Antibacterial effects and physical properties of a glass ionomer cement containing BioUnion filler with acidity-induced ability to release zinc ion

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BioUnion filler is a bioactive glass particle that releases Zn\(^{2+}\) in an acidic environment. In this study, the ion release, antibacterial, and physical properties of a glass ionomer cement (GIC) incorporating BioUnion filler (CA) were assessed in vitro. The concentration of Zn\(^{2+}\) released from CA into acetic acid was higher than that released into water and its minimum inhibitory concentrations against six oral bacterial species. Moreover, the concentration of Zn\(^{2+}\)-release was maintained during all the seven times it was exposed to acetic acid. Compared to a conventional cement and resin composite, CA significantly inhibited the growth of oral bacteria and hindered their adhesion on the material surface. Thus, our study outcomes show that the release of Zn\(^{2+}\) from CA in the acidic environment does not affect its compressive strength.

Keywords: BioUnion filler, Zinc, Glass ionomer cement, Antibacterial effect, Ion release

INTRODUCTION

Dental caries, a major oral health problem worldwide, is caused by the formation of a cariogenic environment in the oral cavity and depends on many factors including diet and oral hygiene. Moreover, people try to prevent/manage dental caries by overzealous toothbrushing, which could promote gingival recession and elevate the risk of root surface caries in elderly individuals. Resin composites or glass ionomer cements (GICs) are used for the restoration of root surface caries; however, secondary caries is the most common cause of restoration failure.

Since their development in the 1970s, GICs have been widely used for restoration owing to their unique characteristics, such as anti-cariogenic properties and good adhesion to the tooth surface. While some studies reported that F\(^{-}\) ions released from GICs inhibit the growth of oral bacteria, others claimed that such inhibition is inefficient and short-lived. Thus, much effort has been devoted to enhancing the antibacterial effect of GICs, such as by incorporating chlorhexidine, silver nanoparticles, propolis extract, and phoshphopeptide-amorphous calcium phosphate.

BioUnion filler is a bioactive glass particle composed of silicon dioxide (SiO\(_2\)), zinc oxide (ZnO), calcium oxide (CaO), and fluorine. It is capable of releasing Zn\(^{2+}\), F\(^{-}\), and Ca\(^{2+}\) ions. Caredyne-Restore (GC, Tokyo, Japan), a new GIC developed for the restoration of root surface caries, contains BioUnion filler. It was previously reported that Zn\(^{2+}\) release from the BioUnion filler is accelerated in an acidic environment owing to the filler’s solubility in acid. As a known antibacterial component, Zn\(^{2+}\) inhibits the growth of cariogenic bacteria such as Streptococcus mutans. Thus, acidity-induced release of Zn\(^{2+}\) is expected to hinder plaque formation on the surfaces of materials containing BioUnion filler. Hasegawa et al. reported that a GIC containing BioUnion filler inhibited S. mutans biofilm formation by interfering with bacterial adhesion on the surface. Saad et al. reported the inhibition of root dentin demineralization and S. mutans biofilm formation by a tooth surface coating comprising BioUnion filler. However, there has been no investigation on the acidity-induced release characteristics of Zn\(^{2+}\) from a GIC containing BioUnion filler, or its inhibitory effects against S. mutans and other oral bacterial species. It was hypothesized that acidic conditions will induce the GIC containing BioUnion filler to release more Zn\(^{2+}\) and inhibit oral bacteria. However, the incorporation of acid-soluble BioUnion filler might adversely influence on the physical properties of GIC. In this study, we investigated the characteristics of Zn\(^{2+}\) release from Caredyne-Restore under acidic conditions, and evaluated the material’s antibacterial effects against oral bacteria in vitro. In addition, we measured the physical properties of Caredyne-Restore.

MATERIALS AND METHODS

Bacteria
Six oral bacterial species (Streptococcus mutans NCTC 10449, S. sobrinus NCTC 12279, S. oralis NCTC 11427, S. mitis NCTC 12261, Actinomyces naeslundii ATCC 19246, and Fusobacterium nucleatum 1436) were used.

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The first five species were cultured in a brain-heart infusion (BHI) broth (Becton Dickinson, Sparks, MD, USA) and on BHI agar plates (Becton Dickinson), while F. nucleatum was cultured in a Todd Hewitt broth (THB; Becton Dickinson) and on THB agar plates supplemented with 0.1% L-cystein. S. mutans, S. sobrinus, S. oralis, and S. mitis were cultured from a stock culture at 37°C for 24 h under anaerobic conditions, while A. naeslundii and F. nucleatum were incubated at 37°C for 48 h under anaerobic conditions.

