Antimicrobial Activity of Glass Ionomer Cement Incorporated with Chlorhexidine-Loaded Zeolite Nanoparticles

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A functional dental restorative system with antimicrobial properties was developed using zeolite (ZE) nanoparticles (NPs) as a drug delivery carrier. ZE NPs loaded with chlorhexidine (CHX) were prepared using the ionic immobilization method. The resulting CHX-loaded ZE NPs were then incorporated into commercial dental glass ionomer cement (GIC). The average size of the CHX-loaded ZE NPs was about 100 to 200 nm, and the NPs were dispersed homogeneously in the GIC. The in vitro release profile of encapsulated GIC containing CHX showed an early release burst of ∼30% of the total CHX by day 7, whereas GIC containing CHX-loaded ZE NPs showed a sustained release of CHX without the early release burst in a 4-week immersion study. The agar diffusion test results showed that the GIC incorporated with CHX-loaded ZE NPs showed a larger growth inhibition zone of Streptococcus mutans than GIC alone, indicating that this innovative delivery platform potently imparted antimicrobial activity to the GIC. Moreover, these findings suggest that a range of antimicrobial drugs that inhibit the growth of oral bacteria can be incorporated efficiently into dental GIC using CHX-loaded ZE NPs.

Keywords: Zeolite, Chlorhexidine, NPs, Antimicrobial Effect, Glass Ionomer Cement.

1. INTRODUCTION

Glass ionomer cements (GICs) are used in dentistry for dental fillings and luting cements.1 After long-term clinical services, however, microleakage may frequently occur, which eventually results in secondary caries, thus compromising the longevity of the restoration. One of the main causes of secondary caries is demineralization of the tooth structure due to invasion of oral bacteria such as Streptococcus mutans. To overcome this problem, several attempts in developing GIC with antimicrobial activity by the addition of antimicrobial agents such as chlorhexidine (CHX) have been reported.2 However, the release of antimicrobial agents from the material into the surrounding milieu has several common problems: decreased mechanical properties of the carrier material, short-term antimicrobial effectiveness, and potential toxicity if the release is not properly controlled.3

Zeolites (ZEs) are microporous crystalline solids with well-defined structures containing aluminum, silicon, and oxygen in their regular framework. Void spaces within the framework are capable of hosting cations, water, or other organic molecules, which in turn can be selectively exchanged for other species. The antimicrobial activity of GIC can be enhanced by a ZE drug carrier.4

The purpose of this study is to develop CHX-loaded ZE nanoparticles (NPs) and investigate the effect of GIC incorporated with these NPs on the antimicrobial activity against S. mutans.

2. EXPERIMENTAL DETAILS

2.1. CHX-Loaded ZE NPs

H-beta ZE (SiO2:Al2O3 = 98:2 (w/w), Cosmo Catalysts Co., Ltd.) was used as the drug carrier. CHX was incorporated into the ZE by the ionic immobilization method,5 in which the ZE NPs (30 mg) were added to CHX (Sigma Co., USA) solution (30 mg/10 mL). The mixed solution
was stirred at room temperature for 24 h, then centrifuged, washed with distilled water, and air dried.

The morphology and the distribution of CHX-loaded ZE NPs in the GIC specimen were examined using scanning electron microscopy (SEM, JSM-6700F, Jeol, Japan). The chemical structures of the ZE and CHX-loaded ZE NPs were characterized by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (Frontier, PerkinElmer Inc., USA).

GIC specimens were prepared as follows. A conventional powder/liquid type GIC (Fuji II, GC Corp., Japan) was used as the control. Two experimental GIC specimens containing 1% of CHX powder (GIC + CHX) or 1% of CHX-loaded ZE NPs (GIC + CHX-ZE) (by weight) were prepared. The morphologies of the GIC specimens were observed using SEM.

2.2. CHX Release

The disc-shaped GIC specimens were immersed in 3 mL phosphate buffered saline (PBS, pH 7.4). After being stored at 37 °C for 1, 4, 7, 10, 15, 20, and 30 days, concentration of eluted CHX from specimens was determined by high performance liquid chromatography (HPLC, LC-20AD, Shimadzu Co., Japan). An indirect method was employed to determine the drug encapsulation efficiency. The unloaded CHX was quantified by measuring the free drug found in the supernatant of the prepared CHX-loaded ZE NPs by HPLC. The CHX-loaded quantity (total CHX added – free CHX) was determined using the amount of unloaded drug.4

2.3. Antimicrobial Activity

*S. mutans* (ATCC 25175, KCTC 3065) suspension (OD600 = 0.5) was streaked on brain heart infusion (BHI) agar. Then, disc-shaped GIC specimens (8 mm in diameter and 1 mm in thickness) were placed onto the agar plate and incubated over night at 37 °C. The disc-shaped GIC specimens were placed in the wells of a 24-well plate. A 10 μL of bacterial suspension (10⁶ CFU/mL) was placed on the surface of each specimen and incubated for 24 h at 37 °C. Evaporation of the suspension liquid resulted in a layer of bacteria, ensuring direct contact between the bacteria and the specimen surface. The specimens were washed with distilled water, and freeze-dried.

2.4. *S. Mutans* Adhesion to Specimens

The disc-shaped GIC specimens were placed in the wells of a 24-well plate. A 10 μL of bacterial suspension (10⁶ CFU/mL) was placed on the surface of each specimen and incubated for 24 h at 37 °C. Evaporation of the suspension liquid resulted in a layer of bacteria, ensuring direct contact between the bacteria and the specimen surface. The specimens were washed with distilled water, and freeze-dried, and observed by SEM.

