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| Citation | Nano Biomedicine, 3(1), 191-199<br>https://doi.org/10.11344/nano.3.191 |
| Issue Date | 2011-06 |
| Doc URL | http://hdl.handle.net/2115/55136 |
| Type | article |
| File Information | 3-1_191-199.pdf |

*Kaga et al., Nano Biomedicine 3(1), 191-199, 2011*

**ORIGINAL ARTICLE**

# Mechanical Properties and Ions Release of S-PRG Filler-containing Pit and Fissure Sealant

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## Synopsis

The purpose of this study was to clarify the properties of functional filler-containing pit and fissure sealants (FS). Seventy-two specimens were prepared and divided into three groups of three resin sealants ( S-PRG filler-containing FS, DELTON and Teethmate F1-2.0) and one glass-ionomer sealant (Fuji Ⅲ LC). Each of six discs (6 mm in diameter×3 mm in thickness) was used for 24-h, 4-week and 12-week experiments. Diametral tensile strength (DTS) and ion release were measured. S-PRG FS and Delton showed high values of DTS (23.2 MPa and 23.5 MPa, respectively) after 24 hours of storage. The DTS values of each sealant remained relatively constant. A large amount of fluoride was initially released from the sealants. However, fluoride release did not influence on DTS. S-PRG filler-containing FS released large amounts of strontium, boron and fluoride ions. Filler-containing sealants release large amounts of ions, contributing to antibacterial effects.

***Key words:** S-PRG fillers, pit and fissure sealants, diametral tensile strength, ion release*

## 1. Introduction

Caries in pits and fissures account for a major part of dental caries experience in children and adolescents. Sealants have been shown to be effective for inhibiting the progression of caries when applied to incipient lesions of pits and fissures [1, 2]. The preventive benefits of pit and fissure sealants are only guaranteed when sealants are completely retained with an adequate adaptation to the enamel. Fissure penetration ability in complicated fissure morphology would provide a better sealing of sealants for preventing secondary caries [3-5]. However, acid etching of enamel fissures does not always allow sealants to penetrate into the entire pits and fissures. Polymerization shrinkage of materials could also play an important role in sealant adaptation to the enamel surface. Those insufficient factors should be improved by adding functional fillers for preventing bacterial growth and subsequently re-mineralizing the enamel around the sealant in pits and fissures.

It is postulated that sealants possessing antibacterial properties are advantageous. An *in vitro study* demonstrated that uncured monomers eluted from resin sealants showed antibacterial activity [6]. Fluoride release by sealants is able to inhibit the growth of *S. mutans* [7]. Some studies have shown that not only fluoride but also boron [8] and strontium ions [9, 10] inhibit caries-origin bacterial growth. Moreover, ions released from glass-ionomer cement restoration shifts low pH to neutral [11, 12].

Numerous studies have shown caries-protective effects resulting from long-term fluoride release. In fluoride-releasing restorative materials, resin fillings have high strength with low fluoride release, whereas glass-ionomer cement (GIC) fillings have low strength with high fluoride release. However, no positive linear correlation was found between compressive strength and fluoride-release [13]. Cildir *et al.* showed that surface roughness of fluoride-releasing fissure sealants increased over time, whereas compressive strength decreased [14]. Although the clinical success of a dental material is influenced by its fluoride release behavior, decrease in mechanical properties is a possible disadvantage and/or an adverse effect. Fluoride-releasing fissure sealants could act as intra-oral devices for slow release of fluoride from a clinical point of view.

Recently, a functional S-PRG filler-containing fissure sealant (S-FS) has been developed. This study was carried out to determine whether there is any correlation between mechanical properties and release of fluoride or ions in an experiment performed over a long period using specially designed S-FS. The properties of the new sealant were also compared with those of resin fissure sealants and a glass-ionomer cement fissure sealant.