Measurement of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of Zn2+, Ca2+, and F

Measurement of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of Zn2+, Ca2+, and F for oral bacteria

ZnCl2, CaCl2 (Wako, Osaka, Japan), and NaF (Sigma-Aldrich, Tokyo, Japan) were dissolved in distilled water to prepare standard solutions of Zn2+, Ca2+, and F, respectively. The standard solutions were subjected to serial 2-fold dilution to reach a concentration of 4–16,384 ppm. The diluted solutions (50 μL) were dropped into wells of a 96-well microplate. Next, 50 μL of bacterial suspension from the stock culture was incubated for 24 h, adjusted to a density of 2.0×106 colony-forming units (CFU)/mL, and added to the wells, resulting in a two-fold dilution of the original solution. The microplates were then anaerobically incubated at 37°C for 48 h, and the MIC was determined as the lowest concentration required to prevent visible bacterial growth (when turbidity is not observed by visual examination). Then, 100 μL of sample was taken from the wells that showed no visible growth and inoculated on agar plates. After anaerobically subculturing for 48 h at 37°C, plates that showed no bacterial colonies were used to determine the MIC. The experiments were repeated five times.

Specimen preparation

The set specimens were prepared using a GIC containing BioUnion filler (Caredyne-Restore; hereafter denoted as CA). The material details are shown in Table 1. The powder and liquid of CA were mixed in a ratio of 2.3:1 (w/w). The paste was poured into a mold of the same dimension, covered with celluloid strips and a glass slide, and stored at 25°C for 24 h. The specimen was then polished using silicon carbide grinding papers (#120 to #2500; Buehler, Lake Bluff, IL, USA). A conventional GIC (Fuji VII, GC; hereafter denoted as F7) and a resin composite (MI FIL, GC; hereafter denoted as MI) were used as controls. The powder and liquid of F7 were mixed in a ratio of 1.8:1 (w/w), and the set cement was prepared by the same method as described above. To prepare a cured specimen of MI, the single paste was poured into a mold of the same dimension, covered with celluloid strips and a glass slide, cured for 40 s in a light activation unit (Quick Light VL-1, Morita, Kyoto, Japan), and finally polished as described above. Before testing, all specimens were washed with 70 vol% ethanol for 5 min, which is the mildest treatment to achieve disinfection.

Ion release from CA and F7

The characteristics of Zn2+, Ca2+, and F− release from different cements into water and acetic acid were evaluated. The specimens were immersed in either 300 μL of distilled water at pH 7.0 or acetic acid at pH 4.5 in a 48-well microplate (Tissue Culture Plate; VIOLAMO, Osaka, Japan) at 37°C for 24 h and shaken at 100 rpm. Then, 200 μL of the eluate was collected. The eluate was diluted with 4.8 mL of distilled water, and the concentrations of Zn2+ and Ca2+ were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES; iCAP 7000 Series, Thermo Scientific, Cambridge, UK). The concentration of F− was determined using a fluoride ion electrode (6561S-10C, HORIBA, Kyoto, Japan). These experiments were repeated four times.

To evaluate the characteristics of Zn2+ release from CA with repeated exposure to acetic acid, the specimens were immersed in 300 μL of acetic acid (pH 4.5) in a 48-well microplate at 37°C for 1 day, and then immersed in distilled water (pH 7.0) at 37°C for 3 days with the water replaced every day. The procedure was repeated seven times for a total of 28 days. That is, the specimens were immersed in acetic acid on Days 1, 5, 9, 13, 17, 21, and 25, and in distilled water on the other days. A total of 200 μL eluate was collected every day, diluted with 4.8 mL of distilled water, and the concentration of Zn2+
was measured by ICP-OES. All the experiments were repeated five times.

**Elemental analysis of BioUnion filler and CA**
The elemental composition of BioUnion filler was analyzed by field-emission scanning electron microscope/energy dispersive spectroscopy (FE-SEM/EDS; JSM-F100, JEOL, Tokyo, Japan), while particle morphology was observed using FE-SEM at 10 kV. FE-SEM/EDS was also used to analyze elemental composition of the set CA cement before and after it was exposed seven times to acetic acid (described above).

**Evaluation of antibacterial activity of CA, F7, and MI**
Unstimulated human saliva was collected from four healthy donors, and the process was approved by the Ethics Review Committee of Osaka University Graduate School of Dentistry and Osaka University Dental Hospital (Approval number: R1-E52). The collected saliva was filtered twice through a 0.22-micrometer syringe filter, and the specimens were immersed in 1 mL of filtered saliva for 2 h at 37°C.

To evaluate the growth of each species on the surface of set CA treated with human saliva. In this test, 20 μL of BHI broth supplemented with 1% sucrose (Wako) was inoculated on the saliva-treated CA treated with human saliva. The experiment was repeated five times. F7 and MI were used as controls.

