Effects of porous-hydroxyapatite incorporated into glass-ionomer sealants

Yukari SHINONAGA¹, Kenji ARITA¹, Takako NISHIMURA¹, Szu-Yu CHIU¹, Hsiu-Hui CHIU¹, Yoko ABE¹, Mie SONOMOTO¹, Kyoko HARADA¹ and Noriyuki NAGAOKA²

¹ Department of Pediatric Dentistry, Osaka Dental University, 1-5-17 Otemae, Chuo-ku, Osaka 540-0008, Japan
² Laboratory for Electron Microscopy, Okayama University Graduate School of Medicine Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-Cho, Okayama 700-8525, Japan
Corresponding author, Yukari SHINONAGA; E-mail: sinonaga@cc.osaka-dent.ac.jp

The purpose of the present study was to evaluate the mechanical and chemical properties of a novel glass ionomer cement for use as a pit and fissure sealant containing a porous hydroxyapatite, namely, apatite ionomer cement (AIC). Control sealant samples were used Fuji III (GIC-S). The experiment sealant samples (AIC-S) consisted of porous spherical hydroxyapatite (HApS) particles added at 28 wt% to GIC-S powder. The GIC-S and AIC-S samples were evaluated through mechanical strength measurements, scanning electron microscopy observations, energy dispersive X-ray spectroscopy analysis, fluoride ion release tests, and antibacterial tests. The flexural strength of the AIC-S was significantly higher than that of GIC-S for each period, 1 h, 24 h and 1 year. The fluoride release dose for AIC-S was consistently higher than that for GIC-S. In addition, the antibacterial properties of AIC-S were superior to those of GIC-S. The novel AIC-S may be a more suitable sealant material for pits and fissures with intact and/or infected enamel.

Keywords: Glass ionomer cement, Hydroxyapatite, Antibacterial property, Fluoride, Caries prevention

INTRODUCTION

Occlusal surfaces of posterior teeth are the most vulnerable sites for dental caries due to their anatomy, which favors plaque maturation and retention. Although the overall caries rate has fallen for populations in industrialized countries, the rate of caries lesions in pits and fissures has not decreased. Moreover, Ripa observed that although the occlusal surfaces represented only 12.5% of the total surfaces of the permanent dentition, they accounted for approximately 50% of caries in school children.

A fissure sealant is a material that is placed in the pits and fissures of teeth in order to prevent or arrest the development of dental caries. Two predominant sealant materials currently in use are resin-based (RB) sealants and glass ionomer cement (GIC) sealants. Glass ionomer cement was developed in the 1970's as an alternative sealant material to RB composite. RB sealants are bonded to the underlying enamel by the use of the acid etch technique. However, GIC adheres to enamel by chemical bonding, so that the sealant application procedure does not require acid etching of the tooth surface or the use of an adhesive. GIC releases fluoride, which is considered to be a major advantage of the material. One of the main clinical advantages of GIC is its ability to bond chemically to dentin and enamel without the use of the acid-etch technique, which makes GIC less vulnerable to moisture. This, in conjunction with active fluoride release into the surrounding enamel, has led to the development and evaluation of GIC as an alternative fissure sealant system, particularly for cases in which moisture control is difficult to achieve. When applied, GIC is not as sensitive to humidity and fluids as RB materials. Several studies have demonstrated that RB sealants are able to stop further progression of carious lesions in pits and fissures, including dentin lesions. The rationale for this approach is that the placement of a sealant isolates the carious lesion from the surface biofilm. This suggests a therapeutic use for sealants in addition to a preventive use. Moreover, a systematic review through a meta-analysis indicated that GIC and RB fissure sealants were equally effective in caries prevention.

However, GIC offers mechanical properties and wear resistance that are inferior to composite resins. Accordingly, several researchers have attempted to overcome these poor mechanical properties. In these approaches, the addition of substances as reinforcements to GIC did not compromise its fluoride release or compressive strength. Recent reports of the addition of hydroxyapatite (HAp) into commercially prepared GIC showed improvement in the cement's mechanical properties, i.e., compressive and flexural strength with good bonding and fluoride properties. Moreover, our previous study demonstrated that the addition of HAp particles with highly reactive properties, such as a high specific surface area, can enhance the mechanical and chemical properties of conventional GIC for dental restoration.