## 2. Materials and Methods

### *1) Pit and fissure sealant materials*

Three light-cured resin fissure sealants and one glass-ionomer cement pit and fissure sealant used in this study are shown in Table 1. Shofu Inc. (Kyoto, Japan) developed surface reaction-type pre-reacted glass-ionomer (S-PRG) fillers. The principal concept and production process of S-PRG fillers have been described in detail in a report by Fujimoto *et al.* [15]. S-PRG-containing fissure sealant (S-FS) is resin-based fissure sealant containing S-PRG filler (product No. SI-R 20901) with an average particle size of approximately 1.0 μm. DELTON FS+ (DE) is resin F-S containing barium alumino fluoroboro silicate glass fillers. Teethmate F-1 2.0 (TF) is resin F-S containing few fillers. Fuji Ⅲ LC (Ⅲ LC ) is glass-ionomer cement (GIC) formulated for fissure sealing used by mixing fluoroalumino-silicate glass fillers and metacrylate poly-acrylic acid.

### *2) Sample preparation*

Seventy-two specimens for each material were prepared for diametral tensile strength (DTS) tests and fluoride release measurements. Each resin material was placed in a stainless steel mold (6 mm in diameter × 3 mm in thickness) and then covered with polyethylene sheets and pressed between two glass sheets to the thickness of the mold and cured by a light illumination unit for 30 seconds from each side ( Blue shot, Shofu Ltd. Kyoto, Japan). Then discs were removed from the mold and light-cured for a further 30 seconds for each side in direct contact with the discs. Fuji Ⅲ LC was mixed with powder and liquid according to the manufacturer's instructions and light-cured by the same method as that used for resin sealants. The thicknesses and diameters of specimens were measured with a digital caliper. Then each specimen was transferred to a plastic tube containing 3 ml of distilled water. All samples and containers were stored in an incubator at 37℃ and used for each experiments.

### *3) Diametral tensile strength tests*

Figure 1 shows the procedures for the diametral tensile strength (DTS) test and fluoride release measurement. Seventy-two specimens were prepared and divided into three groups of failure at 24 h, 4 weeks and 12 weeks of each material for measurements of DTS and constant fluoride release. Each for six discs (6 mm in diameter × 3 mm in thickness) was light-cured and stored in 3 ml of distilled water at 37℃. The first group of 24 specimens for each material was compression-loaded until failure for the DTS test after 24 h of storage. The second group of 24 specimens, for which distilled water was changed every week, was loaded after 4 weeks of storage. In the last group of 24 specimens, distilled water was changed every week, and the amount of fluoride released was measured every week for 12 weeks. Then specimens were loaded for the DTS test at 12 weeks. DTS was determined by loading until failure using a universal tensile test machine (Instron 3345) with a crosshead speed of 1.0 mm / min, and the initial compressive strength values were determined.

### *4) Fluoride ion measurements*

The third group of 24 specimens for each material scheduled for failure at 12 weeks was used for constant fluoride ion release measurement. Specimens were incubated at 37℃ with 3 ml of distilled water and the amount of fluoride release in distilled water was measured every week for 12 weeks.

Each specimen was clasped with a pair of clean metal forceps and rinsed with 1 ml of distilled water. After that, each specimen was dried for one minute on absorbent paper, placed in a new tube containing 3 ml of fresh distilled water, and incubated for one week at 37℃. Then, the amount of fluoride released from each specimen into 3 ml of distilled water was measured.

Fluoride release was analyzed using a fluoride ion-selective electrode (Orion 9609 BNWP, Thermo Fisher Scientific) connected to an ion analyzer (Orion 2115010 Dual Star pH/ion-meter, Thermo Fisher Scientific). The fluoride electrode was maintained according to the manufacturer's instructions, and the electrode was recalibrated with standard solutions at the start of every measurement. The required concentrations of standard solutions, 0.1 ppm, 1 ppm, 10 ppm and 100 ppm, were prepared from a 100-ppm F stock solution (Orion 940907, Thermo Fisher Scientific). Fluoride ion concentration for each incubation period was determined by adding 0.3 ml of Total Ionic Strength Adjustment Buffer (TISAB III, Orion 940911, Thermo Fisher Scientific) to each test tube. The TISAB buffer was added to provide a constant