**Evaluation of physical properties of CA and F7**
The shear bond strength, setting time, acid erosion, and compressive strength of CA were measured. F7 was used as the control. To measure the shear bond strength, the crown of bovine incisors was embedded in an acrylic resin (UNIFAST II, GC) with the buccal surface facing upward. The surface was planarized with 120 grit silicon carbide paper to expose the flat enamel and dentin, and polished with 320 grit silicon carbide paper. A cylindrical polyethylene mold with internal dimensions of 2.4 mm (diameter)×3 mm (height) (Ultradent, South Jordan, UT, USA) was set on the exposed enamel/dentin. Subsequently, the powder and liquid of the cement were mixed, and the resulting paste was poured into the mold. The assembly was stored at 37°C and a relative humidity of >90% for 5 min. Then, the mold was removed, and the specimen with attached cement was stored in distilled water at 37°C for 24 h. The shear bond strength test was carried out using a tabletop testing machine (EZ-S, Shimadzu, Kyoto, Japan) at a crosshead speed of 1.0 mm/min. The shear bond strength was calculated by dividing the load by the bonded area (4.52 mm²). Six replicates were measured for each sample.

The setting time, acid erosion, and compressive strength were tested according to International Organization for Standardization (ISO) 9917-1:2007. To measure the setting time, the powder and liquid of the cement were mixed, and the paste was placed in a stainless-steel mold. The entire assembly was then transferred to an incubator (37°C, >90% relative humidity). A Vicat needle with a weight of 400 g and an active tip diameter of 1.0 mm was lowered vertically onto the horizontal surface of the cement. The time from the start of mixing until the setting of the cement (defined as when the needle failed to make an indentation) was considered the setting time. Five measurements were made for each sample.

To measure acid erosion, a polymethyl methacrylate (PMMA) disc (30 mm diameter, 5 mm thickness) with a hole (5 mm diameter, 2 mm depth) in the center was prepared. The hole was filled with the cement, covered with a polyester sheet, pressed firmly, and clamped. At 180 s after the end of cement mixing, the entire assembly was transferred to an incubator (37°C, >90% relative humidity). After 24 h, the clamp and polyester sheet were removed, and the specimen was polished with 1200 grit abrasive paper until a flat surface was obtained. The initial depth was measured at five points near the center.
Table 2 Minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of Zn$^{2+}$, Ca$^{2+}$, and F$^{-}$ on oral bacteria

| Bacterial Species | MICs | MBCs |
|-------------------|------|------|
|                   | Zn$^{2+}$ | Ca$^{2+}$ | F$^{-}$ | Zn$^{2+}$ | Ca$^{2+}$ | F$^{-}$ |
| S. mutans         | 64    | 512   | 128    | 512      | 4,096     | 2,048    |
| S. sobrinus       | 128   | 512   | 256    | 512      | 8,192     | 4,096    |
| S. oralis         | 64    | 256   | 256    | 1,024    | 8,192     | 8,192    |
| S. mitis          | 64    | 1,024 | 128    | 512      | 8,192     | 1,024    |
| A. naeslundii     | 128   | 512   | 256    | 512      | 4,096     | 4,096    |
| F. nucleatum      | 128   | 512   | 256    | 1,024    | 2,048     | 2,048    |

Table 3 Concentrations of Zn$^{2+}$, Ca$^{2+}$, and F$^{-}$ released from CA and F7 into water and acetic acid

| Cement | Ion     | Water (pH 7.0) | Acetic acid (pH 4.5) |
|--------|---------|----------------|----------------------|
| CA     | Zn$^{2+}$ | 3.6±0.9        | 170.4±7.0            |
|        | Ca$^{2+}$ | 2.7±1.4        | 26.4±2.6             |
|        | F$^{-}$  | 160±10.7       | 110.2±6.0            |
| F7     | Zn$^{2+}$ | n.d.           | n.d.                 |
|        | Ca$^{2+}$ | n.d.           | n.d.                 |
|        | F$^{-}$  | 89.9±1.4       | 50.8±7.0             |

Mean±S.D. (ppm)
n.d.: not detected
**Ion release property**

The concentrations of $\text{Zn}^{2+}$, $\text{Ca}^{2+}$, and $\text{F}^{-}$ released from CA and F7 into distilled water and acetic acid solution (pH 4.5) are shown in Table 3. The concentration of $\text{Zn}^{2+}$ released from CA into acetic acid (170.4±7.0 ppm) was significantly higher than that released into water and the MIC values against the examined bacterial species (64–128 ppm) ($p<0.05$, ANOVA, Tukey’s HSD test). In contrast, the concentration of $\text{F}^{-}$ released from CA into acetic acid (110.2±6.0 ppm) was significantly lower than that released into water and the corresponding MICs ($p<0.05$). The concentrations of $\text{F}^{-}$ released from F7 into water and acetic acid were also significantly lower than the corresponding MICs ($p<0.05$).

