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Preparation of zinc oxide ceramics with a sustainable antibacterial activity under dark conditions

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Abstract

Fabrication of ZnO ceramics with a sustainable antibacterial activity even in the dark has been conducted. Fine ZnO powders were hydrothermally treated in 0.5–3 mol ml^{-1} $Zn(NO_3)_2$ aqueous solutions at 110–180 °C for 3–20 h. After an uniaxial pressing of the ZnO powders thus prepared, they were sintered at 400–600 °C for 1 h in air. Sustainability in antibacterial activity was evaluated using a colony count method with *Escherichia coli* bacteria on nutrient agar medium (36 °C/24 h) in a Na–P-buffer solution. The best data was attained for the ZnO ceramics prepared from the following conditions: a 3 mol ml^{-1} zinc nitrate solution for the hydrothermal treatment at 120 °C for 7 h and sintering in air with a step-by-step pattern (470 °C/1 h–485 °C/1 h–500 °C/1 h). ESR and chemical photoluminescence analyses have cleared that radical oxygen of super-oxide ($^{\bullet}O_2^-$) originated from the surface of ZnO might exhibit an antibacterial activity even under the dark condition.

Keywords: A. Sintering; B. Chemical properties; C. ZnO; D. Biomedical applications

1. Introduction

Since the 1980s, methicillin-resistant *Staphylococcus aureus* (MRSA) commonly linked with both hospital-associated infections and new community-acquired strains, which is brought by a variety of disease-causing bacteria, such as *Enterococcus*, *Staphylococcus*, and *Streptococcus*, has been thought to be a serious threat to public health worldwide [1]. Therefore, new strategies need to identify and develop the next generation of drugs or agents to control bacteria infections.

Up to now, several intensive studies have revealed that some metal oxides (TiO_2 [2], SiO_2 [2], MgO [3], CaO [3], CeO_2 [4], and ZnO [2,3,5,6]) show bacteriostatic, antimicrobial, or biocidal action [7]; for example, it is well-known that illuminated suspensions containing TiO_2 are effective at killing *Escherichia coli* (*E. coli*), which activity is originated from its photocatalytic disinfection [8]. However, the disadvantage of utilizing TiO_2 is that UV light is required to activate the photocatalyst and initiate the killing of the bacteria and viruses [9,10].

Among above-mentioned metal oxides, *in vitro* antibacterial activity and efficiency of regular zinc oxides (for examples, high-purity fine powders commercially available) were carefully examined. Sawai and Yoshikawa [3] reported that after quantitative evaluation of antibacterial activities of some metal oxide powders (ZnO, MgO and CaO), ZnO was the most effective for *Staphylococcus aureus*, which might be due to strong affinity to their cells. Sawai et al. [11–16] have investigated the antibacterial behaviors related of ZnO-based substances intensively, *i.e.*, ZnO–CaO solid solutions [11], carbon powders containing ZnO [12], mixtures of ZnO and MgO powders [13], developing a quantitative evaluation method [14], and identification of reactive oxygen species (ROS) generated from ZnO [15,16].

Recently, eco-toxicity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms has been examined precisely by Jones et al. [6] and Adams et al. [2]. They reported that the antibacterial behavior of ZnO increased with increasing nanoparticles concentration and with decreasing particle size; the particle concentration was observed to be

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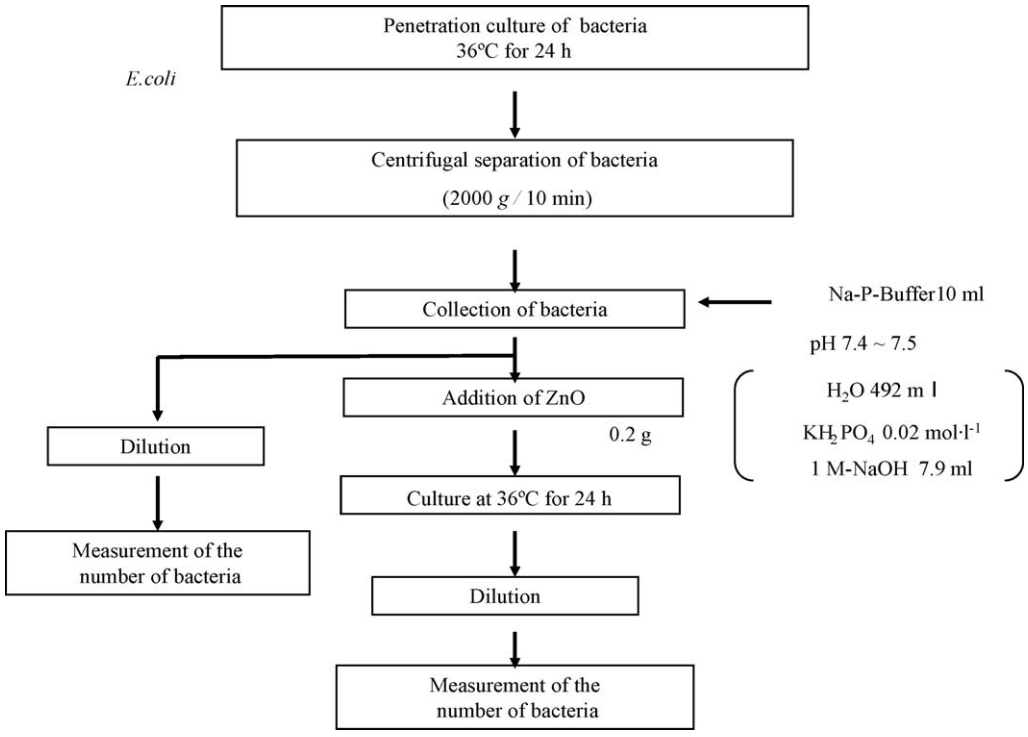

Fig. 1. Antibacterial test process.

more important than the particle size. And that the use of dispersant did not affect much on the antibacterial activity of ZnO nanofluids but enhances the stability of the suspensions. In addition, they described that the presence of ZnO nanoparticle damaged the membrane wall of the bacteria. Adams et al. [2] investigated the comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions and reported that the presence of light was a significant factor under the most test conditions, which might be due to the role in promoting generation of ROS, such as radical oxygen of super-oxide ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2). On the other hand, bacteria growth inhibition was also observed under dark conditions, indicating that undetermined mechanisms additional to photocatalytic ROS production were responsible for toxicity. Among these antibacterial materials-related literatures, little information is available about fabrication of metal oxide ceramics; this might be due to the heating process, which is required for conventional ceramic processing to fabricate bulk materials by sintering fine powders, resulted in the degradation of their antibacterial behaviors [17]. Therefore, there has been no report on the fabrication of metal oxide ceramics with a strong antimicrobial activity.

