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Effect of mixing ratio on bactericidal action of MgO–CaO powders

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MgO–CaO powders were prepared with different molar ratios (MgO/CaO) at 1400 °C for 2 h in air. By using the powder samples obtained, the change in bactericidal effect as a function of MgO–CaO composition was studied by colony count method. From the XRD measurements, it was found that CaO solid solution was formed by the replacement of Mg^{2+} ion with larger Ca^{2+} ion. However, no formation of MgO solid solution was observed. The average particle size and the specific surface area of the samples used in this study were about 0.2 μm and 10.5 m^2g^{-1}, respectively. The pH values of physiological saline containing powder samples increased with the increase of CaO content, and the value reached 12.1 in sample with the molar ratio (MgO/CaO) of 0.25. From the results of bactericidal tests for *Staphylococcus aureus*, it was found that the bactericidal effect increased with the increase of CaO content in the samples.

1. Introduction

Microbial contamination has produced various problems in industry and other vital fields, such as deterioration, degradation, infection, etc. [1, 2]. In order to solve these problems, organic compounds have been used as conventional antibacterial agents for the elimination of microbial pollution, such as sodium hypochlorite, tetra-alkylammonium, etc. [3, 4]. However, almost all parts of the organic agents are harmful to human body and environment [5, 6]. Therefore, new pasteurization and antibacterial techniques have been demanded and studied.

Recently, the occurrence of antibacterial activity by using ceramic powders has been pointed out with much attention as a new technique that can substitute for conventional ones using organic agents. Ceramic powders of zinc oxide (ZnO), calcium oxide (CaO) and magnesium oxide (MgO) were found to show a marked antibacterial activity without the presence of light [7–10]. The use of these ceramics has the following advantages: mineral elements essential to the human body and strong antibacterial activity in small amount without the irradiation of light [11, 12]. However, it is not yet clear what the antibacterial activity is influenced by the mix of these powders that show the strong activity.

In the present work, MgO–CaO powders were prepared with the different molar ratios (MgO/CaO) at 1400 °C for 2 h in air. After preparing the slurries of the powders obtained, the change in antibacterial activity, i.e. bactericidal effect, as a function of MgO–CaO composition was studied by colony count method.

2. Experimental

2.1. Preparation of MgO–CaO powders

The procedure employed in the present work for preparing MgO–CaO powders is shown in Fig. 1. The raw materials, MgO (purity: 99.9%) and CaO (purity: 98.0%), were mixed with different molar ratios (MgO/CaO). The mixed powders were heated at 1400 °C for 2 h in air and then they were granulated by planetary ball mill. The as-prepared powders were suspended with physiological saline in the concentration of 10 mg cm^{-3} and they were used in antibacterial tests. The sample code and the chemical composition of powder samples used in this study are listed in Table I.

In order to examine the crystal structure of powder samples, X-ray diffraction measurement (XRD: RINT-2500 VHF) was carried out.

2.2. Tests of bactericidal effect

Staphylococcus aureus 9779 (hereafter, *S. aureus*) was used as test bacterium and obtained from the Tokyo Metropolitan Research Laboratory of Public Health. The bacterium was cultured in brain heat infusion broth (BHI: Eiken Chemical, Co.) at 37 °C for 24 h on a reciprocal shaker. The bacterial culture was suspended in a sterile physiological saline with a final concentration of about 10^6 CFU cm^{-3} (CFU: colony forming unit). The solution of bacterial suspension was added into the saline containing powder samples with a concentration of 10 mg cm^{-3} and then stirred at 37 °C for various times. After sampling the bacterial suspension of 0.1 cm^{-3}

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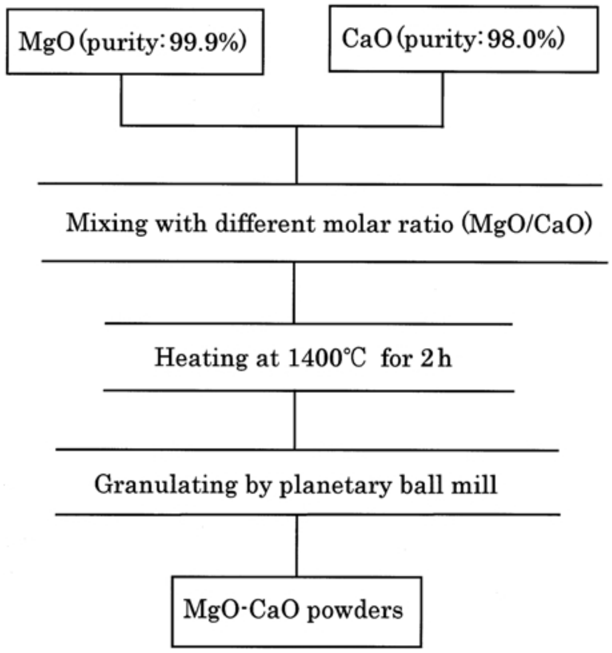

Figure 1 Preparation procedure of MgO–CaO powders.

