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Antibacterial activity of ZnO powder with crystallographic orientation

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Abstract ZnO powder with crystallographic orientation was prepared from the mixed aqueous solution of zinc chloride, tri-ethanol amine and thio-urea. From X-ray diffraction measurement, as-prepared powder was found to have the orientation along *a–b* axes of hexagonal structure, and a needle-like shape with the aspect ratio of 5 was observed by scanning electron microscope, indicating that as-prepared powder had crystallographic orientation. In the tests of antibacterial activity by colony count method, ZnO powders with and without crystallographic orientation were used in present work. Survival ratio of bacteria decreased with increasing powder concentration, i.e., increase in antibacterial activity. The antibacterial activity in ZnO powder with crystallographic orientation was weaker than that in commercial ZnO powder without orientation at same powder concentration. Regarding specific surface area of the powders used in antibacterial tests, however, antibacterial activity in powder with orientation was found to be similar to that without orientation; that is, the crystallographic orientation of ZnO did not affect antibacterial activity. The activity toward *Staphylococcus aureus* was stronger than that toward *Escherichia coli*, irrespective of the kind of powders.

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1 Introduction

Some oxide ceramics, calcium oxide (CaO), magnesium oxide (MgO) and zinc oxide (ZnO), have been known to show a marked antibacterial activity in small amount of powder and without the presence of light, which has been pointed out with much attention as a novel technique that can substitute for conventional ones using organic compounds, such as quaternary ammonium salt and chlorine disinfectant [1–10]. Sawai et al. and Yamamoto et al. reported that main chemical species contributing to occurrence of the antibacterial activity were assumed to be active oxide, hydrogen peroxide (H_2O_2) and super-oxide (O_2^-), generated from the surface of these ceramics[11, 12]. Among these ceramics, especially, ZnO has several advantages; showing a marked antibacterial activity in neutral region (pH 7) and being a mineral element essential to human being. Therefore, there are many reports on antibacterial activity of ZnO [13–19]. In order to efficiently utilize the generated H_2O_2 on occurrence of the antibacterial activity, Yamamoto et al. developed the activated carbon sphere dispersed with nano-size ZnO so far [20]. And also, antibacterial activity has been clarified to be dependent on particle size, specific surface area and crystallinity of ZnO; that is, the activity becomes strong with increasing specific surface area, and decreasing particle size and crystallinity [13–15]. In previous reports described above, however, it was not yet clear how change in antibacterial activity is expected by the crystallographic orientation of ZnO. In other words, it is important to ensure the powder characteristics of ZnO on antibacterial activity, in order to know antibacterial mechanism of ZnO.

In present work, ZnO powder with crystallographic orientation was prepared from the mixed aqueous solution of zinc chloride, tri-ethanol amine and thio-urea, which has

shape with the aspect ratio of 5. Antibacterial activity of the obtained ZnO powder was evaluated by colony count method, with emphasis on the orientation of ZnO on the activity.

2 Materials and methods

2.1 Preparation of ZnO with crystallographic orientation

In Fig. 1, the block diagram for preparing ZnO powder with crystallographic orientation was shown. Zinc chloride (Nacalaitesque, purity; 98%) ($ZnCl_2$), tri-ethanol amine (Nacalai tesque, purity; 98%) ($C_6NH_{15}O_3$: hereafter, TEA) and thio-urea (Nacalaitesque, purity; 98%) ($C_2N_2H_4S$: hereafter, TU) were used as starting raw materials to prepare ZnO powder with crystallographic orientation. These raw materials were dissolved separately into distilled water at the concentration of 0.2 mol dm^{-3}. $ZnCl_2$ aqueous solutions were mixed with TEA aqueous solution at a molar ratio (TEA/$ZnCl_2$) of 2, and the mixed solution was stirred at room temperature for 24 h. After TU aqueous solution was added into the mixed solution at a molar ratio (TU/$ZnCl_2$) of 1, aqueous solution containing three raw materials was refluxed at its boiling point for 3 h, resulting in a hydrolysis reaction of TU, forming crystalline precipitate. Then, precipitate was obtained, and washed with distilled water after separating the precipitate from the solution. The precipitate, ZnO powder with crystallographic orientation, after keeping at 70 °C for 15 h, was used in antibacterial test. Yield of the ZnO obtained by this preparation procedure was approximately 85%.