On the other hand, for corresponding to an increasing demand for home appliances, health-related and industrial applications, new antimicrobial or antibiotic materials being active and sustainable under dark conditions are much required. Even though, ZnO nanoparticles show the growth inhibition of bacteria in the dark, our preliminary experimental results indicated that the repeated-use of ZnO reduced their antibacterial activity remarkably.

The purpose of this study was to fabricate new porous bulk ZnO ceramics having a strong inhibition of bacteria growth in the dark sunshade from a viewpoint of (i) bulk materials with an easy-handling and a biochemical (biomedical) safety and (ii) a sustainable antibacterial activity.

2. Experimental procedure

2.1. Antibacterial test

Sustainability of antibacterial activity for various kinds of ZnO materials in the dark was evaluated with a colony count method using *E. coli* bacteria (*E. coli* IFO 3972 (ATCC 8379)) on nutrient agar medium in a Na–P-buffer solution as shown in Fig. 1. Penetrated culture of bacteria was grown at 36 °C for 24 h in a laboratory incubator. Then, bacteria were separated from the culture centrifugally at $2000 \times g$ (a gravitational constant) for 10 min. A 10 ml of Na–P-buffer aqueous solution containing KH_2PO_4 and NaOH, with a pH value of 7.4–7.5, was added to a small amount of bacteria collected. Test samples, 2.0×10^{-4} kg of ZnO powders, were added into the mixed medium containing the Na–P-buffer solution, and then, they were cultured at 36 °C for 24 in the dark sunshade. After the culture, the medium was separated into two parts: one is a small amount of medium and the other is large amount of residue. The former was diluted by the factor of 10^6 and then the number of colony (N) was countered. Antibacterial activity f was defined as the following equation: $f = -\log(N/N_0)$, where N_0 the number of the colony before the addition of ZnO, *i.e.*, the number of bacteria just started the test; $N_0 = 10^7$ ml^{-1} or 10^6 ml^{-1} was utilized. If the value of f is 7 under the condition of $N_0 = 10^7$ ml^{-1}, all the colonies were perfectly disappeared after the antibacterial activity test. The latter wet medium containing both ZnO and *E. coli* bacteria were separated by a

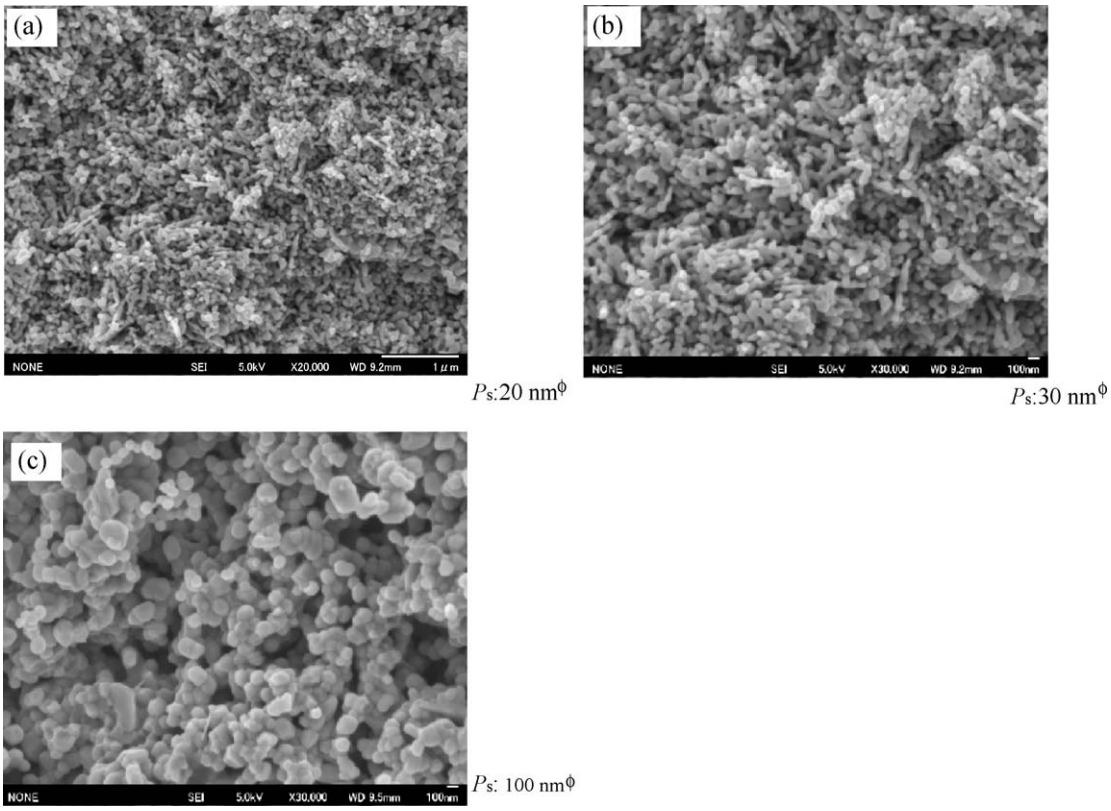

Fig. 2. FE-SEM photographs of ZnO powders: (a) as-received powder of ''NANO FINE'', (b) after sintering (Pt.2) without hydrothermal treatment, and (c) after sintering (Pt.2) using the hydrothermally treated (3 M/120 °C/7 h) powder.

laboratory centrifuge (''KN-70'' with a rotating speed of 2000 rpm (centrifugal force about <740 gravitational acceleration *g*), Kubota Co. Ltd., Tokyo, Japan) for 10 min. After the centrifugation, the supernatant solution was pipetted off. The precipitated ZnO was wrapped with an Al foil and then they were treated for heat sterilization at 190 °C for 2 h to disinfect active *E. coli* bacteria adhered to the ZnO powder. The resulting ZnO powder was again used to evaluate the sustainability of antibacterial activity for the ZnO materials prepared in the present study.

