

# Influence of Carbon Film on the Antibacterial Characteristics of Ceramics

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## Introduction

It has been known that ceramics, such as CaO, MgO, and ZnO, have strong antibacterial activity without the presence of light. Moreover, we developed the activated carbon dispersed with nano-size ceramic particles, which showed the synergistic effect of adsorption ability and growth inhibition to bacteria. In CaO and MgO, however, antibacterial activity decreases with the hydration reaction with moisture. On the other hand, ZnO is easily dissolved under alkaline and acidic region, and it results in a loss in the antibacterial activity. In order to show antibacterial activity in various environments, it needs to improve the hydration reaction and the solubility, which is important to protection from harmful bacteria to human being. Inagaki *et al.* reported that carbon-coated TiO<sub>2</sub> could be prepared by pyrolysis of poly (vinyl alcohol) and the carbon film formed on the surface of TiO<sub>2</sub> had pores with the diameter of nano size [1]. If the hydration reaction of the solubility of antibacterial ceramics can be improved by coating the surface with carbon film, CaO and ZnO might be possible to use as antibacterial agents in various environments. In present work, carbon film was coated on the surface of CaO and ZnO particles in order to improve resistance for hydration reaction and solubility of the particles. Antibacterial activity of carbon-coated ceramics was studied by using *Escherichia coli* and *Staphylococcus aureus*.

## Experimental

Ceramic particles, such as CaO and ZnO, and poly (vinyl alcohol) (hereafter, PVA) with polymerization degree of 2000 were used as starting materials. After ceramic particles and PVA were mixed at a mass ratio (ceramic/PVA) of 1, the mixture was heated at 700°C for 3h in an argon gas to prepare ceramic particles coated with carbon. Crystal structure of the particles with and without carbon was examined by x-ray diffraction measurement (XRD). The amount of carbon coated on the surface of particles was determined by oxidizing at 1000°C in air. The specific surface area of the carbon-coated ceramics was measured by analyzing the adsorption isotherms of N<sub>2</sub> at -196°C (BET).

*Escherichia coli* (hereafter, *E. coli*) and *Staphylococcus aureus* (hereafter, *S. aureus*) were used as test bacteria in this study, because these bacteria have difference of the structure in cell wall. These bacteria were incubated in Brain Heart Infusion broth at 37°C for 20h, and suspended into sterile saline at approximately 10<sup>3</sup> CFU cm<sup>-3</sup> (CFU: Colony Forming Unit). Antibacterial activity of ceramic particles was evaluated based on the minimal inhibitory concentration (MIC) calculated from change in electrical conductivity with bacterial growth [2]. The conductivity change was monitored during incubation at 35°C for 48h, which was used as the control in the case of no addition of ceramic particles. That is, ceramic particles when the value of MIC is small can understand to have a strong antibacterial activity.

## Results and discussion

Crystal structure of the ceramics coated with and without carbon was determined by XRD. The diffraction peaks corresponding to cubic type were detected in original CaO, and no change of the peaks was observed after carbon coating. In original ZnO coated with and without carbon, the diffraction peaks corresponding to hexagonal type were detected. By XRD, it was found that carbon coating did not affect the crystal structure.

Ceramic particles coated with carbon were oxidized at 1000°C, and the amount of carbon coated on the surface of ceramic particles was calculated from the mass loss after oxidizing. The amount of carbon coated on the surface of CaO was about 5 mass% which was similar to the amount formed on the surface of ZnO. Particle size distribution of carbon-coated CaO was similar to that of original CaO, indicating average particle size of 11 μm in narrow distribution. In original ZnO, the average particle size was 5.0 μm. Carbon-coated ZnO, however, showed the particle size distribution ranging from 0.2 to 50 μm. ZnO is reduced under an existence of carbon, and a part of ZnO may evaporate. The evaporated ZnO resulted in the production of small particle. Also, ceramics are often aggregated by

heating at high temperature. Change of the particle size in ZnO with and without carbon was anticipated to be due to the re-deposition of evaporated ZnO and the aggregation among carbon-coated ZnO.

Table 1 summarized specific surface area and MIC of the samples used in this study. Specific surface area of the ceramic particles coated with carbon was larger than that of original particles. Regarding the particle size distribution, the reason that the surface area increased in carbon-coated CaO was presumed to be the existence of pores in carbon film formed on the surface of CaO, indicating no change in the distribution of particles before and after coating. Since carbon-coated ZnO had wide distribution, the increase of the surface area was assumed to be due to either the change in particle size distribution or the formation of pores in carbon film.

The value of MIC in CaO coated with and without carbon showed 3.8, irrespective of the kind of bacteria, indicating that the formation of carbon film on CaO did not affect antibacterial activity. Occurrence mechanism of the antibacterial activity in CaO has been known to be due to the generation of super-oxide,  $O_2^-$ , from the surface of CaO and the increase of pH value with the hydration reaction of CaO [3]. These two factors contribute identically to antibacterial action of CaO regardless of the kind of bacterial cell. In CaO, therefore, it was presumed that no carbon coating affected antibacterial activity. On the other hand, the MIC in carbon coated ZnO showed the larger value than that in original ZnO. Yamamoto et al. have found the generation of  $H_2O_2$  from the surface of ZnO and clarified that the generated  $H_2O_2$  was effective for growth inhibition of bacteria [2]. The reason that carbon-coated ZnO showed the lower antibacterial activity than original ZnO was assumed to be due to the decrease in the generating amount of  $H_2O_2$  by carbon coating. Antibacterial action of  $H_2O_2$  against *E. coli* is lower than that against *S. aureus*. The reason for less affecting the antibacterial activity against *E. coli* seems to be due to a low sensitivity toward  $H_2O_2$ .

**Table 1.** Specific surface area and MIC of the samples used in this study.

| Bacteria         | Sample            | Specific surface area<br>( $m^2 g^{-1}$ ) | MIC<br>( $mg cm^{-3}$ ) |
|------------------|-------------------|---|-------------------------|
| <i>S. aureus</i> | ZnO               | 1.2                                       | 1.0                     |
|                  | Carbon-coated ZnO | 8.0                                       | 1.5                     |
|                  | CaO               | 1.4                                       | 3.8                     |
|                  | Carbon-coated CaO | 23.1                                      | 3.8                     |
| <i>E. coli</i>   | ZnO               | 1.2                                       | 32                      |
|                  | Carbon-coated ZnO | 8.0                                       | 85                      |
|                  | CaO               | 1.4                                       | 3.8                     |
|                  | Carbon-coated CaO | 23.1                                      | 3.8                     |

MIC: Minimal Inhibitory Concentration

### Conclusions

Antibacterial activity of carbon-coated CaO and ZnO was studied by using *Escherichia coli* and *Staphylococcus aureus*. In antibacterial tests, it was found that antibacterial activity of carbon-coated ZnO was weaker than that of original ZnO, irrespective of the kind of bacteria. However, antibacterial activity of carbon-coated CaO was similar to that of original CaO; that is, formation of the carbon film did not affect the antibacterial activity.

### References

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