Enamel Surface with Pit and Fissure Sealant Containing 45S5 Bioactive Glass

S.-Y. Yang1,2, J.-S. Kwon1,2, K.-N. Kim1,2, and K.-M. Kim1,2

Abstract
Enamel demineralization adjacent to pit and fissure sealants leads to the formation of marginal caries, which can necessitate the replacement of existing sealants. Dental materials with bioactive glass, which releases ions that inhibit dental caries, have been studied. The purpose of this study was to evaluate the enamel surface adjacent to sealants containing 45S5 bioactive glass (BAG) under simulated microleakage between the material and the tooth in a cariogenic environment. Seals containing 45S5BAG filler were prepared as follows: 0% 45S5BAG + 50.0% glass (BAG0 group), 12.5% 45S5BAG + 37.5% glass (BAG12.5 group), 25.0% 45S5BAG + 25.0% glass (BAG25.0 group), 37.5% 45S5BAG + 12.5% glass (BAG37.5 group), and 50.0% 45S5BAG + 0% glass (BAG50.0 group). A cured sealant disk was placed over a flat bovine enamel disk, separated by a 60-µm gap, and immersed in lactic acid solution (pH 4.0) at 37 °C for 15, 30, and 45 d. After the storage period, each enamel disk was separated from the cured sealant disk, and the enamel surface was examined with optical 3-dimensional surface profilometer, microhardness tester, and scanning electron microscopy. The results showed a significant increase in roughness and a decrease in microhardness of the enamel surface as the proportion of 45S5BAG decreased (P < 0.05). In the scanning electron microscopy images, enamel surfaces with BAG50.0 showed a smooth surface, similar to those in the control group with distilled water, even after prolonged acid storage. Additionally, an etched pattern was observed on the surface of the demineralized enamel with a decreasing proportion of 45S5BAG. Increasing the 45S5BAG filler contents of the sealants had a significant impact in preventing the demineralization of the enamel surface within microgaps between the material and the tooth when exposed to a cariogenic environment. Therefore, despite some marginal leakage, these novel sealants may be effective preventive dental materials for inhibiting secondary caries at the margins.

Keywords: microleakage, tooth demineralization, microhardness, surface roughness, caries resistance, SEM

Introduction
The use of pit and fissure sealants on patients who are at high risk of occlusal caries has been verified to be cost-effective for oral health care (Weintraub 2001). However, clinical use of these sealants has long been limited because secondary caries has occurred around the material-tooth interface (Cehreli and Gungor 2008) due to either partial detachment of the material or marginal microleakage (Hevinga et al. 2007). Numerous studies have focused on composite resins that have a cariostatic ability to neutralize the acidic environment (Wiegand et al. 2007; Mehdawi et al. 2013; Melo et al. 2013; Zhou et al. 2013) or to promote the remineralization of the tooth around the area (Cheng et al. 2012).

45S5 bioglass has been used in bone-filling material in the medical field due to its benefits in terms of bone regeneration and its biocompatible properties (Hench et al. 1971; Hench and Paschall 1973). Moreover, numerous articles have demonstrated that dental materials with 45S5 bioactive glass (45S5BAG) have the potential to remineralize a demineralized tooth structure (Vollenweider et al. 2007; Wang et al. 2011; Jones 2013). This glass also possesses antibacterial attributes that are attributed to the high aqueous pH value caused by the release of alkali ions (Hu et al. 2009). Most recently, we reported that sealants containing 45S5BAG filler can increase the pH level of a lactic acid solution and that their mechanical properties match or exceed some commercial sealants (Beun et al. 2012; Yang et al. 2013). Consequently, our previous study suggested that 45S5BAG filler has a key role as a caries-inhibiting material.

Here, we wanted to determine whether the 45S5BAG filler can actually prevent tooth demineralization under cariogenic conditions rather than simply neutralizing acid solutions. Therefore, more detailed research with particular methods was carried out in this study to evaluate the effects of sealants containing 45S5BAG on demineralization resistance under simulated clinical conditions.

The purpose of this in vitro study was to evaluate the effects of sealants containing 45S5BAG filler on the inhibition of enamel demineralization by simulating microleakage of 60-µm
gaps that occur over a prolonged period (15, 30, and 45 d) in a cariogenic environment. The null hypotheses were that 1) increasing the 45S5BAG filler contents would not result in significant differences in the inhibition of enamel demineralization with the same exposure time in a cariogenic environment and 2) increasing the exposure time (15, 30, and 45 d) in the cariogenic environment would not affect the inhibition of enamel demineralization with the same 45S5BAG filler content.

