Antimicrobial Evaluation and ESR Measurement of Aluminum Anodic Oxide Films Containing an Iodine Compound

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The hydroxyl radical generated from aluminum anodic oxide film by UV irradiation was evaluated using ESR measurement and its antimicrobial characteristics was also investigated using film covering method with photo irradiation. DMPO-OH adduct was detected using ESR with DMPO (5,5-Dimethyl-Pyrroline-N-Oxide) as a spin trap reagent when aluminum anodic oxide film was irradiated by UV. It found from this result that hydroxyl radical generate from aluminum anodic oxide film by UV irradiation and that the aluminum anodic oxide film occur antimicrobial action by photo irradiation. Further the aluminum anodic oxide film impregnated with iodine compound showed high antimicrobial action in spite of photo irradiation.

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1. Introduction

Aluminum anodic oxide film is an oxide layer on aluminum substrate obtained by an anodic oxidation treatment and it has been mainly used for pan and kettle, etc. Aluminum anodic oxide film has a special geometrical structure and it is hexagonal cylindrical cells structure like a honey-comb, which is called "Keller's model".

It can be expected that aluminum anodic oxide film may show photocatalytic property in spite of an insulator (band gap is 10eV) since aluminum anodic oxide film is considered to be n-type semiconductor with electrons resulting from excess aluminum. In fact, photo-electric effect has been observed in aluminum anodic oxide film[1]. On the other hand, in order to expand the industrial uses of iodine being an abundant underground resource in Japan, we have developed an aluminum coating impregnated with iodine compound as a new antimicrobial material[2].

In this study, active radical species generated via photocatalytic reaction from aluminum anodic oxide film with and/or without iodine compound by UV irradiation was detected by electron spin resonance (ESR) using 5,5-dimethyl-pyrroline-N-oxide (DMPO) as a spin trap reagent and the antimicrobial characteristics of aluminum anodic oxide film with and without iodine compound were also investigated by photo irradiation.

2.Experimetal procedure

2.1 Anodic oxidation treatment of aluminum

Aluminum plate (A1100, purity 99 %) was used as substrate. The aluminum plate was degreased and etched beforehand, and anodized in 20 mass% H_2SO_4. In this study, anodic oxidation treatment time, temperature and current density were 25 min, 10 □ and 3.0 A/dm^3, respectively.

2.2 Iodine impregnation treatment of aluminum anodic oxidation coating

The oxidative treated aluminum plate was placed in aqueous solution containing PVPI (povidone-iodine (poly (1-(2-oxo-1-pyrrolidinyl) ethylene) iodine complex)) of 1000 ppm and was electrodeposited under the condition of the constant voltages of 150 V for 4 minutes in order to impregnate with iodine compound into the micro pores. The obtained sample was ultrasonically washed for 3 minutes in ethanol, and was dried at 100 □.

2.3 ESR measurement

ESR measurement was performed by FA-200 (JEOL, Japan). Radicals were detected by means of the

spin-trap method. 5,5-Dimethyl-Pyrroline-N-Oxide (DMPO) was used as a spin-trap reagent. Aluminum anodic oxide film was immersed in the mixture of 2 cm^3 water and 20 mm^3 DMPO. ESR spectra of aluminum anodic oxide film was measured after irradiated by 500 W Xe lamp (SX-UI 500XQ, Ushio, Japan, UV intensity is 17 mW/cm^2).

2.4 Evaluation of antimicrobial characteristics

2.4.1 Bacteria and pre-culture

The bacteria used in this work were *Escherichia coli* (*E. coli*, IFO 3972) as a typical Gram-negative bacterium and *Staphylococcus aureus* (*S. aureus*, IFO 12732) as a typical Gram-positive bacterium. Each of the bacterium was pre-cultured at 37 □ for 18 hours in a culture dish placed an agar culture medium. Their cells were collected by using the centrifuge, and then washed with sterilized physiological salt solution and pure water.

2.4.2 Antimicrobial evaluation (Film covering method with photo irradiation)

The sample (50 mm×50 mm×1 mm) was placed in a sterilized culture dish and bacteria suspension of 0.4cm^3 was contacted on the sample surface and covering film was put on the bacteria suspension and the sample. The culture dish contained the sample and the bacteria suspension was cultured at 37℃ for 24 hours under the condition of relative humidity above 90 % with and without photo irradiation. After the culture, the bacteria adhered to the sample and the film were washed out with SCDLP culture medium of 10cm^3 and the survival colony numbers of the bacteria were counted by plate culture method with SA culture medium after the culture at 37 ℃ for 24 hours.

