ORIGINAL

Effect of Conventional Glass-Ionomer Cement with Zinc Oxide in Inhibiting Dentin Demineralization *In Vitro*

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Abstract: The aim of this study was to enhance the demineralization-inhibiting effect in dentin using a mixture of zinc oxide and conventional glass-ionomer cement (GIC). In the experimental group, cavities in bovine dentin were filled with GIC mixed with zinc oxide (GIC-Z group). In the control group, cavities were filled with GIC alone (GIC group). Specimens were immersed in acidic solution at 37°C for 0, 3, 6 or 9 days. Dentin was then observed under contact microradiography (CMR) for demineralization. For day 9 specimens, mineral loss was measured and specimens were observed under scanning electron microscopy (SEM). In addition, ordinary dentin samples were also observed for normal dentinal tubules. Electron probe microanalysis (EPMA) was used to investigate zinc infiltration into day 9 specimens in the GIC-Z group. Concentrations of Mg²⁺, Ca²⁺ and F⁻ in the acidic solution were measured with day 9 specimens in both GIC-Z and GIC groups. Mechanical properties of compressive strength and disintegration rate were compared between the GIC-Z and GIC cements.

On CMR images, subsurface demineralized lesions were observed in days 3, 6 and 9 specimens. Total mineral loss did not differ substantially between day 3 and day 6 specimens. Mineral loss was slightly reduced with the day 9 specimen in the GIC-Z group. Scanning electron micrograph of the polished surface before acid treatment showed that all dentinal tubules were open. In micrograph of day 9 specimen in the GIC group, many dentinal tubules were closed. In micrograph of day 9 specimen in the GIC-Z group, most dentinal tubules were closed. By EPMA analysis, intensity of Zn was highest at the dentin face in the GIC-Z group. Concentrations of Mg²⁺ and Ca²⁺ in the acidic solution were significantly lower in the GIC-Z group than in the GIC group. No difference in F⁻ concentration was found between the two groups. Mechanical properties were not significantly different between the two groups.

In conclusion, addition of zinc oxide to GIC contributes to inhibit dentin demineralization without major changes in mechanical properties compared to GIC.

Key words: glass-ionomer cement, zinc oxide, dentin demineralization

Introduction

In today's aging Japanese population, the incidence of root caries is on the rise. Conventional glass-ionomer cement (GIC) and composite resin represent the leading restorative materials for

treating this kind of caries. Of the two, GIC is better for inhibiting secondary caries, as it contains fluoride^{1,2,3)}. Fluoride is essential for decreasing the cariogenic activities mediated by biofilm^{4,5)} and enzymes⁶⁾. Furthermore, fluoride retards demineralization by increasing mineralization. Consequently, GIC represents a good dental material for preventing or reducing caries, including arrested and secondary caries in clinical situations. Conversely, zinc oxide

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Fig. 1.

Preparation of dentin specimen. Enamel was ground cervicolabially with #1200 waterproof silicon carbide paper. Dentin was exposed in areas of 7×7 mm² and then covered with inlay wax. A cylindrical cavity (2 mm in diameter and 1 mm in depth) was prepared in the center, and the cavity was filled with GIC-Z or GIC and coated with cavity varnish. After immersed in distilled water for 24 hours, the specimen was polished and cleaned by ultrasonication and immersed in a pH 5.0 acidic solution at 37°C.

is suitable for lining⁷⁾, pulp capping⁸⁾, sealing^{9,10)} and root canal filling¹¹⁾. Recently, toothpaste containing both zinc oxide and fluoride has been developed to prevent root surface and dentinal caries¹²⁾.

This study investigated the effects of mixing GIC with zinc oxide (GIC-Z) on dentinal caries and the degree of inhibition of dentinal demineralization.

Materials and Methods

Materials

Powdered zinc oxide (Wako Pure Chemical Industries, Tokyo, Japan) mixed with GIC (Fuji Ionomer Type II; GC Corporation, Tokyo, Japan) to 5 wt% was used in the experimental group (GIC-Z group). GIC alone was used in the control group (GIC group). In a preliminary study, 10 wt% of zinc oxide was used, but this proved difficult to handle, and 5 wt% was used in subsequent experiments.