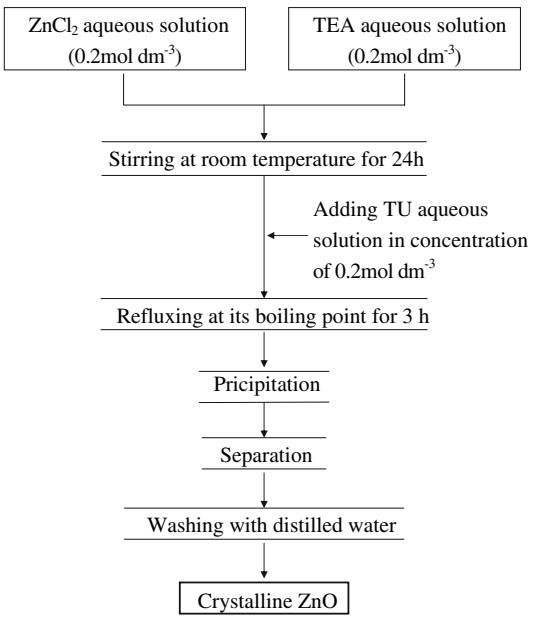

Fig. 1 Block diagram for preparing ZnO with crystallographic orientation

The crystallographic orientation of the ZnO powder obtained in this study was confirmed by X-ray diffraction measurement (XRD; RIGAKU, RAD-C SYSTEM). Energy-dispersive X-ray analysis (EDX) installed on a field emission-scanning electron microscope (FE-SEM; HITACHI, S-4500) was carried out to determine the impurity concentration in the as-prepared ZnO powder. As-prepared ZnO was observed by FE-SEM, which the specific surface area was determined by measuring the adsorption isotherms of N_2 at –196 °C (BET; BELSORP-mini).

Commercial ZnO powder (hereafter, com-ZnO: Kanto Chemical, Co., Purity; 99%) was also used as a reference material in order to compare antibacterial activity between as-prepared ZnO and com-ZnO, which the characteristics were measured by XRD, FE-SEM and BET.

2.2 Antibacterial test

Escherichia coli 745 (hereafter, *E. coli*) as a gram-negative bacterium and *Staphylococcus aureus 9779* (hereafter, *S. aureus*) as a gram-positive bacterium were used as test bacteria, because the occurrence of antibacterial activity may affect the kind of bacteria. Figure 2 shows the procedure of antibacterial test. *E. coli* and *S. aureus* were cultured at 36 °C for 48 h in a LB medium on a reciprocal shaker, which contains 0.5% yeast extract (Becton, Dickinson and Co.), 1% bactopeptone (Becton, Dickinson and Co.) and 1% sodium chloride (WAKO PURE CHEMICAL IND., LTD, purity; 99.9%) (NaCl). The medium was washed four times with sterile water, and the bacterial