TABLE I Sample code and chemical composition of the samples used

Sample code	Molar ratio (MgO/CaO)
MC-41	4.00
MC-64	1.50
MC-55	1.00
MC-46	0.67
MC-14	0.25

from the solution, the bacterium was cultured at 37 °C for 48 h by using pearl-core plate count agar (PPCA: Eiken Chemical, Co.) as the medium. The colony formed with bacterial growth was counted and the survival ratio of bacterium was calculated.

The bactericidal effect has been known to depend on pH values in the medium [13, 14]. In order to examine the pH values when the powder samples were added into the medium, the samples were dispersed into physiological saline at a powder concentration of 10 mg cm^{-3}. After keeping the dispersed solutions for 24 h, the pH values of physiological saline were measured.

3. Results and discussion

3.1. Powder samples

In Fig. 2, XRD patterns of the powder samples are shown. In the results of XRD measurements, it was found that MgO of the cubic phase with the NaCl type structure coexisted with CaO similar to the structure of MgO. In the powder samples with the molar ratio (MgO/CaO) ranging from 0.25 to 1.00, a diffraction line of CaO with the index of 111 shifted to high-angle side, increasing the amount of CaO. In samples with the ratio above 1.00, however, 111 line corresponding to CaO shifted to low-angle side, and the diffraction angle of CaO in MC-14 sample was comparable with that in MC-41 sample. No changes of diffraction angle in the peaks of MgO were

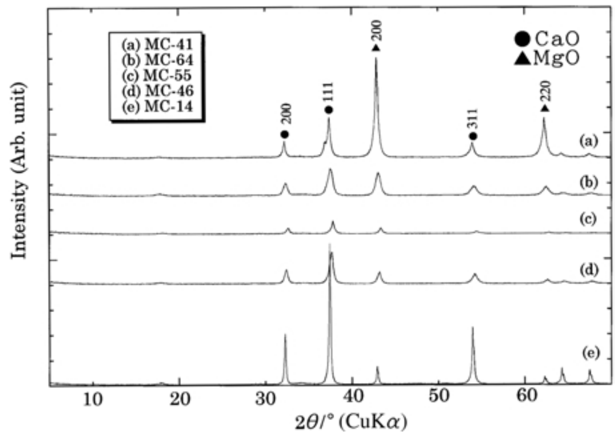

Figure 2 XRD patterns of MgO–CaO powders used in the test of bactericidal effect; the powders being heated at 1400 °C for 2 h in air.

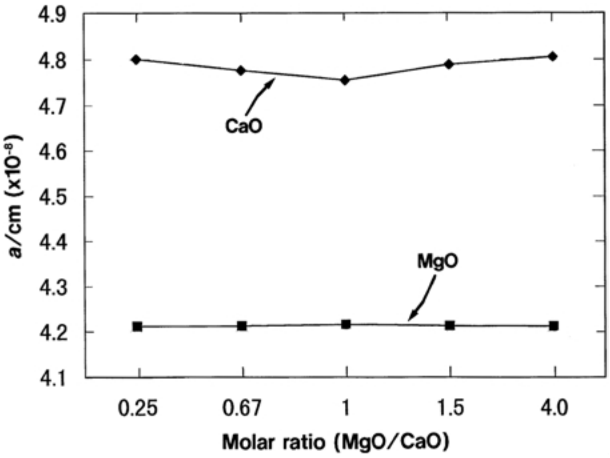

Figure 3 Change of lattice constant with MgO–CaO composition.

observed. From the values of diffraction angle, 2θ, in the observed peaks, the lattice constant of MgO and CaO was calculated, as shown in Fig. 3. The lattice constant of MgO showed a value of about 4.21×10^{-8} cm, irrespective of the molar ratio of MgO–CaO powders. In the case of CaO, however, the sample with a molar ratio of 1.00 showed the minimum value of 4.85×10^{-8} cm. The shift of 111 diffraction line of CaO is considered to be due to the replacement of Mg^{2+} ion (ion radius: 65 nm) with larger Ca^{2+} ion (ion radius: 99 nm). MgO detected in all samples seems to be due to excess MgO in the formation of solid solution. In the case of MgO, it is found that no replacement of Ca^{2+} ion occurs in MgO.