Materials and Methods

Preparation of 45S5BAG Powder and Resin Matrix

SiO2 (Junsei Chemical Co., Tokyo, Japan), Na2CO3 (Duxsan Pure Chemicals Co., Ansan, Korea), CaCO3 (Samchun Pure Chemicals Co., Pyeongtaek, Korea), and P2O5 (Sigma-Aldrich, Steinheim, Germany) powders were weighed to obtain a composition that was identical to 45S5 bioglass (45SiO2, 24.5CaO, 24.5Na2O, 6P2O5 in percentage weight [wt%]). The weighed powders were melted in a platinum crucible at 1400 °C for 4 h and then quenched into a graphite plate mold at room temperature. The melt-derived 45S5BAG was ground with a mortar and pestle. The ground powder was filtered through a 500-mesh sieve (<25 µm) and was not silanized. The mean particle size as measured by a particle size analyzer (Zetasizer Nano ZS90; Malvern Instruments Ltd., Malvern, UK) was 2,047 ± 600 nm in diameter.

A resin matrix was prepared with a mix of 49.5 wt% bisphenol A glycerolate dimethacrylate and 49.5 wt% triethylene glycol dimethacrylate in a 1:1 mass ratio, which was based on the available information on the composition of a commercially marketed sealant (Clinpro Sealant; 3M ESPE, St. Paul, MN, USA). Then, 0.3 wt% camphorquinone and 0.6 wt% 2-(dimethylamino) ethyl methacrylate were added as a photoinitiator and accelerator, respectively. All resin matrix materials were purchased from Sigma-Aldrich. Then, 180 ± 30 nm of silanized dental glass powder (NanoFine NF180; Schott, Landshut, Germany) was selected. The 45S5BAG and silanized dental glass filler were added to the resin matrix in different proportions (Table).

<table>
<thead>
<tr>
<th>Group</th>
<th>BAG0</th>
<th>BAG12.5</th>
<th>BAG25.0</th>
<th>BAG37.5</th>
<th>BAG50.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin matrix</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Content of 45S5BAG filler</td>
<td>0.0</td>
<td>12.5</td>
<td>25.0</td>
<td>37.5</td>
<td>50.0</td>
</tr>
<tr>
<td>Content of silanized dental glass filler</td>
<td>50.0</td>
<td>37.5</td>
<td>25.0</td>
<td>12.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values in percentage weight.
45S5BAG, 45S5 bioactive glass; BAG, bioactive glass.

Preparation of Enamel Disks

A micromotor (Marathon-4; Saeyang Microtech, Daegu, Korea) and a diamond-coated disk (NTI-Kahla GmbH, Kahla, Germany) were used to cut the roots from the bovine anterior teeth. Separated crowns were initially cut into a disk shape 11.0 ± 0.1 mm in diameter and 3.0 ± 0.1 mm in thickness and ground with a water-cooled rotating polishing machine (Ecomet; Buehler Ltd., Lake Bluff, IL, USA) with 600-, 800-, 1,200-, and 2,000-grit silicon carbide paper (Deerfos, Incheon, Korea) to obtain flat enamel disks that were 10.0 ± 0.1 mm in diameter and 2.0 ± 0.1 mm thick. In addition, 3 of the prepared enamel disks were randomly selected for the baseline evaluation prior to the following procedure.

Simulation of Microleakage with Sealants and Enamel Disks

To simulate the microleakage between the sealants and the tooth structure in vitro, 2 polyester films (2.0 mm × 10.0 mm × 60 µm) were placed on each end of the flat enamel disk and covered with an experimental sealant disk. The assembly was wrapped with Parafilm (BEMIS, Neenah, WI, USA) perpendicularly to the long axis of the polyester films to avoid separation of the sealant disk from the enamel disk while leaving both gap surfaces open. Five assemblies were prepared for each group. A schematic diagram of the experimental design is shown in Figure 1.