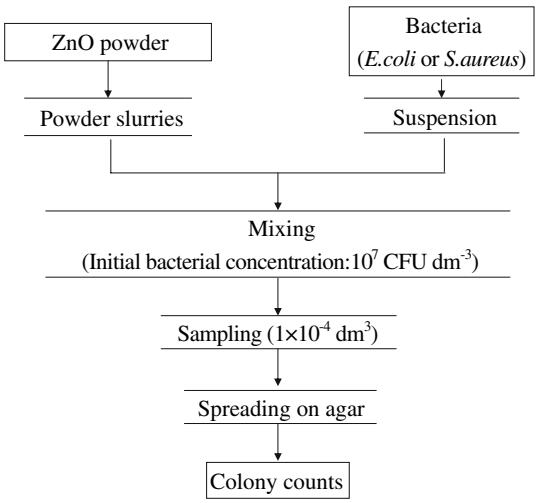

Fig. 2 Procedure of antibacterial test carried out in this work

culture was suspended in sterile water with a final concentration of 10^7 CFU dm^{-3} (CFU; Colony Forming Unit). Subsequently, the solution of bacterial suspension was added into sterile water containing ZnO powders with concentration in the range from 0.16 to 40 g dm^{-3} and then kept at 36 °C for different times on a reciprocal shaker. After sampling the bacterial suspension of 1×10^{-4} dm^3, the bacterial suspension was spread on nutrient agar (NA, Eiken Chemical, Co.) for *E. coli* and pearl-core plate count agar (PPCA, Eiken Chemical, Co.) for *S. aureus*, and cultured at 36 °C for 48 h without the presence of light. The colony formed with bacterial growth was counted. By calculating the ratio (N/N_0) between the viable bacterial counts (N (CFU dm^{-3})) at specified time and the initial counts (N_0 (CFU dm^{-3})) of bacteria, antibacterial activity was evaluated.

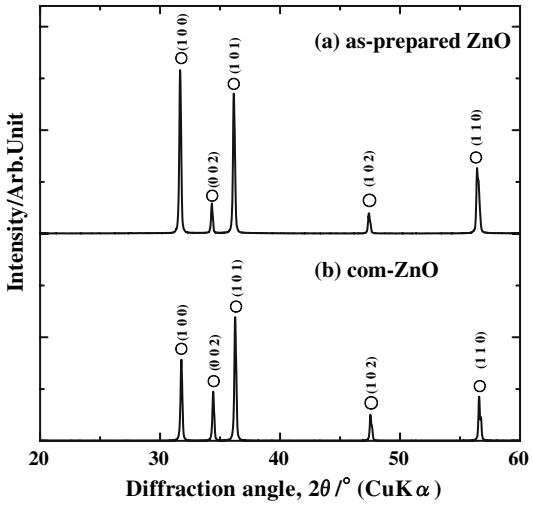

Fig. 3 XRD patterns of (**a**) as-prepared ZnO and (**b**) Com-ZnO

3 Results and discussion

3.1 Powder sample

Figure 3 shows XRD diffraction patterns of the as-prepared ZnO and com-ZnO. By XRD, diffraction peaks corresponding to ZnO with hexagonal-type structure were detected in both samples, whereas the diffraction intensity of as-prepared ZnO was different from that of com-ZnO. Two diffraction lines of as-prepared ZnO with the index of 100 and 110 were high, compared with the same lines of com-ZnO. This indicates that two crystal planes, 100 and 110, in hexagonal-type structure grew. In other words, it is presumed that as-prepared ZnO has crystallographic orientation along *a*–*b* axes in hexagonal structure.

FE-SEM micrographs of as-prepared ZnO and com-ZnO are shown in Fig. 4. Particle shape of the com-ZnO was spherical with a diameter of approximately 0.6 μm. However, as-prepared ZnO had needle-like shape with aspect ratio of approximately 5. Since as-prepared powder was found to have crystallographic orientation by XRD, the reason that as-prepared ZnO shows needle-like shape is presumed to reflect the crystallographic orientation.

Yamamoto et al. [13] have reported that small amount of sulfur was detected as a impurity in crystalline ZnO formed by chemical bath method using TU, because TU is a compound containing sulfur. If as-prepared ZnO obtained by using TU contains sulfur, sulfur has influence which may affect the antibacterial activity. In order to measure the existence of sulfur in as-prepared ZnO, elemental analysis by EDX was performed. By the analysis, it was found that no sulfur was detected in as-prepared ZnO. Therefore, it seems to be not necessary to take into consideration the influence of impurities on the antibacterial activity.

