# 新規シーラント用セメントの化学的および生物学的特性について

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Newly developed glass ionomer cement

[Objectives]

Pit and fissure sealants are recognized as an important adjunct approach to prevent caries. Glass-ionomer cements (GICs) and composites are currently used clinically as pit and fissure sealants. Although the strength properties of GICs are inferior to those of composites, GICs exhibit a number of advantages over composites such as fluoride release, biocompatibility and adhesion. The aim of this study was to investigate the chemical and biological properties of newly developed bioactive cements, modified such that they are largely composed of calcium, phosphate and fluoride. In addition, we investigated whether newly developed bioactive cements have the potential to further protect surrounding hard tissue and enhance remineralization of demineralized tissue by additional ion release.

The research questions are: what effect do the newly developed bioactive cements have on (1) compressive strength; (2) calcium, phosphate and fluoride ion release; (3) fluoride release and recharge; (4) antibacterial effects; (5) inhibition of biofilm formation; and (6) cell proliferation activity.

[Materials]

We developed four types of novel GIC based on Fuji VII® (Fuji VII; GC, Tokyo, Japan) GIC, modified with phosphate and fluoride and including 0 mol% (0YG), 5.4 mol% (5.4YG), 8.9 mol% (8.9YG) and 10.7 mol% (10.7YG) of calcium, respectively. Fuji VII, Fuji III® (Fuji III; GC), BeautiSealant® (BS; Shofu, Kyoto, Japan) and Teethmate F-1® (TM; Kuraray, Tokyo, Japan) were used as control materials.

[Methods]

1. X-ray photoelectron spectroscopy (XPS)

The chemical composition of the surface was analyzed using XPS for all materials.

2. Compressive strength

Compressive strength tests were performed following JIS T6607 methods.

3. Ion release

Specimens (20 mm high × 2 mm diameter) were prepared and stored in water (pH 6.9) or aqueous lactic acid (pH 5.0) or aqueous citric acid (pH5.0). Ion release of calcium, phosphate and fluoride after 24 hours storage were determined using atomic absorption spectroscopy, colorimetry and an ion-specific electrode, respectively.

4. Fluoride releases and recharge

Disks were prepared, allowed to age for 24 hours, then placed into aqueous lactic acid. Fluoride ion release was measured after 1, 3, 6, 12, 24 and 168 hours. The samples were then soaked for 5 min in an aqueous 9,000 ppm fluoride solution, after which fluoride release was remeasured at 1, 3, 6, 12, 24 and 168 hours.

5. Antibacterial effect

Standing specimens were incubated with *S. mutans* (*S. mutans* JCM 5705) in TY medium (tryptic soy broth 30 g/l, yeast extract 5 g/l). Bacterial suspensions were independently incubated with TY medium for 24 hours at 5% $CO_2$ and 37°C. Viability was determined by colony-forming units.

6. Inhibition of biofilm formation

Cell strainers with standing specimens were incubated with *S. mutans* (*S. mutans* JCM 5705) in TY medium containing 0.5% sucrose in a glass-coated dish at 5% $CO_2$ and 37°C. After 24 hours, Calcein-AM staining was performed. Biofilms were imaged on a confocal laser scanning microscope (TE2000-E; Nikon, Tokyo, Japan).

7. Cell proliferation activity

Human gingival epithelial cells (HGEP; CELLnTEC Advanced Cell Systems, Bern, Switzerland) were incubated with CnT-Prime and Epithelial Medium (CELLnTEC Advanced Cell Systems) at 5% $CO_2$ and 37°C. Next, the soaked specimens were moved to cell culture medium. After 24 hours, WST-1 was added to each well. Absorbance was measured after 1 hour.

8. Statistical analysis

The data obtained were compared with those of the control group using one-way ANOVA and Tukey's test.

[Results]

1. X-ray photoelectron spectroscopy (XPS)

It was found that 5.4YG, 8.9YG and 10.7YG contain calcium and increased phosphate and fluoride compared with Fuji VII and Fuji III.

2. Compressive strength

The GIC groups, including the newly developed samples, showed no significant differences in compressive strength after 1 and 7 days; 8.9YG had a significantly higher compressive strength than Fuji VII after 35 days.

3. Ion release

Calcium ion release was observed only in 5.4YG, 8.9YG and 10.7YG. Phosphate ion release was observed in 8.9YG, 10.7YG, Fuji III and BS. The rates of fluoride ion release from newly developed GICs were significantly greater than those of Fuji VII, Fuji III and BS.

4. Fluoride release and recharge

All materials except TM can be recharged with fluoride ions. The initial burst of fluoride ions for TM was significantly higher than in other materials; however, TM has no fluoride recharge capabilities.

5. Antibacterial effect

Compared with the control group, which did not release fluoride ions, all materials showed significantly stronger antibacterial effects.

6. Inhibition of biofilm formation

Compared with the control group, which did not release fluoride ions, all materials were able to inhibit biofilm formation. The newly developed GICs and BS showed less biofilm formation than FujiVII and FujiIII.

7. Cell proliferation activity

No significant difference was found among materials.

[Discussion]

The compressive strength test showed that there is no effect on mechanical properties under the neutral conditions; however, because the newly developed GICs have increased ion uptake under acidic conditions, compressive strength may be decreased in these samples compared to FujiVII and FujiIII. Although our purpose in this study is clinical application as pit and fissure sealant materials, it can be expected that the newly developed GICs have good effectiveness because of their high ion release, even though this decreases their mechanical strength. In addition, the pit and fissure sealants that were observed in the follow-up appointments as partial loss can be retreated.

A higher release of calcium ions from the material was observed under acidic conditions. Previous studies have shown that citric acid is a trioritic acid, which can chelate transition metals. The results indicate that these effects cause collapse of GIC cements.

For caries prevention, fluoride application is used as a means to provide a continuous, low level of fluoride in the oral environment. Studies have suggested that GICs and BS can be expected to provide superior continuous fluoride ion release compared to TM, which has no fluoride recharge capability.

The results of the antibacterial effect and inhibition of biofilm formation tests showed that all materials have antibacterial effects against *S. mutans*. All materials release fluoride ions; thus, it is suggested that the antibacterial effect against *S. mutans* may be caused by the fluoride ion.

[Conclusion]

Three of four newly developed GICs modified with calcium, phosphate and fluoride ions - 5.4YG, 8.9YG and 10.7YG - were found to be superior to other sealant materials.