The release profiles of $\text{Zn}^{2+}$ from CA over 28 days are shown in Fig. 1. On Day 1, $\text{Zn}^{2+}$ release into acetic acid (170.4±7.0 ppm) was significantly higher than that released into water. The $\text{Zn}^{2+}$ release into acetic acid during seven repeated exposures over 28 days remained the same ($p>0.05$, ANOVA, Tukey’s HSD test).

![Fig. 1](image1.png)

*Fig. 1* Release profile of $\text{Zn}^{2+}$ from CA after repeated exposure to acetic acid.

Release profiles of $\text{Zn}^{2+}$ from CA into acetic acid and water on Days 1–28. The bars represent the standard deviations of five replicates.

![Fig. 3](image3.png)

*Fig. 3* FE-SEM images and elemental mapping images of CA before and after repeated exposure to acetic acid.

| Element | Mass%  | Atom%  |
|---------|--------|--------|
| Si      | 9.00±0.04 | 6.76±0.03 |
| F       | 5.21±0.04 | 5.78±0.05 |
| Ca      | 2.95±0.03 | 1.55±0.01 |
| Zn      | 12.69±0.16 | 4.09±0.05 |

![Fig. 2](image2.png)

*Fig. 2* FE-SEM image and elemental mapping images of BioUnion filler.
Elemental analysis
The FE-SEM image of BioUnion filler is shown in Fig. 2. The particles have irregular shapes and a diameter of approximately 7 μm. EDS analysis revealed the presence of Si, F, Ca, and Zn at the approximate ratios of 9.00, 5.21, 2.95, and 12.69 wt%, respectively.

Figure 3 shows the FE-SEM image and EDS elemental mapping images of CA. Si and F are distributed around the glass particles, whereas Zn and Ca are homogeneously distributed in both the particles and the matrix. No differences were observed in the distributions of Zn and Ca before and after it was exposed seven times to acetic acid.

Antibacterial activity
Figure 4 (Day 0) shows the number of viable bacteria after incubation on CA, F7, and MI. Compared with F7 and MI, CA has a significantly lower number of bacterial colonies \((p<0.05,\, \text{ANOVA, Tukey’s HSD test})\). Moreover, the numbers of viable cells on CA are the same or lower

| Table 4  | Physical properties of CA and F7 | CA     | F7     | ISO range (9917-1) |
|----------|---------------------------------|--------|--------|-------------------|
| Setting time (s)       | 165       | 150      | 90–360       |
| Compressive strength (MPa) | 146.8±5.2    | 143.9±8.3  |
| Acid erosion (mm)   | 0.049±0.007 | 0.053±0.008  | <0.17         |
| Shear bond strength Enamel (MPa) | 5.4±0.8      | 5.3±1.3   |
| Shear bond strength Dentin (MPa) | 4.7±0.7      | 4.9±0.9 |

Fig. 4 Inhibitory effects of CA before and after repeated exposure to acid against the growth of (A) \(S.\ \text{mutans}\), (B) \(S.\ \text{sobrinus}\), (C) \(S.\ \text{oralis}\), (D) \(S.\ \text{mitis}\), (E) \(A.\ \text{naeslundii}\), and (F) \(F.\ \text{nucleatum}\). The bars represent the standard deviations of five replicates. *indicates significant differences. CA significantly inhibited the growth of all examined bacterial species compared with F7 and MI \((p<0.05,\, \text{ANOVA, Tukey’s HSD test})\).

Fig. 5 Number of bacteria adhered on CA after incubation for the six bacterial species. The bars represent the standard deviations of five replicates. *indicates significant differences. CA significantly inhibited the adherence of all examined bacterial species compared with F7 and MI \((p<0.05,\, \text{ANOVA, Tukey’s HSD test})\).
than the initial numbers (2.0×10³ CFU).

Figure 4 also shows the number of viable bacteria on CA, F7, and MI collected during immersion in acetic acid and water on Day 4, 8, and 24. Even after six exposures to acetic acid, CA significantly inhibited the growth of all bacterial species compared with F7 and MI (p<0.05, ANOVA, Tukey’s HSD test), indicating maintained antibacterial effect after repeated acid exposure.

Inhibitory effect against bacterial adherence
Figure 5 shows the number of bacteria adhered on CA, F7, and MI after incubation. CA significantly inhibited the adherence of examined bacterial cells compared with F7 and MI (p<0.05, ANOVA, Tukey’s HSD test). Moreover, no colonies of F. nucleatum were observed on CA.

