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Antibacterial Activity of Zinc Oxide and Copper Oxide Nanocomposite

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Abstract: Nanotechnology has recently emerged as a critical antimicrobial strategy. We developed a novel fluoride-containing zinc-copper nanocomposite (FZC). This study aimed to investigate the antibacterial effect of the nanocomposite and its effect on cell activity.A fluoride-containing zinc-copper nanocomposite (FZC) was synthesized according to the previous study and observed SEM.We analyzed the antibiotic susceptibility of S. mutans (ATCC 25175) to different concentrations of FZC. The bacterial were cultured in BHI medium with FZC and incubated for 24 h at 37°C. The antibacterial effect was calculated by the colony-forming units (CFUs). Cell viability assay was measured using hDPSCs cultured with Dulbecco's modified Eagle's medium and 10% aqueous solution. After that, Cell viability was measured by the Cell counting Kit-8. The control BHI medium allowed bacterial growth, whereas Ca $(OH)_2$ and ZCF(350 mg/ml, 87.5 mg/ml, 21.9 mg/ml) allowed no bacterial growth. The cell activity of FCZ was 80% that of the control.The fluoride-containing zinc-copper nanocomposite developed in this study had higher antimicrobial activity than calcium hydroxide. Even if it's diluted 1000 times, it showed a little antibacterial effect. However, it affects cell activity with no lethal effects.

Keywords - *Antibacterial activity, Biomaterial, Cell viability, Nanocomposite*

I. INTRODUCTION

Oral microorganisms cause oral diseases, such as dental caries, periodontal disease, and halitosis. These diseases are also related to severe systemic diseases such as cardiovascular diseases and bacterial endocarditis. Therefore, many antibacterial materials, such as mouth rinses and local periodontal medicines, are used in the clinical setting. Therefore, many antibacterial materials have been discovered and have been used clinically. Some dental materials with antibacterial activity include trace elements, such as Ag, Cu, and Zn. The antimicrobial activity of metals is based on their ability of metal ions to prevent the uptake of trace elements by microbes [1], inhibit enzymes [2], damage microbial cell membranes [3], and enhance reactive oxygen generation [4]. Moreover, several metals exert direct genotoxic activity [5-7]. Silver-and zinc-reinforced glass ionomer cements are used in clinical applications. The addition of Ag or Zn to these materials results in antibacterial activity [8]. Zinc could prevent dental caries by inhibiting the growth of oral bacteria [9, 10].

Additionally, high concentrations of zinc inhibit the activity of matrix metalloproteinases (MMPs), which degrade collagen fibers and protect collagen from MMPs [11, 12]. Biological polymers, such as collagen, form the core of mineralization [13]. Thus, zinc, which protects collagen, enhances dentin remineralization.

Cu reduces the number of bacteria on the tooth surfaces. The suggested mechanism of action of Cu involves the limitation of bacterial growth and inhibition of glycolysis, leading to a decrease in acid production [14, 15].Cu also interferes with glucan formation through glucosyltransferases. This process may contribute to the reduced plaque accumulation. Zinc salt is commonly used clinically, but its effects are limited by poor retention, because its levels decrease drastically within 1 h of intraoral treatment [16]. Some mouth rinses contain copper gluconate, which exhibits antibacterial activity. Adjunctive methods such as mouthwashes are useful for preventing plaque accumulation [17].The antibacterial effects of copper cement and copper varnish on the root surface are similar to those of chlorhexidine [18].Nanotechnology has been introduced in the field of dental materials in recent years, and nanoparticles have been incorporated into the structures of dental composites [19, 20]. Cu nanoparticles have been applied in various fields including biomedical equipment and devices [21].

The antibacterial activity of novel fluoride-containing ZnO–CuO nanocomposites was reported in a previous study. However, the dose-dependent effects remain unclear. Therefore, in this study, we aimed to evaluate the antibacterial activity of a novel fluorine-containing nanocomposite.

II. MATERIAL AND METHOD

2.1 Preparation and observation of nanocomposite

2.1.1 Preparation Fluoride, Zinc, and Copper nanocomposite (FZC)

Fluoride-containing ZnO and CuO nanocomposites were prepared as described previously [22]. First, $ZnCl_2$ (2.0 mmol), $CuSO_4{\cdot}5H_2O$ (1.0 mmol), NaF (1000 ppm), and NaOH (10.0 mmol) were mixed in distilled water. The mixed solution was kept static at 80^0C for 24 h, and then naturally cooled to room temperature. The solutions were centrifuged, washed several times with distilled water and absolute alcohol to remove any residues, and dried at 80 ^{0}C for 12 h.