The purpose of the present study was to evaluate the mechanical and chemical properties of a novel GIC containing porous HAp, referred to herein as apatite ionomer cement (AIC), as a pit and fissure sealant. In the present study, the antibacterial properties of AIC were also investigated.
MATERIALS AND METHODS

Preparation of AIC for pit and fissure sealant
Chemically cured GIC for pit and fissure sealing, Fuji III® (GC Corp., Tokyo, Japan; GIC-S), was used as the control group and base material, where the powder was composed primarily of fluoro-aluminosilicate glass and the liquids were polyacrylic acid and water. To produce powder for the pit and fissure sealing material group (AIC-S), 28 wt% of GIC-S powder was replaced with a powder composed of spherical HAp (HApS; Taihei Chemical Industrial Co., Ltd., Osaka, Japan) particles using a high-speed dispersion technique, and AIC-S samples were made by mixing the AIC-S powder and GIC-S liquid at P/L 1:1.2 as recommended by the manufacturer. Based on preliminary experiments, the most suitable HApS content in the GIC-S powder was determined to be 28 wt%.

Mechanical properties
1. Flexural strength test
Beam-shaped samples (n=6/group) of GIC-S and AIC-S measuring 20 mm×3 mm×3 mm for flexural strength tests were prepared in stainless-steel split mold. Cement mix was condensed into the mold and clamped for 10 min at room temperature, unclamped and maintained for 15 min (short-term test) or 50 min (long-term test17) at 100% humidity at 37°C, and then stored in artificial saliva (Saliveht® Aerosol, TEIJIN Ltd., Osaka, Japan) for 35 min (short-term test), or 23 h or 1 year (long-term tests). The samples for the flexural strength test were then subjected to a three-point bending test using a universal testing machine (AG-1kNX, Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 0.5 mm/min.

2. Compressive strength test
Cylindrical samples (n=6/group) of GIC-S and AIC-S measuring 4 mm in diameter×6 mm in height for the compressive test were prepared in stainless-steel split mold following the procedures outlined in ISO9917:1:2007. After being stored in artificial saliva for 24 h or 6 months, the samples were tested using a universal testing machine (DSS-5000, Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 1 mm/min.

Scanning electron microscopy (SEM) observation and energy-dispersive X-ray spectrometry (EDS) analysis
SEM (SU-70, Hitachi High-Technologies Co., Tokyo, Japan) was used to observe the microstructure of the HApS particles and the fractured surface (after flexural strength measurement) of the set cement. For SEM observations, the specimens were sputter coated with osmium. Moreover, cross-sectional SEM specimens were prepared using a cross-section polisher (SM-09020CP, JEOL) and backscatter electron imaging was performed in a field-emission SEM system (JSM-6701F, JEOL, Tokyo, Japan). Elemental analysis was carried out for the original HApS particles, the glass cores, and the matrix in the GIC-S and AIC-S specimens, and for HApS inside the AIC-S specimens using an EDS detector (IncaPentaFETx3, Oxford Instruments PLC., Oxford, UK) and SEM (SU-70, Hitachi High-Technologies Co., Tokyo, Japan). The pressure voltmeter was set at 20 kV for 300 s.

Measurement of fluoride ion release
For the fluoride ion release measurements, samples (n=6/group) measuring 10 mm in diameter×2 mm in thickness were prepared in plastic split mold. The samples were individually suspended by nylon thread in 8 mL of distilled water in sealed containers and were stored at 37°C. For the measurements, each disk was removed from the water, washed by immersion in 2 mL of water, dried on filter paper, and immediately immersed in 8 mL of fresh distilled water for further equilibration. 1 mL total ionic strength adjustment buffer solution (TISAB III, Thermo Fisher Scientific, Beverly, MA) was added to the water sample. The fluoride ion concentration was measured every day for 30 days and then every 5 days for 90 days using a fluoride electrode (Model 9609 BN, Orion Research, Boston, MA) connected to an ion analyzer (Model 720A, Orion Research, Boston, MA).

