Antifungal Characteristics of Some Metal Plating

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The antifungal effect of metal plating should be clearly understood, because it would bring much useful information for such applications such as housing, food processing, etc. We selected *Aspergilius flavus* as a fungus and investigated what metal powders exhibited an antifungal effect against growth. We found that copper, manganese, silver and cobalt powders had significant antifungal properties. In this study, we applied the similar tests plating metals and compared the results. We also considered the mechanism behind the antifungal behavior.

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INTRODUCTION

Recently, the contamination by fungi has been a serious issue in various fields as well as in our housing which may be obstacles for our daily lives. And the similar problems relating to fungi have been reported also in medical fields. They may lead to deterioration of medical instruments. To protect the growth of harmful fungi, it is very important to remove or avoid the environments where those fungi can grow very easily. Many investigations have been carried out so far. However, there are not so many reports for antifungal metals at this point. If we could find some metals having high antifungal properties and apply them to plating or coating on steels, a new prospects for surface finishing of steels would be brightened. Therefore, we investigated and assessed the antifungal properties of many metal powders applicable to plating and compared the results with those of some plating specimens. And we discussed on what the antifungal property of metals is and also how to make the characteristics of plating metals available.

EXPERIMENTAL

Fungus And It's Culture

As fungus, *Aspergilius flavus* IF04186 was used for this study. Potato dextrose agar (PDA) was used as culture. For the growth of the fungus, dissolved agar was divided into some test-tubes to make slant culture possible. Firstly, agar (19.5g) was dissolved into water (500mL) and heated at 121 degrees Celsius (250 degrees Fahrenheit) for 10 minutes in the high pressure autoclave (Tomy SX-500) to solve the agar completely. Secondly, the agar solution was divided into test-tubes by pipette so that each volume was 7mL. Then all of these test-tubes were put in the autoclave again and heated under high pressure at 121 degrees Celsius (250 degrees Fahrenheit) for 10 minutes. The sterilized test-tubes were put aslope at the angle of 15 – 30 degrees and remained at a certain time until the agar solidified completely. The tops of these test tubes were sealed by polymer films called para-film to prevent dehydration. At the beginning stage, the fungus was powdery. Therefore, the spore was abstracted after the sterilized water was added. The spore was daubed onto the slant culture by platinum loop. All of the inoculation process was carried out in a clean bench (Hitachi CCV clean bench). Then the fungus was grown in an incubator (Sanyo, MIR-262) under a certain condition (Temperature: 28±1 degrees Celsius; 82.4 Fahrenheit ±1 degrees Fahrenheit, Relative humidity: 98%) for several days.

Test for Fungal Resistance of metal powders

Usually, the tests for correlations between fungus and materials can be classified into two types. The first one is called "Tests for Fungal Growth", while the second one "Tests for Fungal Resistance". The former doesn't require any nutrition during the test. On the other hand, the latter gives the fungus nutrition. In this study, we selected the latter, i.e. "Tests for Fungal Resistance" for the assessment of fungus against some metal. The test for fungal resistance of

metal powders was carried out in petri dish with duplicatus agar layers. The lower layer was usual agar, while the upper layer had distributed metal powders. Since the metal powder tends to be deposited at the bottom of agar due to it's heavy density, it had to be distributed in the upper layer. The lower agar layer was formed from 15 mL agar solution in the original test-tube, while the upper one from 5mL solution. A certain amount of various metal powders (grain size: pass 75 micrometers; 0.003 inch, powder contents: 10~100mg) such as Fe, Mn, Mg, Ag, Ti, Co, Al, Zn and Cu were kneaded together with the upper agar layer and heated at 121 degrees Celsius (250 degrees Fahrenheit) for 10 minutes in the autoclave. Five platinum-loop amount of *A.flavus* IF04186 which grew on the slant culture of PDA at 30 degrees Celsius for several days was floated in sterilized water of 10mL and suspended there by ultrasound agitator for 30 seconds. The fungus solution of 0.1mL was inoculated into each upper layer of PDA mixed with metal powder. The fungus in these petri dish were cultured at 30 degrees Celsius in the incubator and the growth processes were taken as photos by a digitizing camera (Casio EX-Z3B) so that the resistance behaviors for metals against the fungus could be compared each other.

Test for Fungal Resistance of Plating (Slide Culture Method)

Various plating metals such as copper, zinc and tin plating were cut into tiny sheets (1 x 1cm: 0.4 x 0.4 inch) and put on the square PDA of 2 x 2cm (0.8 x 0.8 inch) where the fungal solution of 50 μL was inoculated in advance. Each square PDA was put on a slide glass and all of these were put on V-shape glass rods in petri dish with sterilized water. The test for fungal resistance of plating metals against *A.flavus* IF04186 was carried out at 28 degrees Celsius (82.4 degrees Fahrenheit) in the incubator. And the dissolution of metal was measured by X-ray Fluorescent Analysis (Horiba MESA-500W).