Preparation of dentin specimens (Figure 1)

Extracted bovine incisors were stored frozen until use. Enamel was ground cervicolabially using #1200 waterproof silicon carbide paper to expose dentin surface in areas of 7×7 mm². The remaining areas were then covered using inlay wax. Cylindrical cavities (2 mm in diameter and 1 mm in depth) were prepared in the centers of the exposed dentin areas. The cavities were filled with either GIC or GIC-Z, and the fillings were coated with cavity varnish (20% vinyl acetate, 80% ethyl acetate). Specimens were stored in distilled water for 24 h, then the surfaces were polished and cleaned by ultrasonication, and immersed in an acidic solution (pH 5.0; 3.0 mM Ca; 1.8 mM P; 1 wt% carboxymethylcellulose) at 37°C for 0, 3, 6 or 9 days.

Contact microradiography

After dehydration, specimens were embedded in polyester resin (Rigolac; Nisshin EM, Tokyo, Japan). Seven 100- μ m ground sec-

tions were then prepared. Contact microradiography (CMR) of sections together with aluminum step-wedges was conducted using a soft X-ray emitting device (CMR-3; Softex, Tokyo, Japan). Sections were exposed to X ray of 10 kV and 3 mA at a film-focus distance of 44.4 mm for 8 min. Kodak SO-181 film was used and the developer was Kodak D19. Specimens were then fixed and rinsed by conventional methods and observed under optical microscopy. The CMR images were input into computer using a digital-input imaging device (HC-2500; Olympus, Tokyo, Japan). To convert the images into histograms, the highest intensity of black was taken as 0%, and sound dentin as 100%. In the case of day 9 specimens, mineral loss was measured on the surface at distances of 100 and 200 μ m from the cavity margin.

The results were compared statistically by Student's *t* test.

SEM observations

Day 9 specimens were observed under scanning electron microscopy (SEM, JSM-6340F; JEOL, Tokyo, Japan) after Au-Pd coating with a cool sputter coater. In addition, ordinary dentin samples were observed for normal dentinal tubules.

Electron probe microanalysis

An electron probe microanalysis (EPMA)-WDX (X-3010; Hitachi, Tokyo, Japan) was used to investigate zinc-infiltration in five specimens that had been immersed for 9 days. Line analyses were made using an accelerating voltage of 20 kV and a probe current of 1.00×10^{-8} A.

Solution analysis

An atomic-absorption spectrometer (180-60; Hitachi, Tokyo, Japan) was used to measure concentrations of Mg²⁺ and Ca²⁺ in the acidic solutions in which specimens had been immersed for 9 days for both groups. An ion analyzer (Model 290; Orion Research, Beverly, MA, USA) with TISAB III and a fluoride-sensitive electrode (Model 96-09) were used to measure concentrations of F⁻.

The results were compared statistically by Student's *t* test.

Mechanical properties

The mechanical properties of compressive strength and disintegration rate were investigated, following JIS Z 8801.

To measure compressive strength, 5 cylindrical samples (4-mm diameter, 6-mm height) of each cement were prepared using a metal split mold. One hour after mixing, the samples were stored in distilled water at 37°C for 1 week. Compressive strengths of the samples were determined using a universal testing machine (Autograph DCS5000; Shimadzu, Kyoto, Japan) at a cross-head speed of 1.00 mm/min. The results were compared statistically by Student's *t* test.

To measure disintegration, one hour after mixing, 5 specimens (20-mm diameter, 1.5-mm thick) were submerged by pouring 50

Fig. 2. Contact microradiographic images of day 0 specimens in GIC-Z group (a) and GIC group (b). No apparent changes caused by filling are observed in the cavity margin (M) or polished dentin surfaces. Interspace artifact between dentin and filling can be seen with both cements.

Fig. 3. Contact microradiographic images of day 3 specimens in GIC-Z group (a) and GIC group (b). Subsurface demineralized lesions are apparent in both groups. Progression of demineralized lesions into deep regions along the dentin-filling boundary can be seen. Greater mineralization is observed in GIC group than in GIC-Z group.

Fig. 4. Contact microradiographic images of day 6 specimens in GIC-Z group (a) and GIC group (b). Extension of subsurface lesions is seen in both groups. More marked demineralized lesions along the dentin-filling boundary are observed in GIC-Z group than in GIC group.

Fig. 5. Contact microradiographic images of day 9 specimens in GIC-Z group (a) and GIC group (b). A is 100 μ m from the cavity margin (M), and B is 200 μ m from M. In the GIC-Z group, demineralized areas along the dentin-filling boundary have disappeared. The lesion is narrower and less radiolucent than in the GIC specimen.

Fig. 6a. Mineral distribution at 100 μ m from the cavity margin in day 9 specimens. The y-axis is mineral loss (%), and the x-axis is lesion depth (μ m) from the dentinal surface. The area enclosed by the 100% line represents mineral-loss value. Arrow indicates point of maximum mineral loss.