The concentration of bacteria suspension in this test was $1.0\times10^4 \sim 1.0\times10^7$ CFU/cm^3 in the solution that was diluted 500-fold the standard bouillon culture medium with M/15 phosphate buffer (pH7.0).

3. Results and Discussion

3.1 Characterization of aluminum anodic oxide film with iodine compound

In order to impregnate with iodine compound into the micro-pores of anodic oxidation coating, aluminum plate that was anodically oxidized was placed in the aqueous solution contained povidone-iodine as iodine compound, and was electrodeposited under a constant voltage condition.

(a) Anodic oxidation coating (b) Anodic oxidation coating with iodine compound

Fig.1 Sample appearance of anodic oxidation coating impregnated without and with iodine compound.

The appearances of samples of anodic oxidation coating impregnated without and with iodine compound were shown in figure 1. From result of figure 1, the color of the sample contained iodine compound was brown. As for us, generally, as for the color of I_2 it is yellow or brown, but that of the I^- ion which is ionized has known that it is colorless. From this fact, it was thought that iodine compound is present as the form of I_2 in the micropores of anodic oxidation coating.

SEM image of the surface of anodic oxidation coating impregnated with iodine compound is shown in figure 2. The micropores were presented on the surface of anodic oxidation coating and the pore size was approximately 10 nm.

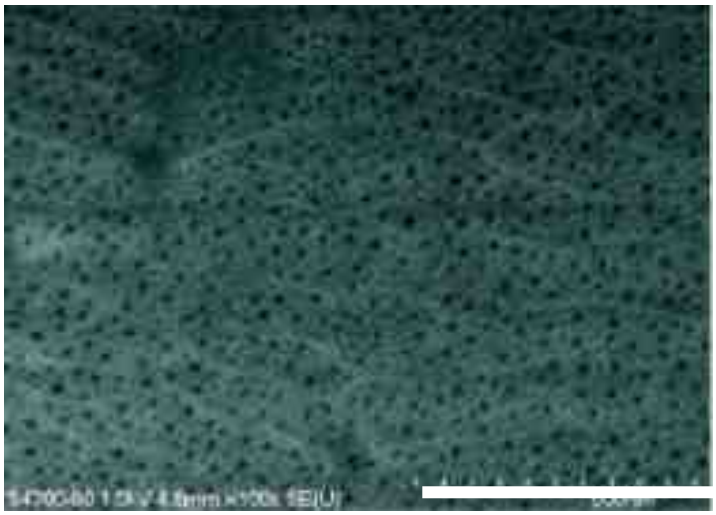

Fig.2 SEM image of the surface of anodic oxidation coating impregnated with iodine compound.