Antibacterial activity test
For the antibacterial activity test, samples (n=6/group) measuring 10 mm in diameter×2 mm in thickness were prepared in plastic split mold. Antibacterial tests were performed using the Adenosine-5’-triphosphate (ATP) luminescence method, which uses the luminescent mechanism of firefly luciferase. Fireflies generate light by creating a reaction between their own ATP and an enzyme called luciferase inside their bodies. The presence of ATP can be considered proof of the presence of a living organism. The amount of bacterial contamination around the specimens was evaluated by measuring the luminescence level by the ATP-luciferase reaction. *Streptococcus mutans ATCC25175 (S. mutans)* was used as the test bacteria. A volume of 10 μL of the stock culture was transferred to 20 mL of Luria-Bertani broth medium and was incubated at 37°C for 18 h. Cells of the resultant culture were corrected and washed twice with physiologic saline by centrifuging at 4,000 rpm for 10 min, and were then resuspended in brain-heart infusion (BHI) medium. *S. mutans* cell suspensions were spectrophotometrically standardized to a concentration of 8×10³ CFU/mL. The samples were immersed in the *S. mutans* suspension (1,000 μL) and were then incubated at 37°C for 4 h. Subsequently, samples were removed from the *S. mutans* suspensions and the *S. mutans* suspensions were diluted with physiologic saline, and the ATP luminescence intensity of the suspension, which was expressed in relative luminescence units (RLUs), was evaluated using a luminometer (Lumitester C-110, Orion Research, Boston, MA).

Statistical analysis
Data were presented as mean±standard deviation. An analysis of variance was performed using the f-test, followed by the Student’s t-test. The significance level was set at 0.05.
RESULTS

Mechanical properties
The results of the flexural strength and compressive strength tests for GIC-S and AIC-S are shown in Fig. 1. The flexural strength of both GIC-S and AIC-S increased with time in each group. The flexural strength of the AIC-S specimens was significantly higher than that of GIC-S for 1 h, 24 h and 1 year after mixing (p<0.01, Fig. 1-A). There was no significant difference in compressive strength between the AIC-S and GIC-S specimens (Fig. 1-B).

SEM observation and EDS analysis
Figure 2-A shows a representative image of a HApS particle, which were used for AIC-S. The particles were...

![Fig. 1](image)

**Fig. 1** Mechanical properties of GIC-S and AIC-S.
(A) Flexural strength of GIC-S and AIC-S specimens stored in artificial saliva solution for 1 h, 24 h, and 1 year after mixing. (B) Compressive strength of GIC-S and AIC-S specimens stored in an artificial saliva solution for 24 h and 6 months after mixing. Data are the mean and standard deviation. ***p<0.001, **p<0.01, NS: no significant difference (Student’s t-test, α=0.05)

![Fig. 2](image)

**Fig. 2** SEM images of HApS, GIC-S and AIC-S.
Representative SEM images of (A) a HApS particle, (B) the surface of a HApS particle, and (C) the inside of a HApS particle. Representative fractured surface of (D) GIC-S and (E) AIC-S specimens stored in artificial saliva for 24 h after mixing. Representative magnified images of fracture surfaces of AIC-S specimens stored in artificial saliva for (F) 24 h and (G) 1 year after mixing.
spherical and were approximately 20 μm in diameter. They had a porous structure consisting of innumerable clumped nano-sized HAp crystals (Figs. 2-B and 2-C). SEM images of fracture surfaces 24 h after mixture for GIC-S and AIC-S are shown in Figs. 2-D and 2-E, respectively. The fracture surface of AIC-S was smooth, although that of GIC-S was irregular. The matrix component was infiltrated into the porous HApS particles, and there was closer adherence among the glass cores, HApS, and cement matrix. In particular, there were few cracks in the intermediate layer between the matrix and the HApS, and the fractured surfaces of HApS were enriched (Fig. 2-E). Furthermore, the intermediate layer had matured for over one year (Figs. 2-F and 2-G). Figure 3 shows compositional SEM images of GIC-S and AIC-S thin sliced section. Reaction layers were observed on the surfaces of the glass cores, and innumerable nano-particles from the HApS were found to be dispersed within the matrix.