It has been reported that antibacterial activity of ZnO increased with an increase of the specific surface area [13]; that is, specific surface area is one of the important factors affecting antibacterial activity. In order to evaluate essential antibacterial activity of ZnO, it is important to

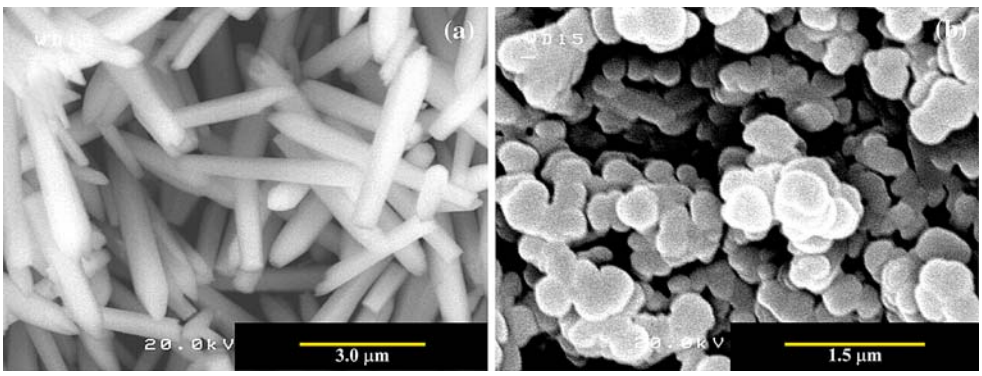

Fig. 4 SEM micrographs of (**a**) as-prepared ZnO and (**b**) Com-ZnO

measure specific surface area of two samples used in antibacterial test. From the measurement of the BET surface area, it was found that the specific surface area of as-prepared ZnO and com-ZnO showed 1.7 and 3.8 $m^2\ g^{-1}$, respectively.

3.2 Antibacterial activity

In colony count method, the powder sample when the values of survival ratio change with a steep decrease at the specified time can be understood to have stronger antibacterial activity. The antibacterial test with *E. coli* was performed in the powder concentration ranging from 0.63 to 40 g dm^{-3}. Figure 5 (a) and (b) show the changes in survival ratio on as-prepared ZnO and com-ZnO, respectively. In the case of as-prepared ZnO (see Fig. 5 (a)), the values of survival ratio decreased with increasing time. At specified time, the values were found to become small with increasing powder concentration, indicating that antibacterial activity increased with increasing powder concentration. The survival ratio in the case of com-ZnO (see Fig. 5 (b)) also decreased with increasing time and powder concentration; that is, the decreasing behavior of *E. coli* in as-prepared ZnO was similar to that in com-ZnO.

Figure 6 (a) and (b) show the changes in survival ratio of *S. aureus* on as-prepared ZnO and com-ZnO, respectively. In the case of as-prepared ZnO shown in Fig. 6 (a), the survival ratio when powder concentration was 0.16 g dm^{-3} was found to decrease 15 min later. In powder concentration above 0.63 g dm^{-3}, however, the ratio decreased steeply in short time, and reached 0.08 at powder concentration of 10 g dm^{-3} after 5 min. On the other hand, the survival ratio in com-ZnO was steeper decrease than that in as-prepared ZnO (see Fig. 6 (b)), which showed 0.04 at powder concentration of 10 g dm^{-3} after 5 min. This result indicates that the increase in powder concentration produces strong antibacterial activity toward *S. aureus*, as well as the activity toward *E. coli*, irrespective of the kind of ZnO powder.

On occurrence of antibacterial activity on ZnO, Yamamoto et al. [12, 15] and Sawai et al. [18] have reported that H_2O_2 generated from the surface of ZnO was one of primary chemical species being effective for antibacterial action. In present work, therefore, it is anticipated that H_2O_2 generated from its surface contributes to the occurrence of antibacterial activity. Since it is reasonable that the powder concentration is comparable with the amount of H_2O_2, the amount of H_2O_2 generated from the surface of ZnO should increase in proportion to increase of the powder concentration and time, as supported by a previous report [12]. The reason that antibacterial activity increased with increasing powder concentration and time is assumed to be due to the increase of H_2O_2 generated from the surface of ZnO.