Exposure of a Microgap between the Sealant Disks and Enamel Disks to Acidic Solution

The interfacial gap between the sealant disk and enamel disk was then filled with lactic acid (Sigma-Aldrich) solution (pH...
4.0) with a micropipette (Pipetman P200; Gilson, Villiers-le-Bel, France). Then, an assembly containing the lactic acid solution was entirely immersed in 10 mL of lactic acid solution at 37 ± 1 °C for 15, 30, and 45 d. The acid solution (pH 4.0) used for immersion of the assembly was refreshed every 24 h for each experimental day. At the same time, the acid solution between the sealant disk and enamel disk was refreshed with a micropipette. For comparison among the experimental groups, enamel disks that had no sealant disks attached, leaving all surfaces exposed, were immersed for 15, 30, and 45 d in either the distilled water (named control group “DW”) or a lactic acid solution of pH 4.0 (named control group “Acid”). The distilled water and lactic acid solution were replaced every 24 h for each experimental day. The enamel disk and sealant disk were detached after each storage period. Separated assemblies were kept in the desiccator at room temperature (25 ± 2 °C) for 48 h without any washing process. Enamel surfaces not covered with polyester film to simulate the microgap model were randomly examined from the center to the edge as follows.

**Enamel Surface Roughness**

The Ra value was measured with an optical 3-dimensional surface profilometer (Contour GT-X3 Base, Bruker, Germany). Five random areas (0.2 × 0.2 mm) of each enamel specimen were measured at 20.0× magnifications in vertical scanning interferometry mode, and the mean Ra value was then calculated. The scan resolution was less than 500 nm laterally and 0.1 nm vertically.

**Enamel Microhardness**

A microhardness tester (Model DMH-2; Matsuzawa Seiki Co., Tokyo, Japan) was used to record the Vickers hardness number (VHN). The microhardness of the enamel surface was randomly measured with a diamond indenter with a 200-g load for 15 s of dwell time. Ten measurements were made on the surface of each enamel specimen and the average value used as the hardness of the particular specimen.

**Enamel Scanning Electron Microscopy Observation**

After roughness and hardness analyses, the opposing sides of the tooth and sealant disk were sputter-coated with platinum in a vacuum evaporator and observed at 2,000× magnification with an scanning electron microscopy (SEM; JEOL JSM-7001F; JEOL Ltd., Tokyo, Japan) with an accelerating voltage of 15.0 kV.

**Statistical Analysis**

The surface roughness and microhardness values of the different groups at the same exposure time were analyzed with 1-way analysis of variance (PASW 18.0; IBM Co., Chicago, IL, USA), followed by Tukey’s statistical test. In addition, the surface roughness and microhardness values at the different exposure times within the same experimental group were analyzed with the same methods ($P = 0.05$).

**Results**

**Surface Roughness**

The baseline for sound enamel before the experimental process in this study had an Ra value ranging from 0.041 to 0.061 µm. Surface roughness of the enamel disks with the experimental groups at 15, 30, and 45 d of exposure to cariogenic conditions are shown in Figure 2A. When the experimental groups within each period were considered, the enamel with BAG0 showed a rougher surface than the other groups containing 45S5BAG ($P < 0.05$). In addition, increasing the 45S5BAG filler proportion significantly decreased the roughness value of the enamel surface ($P < 0.05$). In particular, enamel with BAG50.0 showed no significant difference relative to that of the control group DW ($P > 0.05$). Similar trends were observed in all experimental periods.

When the experimental periods within each group were considered, an increasing trend was noticed in the surface roughness with increasing immersion time in the acid solution for the control group Acid and the BAG0 and BAG12.5 groups. In particular, the roughness value of the enamel surface with BAG0 at 45 d increased nearly 2-fold when compared with the value at 15 d ($P < 0.05$). By contrast, the surface roughness of enamel with BAG50.0 showed a decreasing trend with increasing immersion time ($P < 0.05$). Finally, the surface roughness of enamel with BAG37.5 had no significant change in roughness when compared with the control group DW for each of experimental day ($P > 0.05$).

**Microhardness**

The baseline for sound enamel before the experimental process in this study ranged from 300.1 to 346.8. The surface microhardness levels of the enamel disks with experimental groups at 15, 30, and 45 d of exposure to cariogenic conditions are shown in Figure 2B. When the experimental groups within each period were considered, the VHN values of enamel with BAG0 showed a significantly lower value than the other groups containing 45S5BAG ($P < 0.05$). However, the VHN of enamel with BAG50.0 had no significant difference when compared with the control group DW ($P > 0.05$). Similar trends were observed in all experimental periods.