2.2. Fabrication of ZnO ceramics

From the application viewpoints to develop ZnO ceramics with a high surface area, a fabrication of porous bulk ZnO ceramics with an easy-handling and a biochemical (biomedical) safety was tried as almost the same process as conventional ceramic processing. Fine ZnO powders (Sakai Chemical Co. Ltd., Osaka Japan, ''NANO FINE'') with an average particle size P_s of ∼20 nm (Fig. 2(a)), BET surface area of ∼52 m^2/g, and high purity of 98.5%, were used as the starting material. As the preliminary experiment, they were uniaxially pressed into compacts at 98 MPa and then sintered at various temperatures from 400 to 600 °C for 1 h in air as shown in Fig. 3(i). As will be described later, the best sintering temperature was determined to be 500 °C (see the results shown in Fig. 4). And then two kinds of sintering patterns were selected: (i) a simple linear pattern with a constant temperature increasing rate of 200 °C/h up to 500 °C (Pt.1) and (ii) a step-by-step pattern (470 °C/1 h–485 °C/1 h–500 °C/1 h) (Pt.2), as shown in Fig. 3(i) and (ii), respectively. Bulk ceramics thus fabricated were crushed softly using an alumina mortar and pestle. And then they were passed through #25 and #36 mesh filters with their open sizes of 0.60 and 0.42 mm, respectively. Coarse powders obtained from the residues accumulations between these mesh filters, with their particle diameters between 0.42 and 0.60 mm, were examined for the above-mentioned antibacterial test.

2.3. Hydrothermal treatment

As will be described later, porous ZnO ceramics sintered at 500 °C exhibited an antibacterial activity only a few times of culture tests. Therefore, to improve their antibacterial activity, based on various kinds of preliminary experiments, hydrothermal treatment of ZnO powders were tried, which was originated from the idea that the surface of ZnO could be modified chemically and be functionally graded by the hydrothermal process under a high pressure. Before our study, Xu et al. [18] reported hydrothermal synthesis of zinc oxide powders with controllable morphology, however, they did not investigate nor mention about the antibacterial behavior of these powders.

The hydrothermal treatment in this study is schematically illustrated in Fig. 5. After an uniaxial pressing (98 MPa), fine particle ZnO powder-compacts (20 g, 20 mm in outer diameter of the compacts containing about 0.1 μm-size

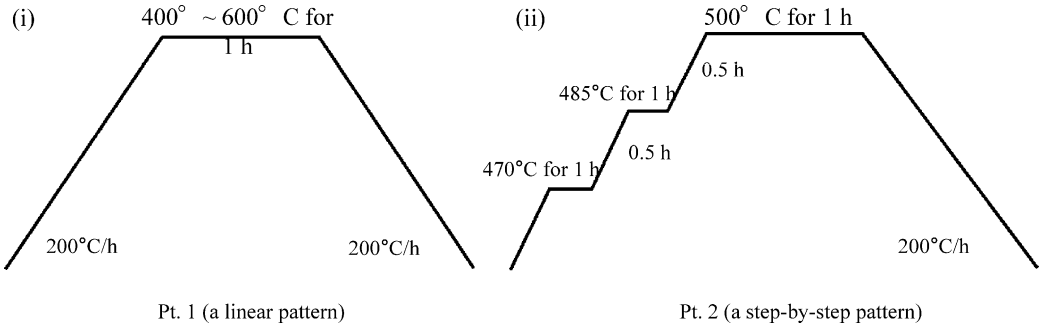

Fig. 3. (i) Sintering patterns of Pt.1 (a linear pattern at 500 °C for 1 h) and (ii) Pt.2 (a step-by-step pattern: 470 °C/1 h–485 °C/1 h–500 °C/1 h).

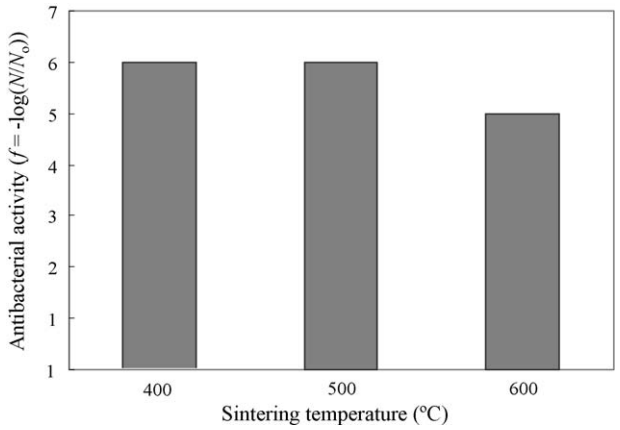

Fig. 4. Antibacterial activity (f) *vs*. sintering temperature; sintering for 1 h in air. The number of the bacteria before testing is 10^7 ml^{-1}.

pores) with an aqueous solution of 100 ml $Zn(NO_3)_2{\cdot}6H_2O$ or $Zn(CH_3COO){\cdot}2H_2O$ were put into a high-pressure stainless container (an inner volume of 115 ml, Fig. 6) and heated for 3–20 h at 110–180 °C; the concentration of aqueous solution was varied from 0.5 to 3 mol l^{-1}. After taken out from the high-pressure vessel, the samples were rinsed with pure water for several times until the pH value of rinsed water reached around 7 and then dried at 120 °C for 10 h in air. Hydrothermally treated ZnO powders (confirmed by X-ray diffraction (XRD) analysis using Cu$K\alpha_1$ radiation with a monochromator under a goniometer-scanning-speed of 0.25°/min, Rigaku Co. Ltd., Osaka Japan, ''Rint 2200'') were also sintered at 500 °C for 1 h in air as described in Section 2.2.

2.4. Characterization of ZnO materials

A field-emission type scanning electron microscope (FE-SEM, JEOL Ltd., Tokyo Japan, ''JSM-7001FD'') was used for microstructural observation; for both crystalline phase identification and determination of unit-cell values a and c of hexagonal ZnO were performed by X-ray diffraction. Interplanar spacings were measured with the aid of an internal standard of high-purity Si, and unit-cell values were calculated by a least-squares refinement.