**Table 1** Materials and compositions

| Name | Chemical Compositions | Manufacturers |
|---|---|---|
| S-PRG filler containing pit and fissure sealant (S-FS) | UDMA,<br>TEGDMA,<br>S-PRG filler | Shofu<br>(Kyoto, Japan) |
| DELTON FS+ (DE) | Bis-GMA, Sodium fluoride,<br>Triethylene glycol dimetharcylate,<br>Barium alumino fluoroboro silicate glass | Dentsply<br>(York, USA) |
| Fuji Ⅲ LC (Ⅲ LC ) | Fluoroalmino silicate glass,<br>Metacrylate polyacrylic acid, water | *GC*<br>(Tokyo, Japan) |
| Teethmate F-1 2.0 (TF) | TEGDMA, HEMA,<br>MDP | Kuraray<br>(Tokyo, Japan) |

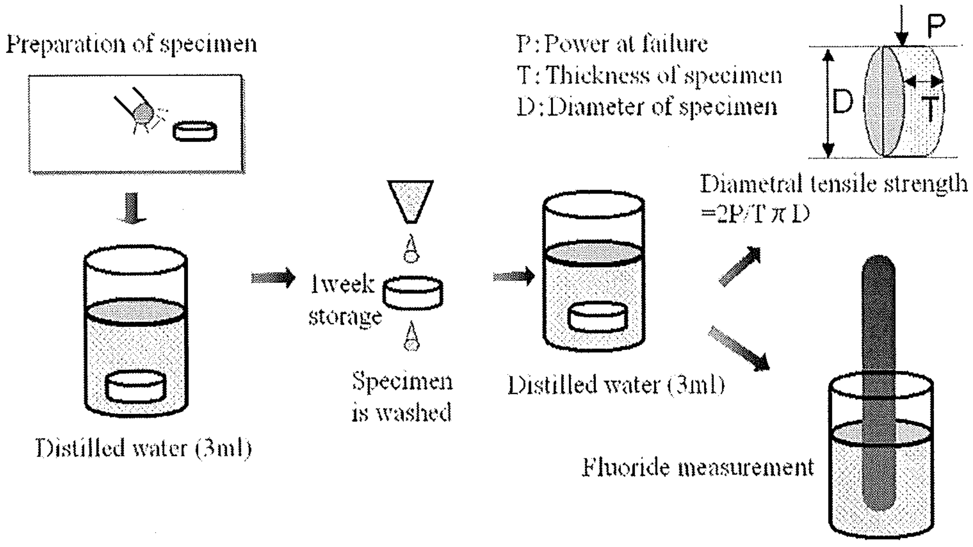

**Figure 1** Procedures for diametral tensile strength test and fluoride release measurements.

background of ionic strength, thereby ionizing the fluoride present in the solution. The fluoride sample containing TISAB III buffer was stirred with a magnetic stirrer for 20 seconds and then the fluoride electrode was positioned immediately in the sample for measurement. The electrode membrane was rinsed well with distilled water and blotted dry between tests. All measured concentrations were presented as parts per million (ppm).

### *5) SEM observation of fractured specimens*

Morphological appearance of the fractured surfaces was observed after DTS tests had been completed for the specimens after 24 h of storage. Fractured specimens were sputter-coated with gold (Ion sputter E-1030, Hitachi Ltd, Tokyo, Japan) and observed by a scanning electron microscope (SEM; S-4000, Hitachi Ltd., Tokyo, Japan).

