melted product was then conventionally quenched onto a graphite plate at room temperature and ground using a mortar and pestle to make fine powder. The ground powder was filtered through a 500-mesh sieve to obtain fine particles less than 25 μm in size and was not silanized for proper ion release from 45S5 BAG in an aqueous environment.

The amorphous structure of the 45S5 BAG powder was identified by X-ray diffraction analysis (XRD, Ultima IV, Rigaku, Tokyo, Japan). A 2θ angle range between 10° and 70° was selected with a scanning speed of 1°/min.

2.2. Preparation of novel pit and fissure sealants

To make the pit and fissure sealants, silanized dental glass powder (180 ± 30 nm; NanoFine® NF180, Schott, Landshut, Germany) was selected, which is used as a conventional glass filler in dental composite resin.

A resin matrix of 49.5% Bisphenol A glycol dimethacrylate (Bis-GMA, Sigma–Aldrich, Steinheim, Germany) and 49.5% Triethylene glycol dimethacrylate (TEGDMA, Sigma–Aldrich, Steinheim, Germany) in a 1:1 mass ratio was mixed with 0.3% Camphorquinone (CQ, Sigma–Aldrich, Steinheim, Germany) and 0.6% 2-(Dimethylamino)ethyl methacrylate (Sigma–Aldrich, Steinheim, Germany) for light polymerization. Five groups were fabricated with varying filler proportions (Table 1).
the entire length of the specimen had been irradiated. The irradiation procedure was repeated on the other side of the material. Then, the specimen was separated from the mold and any flash on the samples was carefully removed with 400 grit abrasive paper.

To investigate the neutralizing ability of each group, lactic acid (Sigma-Aldrich, Steinheim, Germany) solution (pH 4.0) was prepared. Three specimens were immersed in 2.14 mL of lactic acid solution, yielding a specimen/solution ratio of 0.14 cm³/1 mL [17], at a temperature of (25 ± 1) °C. Changes in the acid solution’s pH were determined using a digital pH-meter (Orion 4 Star, Thermo Fisher Scientific Inc., Singapore), which had been calibrated at pH 4.01 and pH 7.00 immediately before use. The pH measurement was performed as soon as the specimens were submerged in lactic acid solution. The pH change of the solution was monitored with a pH electrode for 180 min and data were acquired each minute. Furthermore, the time required for the solution’s pH to rise from 4.0 to 5.5 [18] was recorded for 180 min.

2.4. Three-point flexural strength

A three-point flexural strength was measured according to the method outlined in ISO 4049 (2009). The specimens were prepared via the same method described above for the acid neutralizing property and stored in distilled water at (37 ± 1) °C until the start of testing.

The specimen was fractured at a crosshead speed of 1 mm/min on a computer-controlled flexural strength test apparatus (Instron 5942, Instron, Massachusetts, USA). The maximum load was recorded and the flexural strength (S) was calculated using the following equation: \( S = 3F/(2bh^2) \), where \( F \) is the maximum fracture load, \( b \) is the width of specimen, and \( h \) is height of specimen.

2.5. Water sorption and solubility

A test was performed to measure the water sorption and solubility of the sealant according to ISO 4049 (2009). Specimens (15 ± 0.1) mm in diameter and (1.0 ± 0.1) mm in thickness were prepared. All the specimens were placed in a desiccator maintained at (37 ± 1) °C. After 22 h, the specimens were removed and stored in a desiccator maintained at (23 ± 1) °C for 2 h, and then they were weighted in an analytical balance (accurate to 0.01 mg) (XS105, Mettler-toledo AG, Greifensee, Switzerland) with a reproducibility of 0.1 mg until a constant mass (\( m_1 \)) was obtained. The diameter and thickness of the specimens were measured using a digital caliper (accurate to 0.01 mm) (Mitutoyo, Japan). The mean diameter value of the specimen was calculated by measuring two diameters, and the mean thickness value of specimen was calculated by measuring four equally spaced points on the circumference. These values were then used to calculate the volume (V) of all samples (in 0.01 mm³). Following these procedures, they were stored for 7 days in distilled water at (37 ± 1) °C, blotted until free from visible moisture, waved in the air for 15 s, and weighed for mass (\( m_2 \)). Finally, each disk was placed in a desiccator and weighed daily until a constant dry mass (\( m_3 \)) was obtained. Water sorption and solubility were calculated using the following equations: \( W_{sp} = (m_2 - m_1)/V \), \( W_{sol} = (m_1 - m_3)/V \) where \( W_{sp} \) is the absorption of the test material (µg/mm³) and \( W_{sol} \) is the solubility of the test material (µg/mm³).