The results of EDS analyses of the glass core, matrix, and HApS are listed in Table 1. Fluorine (F), aluminum (Al), silica (Si), and strontium (Sr) from the glass cores were detected inside the HApS in the AIC-S. Conversely, calcium (Ca) and phosphorus (P) from the HApS were detected in the AIC-S matrix.

Fluoride ion release properties
The accumulated fluoride ion release doses for GIC-S and AIC-S are shown in Fig. 4. The accumulated dose was larger for AIC-S than for GIC-S on all of the experimental days. After storage for 90 days, the value for AIC-S (870.9±60.0 μg/cm²) was significantly higher than that for GIC-S (361.5±16.9 μg/cm²) (p<0.001).

Antibacterial activity
The results of the antibacterial tests conducted by the ATP luminescence method are shown in Fig. 5. The luminescence intensity of the S. mutans suspension in contact with both GIC-S and AIC-S decreased significantly after incubation for 4 h, compared with that of the S. mutans suspension before incubation (p<0.001) and that after 4 h of incubation without a specimen (p<0.001). In addition, after incubation for 4 h, the intensity was significantly lower (p<0.01) using AIC-S than that using GIC-S.

Table 1  EDS analysis

<table>
<thead>
<tr>
<th>Element</th>
<th>Glass core</th>
<th>Cement matrix</th>
<th>HApS</th>
<th>Original HApS particle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GIC-S</td>
<td>GIC-S</td>
<td>AIC-S</td>
<td>HApS inside AIC-S</td>
</tr>
<tr>
<td>F</td>
<td>16.16</td>
<td>12.55</td>
<td>6.75</td>
<td>3.09</td>
</tr>
<tr>
<td>Al</td>
<td>15.73</td>
<td>11.39</td>
<td>9.89</td>
<td>5.46</td>
</tr>
<tr>
<td>Si</td>
<td>13.81</td>
<td>10.65</td>
<td>5.28</td>
<td>1.54</td>
</tr>
<tr>
<td>P</td>
<td>0.48</td>
<td>0.41</td>
<td>5.08</td>
<td>9.60</td>
</tr>
<tr>
<td>Ca</td>
<td>0.00</td>
<td>0.00</td>
<td>11.17</td>
<td>40.43</td>
</tr>
<tr>
<td>Sr</td>
<td>21.06</td>
<td>13.36</td>
<td>11.57</td>
<td>7.48</td>
</tr>
</tbody>
</table>

Elemental compositions (mass %) of each part of the GIC-S and AIC-S and of the HApS particle.
DISCUSSION

Recently published systematic reviews recommend pit and fissure sealants for high-caries-risk children\textsuperscript{21,22}. However, there is concern that bacteria could be trapped within sealed lesions, which could cause dental caries\textsuperscript{23}. Therefore, the fluoride release properties and the antibacterial activity can be considered to be the most important requirements for materials that are to be used as pit and fissure sealants. In particular, GICs have been considered for use as pit and fissure sealants because they release active fluoride ions into the surrounding enamel and exert a cariostatic effect, even after having been removed\textsuperscript{24}. In the present study, it was found that AIC-S offers improved fluoride ion release properties and antibacterial activity, despite being produced by simply adding HAp to GIC-S (Figs. 4 and 5). Moreover, fluoride components into AIC-S were present only in GIC-S powder, i.e., fluoro-aluminosilicate glass, which formed the basis of AIC-S powder. In other words, AIC-S releases more than twice the fluoride released by GIC-S for 90 days, despite the lower fluoride content in AIC-S (Fig. 4). It has been reported that the fluoride released from GIC is mainly from the GIC matrix\textsuperscript{25,26}. SEM observations of the AIC-S fractured surface in the present study (Figs. 2-F and 2-G) indicated that the matrix had infiltrated the HAp\textsubscript{S} particles. It is also possible that the HAp, glass cores, and matrix may react with each other, because the SEM image of the thinly sliced AIC-S section (Fig. 3) and the results of the EDS analysis indicated that the hydroxyapatite became dispersed, dissolved, and then released Ca and P ions into the AIC-S matrix. Conversely, F, Al, Si, and Sr ions from the glass cores were present inside the HAp\textsubscript{S} in the AIC-S (Table 1). These findings suggest that fluoride release occurs in both the matrix and the HAp\textsubscript{S}. However, some researchers have concluded that fluoride release might depend on GICs surface degradation or dissolution of glass-coro\textsuperscript{27,28}. We have not investigated relevance between fluoride release and solubility of AIC-S yet. It was thought that it would be confirmed as future area for our research.

