Form 3

Abstract of Thesis

Name (Deng Fan)	
Title	Development of novel zinc ion-releasing glasses for conferring antibacterial activities to dental materials （歯科材料への抗菌性付与を目的とした新規亜鉛イオン徐放ガラスの開発）

[Objective]

Several attempts have been made to provide ion-releasing glasses with antibacterial effects by adding zinc (Zn), thereby endowing dental materials with antibacterial properties. However, trials to control ion release from the glasses to make it clinically effective, considering the usage conditions of dental materials, are limited. Silicate-based glass is known to dissolve in acids while it is relatively resistant to dissolution in neutral or slightly alkaline environments. On the other hand, phosphate-based glass dissolves rapidly in wet conditions regardless of pH. Therefore, by utilizing these glass technologies, it is possible to fabricate Zn-ion releasing glasses that enable dental resins to exhibit antibacterial effects on demand in response to acidity or resin-based liners to exert immediate cavity disinfecting effects. The purpose of this study was to develop two different types of glass particles; acidity-responsive Zn^{2+}-releasing glass and rapidly soluble Zn^{2+}-releasing glass, and to evaluate their ion releasing properties and antibacterial effects. Additionally, dental resins incorporating each glass were fabricated and their effectiveness was investigated.

[Materials and Methods]

EXPERIMENT 1: Fabrication of acidity-responsive Zn^{2+}-releasing glass and incorporation into dental resins

Silicate-based glasses containing Zn (AGs) were fabricated by the melt-quenching method. Three types of glasses were arranged in ascending order of Zn content (coded AG-1, AG-2, and AG-3). High-resolution imaging of each particle surface and its elemental mapping were obtained by FE-SEM/EDS. The particle size distribution of each glass was determined by a particle size analyzer. The glass structure and elemental composition was analyzed by XRD and XRF, respectively. BioUnion filler, which is the glass powder incorporated in a commercially available glass-ionomer cement, was used as a control (Cont).

1. Evaluation of solubility and ion-releasing properties of AGs: The solubility and the concentrations of Zn^{2+} and F^- released from AGs under different pH conditions (pH 7.0, 6.0, or 5.0) were measured by weight measurement and ICP-OES.
2. Measurements of MICs and MBCs of Zn^{2+} and F^- against *Streptococcus mutans*: The MIC and MBC values of Zn^{2+} and F^- against *S. mutans* NCTC10449 were measured by broth dilution method.
3. Evaluation of antibacterial activity of AGs: The glass particles were immersed in *S. mutans* suspension (1×10^6 CFU/mL) with pH adjusted to 7.0, 6.0 or 5.0. After 24-h anaerobic incubation, the number of bacteria was counted.
4. Evaluation of on-demand ion-releasing and antibacterial activity of AGs: The glass particles were immersed in acetate buffer (pH 5.0) for one day. The solution was then replaced with HEPES (pH 7.0) and stored for three days. Every 24 h, the buffer solution was replaced, and the concentration of Zn^{2+} was measured. This procedure was repeated until Day 9 with three-time exposures to acetate buffer. The glass particles collected on Day 8 were used to evaluate the antibacterial effects against *S. mutans* adjusted to pH 7.0 or 5.0, employing the method described in section 1-3.
5. Fabrication of dental resins incorporating AG and evaluation of antibacterial activity: The experimental resins were prepared by mixing AG-3 with monomers and the initiator, and polymerized by light-curing. The weight percentage of AG-3 in the resins was 10%, 20%, or 30%. The pH-adjusted *S. mutans* suspension (pH 7.0, 6.0, or 5.0) was inoculated on each cured specimen and the number of viable bacteria was counted after anaerobic incubation for 24 h. A cured resin without AG-3 was used as a control.
6. Evaluation of on-demand ion-releasing and antibacterial activity of dental resin incorporating AG: The experimental resin incorporating 30% AG-3 was exposed to acetate buffer and HEPES using the same method as described in section 1-4, and the concentrations of Zn^{2+} in the eluates were measured. The antibacterial activity of the experimental resin after two cycles of exposure to acetate buffer and HEPES was evaluated by the method described in section 1-4.
7. Evaluation of anti-biofilm effects of dental resin incorporating AG: The experimental resin incorporating 30% AG-3 after the third exposure to acetate buffer was treated with saliva and immersed in *S. mutans* suspension for 12 h. After sequentially treated with BHI with or without sucrose, the biofilm formed on the specimen surface was analyzed by using SEM and CLSM with LIVE/DEAD staining.

EXPERIMENT 2: Fabrication of rapidly soluble Zn^{2+}-releasing glass and incorporation into dental resin

Phosphate-based glass containing Zn (RG) was fabricated by the melt-quenching method. The characteristics of RG were determined by the same methods described in Experiment 1.

1. Evaluation of solubility and ion-releasing property of RG: The solubility and ion-releasing property of RG were

evaluated by the same methods described in section 1-1.