Physical properties
Table 4 lists the physical properties of CA. The setting time, compressive strength, and acid erosion of CA fulfilled the requirement described in the ISO 9917-1. The shear bond strengths of CA to enamel and dentin were 5.4±0.8 and 4.7±0.7 MPa, respectively. CA and F7 displayed no significant differences in their setting time, compressive strength, acid erosion, and shear bond strength to enamel and dentin.

Figure 6 shows the compressive strengths of CA specimens collected on Day 0, 8, and 28 (i.e., after 0, 2, and 7 exposures to acetic acid, respectively). No significant differences were observed in the compressive strengths of the three groups. Even after being exposed to acetic acid seven times, the compressive strength of CA fulfilled the requirement described in the ISO 9917-1 (100 MPa).

DISCUSSION
BioUnion filler is a bio-functional multi-ion-releasing glass particle composed of SiO₂, ZnO, CaO, and F[15]. It has a silicon-based glass structure and is capable of releasing Zn²⁺, Ca²⁺, and F⁻. To determine the inhibitory effect of BioUnion filler, the MICs and MBCs of Zn²⁺, Ca²⁺, and F⁻ ions against six bacterial species were measured by a microdilution assay. Hernández-Sierra et al. reported an MBC of 500 μg/mL (500 ppm) for Zn against S. mutans[20]. Pizzey et al. reported an MIC range of 0.125–0.50 mmol Zn/mL (125–500 ppm) and an MBC range of 1–8 mmol Zn/mL (1,000–8,000 ppm) against S. mutans, S. sobrinus, S. sanguinis, and A. naeslundii[21]. In the present study, the MIC of Zn²⁺ for the examined bacterial species ranged from 64 to 128 ppm, while the MBC ranged from 512 to 1,024 ppm. These values are similar to those reported in the literature. Moreover, Zn²⁺ showed lower MICs and MBCs than Ca²⁺ and F⁻ against the examined bacterial species. Calcium compounds such as calcium hydroxide raise the pH and inhibit oral bacteria when in contact with the aqueous environment[22]; however, the antibacterial activity of Ca²⁺ itself is not strong. In this study, we confirmed that the MICs and MBCs of Ca²⁺ against the examined bacterial species were lower than those of Zn²⁺ and F⁻. Several studies have reported the inhibitory mechanism of F⁻ on bacterial metabolic activity[23]. Takahashi et al. reported that F⁻ inhibits enolase, which catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the Embden-Meyerhof-Parnas pathway in bacterial glycolytic metabolism[24]. Zn²⁺ demonstrates antibacterial activity against both gram-positive and gram-negative bacteria[25]. Although the detailed mechanism has not been fully elucidated, one study reported that Zn²⁺ can penetrate and disturb the cell membranes while preserving the integrity of bacterial genome[26].

It has been previously reported that acidic conditions promote the release of Zn²⁺ from BioUnion filler owing to the filler’s solubility in acid[16]. To evaluate this unique characteristic after the filler is incorporated in a GIC, the release of Zn²⁺, Ca²⁺, and F⁻ from CA was monitored. The concentration of Zn²⁺ released into acetic acid (170.4±7.0 ppm) was higher than the MIC (64–128 ppm) against the examined oral bacterial species, whereas the concentration of Zn²⁺ released into water was lower than this range. For Ca²⁺ and F⁻, their releases into acetic acid were lower than the respective MICs.

An on-disc culture assay was conducted to examine the antibacterial activities of CA, F7, and MI against the six species of bacteria. Before the on-disc culture assay, each bacterial suspension in 10 mL broth was adjusted to a density of approximately 1.0×10⁶ CFU/mL. The numbers of S. mutans, S. sobrinus, S. oralis, and S. mitis after 24-h incubation reached (7.3±1.6)×10⁸, (7.2±1.7)×10⁸, (6.5±1.5)×10⁸, and (6.3±1.4)×10⁸ CFU/mL, respectively.
it took 48 h for their cell number to reach (3.4±1.2)×10⁸ CFU/mL, respectively. However, the growth of anaerobic A. naeslundii and F. nucleatum was comparably slow, and it took 48 h for their cell number to reach (3.4±1.2)×10⁸ and (2.7±0.8)×10⁸ CFU/mL, respectively. To uniformly compare the activity of CA, F7, and MI against the six species, the final bacterial concentration was set to approximately 10⁸ CFU/mL (6.3 log₁₀CFU in 20 μL). Therefore, the on-disc culture assay employed 24 h incubation for the four streptococci and 48 h incubation for the other two species. The results indicate that Zn²⁺ released from CA can effectively inhibit the growth of all six tested bacterial species. In these experiments, each bacterium grew in number on MI after incubation (for 24 or 48 h), i.e., from 4.3 to 5.4–6.9 log₁₀CFU, which is similar to the growth rates when these bacteria were incubated without any cement specimen. Moreover, the release of Zn²⁺ from CA during repeated exposure to acid was evaluated by alternating immersion in acetic acid for 1 day and in distilled water for 3 days. Each exposure to acetic acid triggered the release of Zn²⁺, and even after 7 repeated acid exposures the Zn²⁺ concentration remained above its MIC against the six bacterial species. Therefore, CA is capable of releasing Zn²⁺ with antibacterial effects over a long period.