2.1.2 Scanning electron microscopy (SEM) observation of nanocomposite

The samples were then mounted on an aluminum stub with uncoated carbon tape for SEM. The particle morphology and size distribution were analyzed at magnifications of 60 K and 110 K using an S-4800 (Hitachi) scanning electron microscope at 5 kV. EDS spectra of samples, 100 µm x 100 µm area, were recorded by using a S-2380N (Hitachi) scanning microscope system with a Genesis G4000 (EDAX Japan) detector at 15 kV. The resulting X-Ray spectra were used for identification of the minerals and particle compositions. The elemental analysis % weight of samples was determined by applying the ZAF correction method.

2.2. Antibacterial effect analysis

In all experiments, *S. mutans* (ATCC 25175) was grown anaerobically at 37°C in brain heart infusion medium. Overnight cultures of test bacteria were diluted and adjusted in fresh media to 5×10^5 cells/mL, and the antibiotic susceptibility of *S. mutans* to six different concentrations (350, 87.5, and 21.9 mg/ml,5.5 mg/ml,1.4 mg/ml, 0.4 mg/ml) of FZC using Ca $(OH)_2$ (350 mg/ml) as the positive control and BHI medium only as a negative control. The bacterial suspension (1 µl), 95 µl BHI medium, and 100 µl BHI medium were mixed and incubated for 24 h at 37°C in 96-well microliter plates. The colony numbers from all plates were counted, and the CFUs were calculated. Bacterial growth in each group was analyzed using the Games-Howell test ($p < 0.05$).

2.3 Cell viability assay

Acetate buffer was freshly prepared and titrated to the desired pH level of pH5.5, respectively. The previously prepared FZC nanocomposite powder was adjusted to 0.1% in 10 ml. The products were separated by centrifugation and the supernatant was used as an aqueous solution. hDPSCs (PT-5025) were obtained from Lonza. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 50 U/mL penicillin, and 50 g/mL streptomycin (Gibco, Grand Island, NY, USA). The 96-well plates were inoculated with cells at an initial density of 1.0×10^3 cells/well.All cells were cultured at 37°C in a humidified incubator with 5% CO2 and

95% air. Cells from passage four were used in this experiment. All experiments were repeated at least three times to ensure reproducibility. Material cytotoxicity was assessed using a cell counting Kit-8 assay (CCK-8; Dojindo, Kumamoto, Japan). The materials were incorporated into DMEM with different concentrations of aqueous solutions. The cells were cultured in 96-well plates containing DMEM (10% FBS and 1% antibiotics) and aqueous solutions of different concentrations were added on day 1. The CCK-8 reagent was added on day three. After addition of CCK-8 (10 μL/well), the cells were incubated for 105 min. The absorbance was read at 450 nm using an iMark microplate reader (Bio-Rad, Hercules, CA, USA).

III. RESULTS

SEM images are shown in Fig. 1. The FZC nanocomposites exhibited granular-like microstructures with diameters of approximately 100 nm.