2. Measurements of MICs and MBCs of Zn^{2+} against oral bacteria: The MIC and MBC values of Zn^{2+} against five species of oral bacteria (*L. casei, A. naeslundii, E. faecalis, F. nucleatum,* and *P. micra*) detected in deep caries were measured by broth dilution method.
3. Evaluation of antibacterial activity of RG: Suspension of each of five bacteria described above was prepared (1×10^6 CFU/mL) and incubated in the presence of RG. After anaerobic incubation for 24 h, the number of viable bacteria was counted.
4. Fabrication of dental resin incorporating RG and evaluation of inhibition of bacteria in dentinal tubules: The experimental resin was prepared by mixing RG with monomers and the initiator. The weight percentage of RG in the resin was 10%. The dentin block obtained from human tooth were infected with *L. casei* or *S. mutans*, and the experimental resin was applied on the surface. After light-curing of resin, the dentin block was sectioned and stained with LIVE/DEAD staining kit to evaluate viability of bacteria in dentinal tubules through CLSM observation. A dental resin without RG was used as a control.

[Results and Discussion]

EXPERIMENT 1

1. The morphology of each AG particle was irregular in shape. The EDS mapping confirmed that Zn contents in the glass particle increased from AG-1 to AG-3. The XRD analysis indicated the amorphous structure of each glass. The median diameter of each particle was approximately 11 μm. The XRF analysis confirmed the Zn content of three types of AGs at a mol fraction of approximately 25.3, 34.6, and 42.7%, respectively.
2. As the pH value decreased, AGs demonstrated significantly greater solubility and Zn^{2+}-release ($p<0.05$). The concentrations of Zn^{2+} released at pH 5.0 significantly increased in the order of Cont, AG-1, AG-2, and AG-3 ($p<0.05$).
3. For Zn^{2+}, the MIC and MBC for *S. mutans* were 125 and 250 ppm, respectively. For F^-, the MIC for *S. mutans* was 125 ppm, whereas the MBC was greater than 500 ppm.
4. Inhibition of *S. mutans* by AGs was significantly greater with pH decrease ($p<0.05$). The inhibition of AG-2 and AG-3 for *S. mutans* was more effective than AG-1 and Cont ($p<0.05$).
5. Exposure to acids increased Zn^{2+}-release from AG-2 and AG-3. After three-time exposures to acid, the concentrations of Zn^{2+} released from AG-2 and AG-3 were greater than the MBCs of Zn^{2+}. After repeated exposure to acid, AG-2 and AG-3 demonstrated antibacterial effects against *S. mutans* under acidic conditions. The inhibition of *S. mutans* by AG-3 was significantly greater than by Cont, AG-1, and AG-2 ($p<0.05$). Based on these results, AG-3 with 42.7 mol% Zn was selected to be incorporated in dental resins.
6. At pH 5.0 and 6.0, the number of viable cells on the experimental resin incorporating 30% AG-3 was significantly smaller than those on both the control and the experimental resins containing 10 and 20% AG-3 ($p<0.05$). This reduction was also significant compared to the initial number of bacteria, indicating that the experimental resin containing 30% AG-3 exhibited bactericidal effects under acidic conditions.
7. The concentrations of Zn^{2+} released from dental resin incorporating 30% AG-3 increased repeatedly in response to exposure to acids. Its killing effects against *S. mutans* were maintained under acidic conditions even after repeated exposures to acid.
8. The dental resin incorporating 30% AG-3 exhibited a greater percentage of membrane-compromised bacteria, which are likely dead, with a reduced biofilm thickness when compared with the control resin without AG-3.

EXPERIMENT 2

1. RG particle demonstrated irregular shape and amorphous structure. The median particle diameter was approximately 12 μm. XRF analysis confirmed the Zn content of RG at a mol fraction of 11.9%.
2. Across all three pH conditions, the solubilities of RG after 24 h were over 90% and the concentrations of Zn^{2+} released were beyond 5900 ppm.
3. For the five species, the MIC values ranged from 62.5 to 1000 ppm and the MBC values ranged from 125 to 4000 ppm.
4. RG exhibited bactericidal effects against all bacterial species.
5. The CLSM images revealed that dental resin incorporating 10% RG exhibited complete eradication of bacteria in dentinal tubules.

[Conclusion]

Acidity-responsive and rapidly soluble Zn^{2+}-releasing glasses were successfully fabricated. The acidity-responsive Zn^{2+}-releasing glass effectively released Zn^{2+} under acidic conditions, achieving exhibition of on-demand killing effects against bacteria in the dental plaque when incorporated into resins. The rapidly soluble Zn^{2+}-releasing glass released high concentration of Zn^{2+} in a short period at neutral pH, being useful for killing bacteria in dentinal tubules when incorporated into resins. These findings indicated that the two novel glasses developed in this study enabled the controlled release of Zn^{2+}, allowing for conferring possible clinically effective antibacterial activities to dental materials under their usage conditions.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、ケイ酸塩系およびリン酸塩系の 2 種の亜鉛含有ガラスを新規に作製し、それら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。さらに、各ガラスを配合した歯科用レジンを試作し、その有効性を検討したものである。

その結果、ケイ酸塩系の亜鉛含有ガラスおよびそれを配合した試作レジンは、酸性条件で効果的に亜鉛イオンを徐放することで、歯肉縁上プラーク内の細菌に対して抗菌効果を発揮することが明らかとなった。また、リン酸塩系の亜鉛含有ガラスおよびそれを配合した試作レジンは、高濃度の亜鉛イオンを短期間に放出することで、象牙細管内の細菌に対して即時殺菌効果を発揮することが立証された。

以上の研究成果は、酸応答性あるいは速溶性の亜鉛イオンの徐放により、臨床的に有効な抗菌性を発現できる新たな歯科材料技術の確立に成功したことを示すものであり、本研究は博士（歯学）の学位論文として価値のあるものと認める。