GICs are formed by an acid-base reaction between a basic fluoroaluminosilicate glass and polyacrylic acid²⁷. The aluminosilicate glass contains Ca²⁺, Al³⁺, and Si⁴⁺ which are linked to each other by bridging oxygens. When aluminosilicate glass and polyacrylic acid are mixed, the protons of polyacrylic acid cause hydrolysis of the glass network. Consequently, Ca²⁺ and Al³⁺ are released and bind with the carboxyl groups of polyacrylic acid to form a matrix. A silica gel layer is produced around the remaining glass particles to impede further glass degradation²⁸. Ca²⁺ and Al³⁺ in the matrix can be released into an acidic solution via ion exchange with H⁺²⁹. In the case of CA, Zn²⁺ and Ca²⁺ are also released from the BioUnion filler upon mixing with polyacrylic acid. The EDS analysis showed homogeneous distribution of Zn and Ca in both the particles and the matrix, indicating that Zn²⁺ and Ca²⁺ released by BioUnion filler upon mixing with the liquid of CA were either bound to the polyalkenoate groups or trapped in the matrix of CA. Moreover, repeated exposure to acetic acid did not affect the distribution of Zn and Ca, suggesting that these ions were released from both the filler and the matrix into distilled water and acetic acid.

As a complex mixture of different bacterial species, oral biofilms are formed in a specific sequence of events, starting with attachment of the initial colonizing bacteria (streptococci, etc.) to the tooth surface with the acquired pellicle, followed by the coaggregation of late colonizers³⁰. During biofilm formation, F. nucleatum acts as a bridging organism connecting the early and late colonizing bacteria³¹. S. mutans, S. sobrinus, and other bacteria initiate the formation of dental caries by producing glucosyltransferase, which catalyzes the synthesis of extracellular glucans from sucrose and acid³², whereas A. naeslundii is the pathogen responsible for root surface caries³³. To evaluate the antibacterial effects of CA, suspensions of the six bacterial species containing sucrose were incubated on the set cement. The results confirmed that CA inhibits the growth of all six bacterial species more effectively than the control cement and resin composite. The pH changes were also monitored during incubation of bacterial suspension containing 1% sucrose but no solid specimen. After incubation for 24/48 h, the pH of S. mutans, S. sobrinus, S. oralis, S. mitis, A. naeslundii, and F. nucleatum suspensions gradually decreased to 4.3 (24 h), 4.2 (24 h), 4.5 (24 h), 4.4 (24 h), 4.6 (48 h), and 4.7 (48 h), respectively. Comparison of these changes with the pH changes during incubation with CA revealed that the inhibitory effects of CA are due to Zn²⁺ release triggered by the acids produced by these bacteria. Moreover, the numbers of viable cells of S. mutans, S. sobrinus, S. mitis, A. naeslundii, and F. nucleatum decreased after incubation on CA, even though the measured Zn²⁺ release into acetic acid is lower than the corresponding MBC range. This could be due to the much smaller volume of bacterial suspensions used (20 μL), compared with that of acetic acid (300 μL) used for immersion. Nonetheless, this on-disc culture assay revealed that the acidity-induced release of Zn²⁺ from CA was responsible for its inhibitory effects against oral bacteria. Moreover, CA demonstrated stronger antibacterial effects against all six bacterial species than F7 and MI. The results indicate a long-lasting antibacterial effect of CA against oral bacteria.

Ion-releasing inorganic fillers with antibacterial activity have been intensively studied for application in restorative and preventive treatments. The eluate from these materials could suppress the adherence of oral bacteria, thereby inhibiting oral biofilm formation on the surface of restorative materials³⁴. In this study, the number of attached cells after incubation in bacterial suspensions was significantly lower on CA than that on F7 and MI. This suggests that the ability of CA to release Zn²⁺ was responsible for the inhibition of bacterial growth as well as bacterial adhesion, which could retard oral biofilm formation. Furthermore, fewer F. nucleatum cells were observed on CA, F7, and MI. As this bacterium serves as a “bridging organism” between early and late colonizers³⁵,³⁶, its reduction indicates inhibition of bacterial adhesion to the specimen surfaces.

