Abstract

Application of glass ionomer cement containing a newly developed adhesive monomer to indirect pulp capping material

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Kohei Sato
Division of General Dental Sciences 1,
Department of Oral Rehabilitation,
School of Dentistry,
Health Sciences University of Hokkaido

[Introduction]

In dental practice, pulp removal may be required when caries reaches the pulp. However, since unmyelinated teeth often cause fractures and tooth extraction, preservation of the pulp is important for prolonging the life of the tooth. Indirect pulp capping is one of the pulp-conserving therapies, and calcium hydroxide preparations are recommended as pulp capping materials to be used. There are issues such as the need for temporary restoration of composite resin (CR) and glass ionomer cement (GIC).

In recent years, 4-MET-Ca (calcium 4-Methacryloxyethyl trimellitate, CMET) has been developed as a new adhesive monomer, and when blended with a bonding material, it improves initial adhesiveness to dentin, improves long-term durability, and has the ability to induce calcification. Etc. have been reported.

On the other hand, GIC was a material that was used less frequently than CR from the viewpoint of aesthetics and adhesiveness, but with the increase in root surface caries in recent years, it has been regaining attention as a material with anti-cariogenic properties and acid resistance.

In this study, we investigated the material and biological properties of a pulp capping material with excellent sealing properties and calcification-inducing ability by adding his CMET to a conventional GIC.

[Material and method]

1. Material properties

1) Materials

In the experiment, Fuji VII was used as the conventional GIC, and CMET and 4-MET were used as the adhesive monomers. C-GIC added 10 to 20% (w/w) of CMET to GIC, and 4M-GIC added 10 to 20% (w/w) of 4-MET to GIC. The control was GIC.

2) Measurement of pH and eluted ions

Various disc-shaped samples were prepared, immersed in ultrapure water for 1 day, and then the pH of the eluate and the amount of each ions (Si, Al, Sr, Ca, F) were measured (n=5).

3) Evaluation of compressive strength

Various columnar samples were prepared, immersed in distilled water for 1, 7, and 28 days, and then the compressive strength was measured (n=6).

4) Evaluation of shear bond strength and observation of fracture surface

The central part of the crown of a caries-free human-extracted molar (n=75) was cut in the axial direction, and various cement hardened bodies were planted on the tooth surface. After being immersed in distilled water for 1, 7 and 28 days, a shear bonding test was performed (n=10), and the fracture surface was observed by SEM.

5) Antibacterial test

Antibacterial tests were conducted on four bacterial species, *S. aureus, S. mutans, A. viscosus,* and *C. albicans,* using various cement eluates, and the viable bacteria count was measured.

2. Biological properties

1) Preparation of eluate

Disc-shaped samples of 20% C-GIC, 18% 4M-GIC, and GIC were immersed in ultrapure water for 1 day, and various eluates after sterilization filtration were used for the experiment. GIC were used as the control.

2) Cell culture

Human dental pulp stem cells (hDPSCs) were cultured in DMEM containing 10% FBS under 37℃, 5% CO_2 and 95% Air, and the cells of the 4th passage were used for the experiment. In the differentiation-inducing medium, β-glycerophosphate

(10 mM) and ascorbic acid (50 μg / mL) were added to DMEM containing 10% FBS when the cells were confluent, and the medium was changed every 3 days.

3) Observation of cell morphology

Various eluates were set to 10, 20, 30, 40, 50% (v/v) and observed days 3 of sowing.

4) Evaluation of cell proliferation ability

In the cytotoxicity test, various eluates were set to 10, 20, 30, 40, 50% (v/v) and measured on the 3rd day of seeding (n=6). In the cell proliferation test, the eluate was set to 1, 2, 5, 10, 15, 20% (v/v) and measured on days 5 of seeding (n=6).

5) Evaluation of ALP activity

Various eluates were set to 2.5, 5.0, 10% (v/v) and measured on days 9, 12, and 15 of sowing (n=3).

6) Evaluation of Alizarin red staining

Staining and quantitative analysis of Ca were performed 21 and 28 days after sowing at the same concentration as ALP activity (n=3).

7) Statistical processing

Compressive strength and shear strength were performed by Tukey's method after two-way ANOVA, and antibacterial test was performed by Games-Howell method after one-way ANOVA. For others, after one-way ANOVA, statistical analysis was performed by Tukey's method under the condition of significance level of 5%.

