Antibacterial characteristics of carbon-coated CaCO_3/MgO powder led by the pyrolysis of poly(vinyl alcohol)-dolomite mixture

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In order to reduce oral diseases with bacterial growth, carbon-coated CaCO_3/MgO powder possessing antibacterial activity was obtained by the pyrolysis of poly(vinyl alcohol)-dolomite mixture at 700 °C in an argon gas. Evaluation of the antibacterial activity was performed by colony count method, using *Escherichia coli* and *Staphylococcus aureus*. CaCO_3 and MgO were formed at the temperature range from 400 to 700 °C. At 800 °C, CaO was produced by the calcination of CaCO_3 . The powders after PVA pyrolysis turned into black, i.e., the formation of carbon on its surface. In the case without PVA, the formation temperature of CaCO_3/MgO exceeded 100 °C than the mixture of dolomite and PVA. Antibacterial activity of the carbon-coated CaCO_3/MgO powder against *Staphylococcus aureus* was found to be stronger than against *Escherichia coli*. Comparing with CaCO_3/MgO powder without carbon, it was found that the antibacterial action was reduced by forming carbon on its surface.

KEYWORDS : Carbon coating, Particles, Pyrolysis, Dolomite, Antibacterial activity

1. Introduction

Radioactive ray and chemotherapy are performed to treat cancer at face outskirts, and it results in complication in oral cavity so that cells are damaged. One of the factors to cause the complication is bacterial growth, such as harmful bacteria, *Bacterionema matruchotii*, *Streptococcus mitis* and *Porohyromonas gingivalis*. These bacteria live in oral cavity, which may form plaque on the surface of tooth, and cause inflammation in oral cavity¹⁾. Therefore, sterilization processes are required to be adequate in primary care to maintain the healthy oral condition. Removing harmful bacteria living in plaque is essential generally to use tooth paste containing abrasives, such as calcium carbonate and hydroxyapatite^{2),3)}. However, the abrasives can not completely remove plaque. Bacterial extinction remaining in plaque may occur by abrasives possessing antibacterial activity which contributes to the reduction of oral diseases.

Calcium magnesium carbonate ($\text{CaMg}(\text{CO}_3)_2$) is a double salt

consisting of calcite (CaCO_3) and magnesium carbonate (MgCO_3), and has been used as one of food additives, because of safe for ingestion to human being. No these carbonates exhibit any antibacterial activity, which is included in toothpaste in order to remove plaque formed on the surface of tooth. On the other hand, calcia (CaO) and magnesia (MgO) exhibit strong antibacterial activity without the presence of light⁴⁾⁻⁶⁾, due to the generation of super-oxide anion, O_2^- , on the surface of these powders. Stoimenov *et al.*⁷⁾ prepared nano-scale MgO powder, which showed the pronounced bactericidal action against vegetative cells and spores of bacteria. Koper *et al.*⁸⁾ also reported that the nano-scale MgO powder taking place active forms of halogens inactivated viruses and aflatoxins.

Inagaki *et al.*⁹⁾ and Morishita *et al.*¹⁰⁾ prepared carbon-coated ceramics through pyrolysis of poly(vinyl alcohol). It is generally known that carbon adsorbs the large amount of bacteria¹¹⁾. If the carbon-coated powders consisting of MgO and CaCO_3 are obtained by the pyrolysis of poly(vinyl alcohol)-dolomite mixture, it may be

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possible to remove large amount of bacteria and plaque found on the surface of tooth, inhibiting oral diseases with harmful bacterial growth.

In present work, carbon-coated CaCO_3/MgO powder is obtained by the pyrolysis of poly (vinyl alcohol)-dolomite mixture. Antibacterial activity is examined in the obtained powder by colony count, which compares the powder coated with carbon to that without carbon.