### *6) Element analysis by ICP*

Three discs of 13 mm in diameter × 1 mm in thickness for each sealant were light-cured by the same procedure as that described above. Specimens were incubated in 5 mL of distilled water for 24 h at 37℃. Element analysis of ions (silicon, strontium, aluminum, barium, boron, phosphate and calcium) released from the S-PRG filler-containing sealant and other sealants was performed using inductive coupled plasma atomic emission spectroscopy (ICP-AES; ICPS-8000, Shimadzu Co., Kyoto, Japan). Analysis was conducted after preparation of calibration curves corresponding to each element (standard solution concentrations: 0, 2.0, 20 and 50 ppm for silicate; 0, 0.5, 5 and 20 ppm for strontium; 0, 0.5, 5 and 20 ppm for aluminum; 0, 0.5, 5 and 20 ppm for barium; 0, 0.5, 5 and 20 ppm for boron; 0, 0.5, 5 and 20 ppm for phosphate; and 0, 0.5, 5 and 20 ppm for calcium). For each ion, the amount released into solution was expressed in ppm. ICP was not used for analysis of fluoride; fluoride was measured using an ion analyzer and fluoride electrode by the same procedure as that described above.

### *7) Statistical analysis*

The differences in DTS values were analyzed by two-way ANOVA and Tukey's test with a p-value of less than 0.05 regarded as significant. Fluoride ion release measurements were statistically analyzed by one-way ANOVA followed by the Tukey-Kramer post-hoc test at a significance level of 0.05.

## 3. Results

Figure 2 shows the mean values and 1 SD of DTS. Sealants with the same letter are not significantly different in DTS values throughout the storage periods of 24 h, 4 weeks and 12 weeks. The DTS values of each sealant remained relatively constant. Comparisons of DTS with S-FS and DE in the measurement intervals showed that S-FS in 4 weeks was lower than DE. DTS values of S-FS and DE were significantly higher than those of TF and Ⅲ LC in all storage periods ($p < 0.05$). TF was lowest in resin sealants. Ⅲ LC showed statistically lower DTS values than those of the resin sealants ($p < 0.05$).

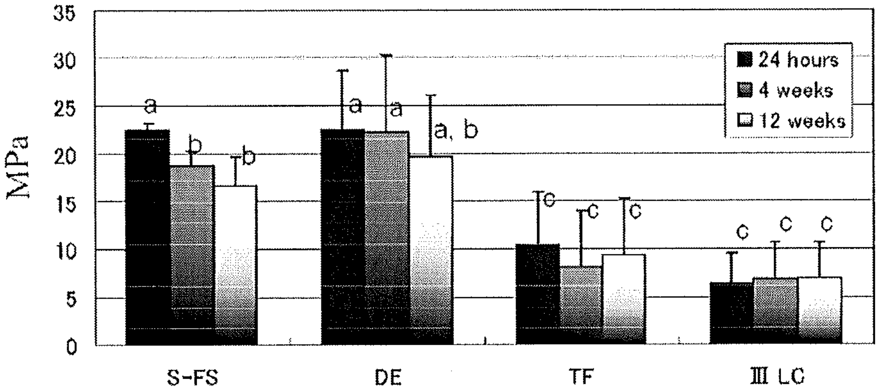

**Figure 2** DTS of S-FS, DE, TF and Ⅲ LC after aging for 24 hours, 4 weeks and 12 weeks. The same letters indicate no significant difference by statistical analysis.

Figure 3 shows fluoride release of sealants. All sealants released fluoride at each of the time intervals until 12 weeks. The largest amount of fluoride was released during the first week, and the amount released sharply dropped in the second week. The amount released then remained small and constant after about three weeks. Fluoride release of Ⅲ LC was highest in the first week and decreased until 3 weeks and was higher than that of resin sealants at the all intervals. There were significant differences between resin-based fissure sealants and Ⅲ LC in the amounts of fluoride released (Tukey-Kramer post-hoc test, $p < 0.05$). Then fluoride release