Regarding the antibacterial properties, although the GIC-S did inhibit the increase in \emph{S. mutans}, the antibacterial properties of AIC-S were superior (Fig. 5). Loyola-Rodrigez and Garcia-Godoy\textsuperscript{23} suggested that the bacterial inhibition activity was associated with sealant fluoride release after setting. Our present study revealed an interaction between the fluoride release properties and the antibacterial activity.

Retention is a major problem with GIC sealants, but if this concern can be resolved, such sealants may offer advantages in terms of fluoride release. Many researchers have reported that the addition of reinforcements improves the flexural strength of GIC. However, most studies have reported a decrease in compressive strength or fluoride release properties\textsuperscript{10,13}. In the present study, the compressive strength of AIC-S was the same as that of GIC-S for 6 months, and progressively increased for both materials (Fig. 1-B). Moreover, the flexural strength of the AIC-S was significantly higher than that of the GIC-S for all periods, 1 h, 24 h and 1 year (Fig. 1-A). This suggests that AIC-S may be more suitable as a pit and fissure sealant when applied under occlusal pressure than GIC-S. In the present study, the elemental compositions of the AIC-S and GIC-S matrices were found to be different (Table 1). In particular, F, Al, Si and Sr, which were originally contained in the GIC-S glass cores, were present in the AIC-S matrix. Moreover, the SEM images indicated that the AIC-S matrix had infiltrated the HAp\textsubscript{S} particles. HAp acts as a drug delivery carrier due to its superior adsorptive properties\textsuperscript{27,28}. This may also be the reason why the AIC-S matrix infiltrates the HAp\textsubscript{S} particles. This infiltration caused the compressive strength of the
HApS particles to increase and become comparable to that of the GIC-S matrix. In contrast, the micro-compressive strength of individual HApS particles is only about 0.6 MPa, which is extremely low compared to the value of approximately 180 MPa for fluoroaluminosilicate glass particles. In addition, HAp also has the ability to not only adsorb but also release several substances. Shiozawa also has the ability to not only adsorb but also release Ca and P ions from dentin substance, HAp. The EDS analyses and SEM observations in the present study revealed that both Ca and P ions were released into the matrix from HAp in AIC-S and that the glass cores and the matrix adhered more strongly to each other in AIC-S than in GIC-S. It was suggested that there were reacted layers between matrix and HApS like the intermediate layers between GIC matrix and dentin substance. These results indicate that the glass cores, matrix, and HAp were unified in AIC-S by ion transport among them, which caused the flexural strength of AIC-S to become larger than that of GIC-S, and the compressive strength to become comparable.

CONCLUSION
A novel GIC containing porous HAp, “apatite ionomer cement (AIC)” was investigated as a sealant material for pits and fissures with intact enamel, and for arresting caries. Based on the measured strength, fluoride release properties, and antibacterial activity of AIC, it was found to be a highly promising replacement for GIC as a sealant material.

ACKNOWLEDGMENTS
The present study was supported by the Japan Science and Technology Agency (JST) and the Japan Society for the Promotion of Science (JSPS) through a Grant-in-Aid for Scientific Research (C) #24592975. The authors would like to thank Mr. Katsuhito Kato at the Research & Development Dept., GC Corporation, Tokyo, Japan, for the SEM observations and helpful discussions. No potential conflicts of interest were disclosed.

REFERENCES
26) Meryon SD, Smith AJ. A comparison of fluoride release from