2.6. Statistical analysis

The results of each test were analyzed with one-way ANOVA (PASW 18.0, IBM Co., USA) followed by Tukey’s statistical test at a significance level of 0.05.

3. Results

3.1. 45S5 BAG powder characterization

The 45S5 BAG powder characteristics are shown in Fig. 1, which confirmed that the 45S5 BAG powder exhibited characteristics of amorphous glass.
3.2. Acid neutralization property

The results of changing the pH of the lactic acid solution are shown in Fig. 2. The pH value of the solution in BAG0, which had no 45S5 BAG, slightly increased initially for 3 min. However, after 180 min of immersion, the final pH was 4.2. Compared to the final pH results of the other groups, this value was the closest to the initial pH of 4.0. In contrast, each group that contained from 12.5% to 50% of 45S5 BAG exhibited increasing final pH values as more 45S5 BAG was added. In particular, the pH of the solution in BAG50, which contained 50% 45S5 BAG, became (8.6 ± 0.3) after 180 min. This final pH was higher than those of any other groups (P < 0.05). Therefore, it was confirmed that the 45S5 BAG filler had a strong ability to neutralize the acid and increase the pH.

Increasing the 45S5 BAG filler proportion significantly decreased the time to raise the pH from 4.0 to 5.5. In particular, BAG50 took (22.6 ± 4.4) min to reach pH 5.5, which was much faster than (157.8 ± 22.1) min for BAG12.5 and (92.6 ± 15.7) min for BAG25 (P < 0.05). However, (48.6 ± 11.6) min for BAG37.5 was not significantly different from that of BAG50 (P > 0.05). In comparison, BAG0 did not raise the pH to 5.5 until 180 min.

The results showed that with the increasing proportion of 45S5 BAG added, the time to raise the acidic pH was shortened.

3.3. Three-point flexural strength

The results of the three-point flexural strength test are shown in Fig. 3. The results showed a decreasing trend of strength with an increasing proportion of 45S5 BAG added. In particular, BAG50 had strength of (43.9 ± 1.7) MPa, which was significantly lower than (76.1 ± 8.8) MPa for BAG0 without 45S5 BAG (P < 0.05). The results of the flexural strength tests were significantly different among the groups (P < 0.05), except for BAG25 and BAG37.5. BAG25’s strength, (54.0 ± 3.0) MPa, was not significantly different from that of BAG37.5, (53.5 ± 2.8) MPa (P > 0.05).

3.4. Water sorption and solubility

The results for water sorption and solubility are shown in Figs. 4 and 5. One-way ANOVA revealed significant differences for water sorption as well as for solubility (P < 0.05) among the different groups. Water sorption showed an increasing trend with increasing proportion of 45S5 BAG added (P < 0.05), in which BAG50 presented the highest value. The water solubility results were generally similar among the different groups (P > 0.05), except for BAG50 which had a lower solubility (P < 0.05).

4. Discussion

As secondary caries are often an undesired consequence of pit and fissure sealants, it is highly desirable to prevent tooth decay. Numerous researchers have attempted to prevent the formation of secondary caries around the dental pit and fissure sealant [19–21]. Strategies to inhibit secondary caries include the following: 1) remineralization of the demineralized tooth and 2) neutralization of acids that are known to cause tooth demineralization.
Some researchers have studied the remineralization of the demineralized tooth using composite materials that release fluoride, calcium, and phosphate ions [17,18,21]. To achieve such ion release, previous studies have used fluoride [22], hydroxyapatite [23], α-tricalcium phosphate (α-TCP) and β-tricalcium phosphate (β-TCP) as fillers in the composite [24-26]. In accordance with a previous study, incorporating bioactive glass into a composite resin has biomimetic properties when immersed in body fluids, leading to the formation of tooth-like hydroxyapatite. This process has the potential for the subsequent remineralization of the demineralized tooth [27].