Fig. 6b. Mineral-loss values (vol%· μ m) at 100 μ m from the cavity margin in day 9 specimens.

Fig. 6c. Mineral distribution at 200 μ m from the cavity margin in day 9 specimens. No significant difference in mineral loss is apparent between the two groups.

Fig. 7. Scanning electron micrograph of polished surface from specimen before acidic treatment. All dentinal tubules are open, although dentinal fibers are not observed.

Fig. 8. Scanning electron micrograph of day 9 specimen in GIC group. Many dentinal tubules are closed. Several dentinal tubules near the cavity margin remain open.

Fig. 9. Scanning electron micrograph of day 9 specimen in GIC-Z group. Most dentinal tubules are closed, leaving a small number of dentinal tubules still open.

Fig. 10. Intensity of Zn is highest in dentin at the area facing the GIC-Z cement. Left panel shows intensity of Zn obtained with ordinary dentin.

Fig. 11. Scattergrams for Mg^{2+} and Ca^{2+} in GIC-Z and GIC groups. A strong correlation ($r=0.84$, $p<0.18$) between Mg^{2+} and Ca^{2+} concentration is observed in the GIC group but not in the GIC-Z group ($r=0.35$, $p<0.45$).

Fig. 12. Results of compression tests ($n=5$). Compression strength tends to be lower in the GIC-Z group than in the GIC group, but the difference is not significant.

Fig. 13. Results of disintegration analysis ($n=5$). Disintegration rate tends to be lower in the GIC-Z than in the GIC group, but the difference is not significant.

ml of distilled water into the weighing bottle and were stored at 37 °C for 23 h. Then, the specimens were removed from water, and the water was evaporated from the weighing bottle. After cooling to room temperature in a desiccator, the weighing bottle and contents were weighed to within 0.5 mg. The results were compared statistically by Student's *t* test.

Results

Contact microradiography

Figure 2 shows the CMR images obtained from day 0 specimens prior to acid gel treatment. In both GIC-Z and GIC groups, uniformly mineralized dentin and non-mineralized dentinal tubules were clearly observed. In addition, no difference was found between the two groups at the cavity margin or polished dentin surface. The interspace seen between dentin and filling represents an artifact.

Figure 3 shows the CMR images obtained from day 3 specimens. Subsurface demineralized lesions caused by acid gel were observed in both groups. The depth of the lesions was about 70µm from the polished dentin surface, with no significant difference between the two groups. On more detailed examination, however, demineralized lesions had progressed into deep regions along the dentin-filling boundary in both groups. On the other hand, relatively radio-opaque layers were apparent at the outermost surface layer in the GIC group in contact with both acid gel and the dentin-filling boundary. Mineralization was higher in the GIC group than in the GIC-Z group.

Figure 4 shows the CMR images obtained from day 6 specimens. Basic features such as the existence of subsurface demineralized lesions and the outermost relatively mineralized surface layers were almost the same as in day 3 specimens. Under detailed observation, however, subsurface lesions had extended in both GIC-Z and GIC groups. In addition, there were more demineralized lesions along the dentin-filling boundary in the GIC-Z group than in the GIC group.

Figure 5 shows the CMR images obtained from day 9 specimens. In the GIC group, demineralized lesions had grown broader and deeper than in day 6 specimens. In the GIC-Z group, however, the demineralized subsurface lesions were narrower than those in the day 6 specimens. In addition, demineralized areas along the dentin-filling boundary had disappeared. The lesions were narrower and less radiolucent than those of day 9 specimens in the GIC group, and some dentinal tubules were apparent.

Figure 6a shows the mineral loss value (vol%•µm) at 100µm from the margin in day 9 specimens. Mineral loss was slightly reduced in the GIC-Z group. In both groups, mineral loss was maximal at a depth of about 100 µm. Figure 6b shows planar analysis. No significant differences in mineral loss were identified between the two groups (*t*-tests). Demineralization was slightly increased in the GIC-Z group compared to the GIC group. Figure 6c shows mineral loss at a distance of 200µm from the margin.

Table 1. Concentration (mean ±SD) of Mg^{2+} , Ca^{2+} and F^- in the acidic solution.

	GIC-Z group(n=7)	GIC group (n=7)
Mg^{2+} (ppm)	2.11 ± 0.17	3.15 ± 0.88*
Ca^{2+} (ppm)	87.67 ± 12.60	108.13 ± 12.83*
F^- (ppm)	0.414 ± 0.014	0.418 ± 0.041

* $p < 0.05$

Little difference was observed between the groups.