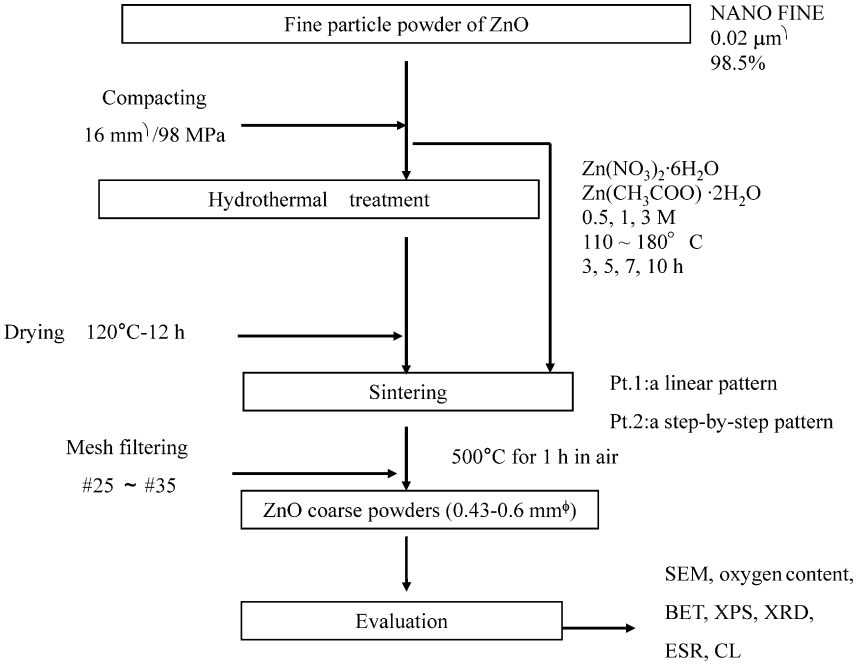

Fig. 5. Preparation process for ceramics *via* hydrothermal treatment.

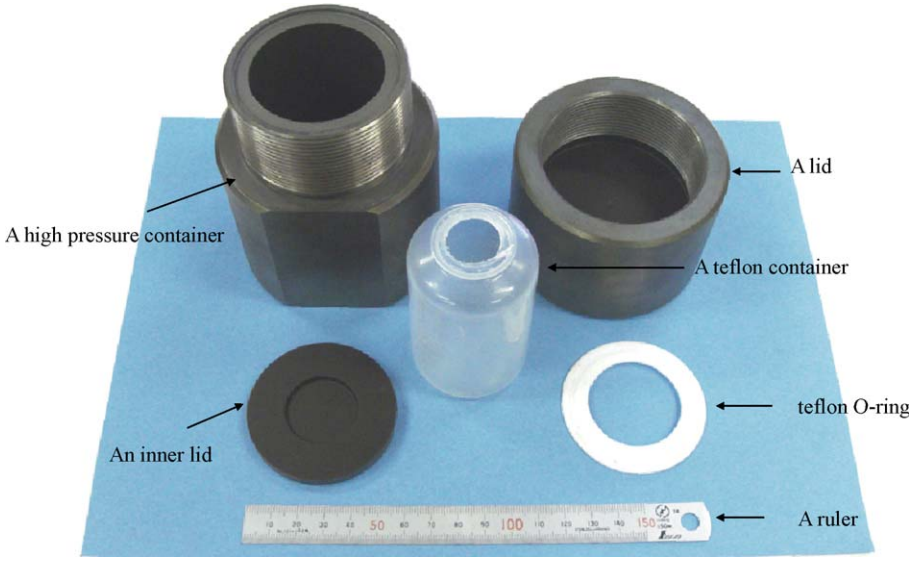

Fig. 6. A photograph of a hydrothermal treatment container.

In addition to these, various kinds of analytical measurements were utilized; excess oxygen contents of both ZnO powders and ceramics were measured by spectrochemical analysis (Horiba Co. Ltd., Kyoto, Japan, ''EMGA-620W/C''), *i.e.*, non-dispersive infrared (IR) absorption spectrum using an inert gas fusion in an impulse furnace, BET surface areas of powders were measured with an absorption measurement system (Shimadzu, Kyoto, Japan, ''ASAP 2010''); X-ray photoelectron spectroscopy (XPS, Shimadzu, ''AXIS-165'') was utilized for evaluation of binding energy of Zn ion at the surface of ZnO particles and ceramics; electron spin resonance spectroscopy (ESR, JEOL Ltd., ''FR80'') was performed on the samples in the dark by a spin-trap method under the conditions: a microwave of 9.226 GHz and an electric power of 10 mW, using a new dimethylpyrroline-*N*-oxide (DMPO)-type spin-trap agents ''CYPMPO'' (Radical Research Inc., Tokyo, Japan) for super-oxide radical anion ($^{\bullet}O_2^-$) and DMPO (Dojindo Laboratories, Kumamoto, Japan) for hydroxyl radical ($^{\bullet}OH$), and scavengers of super-oxide dismutase (SOD, Wako Pure Chemical Industries Ltd., Osaka, Japan) for $^{\bullet}O_2^-$ and dimethyl sulfoxide (DMSO, Sigma Aldrich, Tokyo, Japan) for $^{\bullet}OH$; a chemical photoluminescence (chemiluminescence: CL) method (Tohoku Electric Industries Co. Ltd., Sendai, Japan, ''CLD-100FC'') was used for identification of ROS by detecting luminescence in the mixed solutions of luminol (concentration: 10^{-6} mol l^{-1}) (Nacarai Tesque, Kyoto, Japan) and diluted ZnO suspensions (10^{-11} mol l^{-1}). The diluted ZnO suspension had been prepared by adding the ZnO powder into a carbonate buffer solution (NaOH–$NaHCO_3$) with pH 10.8. Luminescence in the mixed solutions could be detected when the luminol was oxidized by ROS generated from ZnO suspension. In this method, the same reagents as ESR, scavengers such as SOD for $^{\bullet}O_2^-$, DMSO for $^{\bullet}OH$, furthermore, calatase (Sigma Aldrich) for hydrogen peroxide H_2O_2 and sodium azide (NaN_3, Nacarai Tesque) for singlet oxygen $^1O_2$, respectively, were used.