Moreover, based on the experimental periods within each group, the microhardness of all groups except for the control group DW continued to decline with a longer immersion time. However, in the case of the enamel surface with BAG50.0, there was no significant difference in value as compared with the control group DW at all experimental time points ($P > 0.05$) despite an initial drop in value from day 15 to day 30.
SEM Observation

The microstructure images of the enamel disks in the experimental groups at 15, 30, and 45 d of exposure to cariogenic condition are shown in Figure 3. Decreasing the 45S5BAG filler proportion resulted in the formation of a rough and etched pattern. In particular, the enamel surface with BAG0 showed a typical fish-scale appearance, similar to that of the control group Acid, whereas the enamel surfaces with BAG37.5 and BAG50.0 showed no sign of prism outlines, indicating that enamel minerals had been less etched at all experimental days as compared with the enamel with BAG0.

Figure 4 shows that increasing the 45S5BAG filler proportion resulted in more particles of a larger size being embedded in the resin matrix at all experimental days. When the experimental periods within each group were considered, there were no differences among the different exposure times.

Discussion

Although the sealants may strongly bind to the tooth, they do not have the ability to prevent microleakage at the interface between the materials and the tooth structure (Chigira et al. 1994; Geiger et al. 2000). Numerous studies have demonstrated that BisGMA-based sealant materials undergo significant polymerization shrinkage (Irie et al. 2002; Braga et al. 2005). In addition, a microgap could be formed by the partial loss of material from pits or accessory grooves as occlusal forces are applied (Feigal et al. 2000). Consequently, the pit and fissure margins can provide a potential pathway of cariogenic bacterial invasion and biofilm accumulation, resulting in secondary or marginal caries (Hatibovic-Kofman et al. 2001). Therefore, caries-inhibiting materials have been developed for efficient clinical application. Other studies have suggested that the acid-neutralizing property of materials in a cariogenic environment has the potential to reduce the occurrence of dental caries (Itota et al. 2010; Moreau et al. 2011). Based on those results, our present study was designed to evaluate the enamel surface adjacent to a sealant containing 45S5BAG under simulated microleakage conditions that mimic the clinical situation. In previous publications (Hashimoto et al. 2008; Endo et al. 2010), this clinically relevant method was used to examine the ability of materials to mineralize the tooth structure in microgaps, thereby simulating microleakage between the dental materials and the tooth. Therefore, our experimental design applied their modified methods to simulate acid solution–filled gaps to reproduce microleakage between the test materials and enamel surface in vitro. The tooth surfaces were then evaluated with regard to resistance to the acid solution. Initial dental caries occurs when the tooth comes into contact with metabolized acids from oral bacteria, leading to the loss of hydroxyapatite...
mineral from the tooth structure (Yip and Smales 2012). Subsequently, with prolonged exposure to acid, the apatite crystals dissolve, and as this procedure continues, the tooth structure is damaged (McGeouch et al. 2010). In accordance with these changes, in vitro research on demineralized teeth has been reviewed with a variety of evaluation methods (Field et al. 2010). In the current study, surface roughness, microhardness, and image characterization have clearly shown the effects of the experimental material on resistance to enamel demineralization in an acidic environment. The depth of the induced lesions on control group Acid was also examined with SEM by fracturing the tooth disk along the cross section (the images not shown). Despite the limitation of accurate interpretation with SEM images, it was evident that the depths of the induced lesions at 15, 30, and 45 d were approximately 180, 200, and 200 µm, respectively.

The first null hypothesis was rejected. In this study, an enamel surface with an increased 45S5BAG filler proportion showed a strong ability to resist the cariogenic environment. The results showed that the enamel surface with BAG0 was significantly roughened, softened, and etched at each experimental day. Yet, the surface roughness value of enamel with BAG50.0 was significantly lower than in the other groups, and the microhardness values were much higher, indicating that 45S5 BAG is an effective bioactive material for preventing demineralization. Although the volumetric change of each specimen in a cariogenic environment would have given additional information regarding the effect of 45S5BAG, this information was not obtained due to the limitations of the instrument that were used. Still, the results of the SEM imaging suggest that the specimen with a high proportion of the 45S5BAG filler maintained a more sound enamel surface in an acidic environment as compared with enamel with BAG0. According to a previous study (Stoor et al. 1998), the mechanism of the caries-preventing effect of 45S5BAG in a low pH environment is that it can release soluble alkali ions, such as Na⁺ and Ca²⁺, resulting in an increase in the pH value, which is more stable in an acidic solution.