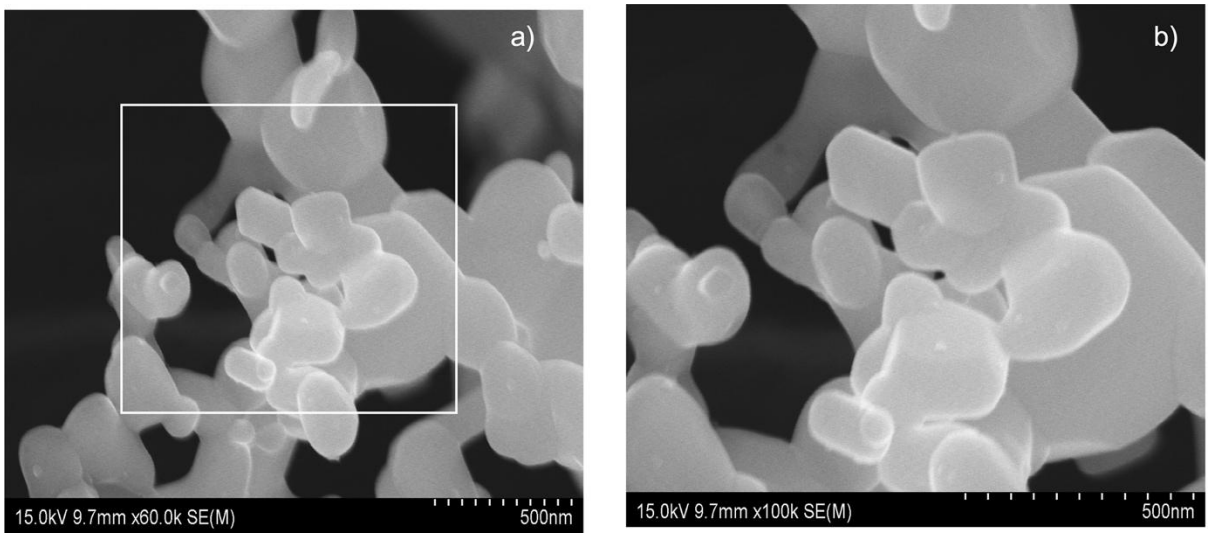

Figure1.SEM images of prepared a) x60K, b) x100K fluorine-containing ZnO–CuO nanocomposite (FZC)

Environmental scanning electron microscopy and energy-dispersive X-ray analysis revealed that the FZC contained 55.5±2.8% Cu, 43.0±2.1%Zn, 1.9±0.8% Cl, and 0.1±0.1% F.

We examined the effect of FZC on *the growth of S. mutans* in suspension (Fig 2).

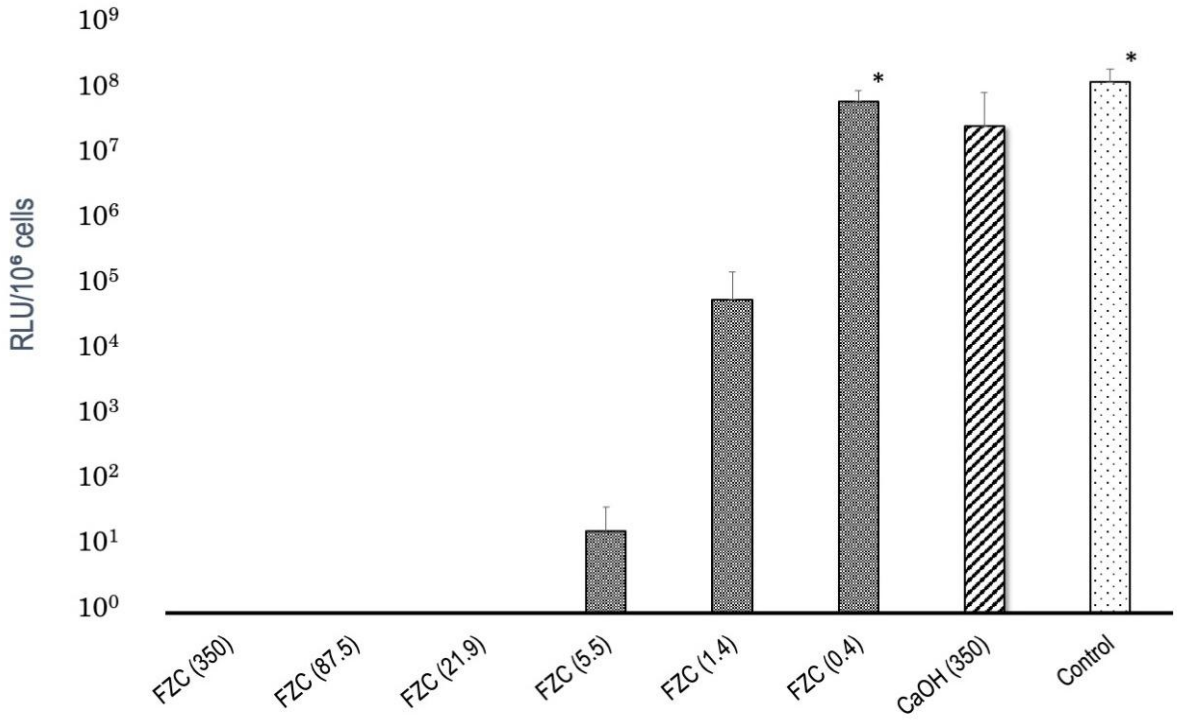

Figure 2. Antibacterial activity of FZC

*p < 0.05; considered statistically significant at Games-Howell test (p < 0.05), n=5.