When the survival ratio of vertical axis shown in Figs. 5 and 6 is converted into logarithmic value, the ratio can be redrawn as linear decrease for time. Therefore, death rate constant, k, can be determined by first-order kinetics like the following formula (1);

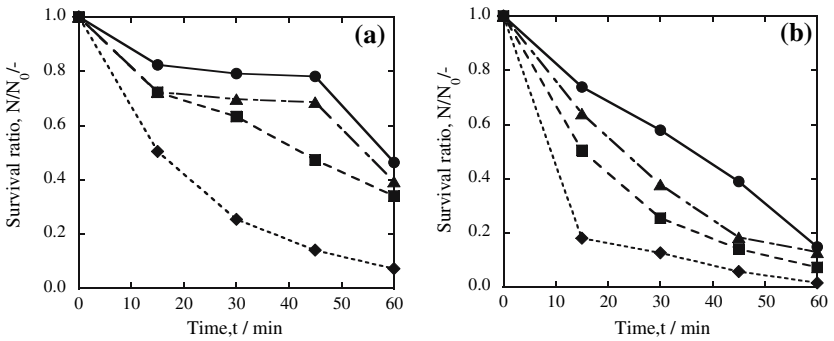

Fig. 5 Change in survival ratio of *E.coli* by using (**a**) as-prepared ZnO and (**b**) com-ZnO. ●, 0.63 g dm^{-3}; ▲, 2.5 g dm^{-3}; ■,10 g dm^{-3}; ◆, 40 g dm^{-3}

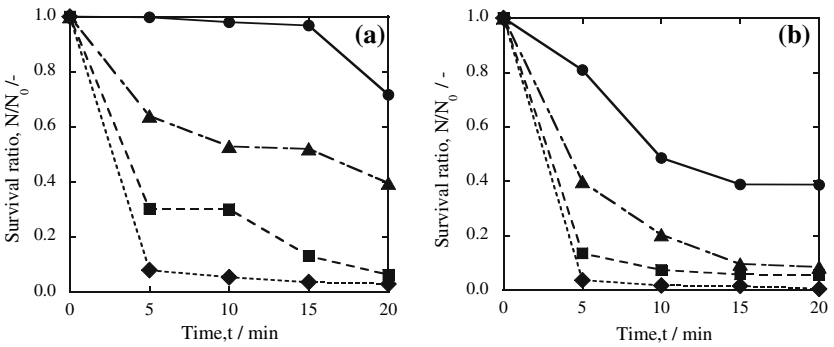

Fig. 6 Change in survival ratio of S.*aureus* by using (**a**) as-prepared ZnO and (**b**) com-ZnO. ●, 0.16 g dm^{-3}; ▲, 0.63 g dm^{-3}; ■, 2.5 g dm^{-3}; ◆, 10 g dm^{-3}

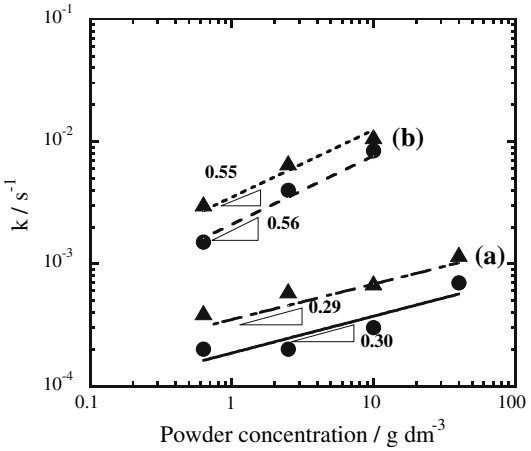

Fig. 7 The relation between death rate constant and powder concentration, sample being ●, com-ZnO and ▲, as-prepared ZnO. (**a**) *E.coli*, (**b**) *S.aureus*