2. Experimental

2.1 Preparation of carbon-coated samples

The natural dolomite powder produced in Philippine was used as a starting raw material, in which the chemical composition was summarized in **Table 1**, being converted as oxide content. Primary inorganic ingredient in the dolomite was calcium and magnesium, including the small amount of silicon, iron, and aluminum as impurities. Prior to the heat-treatment, the dolomite powder was crushed in the mortar made of a high purity alumina, which was a mean particle size of approximately 2 μm . The crushed powder was heated in an argon gas at the temperature range from 400 to 800 $^{\circ}\text{C}$, together with poly (vinyl alcohol) (PVA) with polymerization degree of 2000. The mixing ratio (dolomite/PVA) was 1 in mass ratio. After reaching a specified temperature, the powder was cooled immediately up to room temperature without the keeping time. The carbon-coated samples thus obtained were used to antibacterial assay. CaCO_3/MgO powder without carbon was also prepared by heating dolomite at 800 $^{\circ}\text{C}$ in air, in order to compare with antibacterial activity of the carbon-coated CaCO_3/MgO powder.

The powder samples obtained at each temperature were analyzed by X-ray diffraction (XRD ; RIGAKU, RAD-C SYSTEM), in order to confirm the formation phase. The amount of carbon formed on as-obtained particles was determined from mass loss of the carbon-coated CaCO_3/MgO powder by heating 700 $^{\circ}\text{C}$ for 3 h in air, i.e., burning off of carbon. The specific surface area of the powder samples with and without carbon was determined based on the adsorption-desorption isotherms of N_2 at -196°C (BET ; Bel Japan Inc., BELSORP-mini).

Carbon-coated powder samples were suspended in sterile water at a concentration of 10 g dm^{-3} , and then a vial containing the powder slurries was immersed in a water bath at 36 $^{\circ}\text{C}$. The pH probe was inserted into the powder slurries and continuously monitored until the pH stabilized, at which point the pH of the powder samples was recorded.

2.2 Antibacterial assay

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 to antibacterial assay. The bacteria were stored at -80°C and thawed at 36 $^{\circ}\text{C}$. *E. coli* and *S. aureus* were cultured at 36 $^{\circ}\text{C}$ for 48h on a reciprocal shaker in a LB medium which contains 0.5 % yeast extract (Becton, Dickinson and Co.), 1 % bactopectone (Becton, Dickinson and Co.) and 1 % sodium chloride (NaCl, WAKO PURE CHEMICAL IND., LTD, purity ; 99.9 %). The medium was rinsed four times with sterile water, and the bacterial culture was suspended in sterile water at a final concentration of approximately 10^7 colony forming unit (CFU) dm^{-3} .

The bacterial suspension was added into sterile water containing powder samples in the concentration of 10 g dm^{-3} and then kept at 36 $^{\circ}\text{C}$ for different times on a reciprocal shaker. A volume of 10^{-4} dm^3 of the bacterial suspension was spread on a nutrient agar (NA, Eiken Chemical, Co.) against *E. coli* and a pearl-core plate count agar (PPCA, Eiken Chemical, Co.) against *S. aureus*. The colonies formed on the agar were counted after the incubation at 36 $^{\circ}\text{C}$ for 48 h without the presence of light. The bacterial counts were performed in duplicate on four different occasions and the average values plotted.

3. Results and discussion

3.1 Carbon-coated samples

XRD patterns of the carbon-coated powder samples obtained at different temperatures are shown in **Fig.1**. Diffraction peaks corresponding to dolomite with rhombohedral type are detected in original dolomite, and there were no peaks of other phases, such

Fig.1 XRD patterns of the powder samples obtained by heating PVA-dolomite mixture at the temperature range from 400 to 800 $^{\circ}\text{C}$.

Table 1 Chemical composition of dolomite used in this work.

