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Developing a Testing Method for Antimicrobial Efficacy on TiO_2 Photocatalytic Products

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Abstract

TiO_2 photocatalyst has been known to exhibit a notable disinfecting activity against a broad spectrum of microorganisms. A lot of commercial TiO_2 photocatalyst products have been developed for antimicrobial purposes. However, a standard method has not yet been proposed for use in testing antimicrobial activity. In this study, we developed a TiO_2 photocatalytic adhesion test method with film as the standard testing method for the evaluation of antimicrobial activity. This method was devised by modifying the previous antimicrobial products test method, which has been widely used, and considering the characteristics of TiO_2 photocatalytic reaction. The apparatus for testing the antimicrobial activity was composed of a Black Light Blue (BLB) lamp as UV-A light source, a Petri dish as the cover material, and a polypropylene film as the adhesion film. The standard TiO_2 photocatalyst sample, Degussa P25 TiO_2 - coated glass, could only be used once. The optimal initial concentration of the microorganism, proper light intensity, and light irradiation time were determined to be 10^6 CFU/mL, 1.0 mW/cm^2, and 3 hr, respectively, for testing and evaluating antimicrobial activity on the TiO_2 surface.

Keywords: TiO_2, Photocatalyst, Standardization, Antimicrobial efficacy, Adhesion film

1. Introduction

Numerous studies have utilized and applied the strong oxidizing power of TiO_2 photocatalysts in environmental systems such as air purification, water disinfection and hazardous waste remediation.[1-3] Since the photochemical sterilization of *Escherichia coli* (*E. coli*) using Pt-TiO_2 was first reported by Matsunaga et al. (1985),[4] TiO_2 photocatalysts have also been utilized to disinfect a broad spectrum of microorganisms.[5-9] In an effort to commercialize TiO_2 photocatalysts, many different types of TiO_2-coated materials, such as paper, thin film and glass that exhibit great antimicrobial activities, have been prepared and evaluated.[9-12] In addition, TiO_2 films deposited with antimicrobial metals, such as copper and silver, have been developed to obtain improved antimicrobial activity under mild conditions such as weak light intensity.[13,14]

Although a wealth of TiO_2 photocatalyst coated products containing antimicrobial activity have already been developed and commercialized, there has been no standard method with good experimental rationale suggested for testing antimicrobial activity.[15] The concept of not developing standard methods for testing the antimicrobial activity of TiO_2 photocatalyst was initially suggested by organizations such as the American Society for Testing and Method (ASTM), Japanese Industrial Standard (JIS), and even International Organization for Standardization (ISO), which caused the unnecessary confusion for not only the consumers but also business developers. Thus, the primary goal of this study is to develop a standard testing method for antimicrobial efficacy on TiO_2 photocatalytic products. To achieve this objective, the given standard method for evaluating the antimicrobial efficacy of various products was considered based on the characteristics of TiO_2 photocatalytic reaction.[16] Black Light Blue (BLB) lamp was utilized as UV-A light source and the optimum experimental conditions for initial dosage of microorganism, light intensity and UV irradiation time were determined using a standard testing method with *E. coli*, a well-known indicator microorganism.

2. Materials and Methods

2.1. Experimental Apparatus

A simple scheme of the test method examined in this study was depicted in Fig. 1. The Black Light Blue (BLB) lamp (4 W,

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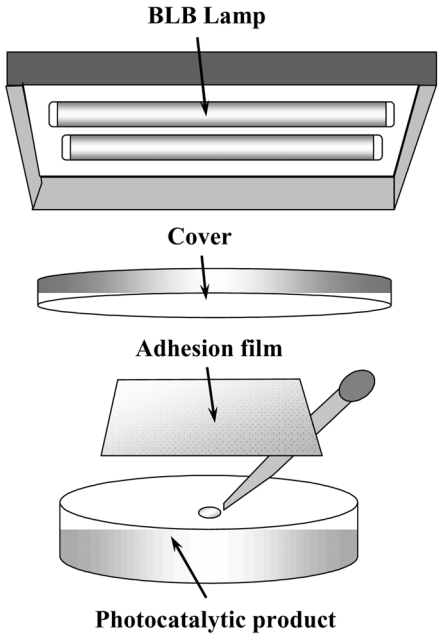


Fig. 1. A schematic of the photocatalytic TiO_2 adhesion test method with film.

Sankyo Denki Co., Japan), which emits wavelengths in the range of 300 to 400 nm, was used for light irradiation and positioned above the cover of the photocatalytic product. The light intensity was adjusted by varying the distance between the BLB lamps and the surface of the product. The light intensity from the BLB lamp on the surface of photocatalytic product was measured by a UV radiometer (UVP. Co., USA). The transmittances of the various covers and adhesion films were analyzed with an UV-vis spectrophotometer (Agilent 8453, Agilent Technologies, USA).