[Result]

1. Material properties

The pH of the C-GIC group and 4M-GIC group decreased in a concentration-dependent manner, and the value of the 4M-GIC group was significantly lower.

In the C-GIC group, the eluted of all ions was significantly higher at the 20% addition amount, and in the 4M-GIC group, the values other than Ca and F were significantly higher at the 20% addition amount. Ca ions was observed only in the C-GIC group.

In the evaluation of compressive strength, it decreased in the C-GIC group and the 4M-GIC group in a concentration-dependent manner.

In the evaluation of shear bond strength, the C-GIC group was significantly higher than the 4M-GIC group, showing the same adhesive strength as the control. In SEM observation, there were many cohesive fractures on the 1st and 7th days of immersion, and mix fractures and interface fractures on the 28th. In the 4M-GIC group, interface fracture occurred on the 1st day of immersion, and mix fracture and cohesive fracture occurred on the 7th and 28th days.

As a result of the antibacterial test, no decrease in viable bacteria count was observed in all cement eluates and no antibacterial property was observed for *C. albicans*. However, it showed significant antibacterial properties against the control group against the other three bacterial species. There was no significant difference between the three cement groups, but the viable bacteria count tended to decrease in the order of 4M-GIC group, C-GIC group, and GIC group.

2. Biological properties

The cell morphology tended to lose the tendency of close adhesion between cells in a concentration-dependent manner in all groups, and this tendency was stronger in the 4M-GIC group. In the cytotoxicity test, toxicity was shown in a concentration-dependent manner in all groups, but it was low in the C-GIC group and significantly lower in 10% C-GIC. In the cell proliferation test, the C-GIC group showed significant proliferation compared to the other groups.

ALP activity tended to increase over time in all groups, and ALP activity on day 15 maintained significantly higher activity in the C-GIC group and GIC group.

As a result of alizarin red staining, Ca deposition tended to increase over time, and on the 28th day, the 10% C-GIC group showed a significant increase in Ca

deposition.

[Consideration]

In the material properties, the pH of the C-GIC group and 4M-GIC group decreased in a concentration-dependent manner, which is thought to be due to the elution of unreacted polyacrylic acid due to the decrease in the powder-liquid ratio (P/L). In previous reports, the eluate pH of CMET and 4-MET alone was 7.02 and 2.16, respectively, suggesting that the acidity of 4-MET affects the pH decrease of the 4M-GIC group. In addition, when the cement mud being cured was acidic, it was considered that the powder particles were eroded and the sustained release of various ions in the C-GIC group and 4M-GIC group increased. In addition, due to the effect of P/L reduction, the compressive strength decreased in both the C-GIC group and the 4M-GIC group in a concentration-dependent manner. This is probably because the amount of glass core decreased and the amount of matrix increased. Shear bond strength was significantly higher in the C-GIC group than in the 4M-GIC group and was comparable to that in the GIC group. This is because in the C-GIC group, Ca ions derived from C-MET may have been involved in the cross-linking reaction to acquire new adhesive strength, and in the 4M-GIC group, Al eluted from the glass by polyacrylic acid, it is possible that a part of this reacted with 4-MET and the normal curing reaction was inhibited. The antibacterial activity of the C-GIC group was significantly higher in *S. mutans* and *A. viscosus* than in the no addition group. No significant difference was observed between the control group and the 4M-GIC group. However, as a result of the addition of CMET lowering the pH of the eluate and increasing the amount of various eluted ions, it is possible that F and Al ions and 4-MET, a resin monomer, inhibited the growth of bacteria.

In biological properties, the 4M-GIC group showed a concentration-dependent suppression of cell proliferation, a tendency for ALP activity and Ca deposition to stagnate, but the C-GIC group adjusted to the optimum concentration showed a tendency to promote cell proliferation, maintain high ALP activity, and increase

Ca deposition. Biological functions such as cytotoxicity, proliferation, and differentiation are known to be affected by various ions and compounds. It is possible that the expression of cytotoxicity in this study was more strongly influenced by the resin monomer 4-MET than by the eluted ions. In addition, the C-GIC group possesses only CMET-derived Ca ions, and its involvement is thought to have affected the low cytotoxicity, high proliferation, and increased Ca deposition amount of the C-GIC group.

[Conclusion]

C-GIC is expected to remineralize dentin as a bioactive material with sustained release of Ca, and has dentin adhesion equivalent to that of conventional GIC and excellent biocompatibility, and protects the pulp. It was suggested that it could be applied as a indirect pulp capping material.