Content (%)	MgO	CaO	SiO_2	Fe_2O_3	Al_2O_3
	18.27	34.49	0.26	0.07	0.07

Fig.2 XRD patterns of the powder samples obtained by heating dolomite at the temperature range from 600 to 800 °C.

as carbonate and oxide coming from impurities. With increasing temperature, these diffraction peaks disappear. Small diffraction peak, 104, corresponding to CaCO_3 is detected at 400 °C, of which the intensity increases with increasing temperature. On other hand, diffraction peaks corresponding to MgO with cubic type is detected at 500 °C. The diffraction intensity of MgO increases with increasing temperature. The increasing behavior of the diffraction intensity on MgO corresponds to that on CaCO_3 . At 700 °C, the peaks of dolomite disappear completely, and the powder sample exhibits the peaks corresponding to two phases of CaCO_3 and MgO. Mass gain of the powder sample obtained at 700 °C exhibited a value of about 5.0 mass %, indicating the formation of carbon on its surface. The color of the powder samples turned into black above 400 °C when dolomite was heated with PVA. At 800 °C, diffraction peaks corresponding to CaO with cubic type are detected, probably due to the thermal decomposition of CaCO_3 to CaO. From these results, it is found that carbon-coated CaCO_3/MgO powder is prepared by heating dolomite with PVA at 700 °C in an argon gas.

Fig.2 shows XRD patterns of the powder samples without carbon obtained at different temperatures. At 800 °C, diffraction peaks corresponding to CaCO_3 and MgO are detected without any peaks of dolomite and CaO, indicating that the formation temperature of CaCO_3/MgO exceeds 100 °C than the mixture of dolomite and PVA. Many researchers have reported on the thermal decomposition of dolomite¹²⁾⁻¹⁵⁾. A decomposition of dolomite to MgO and CaCO_3 begins at about 810 °C, which exhibits an endothermic peak. However, PVA is exothermic pyrolysis during heat-treatment in inert atmosphere. In the case of carbon-coated powder, the

Fig.3 Nitrogen adsorption-desorption isotherms at 77 K for the CaCO_3/MgO powders coated with (Δ) and without (○) carbon.

Fig.4 Survival ratio of *E. coli* on the carbon-coated powder samples obtained at different temperatures. ● : Control (no addition of powder), ▲ : 400 °C, ■ : 500 °C, ◆ : 600 °C, ▼ : 700 °C.

reduction on the formation temperature of CaCO_3/MgO is presumed to be due to the increase in local temperature at the surface of dolomite with the exothermic pyrolysis of PVA.

The nitrogen adsorption-desorption isotherms observed at 77 K for the CaCO_3/MgO powders coated with and without carbon is shown in Fig.3. At low relative pressure, the powder coated with carbon exhibits higher volume than that without carbon, suggesting the formation of meso-pores in the carbon formed on the surface of powder. The BET surface areas of powders coated with and without carbon were 10.5 and 4.0 $\text{m}^2 \text{g}^{-1}$, respectively.

The pH value was 8.56 in original dolomite, which increased with increasing temperature. Carbon-coated CaCO_3/MgO powder exhibited the pH value of 11.2, which corresponded to CaCO_3/MgO

Fig. 5 Survival ratio of *S. aureus* on the carbon-coated powder samples obtained at different temperatures. ● : Control (no addition of powder), ▲ : 400 °C, ■ : 500 °C, ◆ : 600 °C, ▼ : 700 °C.

Fig. 6 Comparison of survival ratio on the CaCO₃/MgO powders coated with and without carbon, indicating survival ratio of (▲) *E. coli* and (●) *S. aureus* on CaCO₃/MgO powder, and that of (△) *E. coli* and (○) *S. aureus* on carbon-coated CaCO₃/MgO powder. The powders coated with and without carbon were prepared at 700 and 800 °C, respectively.

powder without carbon that is obtained by heating dolomite.

3.2 Antibacterial assay

The ordinate is the ratio of the viable bacterial counts (N CFU dm^{-3}) at specified time divided by initial counts (control) of bacteria (N_0 CFU dm^{-3}), which represents the survival ratio. The abscissa shows the incubation time after adding the bacterial suspension into sterile water containing powder samples.

Fig. 4 shows the survival ratio of *E. coli*, using carbon-coated powder samples at a powder concentration of 10 g dm^{-3} . No changes in survival ratio were observed in the carbon-coated powder sample obtained at 400 °C. The survival ratio on the powder samples reduces with increasing temperature. The reducing behavior in survival ratio of *S. aureus* resembled that of *E. coli*, as shown in **Fig. 5**. This indicates enhancement in antibacterial activity with the thermal decomposition of dolomite. In comparison on the survival ratio between *E. coli* and *S. aureus*, the survival ratio of *S. aureus* was less than that of *E. coli*. From the results, the antibacterial action of the powder samples against *S. aureus* was found to be stronger than against *E. coli*.