3. Results and discussion

3.1. Characteristics of ZnO ceramics

As-received ZnO powder (''NANO FINE'') was heated using a linear sintering pattern (Pt.1) at 400, 500, and 600 °C for 1 h in air. Thus obtained ceramics were evaluated from the viewpoints of the antibacterial activity f; Fig. 4 shows the results as a function of sintering temperature. In this case, the number of bacteria before testing $N_0 = 10^7$ ml^{-1} was adopted. The ceramics sintered at 400 and 500 °C, in which the particle size P_s of the latter was ~30 nm, exhibited an f value of ~6 after the 1st antibacterial test, indicating that almost all bacteria were disappeared, only ~10 bacteria were remained after the testing, *i.e.*, antibacterial activity of 99.9999%. However, the ZnO ceramics fabricated at 600 °C indicated a value f of 5, a little lower antibacterial activity than those sintered at 400 and 500 °C. In general, the higher sintering temperature gives ceramics the higher density and strength. Therefore, we selected 500 °C as the sintering temperature for the fabrication of porous ZnO ceramics. Then, sustainable antibacterial activity of ZnO ceramics sintered at 500 °C was examined; the results after the 2nd antibacterial test decreased drastically to a value f of 2, as the same result obtained using as-received ZnO powder. Therefore, a simple ceramic processing using as-received ZnO powder was thought not to produce ZnO ceramics having a sustainable antibacterial activity in the dark sunshade.

3.2. Antibacterial activity of ZnO ceramics via hydrothermally treated powders

In order to enhance the sustainable antibacterial activity of ZnO ceramics, the ZnO powder was modified by hydrothermal treatment in aqueous solutions of zinc nitrate or zinc acetate [19,20]. As described in Section 2.3 (Fig. 5), after the

hydrothermal treatment (HT) ZnO powders were rinsed and then dried. XRD analysis revealed that the HTed sample was single phase of ZnO (JCPDS: #36-1451) and from the FE-SEM observation, ZnO particles, for example, after HT(3M-$Zn(NO_3)_2$/120 °C/7 h), grew from ca. 20 to ca. 30 nm (Fig. 2(b)).

3.2.1. Sintering pattern

HTed ZnO powders were compacted and heated at 500 °C for 1 h in air under two different sintering patterns, *i.e.*, a linear pattern (Pt.1) and a step-by-step pattern (Pt.2) as shown in Fig. 3. The latter sintering pattern gave a better sustainable antibacterial activity; *i.e.*, the antibacterial activity f of ZnO ceramics sintered by Pt.1 using hydrothermally treated ZnO powders in a 3 mol l^{-1} $Zn(NO_3)_2{\cdot}6H_2O$ solution at 120 °C for 3 h, denoted as (Pt.1, HT: 3 M/120 °C/3 h), reached 7 until the 3rd antibacterial test and then dropped to 0, however, that of ceramics made under the condition of (Pt.2, HT: 3 M/120 °C/3 h) could maintain more than 6 up to the 5 th test.

In addition, difference in microstructure of ceramics depended on sintering patterns was investigated from the viewpoint of their mechanical properties. Fig. 2(c) shows an SEM photograph for fracture surface of ZnO ceramics (Pt.2, HT: 3 M/120 °C/3 h), indicating that the porous ceramics composed of homogeneous ZnO grains (∼100 nm). The step-by-step sintering pattern (Pt.2) gave a little higher bending strength to the ZnO ceramics than Pt.1. This difference might be originated from the higher energy totally supplied to enhance the bonding among ZnO grains. However, the difference in sustainable antibacterial activity could not be clearly explained.

3.2.2. Difference in Zn salts

Then the difference in ZnO ceramics (Pt.2) derived from ZnO powders prepared hydrothermally in the $Zn(NO_3)_2{\cdot}6H_2O$ or $Zn(CH_3COO){\cdot}2H_2O$ solutions was investigated; up to the 3rd antibacterial test, there was no difference between them (HT: 1 M/120 °C/10 h), however, nitrate-salt-derived ZnO ceramics gave a higher f values than the acetate-salt-derived ZnO at more than 4 times of test. From these results, hereafter, hydrothermal treatment of ZnO powders was conducted in nitrate-salt solutions.

3.2.3. Solution concentration

Both 1 and 3 mol l^{-1} of $Zn(NO_3)_2{\cdot}6H_2O$ solutions were used for preparation of hydrothermally treated ZnO powders. A higher concentrated nitrate-salt solution resulted in ZnO ceramics (Pt.2, HT: 120 °C/3 h) with a higher f values ($\geq$6) even after the 5th test.

3.2.4. HT temperature

Fig. 7 shows the effect of hydrothermal treatment temperature on the sustainable antibacterial activity of ZnO ceramics (Pt.2, HT: 3 M/3 h). Only HT temperature of 120 °C maintained the f value of 6 even after the 5th antibacterial test; higher HT temperatures than 120 °C tend to decrease the antibacterial activity. From this result, HT temperature was determined to be 120 °C.

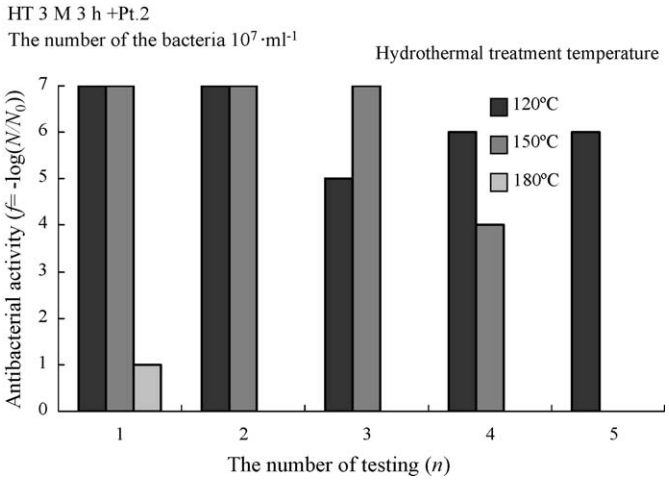

Fig. 7. Antibacterial activity (f) of the ZnO ceramics *vs*. the numbers of testing (n), as a function of HT temperature using 3 mol l^{-1} zinc nitrate aqueous solution for 3 h + sintering (Pt.2). The number of the bacteria before testing is 10^7 ml^{-1}.

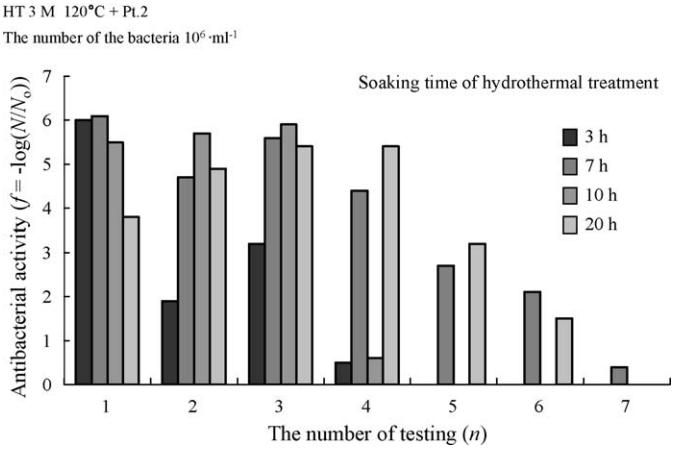

Fig. 8. Antibacterial activity (f) of the ZnO ceramics *vs*. the numbers of testing (n), as a function of soaking time of HT treatment under the conditions (Pt.2, HT: 3 M/120 °C). The number of the bacteria before testing is 10^6 ml^{-1}.