Another strategy to inhibit secondary caries is to develop an acid neutralizing composite. Efforts were made to develop a resin-based material that contained 3-aminopropyltrimethoxysilane-coated glass filler, which was shown to have an acid neutralizing effect. However, this effect was insufficient to produce a cariostatic function due to weak alkalinity [28]. Other studies have developed novel composites that contained nano-sized amorphous calcium phosphate (NACP) filler. These composites showed a capacity for acid neutralization, but their antibacterial effect was quite weak [17]. Therefore, NACP containing composites with antibacterial agents were used to greatly increase antibacterial activity and neutralize acid [29,30].

Accordingly, in this study, novel pit and fissure sealants containing 45S5 BAG filler were developed to prevent secondary caries around the material. A major advantage of the use of the pit and fissure sealant containing 45S5 BAG filler was the resulting increase in the pH of cariogenic acids between the tooth structure and the pit and fissure sealant that would otherwise dissolve the site.

When considering the safety level of local plaque pH in the oral cavity, a pH above 6.0 is considered to be the safe zone, 6.0–5.5 is known as the potentially cariogenic pH, and 5.5–4 is known as the danger cariogenic zone [18,31]. Acids produced by carbohydrate-fermenting acidogenic bacteria include lactic, formic, acetic, and propionic acids [26], and demineralization dominates when the pH is below the critical pH of 5.5 by these acids, which leads to the dissolution of the enamel minerals [24]. To inhibit secondary caries, it would be ideal for the novel pit and fissure sealant to raise the cariogenic pH from 4.0 to a relatively safe pH of 5.5 or above, and quickly neutralize the local acids.

This study demonstrated that when a pit and fissure sealant containing commercially available salinized glass filler was immersed in a lactic acid solution of pH 4.0, it failed to raise the pH to the critical pH of 5.5. It remained near pH of 4.0. In contrast, the pit and fissure sealants containing 45S5 BAG filler were able to raise the pH above 5.5, and the acid neutralization ability rapidly increased with higher BAG filler levels. The pit and fissure sealant containing only 45S5 BAG showed a higher solution pH at 10 min, and it required a much shorter time for the solution to reach the critical pH value of 5.5. Therefore, neutralizing the metabolized acid helps inhibit tooth structure demineralization.

This study demonstrated that the pit and fissure sealant containing 45S5 BAG filler rapidly increased the pH of a lactic acid solution. It was possible to assume that the alkalinity may have been released. 45S5 BAG particles underwent a series of surface reactions that included the release of soluble alkali ions, such as Na⁺ and Ca²⁺ ions, in aqueous environments, resulting in an increase in the aqueous pH value [32]. This conclusion was supported by our results that showed that the neutralizing effect of pit and fissure sealant containing 45S5 BAG filler increased the aqueous pH of acid solution in proportion to the amount of the filler added (Fig. 2). Furthermore, the time required for the pit and fissure sealant with 45S5 BAG filler to increase the pH of the lactic acid solution was visibly faster than that of the pit and fissure sealant with only commercial glass filler.

A separate study indicated that gaps at the margins of commercially available pit and fissure sealants may allow for bacterial invasion and biofilm accumulation. Acid is generated by bacteria and causes demineralization along the restored tooth cavity wall [33]. This study demonstrated that the alkali
ions are released from novel pit and fissure sealant materials, and it is possible that caries progression can be inhibited within these gaps.

The Stephan curve shows that following a glucose mouth rinse, plaque pH decreases to the value corresponding to a cariogenic pH for a minimum 5–20 min. This is followed by a gradual recovery to its starting value, usually over 30–60 min, although this can be longer in some individuals [34]. Therefore, it would be desirable for a specimen to quickly raise the cariogenic pH from 4.0 to 5.5 or above in order to help resist caries. This fact implied that quickly raising the local plaque pH from the cariogenic level to the safety level plays an important role in inhibiting the demineralization of tooth structure.