The particle size and composition of glass fillers affect the physical properties of GICs³⁷. BioUnion filler has a particle size of approximately 7 μm, which is within the range for commercial GICs reported in previous studies³⁸. The setting time, compressive strength, and acid erosion of CA fulfilled the requirements described in the ISO 9917-1. In addition, the physical properties and shear bond strengths of CA were equivalent to those of conventional GIC. Moreover, repeated exposure to acetic acid promoted the release of Zn²⁺ from CA but did not reduce the compressive strength. Overall, the release of Zn²⁺ from the CA matrix did not deteriorate the physical properties. Acidity-induced release of Zn²⁺ from CA is considered to occur through ion exchange between H⁺ and Zn²⁺. In addition, it has been reported
that the ionomer reaction of GICs persists for a long time (known as maturation) and improves their mechanical properties due to the maturation of cements. Therefore, despite the dissolution of BioUnion filler in an acidic environment, the long-term ionomer reaction might be able to maintain the compressive strength of CA even after being exposed to acetic acid seven times. However, further investigation is required to measure the mechanical property of Caredyne-Restore under conditions that more closely resemble the oral environment.

CONCLUSIONS

The release of Zn$^{2+}$ from a GIC incorporating BioUnion filler (Caredyne-Restore) was accelerated under acidic conditions, and the released Zn$^{2+}$ could effectively inhibit the growth and adherence of oral bacteria. The study outcomes provide evidence that Zn$^{2+}$ release from BioUnion filler in acidic environment does not affect the compressive strength of GIC.