3.2.5. Soaking time of HT

ZnO ceramics made from hydrothermally treated powders with various HT soaking time from 3 to 20 h were examined on the sustainable antibacterial activity. In Fig. 8, up to the 3rd test, a little difference from different soaking time was observed, however, more than four-times test, we could find some effect of soaking time on the antibacterial activity. The optimal soaking time was thought to be 7 h.

3.2.6. pH values

During antibacterial test, the pH values of penetrated culture of bacteria were monitored with a slight suspect of their effect on the antibacterial activity. Fig. 9 summarized the antibacterial activity f and pH values as a function of number of testing (n). Here, ZnO ceramics were fabricated under the conditions of hydrothermal treatment in a 3 mol l^{-1} $Zn(NO_3)_2{\cdot}6H_2O$ solution at 120 °C for 7 h and sintering at 500 °C for 1 h in air by the

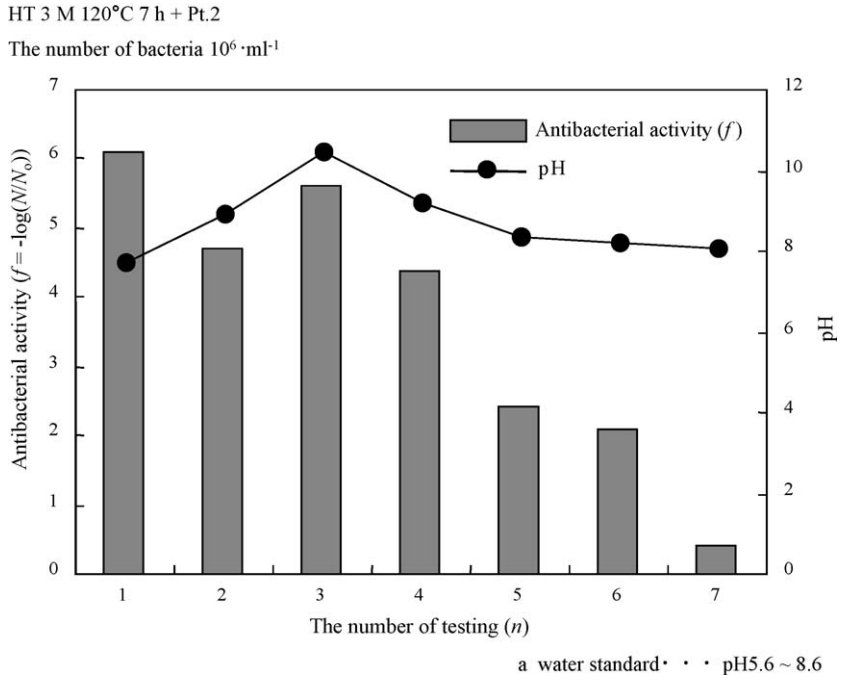

Fig. 9. Antibacterial activity (f) of the ZnO ceramics *vs*. the numbers of testing (n) under the conditions (Pt.2, HT: 3 M/120 °C/7 h).

step-by-step pattern. There is no relationship between f and pH values, as previously reported on the antibacterial behavior of TiO_2 [6].

3.3. Mechanism of antibacterial activity of ZnO in the dark sunshade

To investigate the mechanism of the sustainable antibacterial activity of ZnO ceramics (Pt.2, HT: 3 M/120 °C) under dark conditions, ESR, CL, and the conventional colony count methods were utilized.

3.3.1. ESR study

This measurement was conducted in the sunshade as described before. Fig. 10 shows the ESR spectrum of samples: (a): 1-M DMPO, (b): 1-M DMPO + 10 mass% ZnO powder pulverized from ceramics, and (c): (DMPO + ZnO) + 1.4-M DMSO. DMPO is a trap agent for hydroxyl radical (•OH)

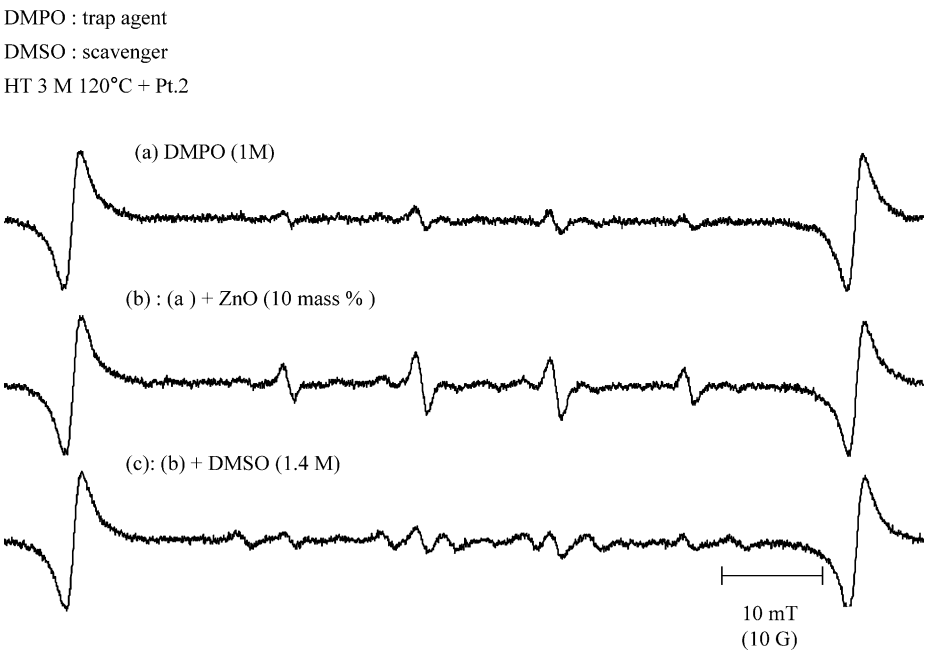

Fig. 10. ESR-confirmation of •OH generation by ZnO. Microwave: frequency f = 9.226 GHz and power = 10 mW.