By XRD, it is found that the amount of MgO increases with the thermal decomposition of dolomite, and also the pH value increases, indicating alkalinity. Alkaline effect should be considered as a primary factor in antibacterial action¹⁶⁾. Occurrence mechanism of the antibacterial activity on MgO has been supposed to be due to the generation of an active oxygen species such as super-oxide anion, O_2^- , on its surface¹⁷⁾. Hewitt *et al.*¹⁸⁾ reported that the sensitivity changes in *E. coli* treated with MgO were consistent with changes induced by active oxygen. In the carbon-coated powder samples obtained in this study, it is reasonable that the amount of MgO in the powder samples increases with the progress of the decomposition

of dolomite, which promote the large amount of O_2^- . Therefore, the reason that the powder samples show strong antibacterial activity with increasing temperature is expected to be due to the increase of the generated amount of O_2^- on the surface of MgO, and an increase of pH value.

The structure and the chemical composition of the cell surface are quite different between *E. coli* and *S. aureus*; that is, the cell surface of *E. coli* consists of the thin layers of lipid A, lipopolysaccharide, and peptidoglycan, but *S. aureus* consists of only a peptidoglycan layer. Membrane functions, activity of enzymes associated with membrane, and maintenance of cell integrity, depend on the structure of the cell surface. *E. coli* shows a comparatively strong resistance for antibiotics, and *S. aureus* is weak in chemical stress¹⁹⁾. In other words, the sensitivity of *S. aureus* to antibiotics is higher than *E. coli*. It is convinced that the impurities included in dolomite do not act as antibacterial species. The reason that the carbon-coated powder samples exhibits stronger antibacterial activity against *S. aureus* than that against *E. coli* is presumed to be due to the high sensitivity to super-oxide anion, O_2^- .

CaCO₃/MgO powder without carbon was prepared by heating dolomite at 800 °C in air, because the diffraction pattern of the powder without carbon is similar to that with carbon obtained by heating with PVA at 700 °C, as shown in **Figs. 1** and **2**. In **Fig. 6**, comparison of the antibacterial activity between CaCO₃/MgO powder with and without carbon is shown, using the powder at a powder concentration of 10 g dm^{-3} . The survival ratio reduces with increasing incubation time, irrespective of the kind of bacteria and powder. In comparison between CaCO₃/MgO powders with and without carbon, the survival ratio on powder with carbon is lower

reduction than that without carbon ; that is, antibacterial activity of the CaCO₃/MgO powder without carbon is stronger than that with carbon. By BET, the formation of meso-pores is recognized at the surface of CaCO₃/MgO powder with carbon. In other words, O₂⁻ is generated through meso-pores, and the powder with carbon should have less surface that O₂⁻ occurs than that without carbon. The reason that antibacterial activity of CaCO₃/MgO powder with carbon is lower than that without carbon is presumed to be due to the reduction of the surface generating O₂⁻.

4. Conclusions

Carbon-coated CaCO₃/MgO powder showing antibacterial activity was obtained by the pyrolysis of poly (vinyl alcohol)-dolomite mixture at 700 °C in an argon gas. Antibacterial activity was evaluated by colony count method, using *Escherichia coli* and *Staphylococcus aureus*. At the temperature range from 400 to 700 °C, CaCO₃ and MgO were formed by the thermal decomposition of dolomite. At 800 °C, CaO was produced by the calcination of CaCO₃. The powders turned into black by PVA pyrolysis above 400 °C, i.e., the formation of carbon on its surface. Antibacterial activity of the carbon-coated CaCO₃/MgO powder against *Staphylococcus aureus* was found to be stronger than against *Escherichia coli*. Comparing with CaCO₃/MgO powder without carbon, it was found that the antibacterial action was reduced by forming carbon on its surface.

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