SEM observations

Figure 7 shows the scanning electron micrograph of the polished surface obtained from a specimen before acidic treatment and filling. All dentinal tubules were open, although dentinal fibers were not apparent. Figure 8 shows the micrograph of day 9 specimen in the GIC group. Many dentinal tubules were closed. However, several dentinal tubules near the cavity margin remained open. Figure 9 shows the micrograph of day 9 specimen in the GIC-Z group. Most dentinal tubules were closed, and only a few dentinal tubules were still open.

EPMA

Figure 10 shows the results of line analysis for Zn obtained from day 9 specimens in GIC-Z group. Within a range of 507±132.5 µm from the cavity margin, the intensity of Zn was highest on the dentin face in the GIC-Z group. This decreased gradually as distance was increased, finally reaching the level of ordinary dentin.

Solution analysis

The results of solution analysis are shown in Table 1. Concentrations of Mg^{2+} and Ca^{2+} were lower in the GIC-Z group than in the GIC group, with significant (*t*-test, $p < 0.05$) differences between the two groups. No difference in F^- concentration was observed between the two groups. Figure 11 shows a scattergram for Mg^{2+} and Ca^{2+} concentrations. A strong correlation ($r = 0.84$, $p < 0.18$) between Mg^{2+} and Ca^{2+} concentrations was observed in the GIC group, and was not observed in the GIC-Z group ($r = 0.35$, $p < 0.45$).

Mechanical properties

The compressive strength was 167.3 ± 23.3 MPa in the GIC-Z group and 182.8 ± 27.2 MPa (Figure 12) in the GIC group, with no significant difference between the groups (*t*-test).

The disintegration rate was 0.84 ± 0.27% in the GIC-Z group, and 1.11 ± 0.40% in the GIC group (Figure 13), again with no significant differences (*t*-test).

Discussion

The present study attempted to determine the extent to which mixing zinc oxide with GIC inhibits dentin demineralization and promotes remineralization.

After dentinal cures are filled with GIC, F^- is gradually released from the cement¹³ and replaces the OH^- in hydroxyapatite, forming fluorapatite. Deposition of fluorapatite on the dentinal surface and on peritubular dentin causes obturation of dentinal tubules^{14,15}. Fluorapatite is an acid-resistant crystal¹⁶. The present result of inhibition of dentin demineralization is attributed to an extremely low concentration of F^- released from the cement.

In the present study, Mg^{2+} concentration was significantly lower in the GIC-Z group than in the GIC group. Moreover, the scattergram demonstrated that the balance between Mg^{2+} and Ca^{2+} had collapsed. Mg^{2+} is not a component of the cement and is present only in dentin^{2,17}. Mg^{2+} concentration is affected by the process of dentinal caries¹⁸. Mg^{2+} in the acidic solution was extracted from dentin, and significantly lower Mg^{2+} in the GIC-Z group demonstrated enhanced inhibition of dentin demineralization in the GIC-Z group compared to the GIC group. Scanning electron micrographs, however, revealed that dentinal tubules were closed in both groups. Dentinal tubules play a very important role in the pathogenesis and progression of dentinal caries¹⁷. Obturation of the dentinal tubules was more marked in the GIC-Z group than in the GIC group, again suggesting greater resistance against dentin demineralization and dentinal caries progression in the GIC-Z group compared to the GIC group. Dissociated Zn^{2+} reportedly replaces Ca^{2+} in hydroxyapatite to form $Zn_3(PO_4)_2$ and $ZnCO_3$, producing deposition in dentinal¹⁹. In the GIC-Z mixture, Zn^{2+} is likely to dissociate from the zinc oxide and promote mineral deposition in the dentinal tubules. F^- also plays a very important role in remineralization. F^- released from GIC is deposited on the surface of dentin, resulting in very high surface concentrations in this experiment. Consequently, the remineralization observed in this experiment is probably attributable to the combined effects of Zn^{2+} and F^- .

These findings show that use of the GIC-Z mixture reduces mineral loss from dentin due to demineralization, and promotes remineralization of the dentin surface compared to GIC alone. Regarding mechanical properties, comparative studies of compressive strength and disintegration rate revealed no significant differences between the cements. The results of both cements achieved ISO9917 standards, although they fell slightly short of ISO7489 standards²⁰. Consequently, although the material can be used for restorations, further studies to strengthen the material need to be considered.

Conclusion

Addition of zinc oxide to conventional GIC enhances the inhibition of dentin demineralization. GIC-Z mixture and GIC alone display no major differences in mechanical properties in

terms of compressive strength and disintegration rate.

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