$$dN/dt = -kN \quad (1)$$

where N is survival ratio (N/N_0), and t is time [21]. Figure 7 shows the relation between k value and powder concentration, which can define having a high antibacterial activity so that k value is high, and the slope of the line means sensitivity between bacteria and H_2O_2. If bacteria are sensitive to H_2O_2, the slope degree appears as the sensitivity change, depending on difference of the cell wall and the killing mechanism of bacteria. In comparison on antibacterial activity between *E. coli* and *S. aureus*, it was found that antibacterial activity toward *S. aureus* was stronger than that toward *E. coli*. On the other hand, antibacterial activity of com-ZnO was found to be slightly stronger than that of as-prepared ZnO, irrespective of the kind of bacteria. The slope in using *E. coli* was the value of approximately 0.3 in both powders. In the case of *S. aureus*, the slope degree of com-ZnO was much similar to that of as-prepared ZnO, being 0.55–0.56, whereas was larger than that in the case of *E. coli*.

It has been well-known that the structure and the chemical composition of the cell wall are quite different between *E. coli* and *S. aureus*; that is, *E. coli* has the cell wall consisting of lipid A, lipopolysaccharide and peptidoglycan, whereas the cell wall of *S. aureus* is made from peptidoglycan mainly. Akiyama et al. [22] reported the effectiveness of ZnO, inhibited the fibrin formation by coagulase of *S. aureus*, as a treatment for atopic dermatitis related to *S. aureus*. This suggests that the effectiveness of ZnO for atopic dermatitis may depend on the strong affinity between ZnO and *S. aureus*. H_2O_2 generated from the surface of ZnO can easily penetrate the cell wall of bacteria [18]. In chemical stress, such as the environment including antibiotics, the tolerance in *S. aureus* is generally weaker than that in *E. coli*. The reason that antibacterial activity toward *S. aureus* was stronger than that toward *E. coli*, therefore, is presumed to be due to the synergistic effect both the sensitivity to H_2O_2 and the affinity for ZnO. From BET measurement, the specific surface area of com-ZnO was about 2 times in comparison with that of as-prepared ZnO, showing 3.8 $m^2\ g^{-1}$ for the com-ZnO and 1.7 $m^2\ g^{-1}$ for the as-prepared ZnO. Since H_2O_2 generates from the surface of ZnO, the generation of H_2O_2 should be dependent on high specific surface area of ZnO, i.e., increase of H_2O_2 with increasing surface area. In comparison of the same bacteria, it was clarified that antibacterial activity of com-ZnO was stronger than that of as-prepared ZnO, as shown in Fig. 7. This result is expected to be caused by difference of the specific surface area between com-ZnO and as-prepared ZnO. That is, it is estimated that the crystallographic orientation along *a–b* axes in ZnO dose not strongly affect antibacterial activity.

In order to confirm effect of the crystallographic orientation on antibacterial activity in detail, it is important to examine antibacterial activity in ZnO with an orientation along *c* axis.

4 Conclusions

ZnO powder with crystallographic orientation along *a–b* axes was prepared from the mixed aqueous solution of zinc chloride, tri-ethanol amine and thio-urea, which showed needle-like shape with the aspect ratio of 5, reflecting the orientation characteristics of the ZnO. In antibacterial tests, it was found that the activity of ZnO increased with increasing powder concentration, irrespective of with and without crystallographic orientation. In comparison of the same bacteria, the antibacterial activity of ZnO without crystallographic orientation was found to be slightly stronger than that of ZnO with orientation, to be due to difference of the specific surface area, irrespective of the kind of bacteria. Antibacterial action toward *Staphylococcus aureus* was stronger than that towards *Escherichia coli*.

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