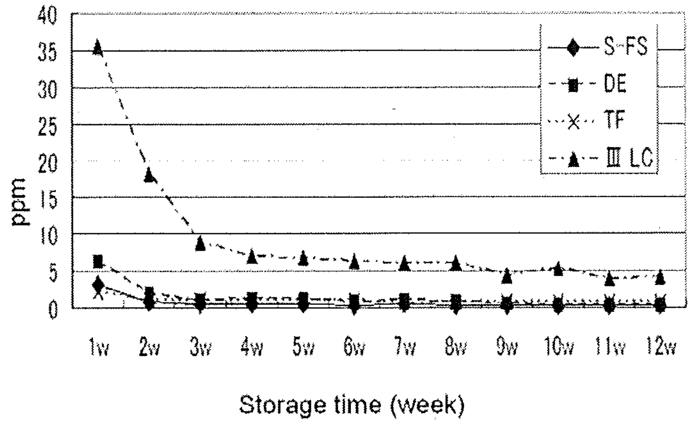

**Figure 3** Concentrations of fluoride released from fissure sealants into distilled water up to 12 weeks.

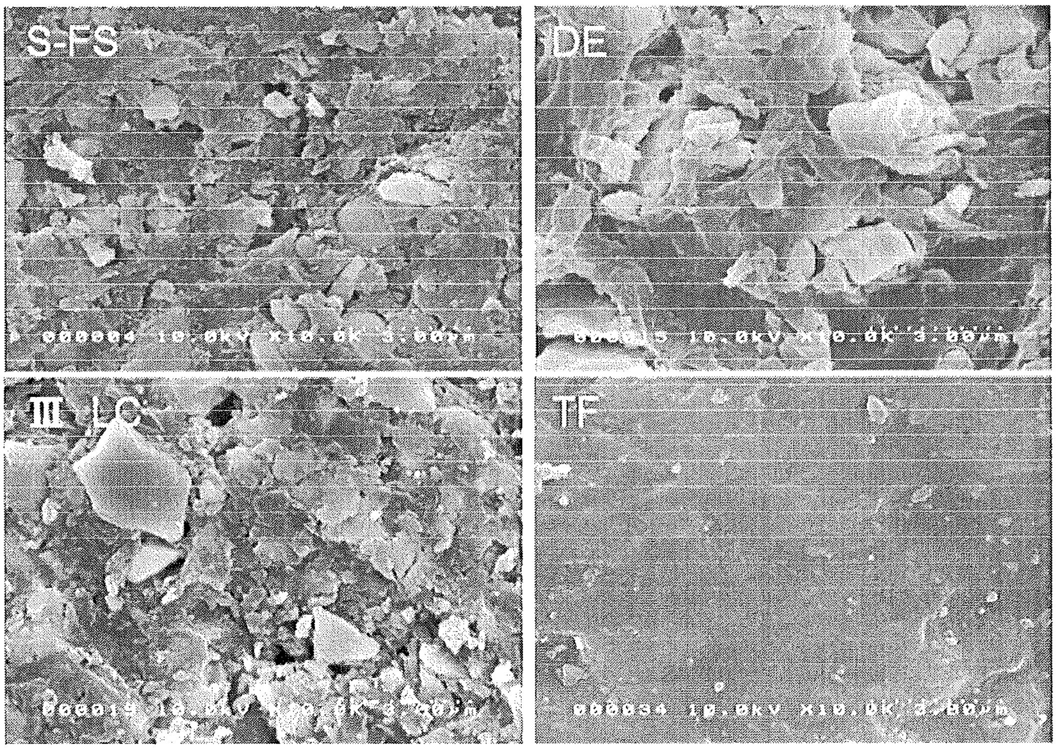

**Figure 4** SEM photographs of fractured microstructures inside each specimen after DTS tests at 24 hours.

was constant up to 12 weeks. Fluoride release of DE was significantly higher than that of S-FS only in the first week ($p < 0.05$). No significant difference was found throughout the experimental period. There was smallest fluoride release by TF from 3 weeks until the end of the experiment.