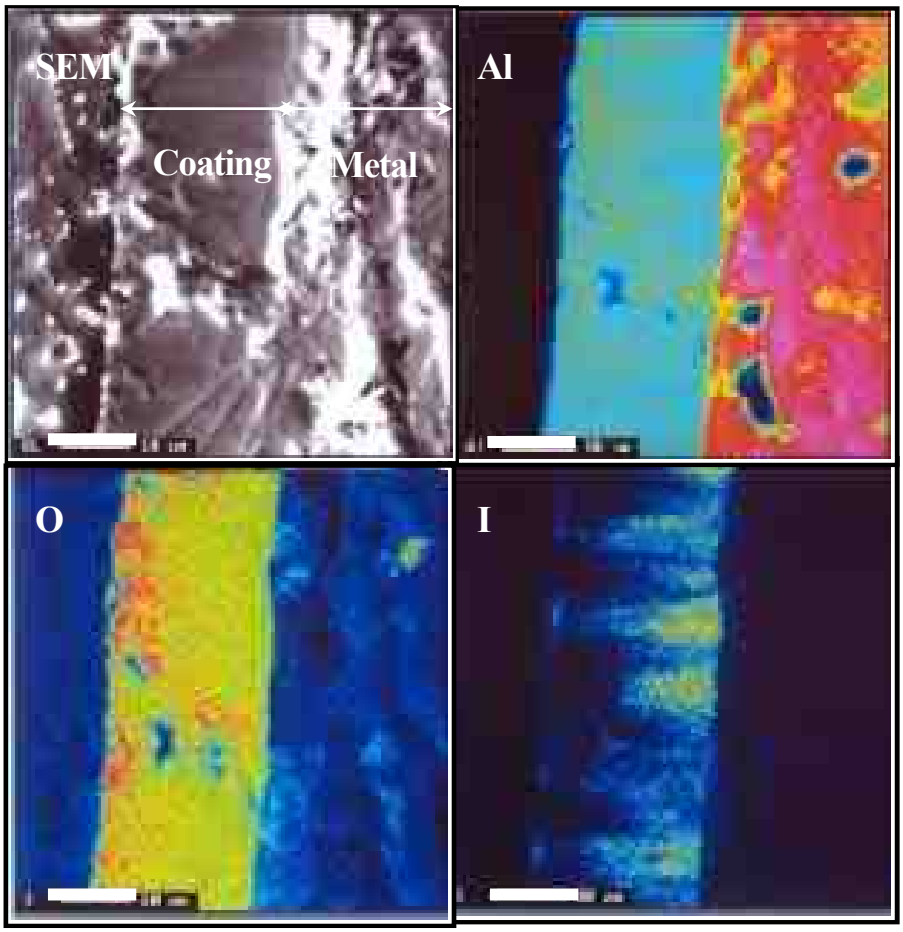

Fig.3 Chemical composition of the cross-section of the coating by EPMA analysis.

Further more, the chemical composition of the cross-section of the obtained coating was analyzed by an EPMA and the result is shown in figure 3. The result of EPMA observation proved that the iodine compound exists mainly in the part of the bottom of the micropore of the anodic oxidation coating.

From the above-mentioned results, it was proved that we could successfully prepare the aluminum coating impregnated with I_2 complex similar to the molecular structure of povidone-iodine using as a starting material.

3.2 Generation of hydroxyl radical from aluminum anodic oxide film

First, the ESR spectrum of DMPO-OH adduct generated from aluminum anodic oxide film by UV irradiation was shown in Figure 4. A typical peak pattern of DMPO-OH which each peak height is 1:2:2:1

was observed. From the pattern and their hyperfine splitting constants (aN=aH=1.49mT), it found that hydroxyl radical generate from aluminum anodic oxide film by UV irradiation.

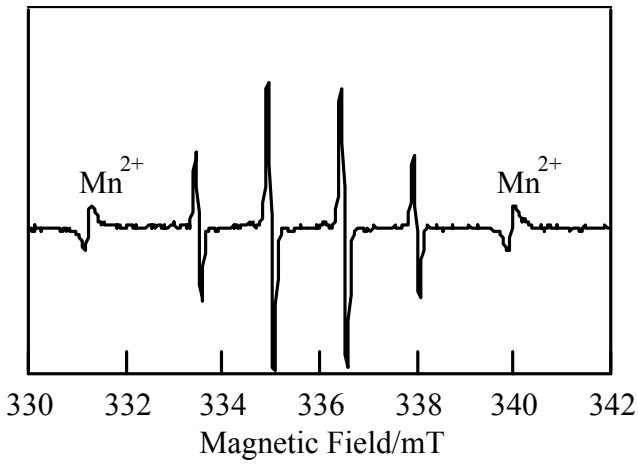

Fig.4 ESR spectrum of DMPO-OH adduct generated from aluminum anodic oxide film by UV irradiation.

Figure 5 shows concentrations of DMPO-OH adduct generated from aluminum anodic oxide film by UV irradiation. The amount of adducts linearly increased with UV irradiation time. Figure 6 shows the dependence of UV irradiation on the concentrations of DMPO-OH adduct. The concentrations of DMPO-OH adduct increased by UV irradiation and the formation of DMPO-OH adduct required UV irradiation.

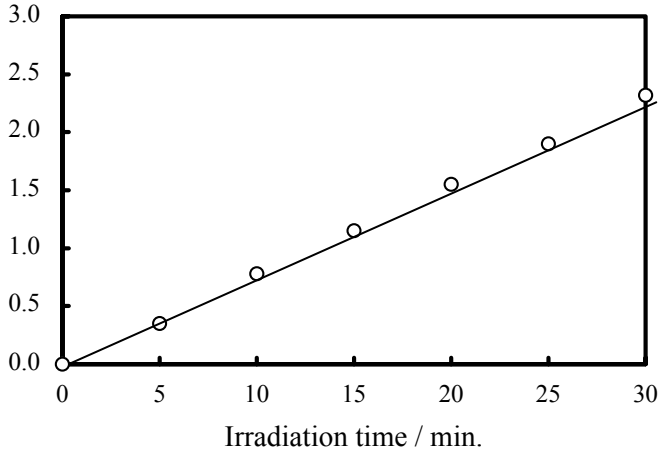

Fig.5 Change of DMPO-OH concentration with irradiation time.