2.5. Compressive Strength and Bond Strength

The compressive strength of the GIC specimens was measured according to ISO 9917-1. Cylindrical specimens (4 mm in diameter and 6 mm in height) were prepared and immersed in distilled water for 24 h prior to testing. Flat human dentin surfaces were produced by wet grinding on 600-grit silicon carbide paper. Cylindrical tubes (internal diameter of 3 mm and a height of 4 mm) filled with freshly prepared GIC materials were placed into the center of the dentin surfaces. The bonded specimens were stored at 37 °C in distilled water for 24 h prior to testing. The compressive strength and shear bond strength tests were performed using a universal testing machine (3366, Instron Co., USA) at a crosshead speed of 1 mm/min.

3. RESULTS AND DISCUSSION

3.1. Materials Characteristics

Since ZE has a relatively high ion-exchange ability and lattice stability, many studies have been done on the structure and its interaction with different cations. In this study, CHX-loaded ZE NPs were prepared using the ionic immobilization method. As shown in Figures 1(a) and (b), the CHX-loaded ZE NPs had sizes ranging from 100 to 200 nm and an irregular spherical shape. Regardless of the CHX binding, the morphology of the ZE NPs was not altered. Figures 1(c) and (d) show the cross-section SEM images of the GIC specimens with and without CHX-loaded ZE NPs. The control GIC showed dense and compact matrix morphology. The GIC + CHX-ZE clearly showed that the CHX-loaded ZE NPs were distributed homogeneously over the GIC specimen. This suggests that the CHX-loaded ZE NPs were fabricated successfully and the NPs were embedded well in the GIC matrix.

Figure 2 shows ATR-FTIR spectra of ZE, CHX, and CHX-loaded ZE NPs. The FTIR spectrum of ZE showed vibration bands at 3,620 and 3,420 cm⁻¹ due to the H₂O interporous structure of O–H stretching and at 1,640 cm⁻¹ due to the H₂O interporous structure of O–H bending. The positions of the vibrational band at 969–461 cm⁻¹ correspond to Si–O stretching vibration. The CHX was associated with the peaks at 1,640 cm⁻¹.
(C=N stretching), 1,500 cm⁻¹ (aromatic C=C bending), 2,540 cm⁻¹ (N–H stretching), and 1,520 cm⁻¹ (N–H bending). Although the CHX peaks were weak in the range from 1,000–1,640 cm⁻¹, the characteristic peaks of ZE and CHX were appeared in the spectrum of CHX-loaded ZE NPs, indicating that CHX had been successfully loaded onto the ZE NPs.

3.2. In Vitro Release

The cumulative release profiles of CHX from the GIC + CHX and the GIC + CHX-ZE specimens are shown in Figure 3. The encapsulation efficiency of CHX to the ZE was more than 70%. The in vitro release profile of CHX from the GIC + CHX specimen showed an early release burst of ∼30% of the total CHX by day 7, followed by the sustained release of the remaining CHX over the next 23 days. However, the release profile of the GIC + CHX-ZE specimen showed a lower amount of CHX released over the study period without an early release burst. This release behavior was not only attributed to the GIC resin that delayed contact of the CHX-loaded ZE NPs with water, but also to electrostatic binding of positive charged CHX molecules onto the negative charged ZE surface, which could partly prevent the eluting of CHX from the ZE in the GIC matrix adsorbed with water. ZEs are based on a three-dimensional framework of SiO₄ and AlO₄ tetrahedra that results in an extended uniform network of channels and pores. Owing to the presence of AlO₄ tetrahedra, the framework is negatively charged. Therefore, these findings clearly indicate that GIC containing CHX-loaded ZE NPs could be used effectively for the sustained releases of antibacterial molecules at an adhesive site.

3.3. Antibacterial Effect

The agar diffusion test results are shown in Figure 4. The CHX powder itself did not show any antimi-
crobial effect. In contrast, the introduction of the GIC + CHX-ZE resulted in a definite antibacterial effect. Figure 5 shows the SEM images of bacteria adhesion on the surfaces of the GIC specimen. A higher...
bacterial adhesion was observed for the control GIC specimen, whereas the bacterial adhesion decreased for the GIC+CHX-ZE specimen, indicating the antibacterial efficiency against *S. mutans*. Thus, the combination system of CHX-loaded ZE NPs and a GIC resin provides an innovative platform for delivering antibacterial molecules in a future treatment of a dental adhesive resin system for enhancing the biological performance.

3.4. Compressive Strength and Bond Strength

The results of compressive strength and shear bond strength tests are shown in Figure 6. In both tests, the two experimental groups (GIC+CHX and GIC+CHX-ZE) did not exhibit significantly different values from that of the control (GIC) (one-way ANOVA, *p > 0.05*), indicating that the addition of the CHX or CHX-loaded ZE NPs did not affect the compressive and bond strength. Takahashi et al.* reported that the incorporation of 1% CHX diacetate into the GIC specimen produced the optimal antimicrobial effects without a significant decrease in compressive strength and dentin bond strength. Therefore, the addition of a small amount (1% by weight) of CHX-loaded ZE NPs does not affect the mechanical properties of the GIC.

4. CONCLUSION

We successfully prepared CHX-loaded ZE NPs to control CHX release from dental GIC. The GIC containing the CHX-loaded ZE NPs exhibited an excellent antibacterial effect against the tested bacteria *S. mutans*. CHX release from the GIC was efficiently controlled for the experimental period (up to 30 days) by the use of CHX-loaded ZE NPs. This incorporation did not affect the compressive strength or bonding properties of the material.

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**References and Notes**


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