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REFERENCES

1) van Houte J. Role of micro-organisms in caries etiology. J Dent Res 1994; 73: 672-681.
2) Imazato S. Bio-active restorative materials with antibacterial effects: New dimension of innovation in restorative dentistry. Dent Mater J 2009; 28: 11-19.
3) Yengopal V, Mckenautsch S. Caries-preventive effect of resin-modified glass-ionomer cement (RM-GIC) versus composite resin: a quantitative systematic review. Eur Arch Paediatr Dent 2011; 12: 5-14.
4) Gluzman R, Katz RV, Frey BJ, McGowan R. Prevention of root caries: a literature review of primary and secondary preventive agents. Spec Care Dentist 2013; 33: 133-140.
5) Ten Cate JM. In vitro studies on the effects of fluoride on demand remineralization. J Dent Res 1999; 69: 614-619.
6) Seppä L, Korhonen A, Nuutinen A. Inhibitory effect on S. mutans by fluoride-treated conventional and resin-reinforced glass ionomer cements. Eur J Oral Sci 1995; 103: 182-185.
7) Prati C, Fava F, Di Gioia D, Selighini M, Pashley DH. Antibacterial effectiveness of dentin bonding systems. Dent Mater 1993; 9: 338-342.
8) Loyola-Rodriguez JP, Garcia-Godoy F, Lindquist R. Growth inhibition of glass ionomer cements on mutans streptococci. Pediatr Dent 1994; 16: 346-349.
9) Meiers JC, Miller GA. Antibacterial activity of dentin bonding systems, resin-modified glass ionomers, and polycyclic-modified composite resins. Oper Dent 1996; 21: 257-264.
10) Yan H, Yang H, Li K, Yu J, Huang C. Effects of chlorhexidine-encapsulated mesoporous silica nanoparticles on the anti-biofilm and mechanical properties of glass ionomer cement. Molecules 2017; 22: 1225.
11) Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. Dent Mater 2006; 22: 647-652.
12) Paiva L, Fidalgo TRS, da Costa LP, Maia LC, Balan L, Anselme K, et al. Antibacterial properties and compressive strength of new one-step preparation silver nanoparticles in glass ionomer cements (NanoAg-GIC). J Dent 2018; 69: 102-109.
13) Elgamily H, Ghallab O, El-Sayed H, Nasr M. Antibacterial potency and fluoride release of a glass ionomer restorative material containing different concentrations of natural and chemical products: An in vitro comparative study. J Clin Exp Dent 2018; 10: e312-e320.
14) Dashper SG, Catmull DV, Liu SW, Myroforidis H, Zalizniak I, Palamara JE, et al. Casein phosphopeptide-amorphous calcium phosphate reduces Streptococcus mutans biofilms development on glass ionomer cement and disrupts established biofilms. PLoS One 2016; 11: e0162322.
15) Imazato S, Kohno T, Teboi R, Thongthai P, Xu HH, Kitagawa H. Cutting-edge filler technologies to release bioactive components for restorative and preventive dentistry. Dent Mater J 2020; 39: 69-79.
16) Liu Y, Kohno T, Teboi R, Kitagawa H, Imazato S. Acidity-induced release of zinc ion from BioUnion$^{2+}$ filler and its inhibitory effects against Streptococcus mutans. Dent Mater J 2020; 39: 547-553.
17) He G, Pearce EI, Sissons CH. Inhibitory effect of ZnCl$_2$ on glycolysis in human oral microbes. Arch Oral Biol 2002; 47: 117-129.
18) Hasegawa T, Takenaka S, Ohsumi T, Ida T, Ohshima H, Terao Y, et al. Effect of a novel glass ionomer cement containing fluoro-zinc-silicate fillers on biofilm formation and dentin ion incorporation. Clin Oral Invest 2020; 24: 963-970.
19) Saad A, Nikaide T, Abdou A, Matin K, Burrow MF, Tagami J. Inhibitory effect of zinc-containing desensitizer on bacterial biofilm formation and root dentin demineralization. Dent Mater J 2019; 38: 940-946.
20) Hernández-Sierra JF, Ruiz F, Pena DC, Martínez-Gutiérrez F, Martínez AE, Guillén Ade J, et al. The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomedicine 2008; 4: 237-240.
21) Pizzey RL, Marquis RE, Bradshaw DJ. Antimicrobial effects of o-cymen-5-ol and zinc, alone & in combination in simple solutions and toothpaste formulations. Int Dent J 2011; 61: 33-40.
22) Siqueira Jr. Jr, Martinelli HA, Santos LE, Menezes MPM, Lopes HP. Antimicrobial activity of calcium hydroxide: A critical review. Int End J 1999; 32: 361-369.
23) Kanapka JA, Hamilton IR. Fluoride inhibition of enolase activity in vivo and its relationship to the inhibition of glucose-6-P formation in Streptococcus salivarius. Arch Biochem Biophys 1971; 146: 167-174.
24) Takahashi N, Washio J. Metabolomic effects of xylitol and fluoride on plaque biofilm in vivo. J Dent Res 2011; 90: 1463-1468.
25) Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: A comparative study. Int J Nanomed 2012; 7: 6003-6009.
26) Ning C, Wang X, Li L, Zhu Y, Li M, Yu P, et al. Concentration ranges of antibacterial cations for showing the highest antibacterial efficacy but the least cytotoxicity against mammalian cells: implications for a new antibacterial mechanism. Chem Res Toxicol 2015; 28: 1815-1822.
27) Wasson EA, Nicholson JW. Studies on the setting chemistry
of glass-ionomer cements. Clin Mater 1991; 7: 289-293.
28) Griffin SG, Hill RG. Influence of glass composition on the properties of glass polyalkenoate cements. Part I: Influence of aluminium to silicon ratio. Biomaterials 1999; 20: 1579-1586.
29) Wilson AD, Crisp S, Ferner AJ. Reactions in glass-ionomer cements: IV. Effect of chelating comonomers on setting behavior. J Dent Res 1976; 55: 489-495.
30) He Z, Huang Z, Zhou W, Tang Z, Ma R, Liang J. Anti-biofilm activities from resveratrol against Fusobacterium nucleatum. Front Microbiol 2016; 7: 1065.
31) Kolenbrander PE, Palmer RJ Jr, Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. Bacterial interactions and successions during plaque development. Periodontol 2000 2006; 42: 47-79.
32) Ghasempour M, Rajabnia R, Irannejad A, Hamzeh M, Ferdosi E, Bagheri M. Frequency, biofilm formation and acid susceptibility of Streptococcus mutans and Streptococcus sobrinus in saliva of preschool children with different levels of caries activity. J Dent Res 2013; 10: 440-445.
33) Jordan HV, Hammond BF. Filamentous bacteria isolated from human root surface caries. Arch Oral Biol 1972; 17: 1333-1342.
34) Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ Jr. Communication among oral bacteria. Microbiol Mol Biol Rev 2002; 66: 486-505.
35) Sawa N. The effect of filler shape and size of resin composite on fracture toughness. J Jpn Conserv Dent 1993; 36: 507-518.
36) Moheet IA, Luddin N, Rahman IA, Kannan TP, Nik Abd Ghani NR, Masudi SM. Modifications of glass ionomer cement powder by addition of recently fabricated nano-fillers and their effect on the properties: A review. Eur J Dent 2019; 13: 470-477.
37) Crisp S, Lewis BG, Wilson AD. Characterization of glass-ionomer cements. I. Long-term hardness and compressive strength. J Dent 1976; 4: 162-166.