In addition to the acid neutralizing property, there is a need for the pit and fissure sealant to have appropriate mechanical properties because the material can encounter mechanical stresses when placed on areas that are subjected to the force of mastication [25]. The results showed that adding 45S5 BAG decreased the flexural strength of pit and fissure sealants. This suggests that the 45S5 BAG powder might be weakly attached to the pit and fissure sealant matrix because of the unsilanized treatment of this filler [35]. Thus, the 45S5 BAG powder acted as a filler that had not adhered to the matrix of pit and fissure sealants, leading to the decreased flexural strength. To reinforce the mechanical strength of pit and fissure sealants that contained only 45S5 BAG filler, the silanized glass filler was added to BAG12.S, BAG2S and BAG37.5. These groups showed significantly higher mechanical strength than BAG50, which did not contain silanized glass filler. Additionally, in accordance with a previous publication [36], these pit and fissure sealants, despite containing 45S5 BAG, possessed mechanical strengths that matched or exceeded those of some commercial pit and fissure sealants.

The bioactivity and degradability of 45S5 BAG are well established in previous studies [37–39]. However, one of the concerns with regard to the 45S5 BAG’s degradability was whether it could be used both in vivo and in clinics or not, as the pit and fissure sealants containing 45S5 BAG may be partially lost. In order to investigate this problem, a previous clinical study has shown that pit and fissure sealants containing BAG had complete retention on the occlusal surface of children even after one year [40]. Even though the BAG used in this study was not exactly same as 45S5 BAG, we can assume that the 45S5 BAG used in our study will have comparable results because of the similar bioactive and degradable properties.

Water sorption and solubility are related mainly to the chemical and dimensional stability of the resin matrix composition in an aqueous environment [41]. Moreover, there are several factors that influence water uptake values, such as resin composite components and filler presence [42]. In the case of BAG50, the highest water sorption values were obtained. It showed an increasing trend with increasing mass fraction of 45S5 BAG filler, which resulted from bioactive glass materials having a hydrophilic characteristic that increases water sorption values [43]. The absorbed water in the pit and fissure sealant can allow surface reactions of 45S5 BAG filler to occur. However, the pit and fissure sealant without 45S5 BAG filler showed the lowest water sorption values, most likely due to the presence of stable silanized dental glass filler, which does not cause water sorption. Solubility results were similar among the different groups, except for BAG50. BAG50, which contained only 45S5 BAG filler, had the lowest solubility value, which may be due to the surface reaction of 45S5 BAG. In a wet environment, 45S5 BAG activates to release ions, which precipitate on the glass surface [44]. In accordance with previous study, in composite materials that contain bioactive glass, precipitation also occurs on the material’s surface [45]. With time, these precipitation layers turn into hydroxyapatite [46], which could enhance the caries-inhibition and remineralizing capabilities between the surface of the pit and fissure sealant and the tooth interface.

In this study, the 45S5 BAG particles were much larger than the glass fillers. The initial aim of this study was to make a hybrid composite that had excellent mechanical strength while having acid neutralizing property, and therefore, micro-sized BAG fillers were mixed with nano-sized glass fillers. It is difficult to predict whether the particle size would have effects on other properties such as wear resistance. However, as shown by the previous study, there were no significant differences in the wear resistance among the nano-filler based composite resins, traditional microhybrid and micro-filler materials [47].

The current study is the first to indicate that a pit and fissure sealant that contains 45S5 BAG filler is a sufficiently promising material for the protection of tooth structure from acid. In this case, there is the potential that the area adjacent to the pit and fissure sealant is buffered with 45S5 BAG in acid attacks, and demineralization could therefore be prevented in secondary caries.

For these reasons, in an aqueous environment, the pit and fissure sealants that contain 45S5 BAG filler are able to increase the pH value, reducing enamel demineralization. Bioactive glasses may even possibly result in a better tooth remineralization.

Further studies are needed to examine the long-term maintenance of a neutralizing effect. In addition, studies that use nano-sized particles rather than the micro-sized bioglass used in this study will be useful due to their large surface area and more favorable neutralizing effect.

5. Conclusion

In this study, pit and fissure sealants that contain 45S5 BAG filler affected the acid neutralizing, mechanical and physical properties relative to pit and fissure sealants without the 45S5 BAG filler. It was noted that this novel material neutralized lactic acid at the cariogenic pH of 4.0 and exhibited appropriate flexural strength, water sorption and solubility for the application to the occlusal surface. Therefore, it was concluded that pit and fissure sealants that contain 45S5 BAG filler are a promising candidates for caries inhibiting dental materials with adequate mechanical and physical properties.
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REFERENCES