RESULTS AND DISCUSSION

Test for Fungal Resistance of metal powders

Various metal powders of 10mg, 50mg and 100mg were mixed into PDA and the growth of the fungus was observed. The PDA without metal powder was observed as control and the results were compared with those for PDAs with metal powders. For the control, the generation of fungus was observed on the third day. On the fourth day, a lot of fungus was found and they continued to grow on the fifth day. Fig.1 shows the result for the control on the fifth day.

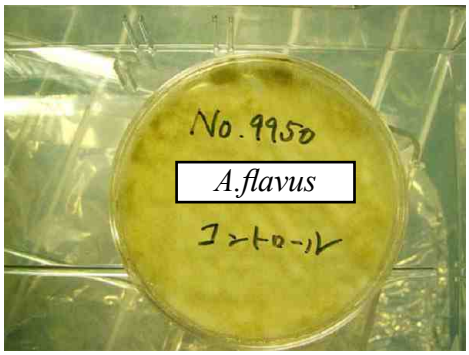

Fig.1 The control's condition with A.flavus on the fifth day.

The similar behaviors were recognized for the PDAs with iron, titanium, zinc, magnesium and aluminum powders. For the PDAs containing these powders, the generation of fungus was observed on the third day like that of control. And it irrupted on the fourth day. These results suggest that these metal powders, iron, titanium, zinc, magnesium and aluminum ones, doesn't show any significant antifungal characteristics within the concentration base range.

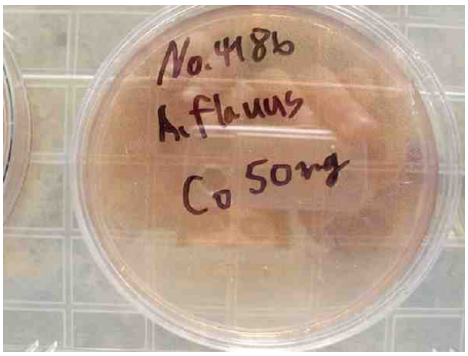

Fig.2 PDA with cobalt powder (50mg) on the fifth day.

For the PDAs containing manganese powder, any difference with the control's results was not observed, when the powder contents were 10 mg and 50mg. Fig.2 shows the results for PDAs containing 50mg cobalt powder, when five days passed after the inoculation. In this case, the generation of A.flavus was not observed at all even on the fifth day (Fig.2), while the control showed the remarkable generation of the fungus on the same day (Fig.1). It indicates that cobalt has very high antifungal characteristics.

Fig.3 shows the results for manganese powder when each PDA contained 50mg. In the case of manganese powder, the growth behavior of *A.falvus* was quite the same with that of the control when the powder contents were 10mg and 50mg. However, the PDA containing 100mg manganese powder didn't show any generation of the fungus. It suggests that manganese also has relatively high antifungal property, even though the extent is slightly weaker than cobalt powder.

When PDA contained manganese powder of 10mg, the behavior was quite the same with that of control. However, the PDA with 50mg manganese didn't show any significant generation, even though the fungus was observed three days

Fig.3 PDA with manganese powder on the fifth day.

after the inoculation. However, the extent of growth was slightly weaker than that of control. When the PDA contained 100mg manganese, the generation of the fungus was not observed at all within 5 days after the inoculation. These results for manganese powder suggest that manganese's antifungal characteristics are weaker than that of cobalt, even though both have high antifungal characteristics generally. The same tendency was observed also for copper powder.

Table 1 summarizes the results for various powders. This table indicates that iron, titanium, magnesium, aluminum and zinc powder didn't show any antifungal properties at all, as already described. On the other hand, cobalt powder showed definite antifungal activity. For manganese and copper, the behavior depended on the powder contents in PDAs. Therefore, the situations are described as exclamation marks in the table.

Table 2 shows the antifungal behaviors for manganese and copper powders. When the contents were 10mg and 50 mg, the behaviors were quite the same with that for the control. However, the PDA didn't show any trails for the generation of the fungus, when the powder content was 100mg.

Table 1 Antifungal activities of various metal powders.

	1st day	2nd day	3rd day	4th day	5th day
Fe, Ti, Mg, Al, Zn	none	none	generation	growth	overgrowth
Co	none	none	none	none	none
Mn, Cu	none	none	!	!	!
Ag	none	none	!	!	!
control	none	none	generation	growth	overgrowth

Table 2 Antifungal activities of manganese and copper powders.