2.2. Preparation of Materials and Photocatalytic Products

All solutions and reagents were prepared with distilled and deionized water (Barnstead NANO Pure, USA) and analytical-grade chemicals were used without further purification (Sigma-Aldrich Co., USA). All glassware was washed with distilled water and autoclaved at 121°C for 15 min prior to use. For testing antimicrobial activity in the standard TiO_2 film samples, the commercial Degussa P25 TiO_2 (P25) powder was used as a photocatalyst. The substrate glasses (50 mm × 50 mm) were coated with a P25 film following the procedures described in previous studies.[17,18] One gram of P25 powder was vigorously mixed with 10 g of 50% carbowax binder (polyethyleneglycol) aqueous solution. The mixed TiO_2 paste was deposited onto the substrate glass with two tracks of one layer of Scotch Magic Tape having 20 μm of thickness and dried under air for 30 min. Then the TiO_2-coated glass plates were heated at 450°C for 30 min to burn off the organic binder. The commercial samples obtained from the Korean Agency for Technology and Standards, were cut into squares of 50 mm × 50 mm and assessed after sterilizing the surface of the product with cotton soaked with 70% ethanol.

2.3. Culture and Analysis of Bacteria

For all experiments conducted in this study, *E. coli* was employed as the indicator microorganism for pathogenic bacteria. *E. coli* (ATCC strain 8739) was inoculated in 50 mL of Tryptic Soy Broth (Difco Co., Detroit, Mich.) medium in a 200 mL flask and grown for 18 hr at 37°C. The bacteria were harvested by centrifugation at 1000 × g for 10 min and washed twice with 50 mL of phosphate buffered saline (PBS, 150 mM, pH 7.1). The *E. coli* stock suspension was prepared by resuspending the final pellets in 50 mL of phosphate buffered saline solution. The initial populations of *E. coli* ranged from approximately 1×10^6 ~ 1×10^8 CFU/mL after diluting the stock suspension. The concentrations of cells was determined by the spread plate method, in which the cells are plated on nutrient agar, incubated at 37°C for 24 hr, and the number of viable colonies are counted.[19,20]

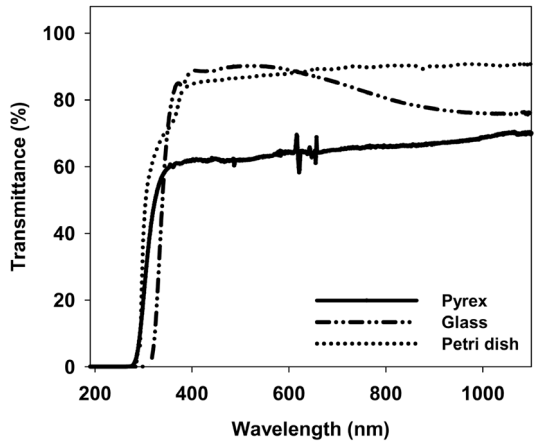
2.4. Experimental Procedures

The TiO_2 - coated glass plate was placed on a Petri dish, and then 0.5 mL of the *E. coli* suspension was poured onto it. The adhesion film was put on the suspension to facilitate the attachment of microbial cells to the TiO_2 surface and the cover was used to maintain the humidity at more than 90% (Fig. 1). The adhesion film was carefully pressed on the glass to prevent the *E. coli* suspension from spilling over the edge of the glass. After illuminating this system with BLB lamps for a predetermined time, 4.5 mL of phosphate buffered saline solution was poured into the Petri dish containing the TiO_2 - coated glass plate and adhesion film. The lamps were stabilized for approximately 30 min prior to sample illumination. The temperature of the reaction plate was held at 25°C throughout the experiment with a cooling fan. Microbial cell were detached from the photocatalytic glass plate by pipetting. This was followed by removing 1 mL of the sample. 0.1 mL of solution was withdrawn from the sample and was diluted to 1/1, 1/10, and 1/100. To count the number of viable cells from each diluted solution, triplicate plates were used. Selected experiments were repeated three times and the average value and statistical deviation were shown in the figures.