Figure 4 shows representative regions of the fracture surfaces of sealants after DTS tests at 24 h of storage. Cracks in the microstructures were caused by dehydration during preparation for SEM analysis. Different shrinkage rates of fillers and resin matrix caused detachment of fillers from the matrix resin. Therefore, glass filler particles appeared to be more loosely bonded to the matrix in S-FS and DE. Microstructure porosity or voids were observed. Approximately 40% of the filler particles in S-FS were around 1 μm in diameter. DE also contained a large amount of particles of different sizes in resin matrix. TF contained small particles dispersed in the polymer matrix. Ⅲ LC exhibited a more tightly integrated glass particle-polymer matrix surface showing typical features of light-cured glass-ionomer cement compared to S-FS and DE. Different particle sizes of alumino-silicate glass were observed in the microstructures.

Table 2 shows results of analysis by ICP of ions released from sealants. Data for silicate, strontium, aluminum, barium, boron, titanium, phosphate, calcium ions and their concentrations released in distilled water are shown. S-FS released the largest amount of boron (4.3 ppm), followed by DE (1 ppm boron) and Ⅲ LC (0.7 ppm boron). S-FS released 2 ppm of strontium. Ⅲ LC released a large amount of strontium (6.4 ppm) and DE released no strontium. On the other hand, DE released 3.9 ppm of barium. Ⅲ LC released large amounts of silicate and aluminum, which are structure constituents of Ⅲ LC. Fluoride release was highest in Ⅲ LC, followed by DE and S-FS. TF released the smallest amount of fluoride.

**Table 2** Amounts of ions released from sealants

| Sample | Elements (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Si | Sr | Al | Ba | F | B | P | Ca |
| S-FS | 0.3 | 2 | 0.4 | 0 | 6.1 | 4.3 | 0.0 | 0.2 |
| DL | 0.2 | 0.0 | 0.4 | 3.9 | 9.2 | 1.0 | 0.0 | 0.1 |
| TF | 0.2 | 0.4 | 0.2 | 0 | 2.7 | 0.0 | 0.2 | 0.4 |
| ⅢLC | 33.4 | 6.4 | 10.4 | 0 | 15.8 | 0.7 | 1.5 | 0.0 |

## 4. Discussion

For caries prevention programs in pediatric dentistry, fluoride-releasing fissure sealants are used as a means to provide a continuous low level of fluoride in the oral cavity [16-18]. Fluoride-containing fissure sealants have been shown to significantly reduce demineralization in adjacent enamel compared to the effects of fissure sealants not containing fluoride [18, 19]. In the present study, all of the fissure sealant materials released fluoride. Throughout the study, glass-ionomer fissure sealants released more fluoride than did resin-based fissure sealants. This difference in fluoride release arose from differences in material formulation and contents of ion-releasing particles as well as the amount of fluoride incorporated in the material. Filler composition and particle size also have a significant influence on fluoride release [20-23]. In previous studies, a "burst effect", in which a large amount of fluoride was released during the first 24 h, was observed, and this was followed by a sharp drop in the amount of fluoride released on the second day and then a gradual decrease [14, 24-28]. In our study, the amount of fluoride released was largest in the first week. Then, the amount of fluoride release was gradually decreased. Among sealants tested, Ⅲ LC released a significantly larger amount of fluoride than the amounts released by resin sealants. The burst effect of fluoride release in sealants may have some beneficial biological effects, such as a bactericidal effect, immediately after sealant application.

Long-term gradual fluoride release is likely to result in deterioration of the mechanical properties of the material. In the process of mastication, compressive strength and surface roughness are very important for sealants properties. Therefore, it is important to determine the correlation between decrease in mechanical properties and long-term fluoride release. Results of DTS tests in the present study showed that long-term fluoride release from sealants did not reduce the mechanical properties. Clidir *et al.* [14] reported significant reduction in compressive strength of sealants after 24 h to 12 weeks of storage. However, no positive correlation exists between compressive strength and fluoride release in resin-based and glass-ionomer fissure sealants [14, 29, 30]. GIC fissure sealants have lower mechanical properties with a high rate of fluoride release, whereas, resin-based fissure sealants are mechanically stronger and release a small amount of fluoride [14]. Although Ⅲ LC released a constant high level of fluoride, DTS remained constant until 12 weeks. However, it was not clear in this study whether filler addition in resin sealants improved the mechanical strength or not.