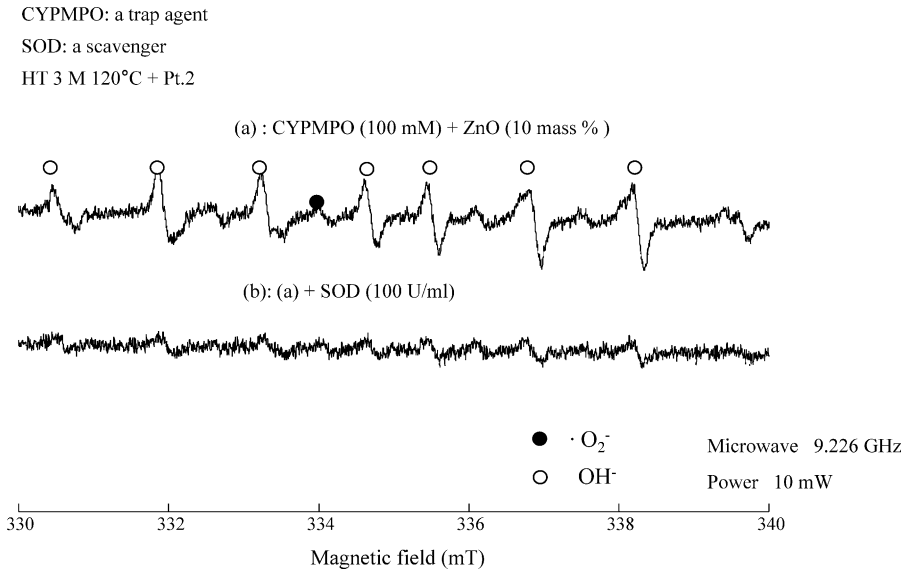

Fig. 11. ESR-confirmation of $^{\bullet}O_2^-$ generation by ZnO. Microwave: frequency $f = 9.226$ GHz and power = 10 mW.

(Fig. 10(a)) and when ZnO ceramic powder was added, the intensities of 4 spectra in the middle range were increased a little (Fig. 10(b)), however, when 1.4-M DMSO, a scavenger of $^{\bullet}OH$, was added again to sample (b), 4 spectrum were reduced (Fig. 10(c)), indicating that ZnO ceramics have generated $^{\bullet}OH$. In Fig. 11(a), ESR 7 spectra originated from $^{\bullet}OH$ were observed. At the same time one spectrum for radical oxygen of super-oxide ($^{\bullet}O_2^-$) in the sample containing of 100 mM CYPMPO, a newly developed trap agent for $^{\bullet}O_2^-$, was also detected. When an SOD scavenger of $^{\bullet}O_2^-$ was added into sample (a), all intensities of spectra were decreased clearly. From these ESR results (Figs. 10 and 11), it should be stated that generations of $^{\bullet}OH$ and $^{\bullet}O_2^-$ from the ZnO have been confirmed, respectively.

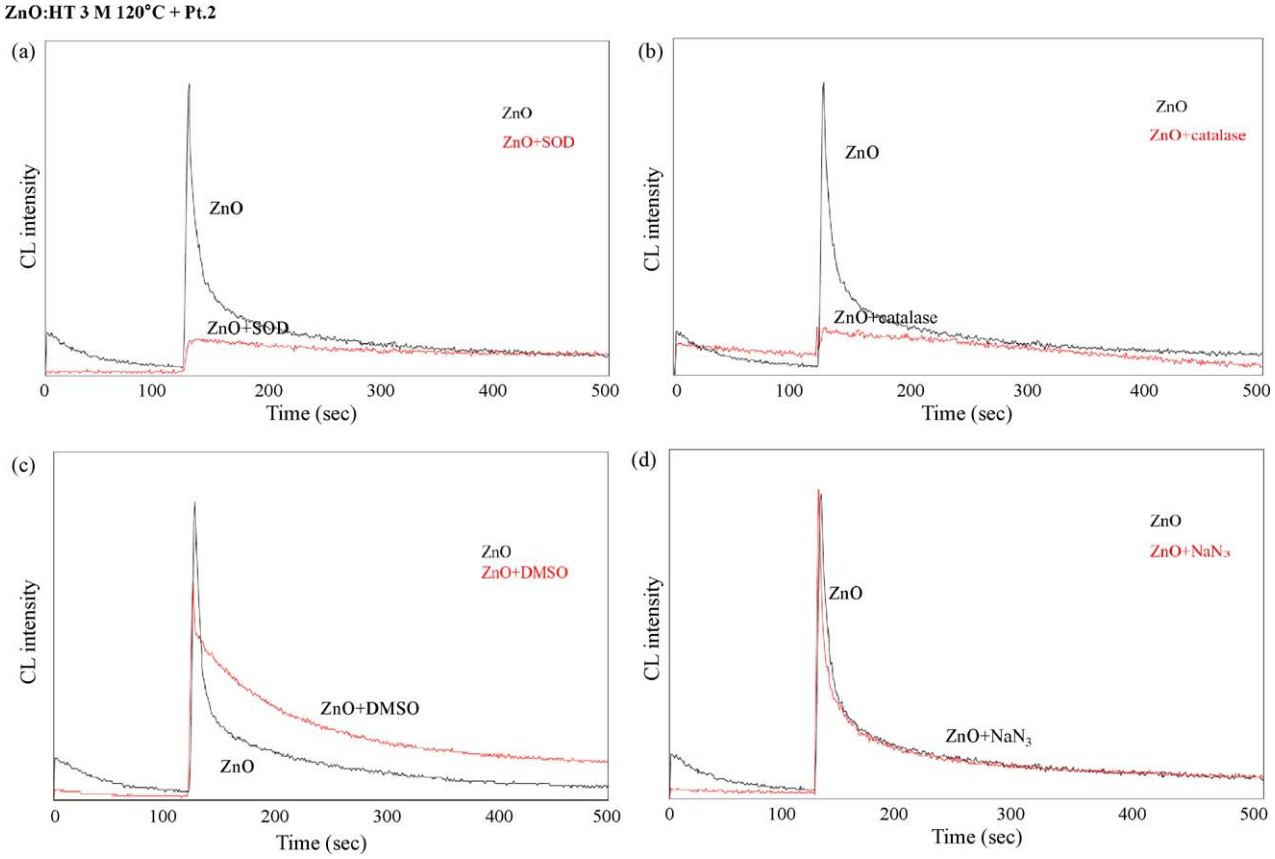

Fig. 12. CL profiles of ZnO (Pt.2, HT: 3 M/120 °C/7 h): (a) with SOD, (b) with catalase, (c) with DMSO, and (d) with NaN_3.