	1st day	2nd day	3rd day	4th day	5th day
10mg	none	none	generation	growth	overgrowth
50mg	none	none	generation	growth	overgrowth
100mg	none	none	none	none	none
control	none	none	generation	growth	overgrowth

Table 3 shows the results for silver powder. In this case, less silver content could prevent the generation of the fungus. It shows the strength of silver's antifungal activity.

These tables suggest that manganese, silver, cobalt and copper have high antifungal characteristics and that the extent of antifungal characteristics increased in this order: silver < copper, manganese < cobalt. On the other hand, it suggests that magnesium, titanium, zinc, aluminum and iron don't show any significant antifungal characteristics at all.

Table 3 Antifungal activities of silver powder.

	1st day	2nd day	3rd day	4th day	5th day
5mg	none	none	generation	growth	overgrowth
10mg	none	none	generation	growth	overgrowth
25mg	none	none	none	none	none
control	none	none	generation	growth	overgrowth

Test for Fungal Resistance of Plating

Based on these results mentioned above, we carried out the tests for fungal resistance of plating by using slide culture technique. The results of observation for the growth behaviors of the fungus, *A.flavus* were shown in Table 4. The specimens are a steel plate (SS400, plate 1), a copper plated steel(plate2), a tin plated steel (plate 3), a tin plated steel bound by a platinum wire(plate4), a tin plate attached by copper plate (plate5) and copper plate bound by a platinum wire (plate6). And the growth of the fungus for each specimen was rated on a scale of A to E. A corresponds to no trails. B means the very small amount of generation for the fungus, while C the small amount of generation. D means the plenty of generation and E corresponds to big-bang overgrowth.

The steel plate was used as control in the series of experiments. Also in this case of plating test, the generation of the fungus was observed on the third day after the inoculation. Unfortunately, we could not find out any appropriate antifungal plating specimens which could prevent the generation of the fungus completely. However, we could get a fix on how various metal plating showed different behaviors for antifungal characteristics. Plate1(JIS SS 400) as control didn't show any generation of the fungus till the third day, when the fungus was generated explosively. Plate 2 (copper plating) also showed the generation of the fungus on the third day. However, the extent of the generation was milder than that of the control. It suggests

that copper could hinder the generation of the fungus to some extent, even though it could not prevent the generation completely. The pure tin plating behaved quite the same with the steel substrate (Plate3).

Table 4 Antifungal activities of various plating specimens.

	1st day	2nd day	3rd day	4th day	5th day
plate1	A	A	E	E	E
plate2	A	A	C	E	E
plate3	A	A	E	E	E
plate4	A	A	B	D	E
plate5	A	A	D	E	E
plate6	A	A	C	D	E

However, it could hamper the growth of the fungus when it was coupled with tin (Plate 4) or a copper plated specimen (Plate 5). These results suggest that the dissolution of tin and it's acceleration by the formation of galvanic cells was a key factor for the prevention of the fungal growth. The same phenomenon was observed also for copper plated specimens, even though the extent was not so remarkable. The silver plate as reference also showed the slight prevention of the fungal growth.

Even though we could not observe the complete prevention against the growth of the fungus, we could fix how various metal plating inhibited it. The mechanism of the retardation can be attributed to the two key factors as follows - The dissolution of metals into ions in the environment and their interactions with the fungus. Since the powders tend to dissolute very easily, it could show the complete prevention against the fungal growth. On the other hand, the practical plated specimens are difficult to dissolve in to PDAs due to their dimensional characteristics. It is very important for us to take their intrinsic antifungal characteristics and their practical dissolution as ions into account at the same time, when we devise antifungal plating in the future.

CONCLUSIONS

Antifungal activities of various metal powders applicable to plating were investigated. Then some of those plating specimens were also investigated for their antifungal properties. *A.flavus* was chosen as the fungus in this study.

(1) Magnesium, titanium, zinc, aluminum and iron powders did not show any significant antifungal characteristics at all.
(2) Manganese, silver, cobalt and copper powders had high antifungal characteristics and that the extent of antifungal characteristics increased in this order: silver < copper, manganese < cobalt.
(3) Tin plated specimen didn't show any significant antifungal activity. However, the galvanic couple with noble metals increased the antifungal properties, even though it didn't lead to any remarkable inhibition against the fungus.
(4) The increase by the galvanic couple suggests that the dissolution of metal into the ion state is critical to the appearance of metal's antifungal characteristics.