The positive control group showed significant antibacterial activity compared with that of the control group. The controls with BHI medium allowed bacterial growth wherein FZC at a concentration of 350 mg/ml, 87.5 mg/ml, and 21.9 mg/ml inhibited bacterial growth. at a 5.5 mg/ml concentration of 1.4 mg/ml showed some bacterial growth. There are no significant differences between FZC at a 0.4 mg/ml concentration of bacteria growth and the control group. FZC showed antibacterial activity at a significantly lower concentration than calcium hydroxide. Calcium hydroxide is commonly used in root canal treatment and exhibits a robust antibacterial effect owing to its strong alkalinity.Changes in cell viability in response to different concentrations of FZC were assessed using a CCK-8 assay. As shown in Fig 3, FZC inhibited cell viability, and its ratio was almost 80% compared with control.

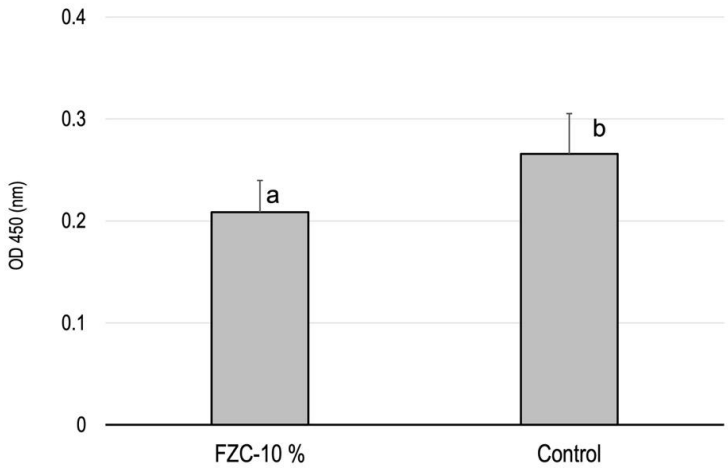

Figure 3. CCK-8 cell viability10% of aqueous solution of FZC and medium without the materials was used as a control. Different lowercase letters indicate a significant difference ($p < 0.05$), n=6.

IV. DISCUSSION

This study showed that FZC has a higher antimicrobial activity than calcium hydroxide. Even when diluted 1000 times, it showed little antibacterial effect.

It has been reported that the antimicrobial activity of Ca $(OH)_2$ is caused by the release of hydroxyl ions in an aqueous environment, which has an extreme oxidizing power.[23] The free radicals of hydrogen ions are highly reactive with various biological tissues; therefore, they react indiscriminately with biological tissues surrounding the calcium hydroxide [24].Against bacteria, hydroxide ions can damage bacterial cytoplasmic membranes, proteins, or DNA, thereby killing the bacteria[25].

The antimicrobial effect of metal nanoparticles such as zinc and copper are also due to the bactericidal effect of the active oxygen produced by interaction with bacteria [3].The ZnO–CuO nanocomposite exhibits better photocatalytic properties than the respective oxides [26]. In addition, the ZnO-CuO nanocomposite could also generate free radicals, decompose pigments, and kill bacteria effectively. In addition, nanocomposites of ZnO and CuO exhibit antimicrobial activity more than 1000 times higher than that of ZnO and CuO. [27]. A ZnO-CuO nanocomposite coating has also been reported to inhibit bacterial adhesion and coating the tooth surface can inhibit bacterial adhesion[28].

Although coatings of ZnO and CuO nanoparticles have been widely applied, nanoparticles from the coating material pose severe problems to living organisms, including vertebrates. However, little biotoxicity has been reported compared to the antimicrobial properties of ZnO-CuO nanocomposites.

Our results showed that the ZnO-CuO nanocomposites affected cellular activity by approximately 20%. Manteca et al. reported that zinc-copper oxide (ZnO-CuO) nanocomposites exhibit weaker embryotoxicity than ZnO and CuO[29]. Therefore, it is necessary to investigate the composition and concentration of ZnO-CuO nanocomposites that exhibit antibacterial properties without affecting the cellular activity.

V. CONCLUSION

Our ZnO/CuO nanocomposites showed significantly more vigorous antibacterial activity than calcium hydroxide.

The nanocomposite also showed some cytotoxicity, reducing the activity of cells by about 20%.

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