Table 1
Characteristics of ZnO ceramics.

Sample	Excess oxygen contents δ	$ZnO_{1+\delta}$	Lattice a (nm)	Parameters c (nn)	Lattice volume v (nm^3)	Binding energy (eV)	Surface area ($m^2\ g^{-1}$)
NANO FINE ZnO	−0.0400	$ZnO_{0.960}$	0.3248	0.5205	0.047554	89.42	52.0
After sintering (Pt.2)	−0.0758	$ZnO_{0.924}$	0.3236	0.5185	0.047022	89.04	9.27
HT 3 h + Pt.2	−0.0316	$ZnO_{0.968}$	0.3244	0.5195	0.047345	89.67	1.78
HT 5 h + Pt.2	−0.0247	$ZnO_{0.975}$	0.3249	0.5206	0.047592	89.82	0.374
HT 10 h + Pt.2	−0.0121	$ZnO_{0.989}$	0.3243	0.5195	0.047316	89.88	–
cf.: ZnO (JCPDS#36-1451)			0.3250	0.5206	0.047621	Zn (ZnO) 3p 89.0	

3.3.2. CL measurement

Chemical photoluminescence (chemiluminescence: CL) profiles for ZnO samples containing some kinds of scavengers are presented in Fig. 12(a)–(d): (a) ZnO with a scavenger SOD for $^{\bullet}O_2^-$, (b) with catalase for H_2O_2, (c) with DMSO for $^{\bullet}OH$, and (d) with NaN_3 for $^1O_2$. By comparing these four data, it might be concluded that with the addition of scavenger SOD and catalase to ZnO, the Cl intensities were much suppressed, suggesting that both $^{\bullet}O_2^-$ and H_2O_2 were generated in the dark sunshade. However, as a little decrease in the CL peak was also observed in the sample (c) containing ZnO with DMSO; it was indicated that generation of $^{\bullet}OH$ by the ZnO was not necessary a negative phenomena. From these, it might be stated that from the CL measurements ZnO ceramics could generate $^{\bullet}O_2^- > H_2O_2 > {}^{\bullet}OH$.

3.3.3. Colony count methods

Antibacterial activity f of ZnO ceramics with radical scavengers was investigated. Fig. 13 summarized the f values in relation to the combination of ZnO and scavengers, proving clearly that with the addition of SOD or both SOD and catalase, the f values were much decreased than those with the single addition of DMSO or catalase, *i.e.*, SOD suppressed the generation of $^{\bullet}O_2^-$ from ZnO.

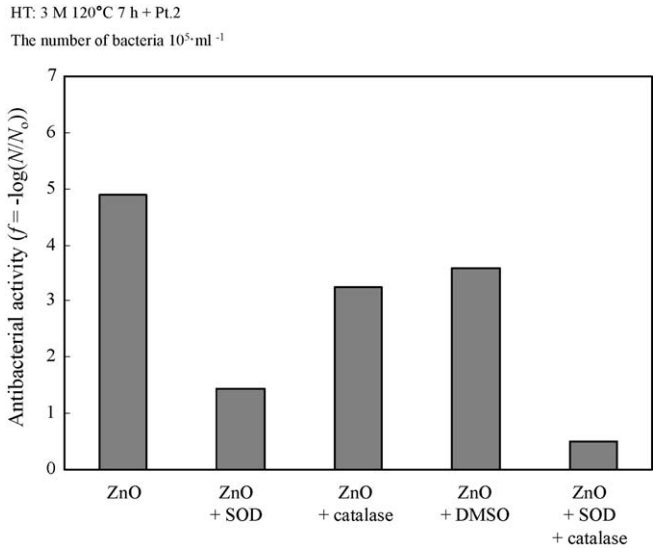

Fig. 13. Antibacterial activity (f) of the ZnO ceramics, fabricated under the conditions (Pt.2, HT: 3 M/120 °C/7 h), depressed by adding radical scavenger.

From these three experimental data, we might reach the conclusions that hydrothermally treated ZnO could generate $^{\bullet}O_2^-$ repeatedly in the dark sunshade. This ROS generation resulted in the sustainable antibacterial activity of ZnO ceramics.

3.3.4. Other characteristics of porous ZnO ceramics

Table 1 summarized the physical properties of ZnO materials prepared in the present study: oxygen contents δ measured by spectrochemical analysis, BET surface areas, lattice constants (a and c) of hexagonal ZnO by XRD, and binding energy determined by XPS. These parameters were measured and considered for the first time to have much relationship between the antibacterial activity or the sustainability of ZnO, however, we could not find any relationship among them.

4. Conclusion

Different from the antibacterial activity of TiO_2 under UV light, newly developed ZnO ceramics have been found to exhibit the sustainable antibacterial activity even in the dark sunshade. These ceramics were fabricated by sintering ZnO powders at low temperature of 500 °C for 1 h in air, in which powders were prepared by treating ZnO hydrothermally in a 3 mol l^{-1} zinc nitrate solution at 120 °C for 7 h. The sustainable antibacterial activity of thus fabricated ZnO ceramics might be originated from the generation of super-oxide anion ($^{\bullet}O_2^-$). Based on this study, much investigation for new applications using its antibacterial activity is expected to start in future.

Acronyms

ROS	reactive oxygen species, such as radical oxygen of super-oxide ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2).
DMPO	dimethylpyrroline-*N*-oxide, a trap agent for hydroxyl radical ($^{\bullet}OH$).
CYPMPO	a newly developed dimethylpyrroline-*N*-oxide (DMPO)-type spin-trap agent for radical oxygen of super-oxide ($^{\bullet}O_2^-$).
SOD	super-oxide dismutase, an scavenger of $^{\bullet}O_2^-$.
DMSO	dimethyl sulfoxide, a scavenger of $^{\bullet}OH$.
CL	chemical photoluminescence (chemiluminescence).
HT	hydrothermal treatment.
Pt.1	a linear sintering pattern with a constant increasing temperature rate.

Pt.2 a step-by-step pattern with a step-by-step increasing temperature during heating process.
ESR electron spin resonance.

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