An increase in fluoride concentration in saliva and in adjacent dental hard tissues would be of some benefit to sites at risk of recurrent caries as well as in preventing further demineralization [31, 32]. In the oral cavity, some fluoride release occurs from the oral fluid for ionic substitution of the mineral phase of enamel. Remineralization is precipitated by the existence of calcium and phosphate, and this specific biomechanics is promoted by the application of fluoride [33, 34].

The amounts of ions released from sealants were measured by ICP in the study. S-FS released considerable amounts of boron followed by fluoride and strontium into distilled water after 24 h of storage. S-PRG filler is produced by the reaction between glass powder and polyacrylic acid and forms a gel phase on the glass core of the filler surface. Soluble ions were discharged to the gel phase, which is the source of ion release [15]. S-FS and Ⅲ LC released strontium. Guida *et al.* [9] reported that the antibacterial effect in the use of GIC is associated more with strontium release than with fluoride release or with a synergistic process involving strontium and fluoride ions. Strontium ions released from RMGIC are rapidly exchanged for calcium ions in the superficial enamel. Synergistic effects with fluoride and strontium ions could promote antibacterial activity [10]. When incorporated together, strontium and fluoride improved the crystallization of carbonated apatite and markedly reduced its acid reactivity [35]. That is, strontium had the capacity to enhance enamel remineralization when used in conjunction with fluoride. Therefore, release of a large amount of strontium from sealants is important for prevention of caries.

S-FS released the large amount of boron among tested sealants. In an *in vivo* study using rats, boron showed antibacterial and anti-inflammatory properties [8]. An important role of boron in plaque as an antibacterial effect should be recognized. Nakajo *et al.* [36] reported that Si, Al and F released from GIC inhibited the fall in pH and acid production as well as streptococci growth. Thus, ion release is very important. Both bacterial growth and subsequent bacterial acid production would be suppressed by ions released adjacent to a functional filler containing sealant, and then a cariostatic resistance environment would be established. Ion release is also possibly effective for shifting low pH to neutral pH. Genchou et al. [37] investigated pH changes of sealants for 24-h incubation at 37℃ in 5 ml of pH 4.0 lactic acid solution. They found that pH of S-FS was shifted from 4 toward neutral pH 6.5. Ⅲ LC was pH 5.6, DE was pH 4.7 and TF was pH 2.9. S-FS had the strongest action for shifting acidic pH to neutral pH. The pH shift of S-FS was obtained by the modulatory effect of S-PRG filler on acidic solutions with more ions being released in lactic acid solution like resin-modified GIC [15].

## 5. Conclusion

S-FS shows high strength and constant fluoride ion release. Strontium and boron ions are thought to contribute to the antibacterial effect and re-mineralization together with fluoride released from sealants. Bacterial acid production de-mineralizes enamel and destroys the sealant-enamel adhesive interface resulting in micro-leakage and secondary caries around the sealants. Our results suggest that S-PRG FS plays as an intra-oral device for slow release of fluoride and ions, and pH-controlled buffer for re-mineralization and antibacterial growth.

## 6. Acknowledgments

This study was supported by a Grant-in-Aid for General Scientific Research (C) (No. 22592273) from the Ministry of Education, Science, Sports and Culture of Japan. The authors wish to express their sincere thanks to the staff of Industrial Research Center of Shiga Prefecture for ion analysis by ICP-AES; ICPS-8000, Shimadzu Co.

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(Received: December 25, 2010/
Accepted: February 28, 2011)

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