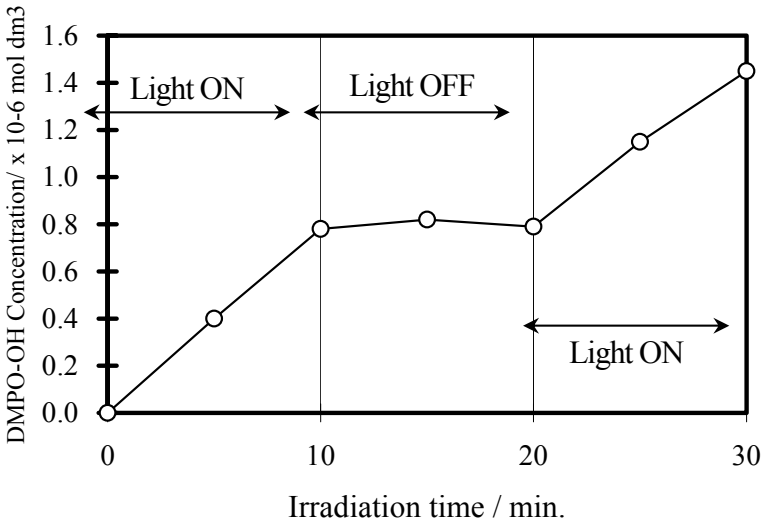

Fig.6 The dependece of UV irradiation on the concentration of DMPO-OH.

Generally, the photocatalytic reaction of TiO_2 can be explained as follows: TiO_2 which is a semiconductor absorbs UV, and produces electron (e^-) and positive hole (h^+). Electron reacts with oxygen, and super oxide anion radical(• O_2^-) is generated. On the other hands, positive hole reacts with adsorbed water and generates hydroxyl radical(• OH). They act as a reaction intermediate and oxidize or reduce the molecules on the surface of TiO_2. It is expected that the same reaction as described above occurred. It is considered to be due to the higher reactivity of • OH with DMPO[$k_{OH}=3.4\times10^9$(mol dm^{-3})$^{-1}s^{-1}$][3] than that with DMPO[k_{O2^-}=18 mol dm^{-3}]$^{-1}s^{-1}$][4]. Moreover, it is reported that disproportionate reaction occurs slowly and it can exist stably under strong alkaline condition[5]. For this reason, DMSO (dimethyl sufoxide) was used instead of distilled water. Figure 7 shows ESR spectrum of DMPO-O_2^- adduct generated from aluminum anodic oxide film by UV irradiation with DMPO and DMSO. From the spectrum pattern in figure 7, it found that super oxide anion radical has generated from aluminum anodic oxide film.

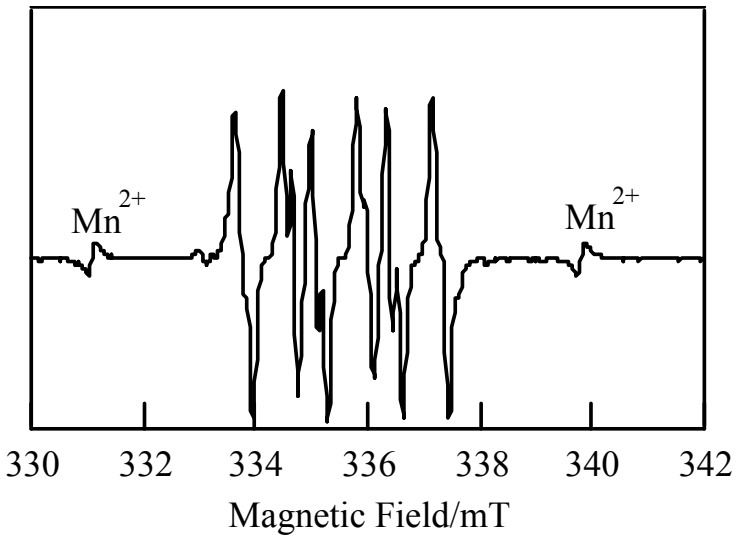

Fig.7 ESR spectrum of DMPO-O2- adduct.