The antibacterial activity is dependent on the particle size of ceramic powders; that is, the activity of powder with small particle size is stronger than those with large particle size [9]. Therefore, it is essential to measure the particle size of powder samples used in this study. After milling the powder samples by planetary ball mill, it was found that the average particle size and the specific surface area of the powders obtained were about 0.2 μm and 10.5 m^2 g^{-1}, respectively.

3.2. Bactericidal effect

Fig. 4 shows the survival ratio of *S. aureus* with incubation time. Hence, the vertical axis, ''N/N$_0$'', represents the ratio between the viable bacterial counts

TABLE II pH values in physiological saline containing MgO–CaO powders

Sample code	pH (−)
MC-41	11.0
MC-64	11.4
MC-55	11.6
MC-46	11.9
MC-14	12.1

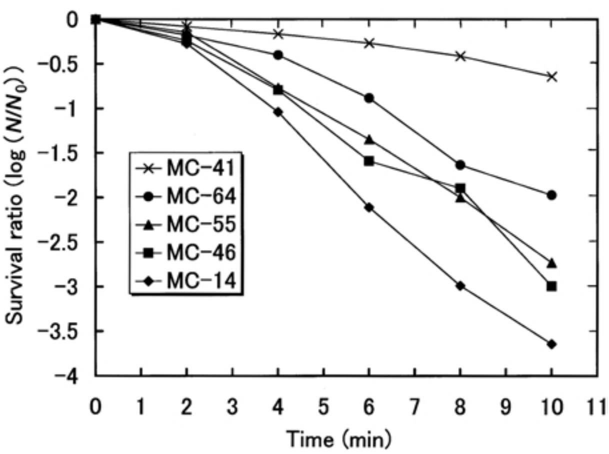

Figure 4 Survival ratio of *S. aureus* in MgO–CaO powders with the powder concentration $10\,mg\,cm^{-3}$.

(N ($CFU\,cm^{-3}$)) at specified time and the initial counts (N_0 ($CFU\,cm^{-3}$)) of *S. aureus*. If the values of N/N_0 are changed with a steep decrease, it can be judged to show the strong bactericidal effect. The values of survival ratio decreased with the increase in incubation time, irrespective of the MgO–CaO composition. The marked decrease of the values was shown in MC-14 sample, that is, the survival ratio of *S. aureus* decreased with the increase of the amount of CaO in mixed powders. This result indicates the increase of bactericidal effect for *S. aureus* observed by increasing the amount of CaO.

The pH values of physiological saline containing powder samples were measured and summarized in Table II. In the case of MC-41 sample, the value was found to be 11.0. With the increase of the amount of CaO, the pH values increased, and it reached 12.1 in MC-14 sample.

The following four factors may affect the bactericidal effect of ceramic powders used in this study: (1) the cations eluted from powder, (2) the mechanical destruction of cell membrane, (3) active oxygen generated from powder, and (4) the pH value [7, 15, 16]. However, Yamamoto *et al.* [17] and Sawai *et al.* [15, 18] reported that factors (1) and (2) had no effect on ceramics. In comparison between pH values and survival ratio, it is found that the bactericidal effect increases with the increase of pH value. In the alkali region, however, Sawai *et al.* [15, 18, 19] reported that the bactericidal effect of CaO on *S. aureus* made some difference compared to that in the case of alkali treatment such as NaOH solution. In fact, the effect contributed to antibacterial activity seems to be not only the pH values but also other factors. They have also found the generation of super-oxide, O_2^-, from the surface of CaO and MgO, and it is considered to be effective for the inhibition of bacterial growth [16]. Therefore, the reason that the MgO–CaO powders showed the strong bactericidal effect with the increase of CaO content is supposed to be due to the generation of super-oxide and the increase of pH value.

4. Conclusion

MgO–CaO powders were prepared with the different molar ratios (MgO/CaO) at 1400 °C for 2 h in air. The change in bactericidal effect as a function of MgO–CaO composition was studied.

In the powder samples, it was found that CaO solid solution was formed by the replacement of Mg^{2+} ion with larger Ca^{2+} ion. However, no formation of MgO solid solution was observed. The average particle size and the specific surface area of the powders obtained were about 0.2 μm and $10.5\,m^2\,g^{-1}$, respectively.

From the results of bactericidal tests, it was found that the bactericidal effect for *S. aureus* increased with the increase in the amount of CaO in the samples. The occurrence of bactericidal effect was supposed to be due to the generation of super-oxide from the surface of powder samples and the increase of pH value in the medium.

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