Form 3

Abstract of Thesis

Name (LIU YUHAN)	
Title	Antibacterial effects of a glass-ionomer cement incorporating bio-active fillers with acidity-induced zinc ion-releasing ability （酸性環境での亜鉛イオン徐放能を備えたバイオアクティブフィラー含有グラスアイオノマーセメントの抗菌効果）

[Objective]

Glass ionomer cements (GICs) have been used for restorations of root surface caries due to their performance under wet conditions and fluoride-releasing property. Although it has been reported that fluoride-release from GICs has the potential to reduce the number of bacteria or interfere with bacterial metabolism in dental plaque, amount of fluoride released from GICs are not sufficient to effectively inhibit bacterial growth.

BioUnion filler (BU) is a glass powder capable of releasing Zn^{2+}, F^{-}, and Ca^{2+}. The most unique property of BU is its ability to release Zn^{2+} actively in an acidic environment. Therefore, once dental plaque is formed on the restoratives incorporating BU, the release of Zn^{2+} is expected to be promoted by acids produced by acidogenic bacteria in dental plaque and induce antibacterial effects. However, such *on-demand* Zn^{2+}-release abilities of BioUnion filler or restorative materials containing BioUnion filler and their antibacterial effects against oral bacteria have not yet been elucidated in detail. The purposes of this study were to investigate the release of ions from BU and a GIC containing BU in an acid environment, and to evaluate their inhibitory effects against the bacteria related to dental plaque formation.

[Materials and Methods]

EXPERIMENT 1. Evaluation of Zn^{2+}-release characteristic and antibacterial activity of BioUnion filler

1. *Characterization of BU*: The elemental composition of BU was analyzed by FE-SEM/EDS. The solubilities of BU in distilled water or acetic acid (pH 4.5) were measured.
2. *Evaluation of ion release from BU*: BU was immersed in distilled water or acetic acid (pH 4.5 or 5.5). After storage for 24 h, the concentrations of Zn^{2+}, Ca^{2+}, and F^{-} in eluates were measured. The fluoroaluminosilicate glass powder of a conventional GIC (Fuji VII, GC Corp.; F7) was used for comparison.
3. *Measurement of MICs and MBCs of Zn^{2+}, Ca^{2+}, and F^{-} for oral bacteria*: The MIC and MBC values of three ions against 6 bacterial species (*Streptococcus mutans*, *S. sobrinus, S. oralis, S. mitis*, *Actinomyces naeslundii*, and *Fusobacterium nucleatum*) were measured with a microdilution assay.
4. *Evaluation of antibacterial activity of BU*: BU or F7 powder was immersed in *S. mutans* suspension adjusted to 10^4 CFU/mL with or without 1% sucrose. After incubation for 24 h, the number of viable bacteria was counted.
5. *Evaluation of Zn^{2+}-release from BU with repeated exposure to acid and inhibition of S. mutans*: BU or F7 powder was immersed in acetic acid (pH 4.5) for 1 day. Then, the particle was immersed in distilled water for 3 days, while replacing water every day. This procedure was repeated three times, and the concentrations of Zn^{2+} in eluates were measured. Each filler was collected on Day 8 after two times exposure to acetic acid, and was used to evaluate the antibacterial effects against *S. mutans* using the same method as described in 1-4.

EXPERIMENT 2. Evaluation of Zn^{2+}-release characteristic and antibacterial activity of a GIC containing BioUnion filler

In this experiment, a GIC containing 35-45% BU (Caredyne Restore, GC Corp.; CA) was examined.

1. *Evaluation of ion release from CA*: CA was immersed in distilled water or acetic acid (pH 4.5). After storage for 24 h, the concentrations of Zn^{2+}, Ca^{2+}, and F^{-} in eluates were measured. F7 was used for comparison. To evaluate Zn^{2+} release from CA with repeated exposure to acid, the immersion procedure in water and acid was repeated seven times for a total of 28 days as described in 1-5, and the concentrations of Zn^{2+} in eluates were measured.
2. *Elemental analysis of CA*: FE-SEM/EDS was used to analyze elemental composition of the set CA cement before and after seven times exposures to acetic acid.
3. *Evaluation of antibacterial activity of CA*: On-disc culture assay was conducted by using six species of bacteria. The set CA disc was immersed in human saliva for 2 h. Each bacterial suspension (20 μL) of six species, adjusted to 10^6 CFU/mL with 1% sucrose, was inoculated on the saliva-treated disc. After incubation for 24 or 48 h, the number of CFU was counted. F7 and resin composites (MI Fil, GC Corp.; MI) were used for comparison. To evaluate inhibitory effect on bacterial growth with repeated exposure to acid, each specimen was collected on Day 4, 8 and 24 using the same method as described in 2-1, and their antibacterial effects against six species were evaluated.

4. *Evaluation of bacterial adherence to CA*: The saliva-treated CA, F7, or MI disc was immersed in each bacterial suspension with 1% sucrose. After incubation, the number of cells adhered to the specimen was determined.
5. *In situ assessments of antibacterial effect of CA*: The disc-shaped specimens were fixed in the region of upper premolars and molars of volunteers using a custom-made acrylic splint. The specimens were collected after 24 h, and the biofilm formed on the surface was observed and analyzed by using a confocal laser scanning microscopy (CLSM) with LIVE/DEAD staining. The number of viable bacteria in the biofilm was determined by colony counting. F7 and MI were used for comparison.