Further, we examined in the same way concerning the aluminum anode oxide film impregnated with iodine compound. The amount of DMPO-OH adduct generated from the aluminum anodic oxidation coatings impregnated with iodine compound almost was the same as that of the aluminum anodic oxidation coatings. Impregnation doing iodine compound on the aluminum anodic oxidation coatings, it was found in the amount of DMPO-OH adduct which it generates from aluminum anodic oxidation coatings that it does not influence.

3.3 Antimicrobial characteristics of aluminum anodic oxide film

The result of the antimicrobial evaluation due to the presence of photo irradiation was shown in table 1 and 2. Table 1 shows the number of surviving *E. coli* under each condition. When photo was irradiated to the specimen, the number of surviving *E. coli* became less than 10. Aluminum anodic oxide film exhibited bactericidal action to *E. coli.* On the other hands, the number of surviving *S.aureus* became also less than 10 by photo irradiation and bactericidal action to *S.aureus* was observed (Table 2). When the number of initial cells is 1.0×10^6 and 1.0×10^7, however, bactericidal action was not observed. From the fact that bactericidal action happens with the surface of the substrate, it was thought that the number of cells which do not contact the surface increased both increase of the number of initial cells. Furthermore, bactericidal action to *E. coli* and *S.aureus* was not observed without photo irradiation

Table 1 The result of the antimicrobial evaluation due to the presence of photo irradiation (*E.coli*.)

Initial Cells Number	Cells number after 24h/ CFU cm^3			
	Dark condition		Light condition	
	Control	Sample	Control	Sample
1.0×10^4	3.4×10^5	1.7×10^5	1.2×10^5	<10
1.0×10^5	1.4×10^5	1.6×10^5	2.0×10^5	<10
1.0×10^6	8.0×10^6	5.3×10^6	2.2×10^6	<10
1.0×10^7	4.0×10^7	2.2×10^7	2.0×10^7	3.0×10^6

Table 2 The result of the antimicrobial evaluation due to the presence of photo irradiation (*S.aureus*.)

Initial Cells Number	Cells number after 24h/ CFU cm^3			
	Dark condition		Light condition	
	Control	Sample	Control	Sample
1.0×10^4	7.0×10^5	2.2×10^5	2.0×10^5	<10
1.0×10^5	2.6×10^5	2.4×10^5	1.0×10^5	<10
1.0×10^6	4.7×10^6	3.5×10^6	2.0×10^6	200
1.0×10^7	6.2×10^7	3.2×10^7	1.5×10^7	5.0×10^6

Table 3 The result of the antimicrobial evaluation due to the presence of photo irradiation using aluminum anodic oxidation coating impregnated with iodine compound (*E.coli*.)

Initial Cells Number	Cells number after 24h/ CFU cm^3			
	Dark condition		Light condition	
	Control	Sample	Control	Sample
1.0×10^4	3.4×10^5	<10	1.8×10^5	<10
1.0×10^5	2.4×10^5	<10	2.0×10^5	<10
1.0×10^6	3.0×10^6	<10	2.8×10^6	<10
1.0×10^7	3.3×10^7	150	2.5×10^7	100

The result of the antimicrobial evaluation due to the presence of photo irradiation using aluminum anodic oxidation coating impregnated with iodine compound which we already have reported[2)] was shown in table 3. From this result, it found that aluminum anodic oxide film impregnated with iodine compound shows high bactericidal action in spite of photo irradiation. When light exists, bactericidal action happens with hydroxyl radical are generated, when light does not exist, it is thought that bactericidal action happens with the iodine compound.

4. Summary

The hydroxyl radical generated from aluminum anodic oxide film irradiated by UV was evaluated by ESR measurement, and its antimicrobial characteristic was also investigated by photo irradiation film covering method. It was found that hydroxyl radical and super oxide anion radical were generated from aluminum anodic oxide film. Generation of DMPO-OH depended on the presence of light and its concentration linearly increased during photo irradiation. The bactericidal activity to *E.coli* and *S.aureus* of aluminum anodic oxide film was exhibited by free radicals generated from aluminum anodic oxide film by photo irradiation. Further it found that aluminum anodic oxide film impregnated with iodine compound shows high bactericidal action in spite of photo irradiation.

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