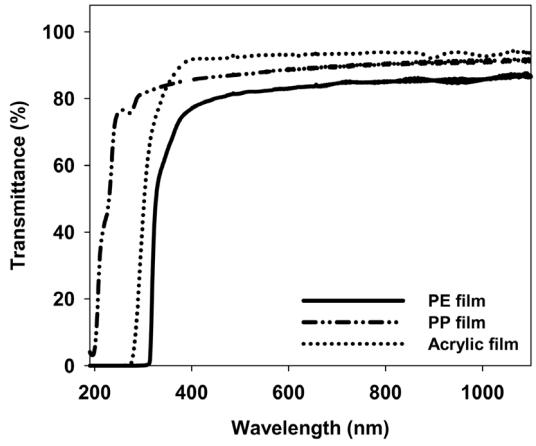
3. Results and Discussion

3.1. Light Transmittance Test of Covers and Films

Three types of covers (Pyrex, glass, and Petri dish) and three types of adhesion films [polyethylene (PE), polypropylene (PP), and acrylic] were considered as candidate materials in selecting the appropriate cover and adhesion film for TiO_2 the photocatalytic adhesion test method. Fig. 2 shows the transmittance profile of three cover materials and adhesion films, in the range of 200 to 1100 nm. As shown in Fig. 2(a), all the cover materials transmitted light above 300 nm, but a different behavior in the profile was observed according to the materials used. The Petri dish was consistently transparent with a transmittance higher than 80% in a range of 300 nm ~ 1100 nm, while the transmittance of glass gradually decreased above 600 nm. The Pyrex



(a)



(b)

Fig. 2. The transmittances of (a) various covers and (b) adhesion films.

exhibited the lowest transmittance among the samples measured. These results suggest that the Petri dish is the most suitable cover material because it had the smallest loss of light intensity.

While all film materials exhibited a similar level of transmittance (as high as 80% above 300 nm) the PP film also transmitted light below 300 nm (Fig. 2(b)). The high transmittance as well as the low price suggests that the PP film is the most appropriate adhesion film.

3.2. Effect of Initial Dosage of Microorganism

Since it is known that the initial concentration affects the inactivation of microorganism we optimized the initial dosage of the microorganism.[21] Fig. 3 shows the comparison of *E. coli* inactivation for 3 hr as initial concentration of *E. coli* varied from 10^6 to 10^8 CFU/mL. The level of *E. coli* inactivation depicted by the difference between the filled bar (0 hr) and the empty bar (3 hr) was largest at 10^6 CFU/mL. The initial dosage below 10^5 CFU/mL was not considered in this study because of difficulty in assessing the inactivation larger than 3 log. The source of this difficulty is related to the two stage dilutions required for analysis of the microorganism (refer to Materials and Methods). Consequently, it was concluded that 10^6 CFU/mL is an appropriate initial dosage of the microorganism because the larger

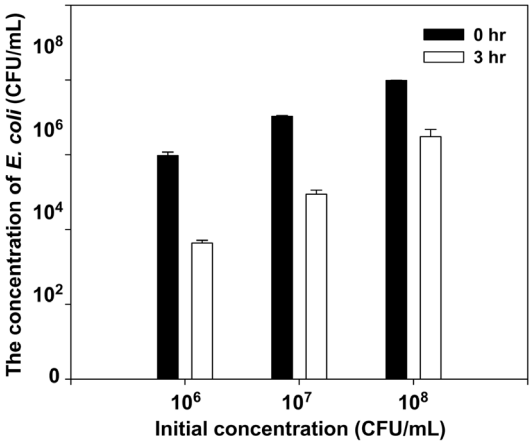


Fig. 3. The effect of the initial concentration of *E. coli* on its inactivation in the photocatalytic TiO_2 adhesion test method.

inactivation makes it easy to precisely estimate the photocatalytic activity of TiO_2.

3.3. Effect of Coating Times of P25-coated Glass

In this system, the P25-coated glass was used as a standard sample to evaluate the antimicrobial activity of TiO_2 with that of commercial products. Since the quantity of P25 could contribute to the efficacy of inactivation, the effect of up to 3 coatings on glass with P25 particles was determined by comparing the efficacy of *E. coli*. As shown in Fig. 4, the number of coatings on glass with P25 particles made no difference on the antimicrobial activity. Therefore, all experiments conducted in this study used one coating of P25 on glass.

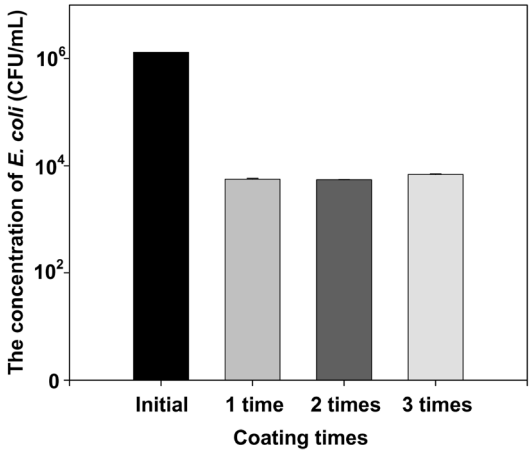


Fig. 4. The effect of the number of P25-coated glass coatings on *E. coli* inactivation in the photocatalytic TiO_2 adhesion test method (t = 3 hr).