Microleakage is not easily identified in a clinical situation (Irie et al. 2002; Rosin et al. 2002; Neves et al. 2014). If certain subjective symptoms are not present—such as white or brown marginal staining, sensitivity to heat or cold, or spontaneous

![Figure 3. Scanning electron microscopy images of the enamel surface. Control groups—lactic acid solution of pH 4.0 (a, h, o) and distilled water (g, n, u)—and BAG0 (b, i, p), BAG12.5 (c, j, q), BAG25.0 (d, k, r), BAG37.5 (e, l, s), and BAG50.0 (f, m, t) test groups were immersed for 15 d (a–g), 30 d (h–n), and 45 d (o–u). BAG, bioactive glass.](image-url)
Enamel Surface with Pit and Fissure Sealant

pain—it is hard to detect microleakage (Iwami et al. 2000). If microleakage is not detected early, does not receive appropriate treatment, or is left untreated for a long time, it can lead to severe caries progression (Murray et al. 2002). Therefore, to validate the effectiveness of sealants containing 45S5BAG, their prolonged durability should be tested.

The second null hypothesis was partially accepted. The effect of the material in a cariogenic environment was tested after 15, 30, and 45 d. This study showed that increasing the immersion time in a cariogenic environment with the same 45S5BAG content induced a slightly decreasing trend of resistance to the acid solution except for the BAG37.5 and BAG50.0 groups. It is interesting to note that the surface microhardness and roughness of the enamel surface with increasing amounts of 45S5BAG were similar to those of the control group DW even with a prolonged immersion time in the acid solution. It may be that this bioactive behavior of 45S5BAG filler is greatly affected by the local microenvironment and therefore modifies the microleakage between the tooth structure and the dental materials. According to the correlation between

<table>
<thead>
<tr>
<th>Before immersion</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
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<tbody>
<tr>
<td>(a)</td>
<td>(f)</td>
<td>(k)</td>
<td>(p)</td>
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<td>(b)</td>
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<td>(e)</td>
<td>(j)</td>
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Figure 4. Scanning electron microscopy images of the sealant surface. BAG0 (a, f, p), BAG12.5 (b, g, l, q), BAG25.0 (c, h, m, r), BAG37.5 (d, i, n, s), and BAG50.0 (e, j, o, t) test groups were immersed for 15 d (f–j), 30 d (k–o), and 45 d (p–t). Images of panels a–e are of the sealant surface before immersion in the acid solution. BAG, bioactive glass.
opposite sides of the tooth and the sealant, as the amount of 45S5BAG in the resin increased, the demineralization on the tooth surface decreased and was very similar to the surface of a sound tooth. In addition, as the exposure time to an acidic environment increased, the demineralization of tooth surface increased, but there were no signs of deterioration on the sealant surface.

Although this study used bovine enamel disks with large surface areas, which may have exposed different enamel layers because of grinding and polishing, such factors are not believed to have influenced acid resistance as previous studies have indicated (Ganss et al. 2000). In addition, the demineralization properties of human teeth are known to be relatively similar to bovine teeth, unlike equine and ovine teeth (Edmunds et al. 1988). Therefore, further studies are needed to examine human teeth for a more precise interpretation.

A study on the effects of experimental materials must be carried out in a clinically relevant situation to obtain a more complete understanding. A previous study of buccolingual sections of sealants that were placed on occlusal surfaces found that the minimum mean width of the gaps was 10.83 µm and the maximum, 64.83 µm (Vineet and Tandon 2000). Therefore, simulation with a 60-µm gap between the sealant and the tooth was used in the current study to mimic the clinical conditions as closely as possible. The surface microhardness of the enamel specimens in the experimental groups containing 45S5BAG showed no significant difference between 30 and 45 d (P > 0.05). For this reason, our study was performed for 45 d before concluding the experiment.

The present study showed an inhibition of enamel demineralization with sealants containing 45S5BAG in a cariogenic environment for the first time. The results of this study provide useful information for the design of in vitro tests of tooth demineralization in microleakage situations. Additionally, sealants containing a 45S5BAG filler will become promising dental materials to prevent marginal or secondary caries at the marginal gap.

Author Contributions
S.-Y. Yang, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; J.-S. Kwon, K.-M. Kim, contributed to conception, data analysis, and interpretation, drafted and critically revised the manuscript; K.-N. Kim, contributed to design, data acquisition, and analysis, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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References