EXPERIMENT 3. Evaluation of physical properties and bonding ability of CA

1. *Physical properties*: The setting time, acid erosion, and compressive strength of CA were examined according to ISO 9917-1. The toothbrush wear of CA was evaluated. F7 was used as a control. The compressive strength of CA and F7 after seven times exposures to acetic acid was also examined.
2. *Bonding ability of CA*: Shear bond strengths of CA to enamel and dentin were evaluated. F7 was used as a control.

[Results and Discussion]

EXPERIMENT 1.

1. EDS analysis revealed Zn, Ca, and F were detected in BU at a mass fraction of approximately 12.7, 3.0, and 5.2%, respectively. The solubility of BU into acid was greater than that into water.
2. The concentrations of Zn^{2+} and Ca^{2+} released from BU into acetic acids were significantly higher than those released into water ($p < 0.05$, Tukey's HSD test). Conversely, the concentrations of F^- released from BU and F7 powder into acetic acids were significantly lower than those into water ($p < 0.05$).
3. The MIC values of Zn^{2+} for the six species ranged from 64 to 128 ppm, whereas the MBC ranged from 512 to 1024 ppm. These values were smaller than those of Ca^{2+} and F^-.
4. Inhibition of *S. mutans* by BU was significantly greater when incubated with sucrose ($p < 0.05$, Tukey's HSD test), reflecting a decrease in suspension pH in response to the addition of sucrose, while no significant difference in the inhibition of *S. mutans* by F7 powder was observed between the incubation with and without sucrose.
5. Exposure to acids increased Zn^{2+}-release from BU, resulting in higher concentrations than MICs against *S. mutans* across three times exposure. BU after repeated exposure to acid demonstrated inhibitory effects against *S. mutans* growth.

EXPERIMENT 2.

1. The concentration of Zn^{2+} released from CA into acetic acid was higher than that into water, and also above the MICs against six bacterial species ($p < 0.05$, Tukey's HSD test), whereas the concentrations of Ca^{2+} and F^- released were lower than the corresponding MICs ($p < 0.05$). The concentration of Zn^{2+}-release was maintained across seven times exposures to acetic acid.
2. Zn and Ca were homogeneously distributed in the matrix of experimental cement, whereas Si and F were densely distributed around the particle.
3. Compared with F7 and MI, CA significantly inhibited the growth of all six species, and also inhibited the bacterial growth after repeated exposure to acid ($p < 0.05$, Tukey's HSD test).
4. CA inhibited the adherence of each bacterial species on the material surface compared with F7 and MI ($p < 0.05$, Tukey's HSD test).
5. *In situ* biofilm formed on CA was significantly thinner than F7 and MI ($p < 0.05$, Tukey's HSD test). The number of surviving cells in the biofilm formed on CA was significantly smaller than that of F7 and MI.

EXPERIMENT 3.

1. The setting time, acid erosion, and compressive strength of CA fulfilled the requirement described in the ISO 9917-1. No significant differences in the setting time, acid erosion, compressive strength, and toothbrush wear were observed between CA and F7 ($p > 0.05$, Tukey's HSD test). No significant difference in the compressive strength of CA was observed between the specimens before and after repeated exposure to acid ($p > 0.05$).
2. No significant difference in the bond strength to enamel and dentin was observed between CA and F7 ($p > 0.05$).

[Conclusion]

The release of Zn^{2+} from BioUnion filler and a GIC incorporating BioUnion filler was accelerated under acidic conditions, and the growth and adherence of oral bacteria were effectively inhibited. Thus, the GIC incorporating BioUnion filler with acidity-induced ability to release zinc ion is able to hinder early-stage biofilm formation on its surface, which is expected to be of benefit in prevention of recurrent caries on root surface.

様式７

論文審査の結果の要旨及び担当者

氏　名　（　Liu Yuhan　）	
論文審査担当者	（職）　氏　名 主査　教　授　今里　聡 副査　教　授　天野敦雄 副査　准教授　野村良太 副査　講　師　峯　篤史

論文審査の結果の要旨

本研究は、亜鉛・カルシウム・フッ素を含むケイ酸塩ガラス（BioUnion filler）と、それを含有するグラスアイオノマーセメントのイオン溶出特性および口腔細菌に対する抗菌効果を評価したものである。

その結果、BioUnion filler およびそれを含有するグラスアイオノマーセメントはともに、酸性環境で効率的に亜鉛イオンを溶出する特性を有し、デンタルプラークの形成に関与する各種細菌に対して抗菌効果を発揮することにより、*in vitro* および *in situ* において硬化セメント表面でのバイオフィルム形成を抑制することが明らかとなった。

以上の研究成果は、酸性環境で抗菌性を発現する新たな歯科用修復材料技術の詳細を明らかにしたものであり、本研究は博士（歯学）の学位授与に値するものと認める。