3.4. Effect of Light Intensity and Irradiation Times

The effects of light intensity and irradiation time on the inactivation of *E. coli* were also evaluated as one of experimental parameters in assessing the antimicrobial activity of photocatalytic TiO_2 samples. Fig. 5 shows the *E. coli* inactivation as the light intensity and the irradiation time varied from 0.13 to 1.0

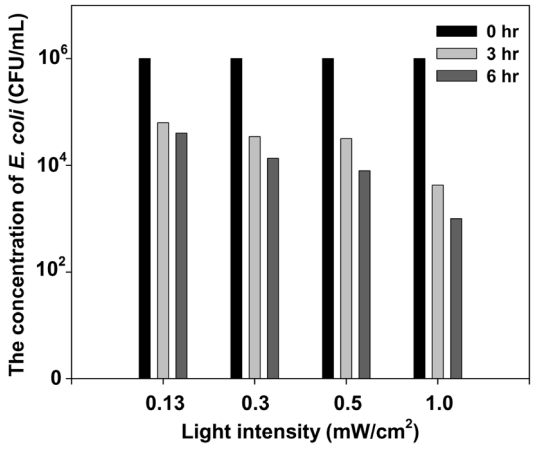


Fig. 5. The effect of UV-A light intensity and irradiation time on *E. coli* inactivation in the photocatalytic TiO_2 adhesion test method ($[E. coli]_0 = 10^6$ CFU/mL).

mW/cm^2 and from zero to 6 hr, respectively. From these experiments it was quite obvious that larger light intensities increased *E. coli* inactivation (Fig. 5). In terms of irradiation time, the first 3 hr achieved much larger inactivation than the subsequent 3 hrs. As a result, the optimal light intensity and irradiation time for the antimicrobial activity of TiO_2 sample were determined to be 1.0 mW/cm^2 and 3 hr, respectively.

3.5. The Application of a TiO_2 Photocatalytic Adhesion Test Method with Film

From the results of Fig. 2 ~ 5, the optimal parameters for testing the antimicrobial activity of photocatalytic TiO_2 samples are as follows: (a) Petri dish as cover material and PP film as adhesion film, (b) an initial dosage of microorganism of 10^6 CFU/mL, (c) a light intensity of 1.0 mW/cm^2, and (d) a rradiation time of 3 hrs. The antimicrobial efficacy under these conditions were assessed with various commercial TiO_2 photocatalytic samples, TiO_2-coated glass, paper, and plastic plates. The equation for determining the antimicrobial efficacy of diverse TiO_2 photocatalytic samples was as follows: (1).[22)]

$$I_r(\%) = \frac{M_a - M_b}{M_a} \times 100 \quad (1)$$

where, I_r : Inactivation ratio

M_a : The initial concentration of microorganism

M_b : The concentration of microorganism on the test sample after 3 hr in photo conditions

Table 1. The antimicrobial efficacy of various commercial TiO_2 photocatalytic samples determined by the photocatalytic TiO_2 adhesion test method with film ($[E. coli]_0 = 10^6$ CFU/mL, t = 3 hr)

Products	I_r (Inactivation ratio)
A (glass)	80%
B (glass)	99.5%
C (paper)	99.8%
D (plastic)	60%

Table 1 shows the diverse levels of antimicrobial activity of commercial TiO_2 samples as determined by equation (1). These results reflect the level of applicability of this method for testing commercial TiO_2 samples. It is worth noting that B (glass) and C (paper) samples coated with TiO_2 showed much better antimicrobial activity in contrast with A (glass) and D (plastic) samples.

4. Conclusions

The conclusions of this study can be summarized as follows:

1. This study suggests for the first time that the TiO_2 photocatalytic adhesion test method with film developed in this study can successfully evaluate the antimicrobial activity of various products coated with TiO_2 when the optimal experimental parameters are used.
2. Petri dish and polypropylene film were determined to be the optimal cover material and adhesion film, respectively for this method.
3. The optimal initial concentration of microorganism, light intensity and light irradiation time for the photocatalytic reaction were 10^6 CFU/mL, 1.0 mW/cm^2, 3 hr, respectively.

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