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Combination of Light Emitting Diode at 375 nm and Photo-reactive TiO_2 Layer Prepared by Electrostatic Spraying for Sterilization

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Abstract – The objective of this work was to increase the efficiency of ultraviolet-light emitting diodes at 375 nm for sterilization. Since TiO_2 had antibacterial properties, which were attributed to the appearance of hydroxyl radicals and superoxide radical anions on the surface species under ultra violet radiation at about 387 nm, photo-reactive layers such as Ag-doped TiO_2 were coated on aluminum substrates by electrostatic spraying. Crystallinity and surface morphology of the coating layer were examined by X-ray diffraction θ-2θ scan and field emission-scanning electron microscope, respectively. In an antibacterial test, we observed above 99% reduction of *Escherichia coli* populations on 3 or 5 mol% Ag-doped TiO_2 layers after irradiation for 2 hrs at 375 nm, while very low inactivation on bare aluminum substrates occurred after irradiation as the same condition.

Keywords: Ag-doped TiO_2, Sterilization, Light emitting diode, Electrostatic spraying

1. Introduction

Indoor air quality, sick building syndrome and control of infection in healthcare facilities have become major public health concerns over the last decade. Air conditioning, which was once a luxury, is now a common feature of most motor vehicles. According to recent research [1], however, the inside of car can contain up to 10-times more pollutants and contaminants than the air in an equal volume of space outside of the car. Manufacturers have worked to overcome this in recent years with improvements in cabin air filters and air ducting. The AC evaporator becomes wet as humid air is passed over its cool surface. This wet environment is a good place for mold and mildew to grow and for dust and pollen to collect, finding their way back into the air system. The smell was caused by fungus, bacteria and other microbes growing on the evaporator case of the air conditioner.

Ultraviolet (UV) disinfection is well-developed sterilization way that has been used in water and air in many countries. Sterilization by UV is friendlier to the environment and inactivates bacteria and viruses [2-4]. For many UV applications, however, there are sustainability issues that arise from current low-pressure lamp in use. Disadvantages of mercury lamps such as mercury waste products, short life span of lamp and lost energy in the form of heat have led researchers to seek a new environmental technology.

Light emitting diodes (LEDs) are much smaller and much lighter than the low pressure mercury lamp and do not contain glass and mercury. Limited research has been performed on the effectiveness of UVA-LED. Most of the data available are for LEDs that emit light of UVC or UVB ranges (200~300 nm), which is more efficient at sterilization than light in the range of UVA (320 ~ 400 nm) [5-6]. In recent, UVA-LEDs are commercialized and verified, while the UVC or UVB-LEDs still need to improve its reliability and to reduce the cost.

The objective of this work was to evaluate the efficiency of UVA-LEDs for sterilization. Specifically, this work evaluated the use of UV-LEDs at 375 nm, for inactivation of *Escherichia coli* (*E. coli*). Furthermore, since TiO_2 had antibacterial properties, which were attributed to the appearance of hydroxyl radicals and superoxide radical anions on the surface species under UV radiation at about 387 nm [7, 8], photo-reactive layers such as Ag-doped TiO_2 were coated on aluminum (Al) substrates.

The number of bacteria which remained after irradiation under UV radiation was counted. To evaluate the sterilization effect by UVA-LEDs, the number of bacteria after irradiating was divided by the number of before irradiation.

2. Experimental

To evaluate bacterial activity, experimental device which contained high power UVA-LEDs at 375 nm (Seoul Optodevice, Korea) was designed as shown in Fig. 1 (a). A UV light source panel was designed with an aluminum base copper clad laminate because of its excellent dissipation, electromagnetic shielding characteristics, dielectric properties and mechanical working properties. Module was designed with an array of four LEDs using a circuit wire-wrapped.

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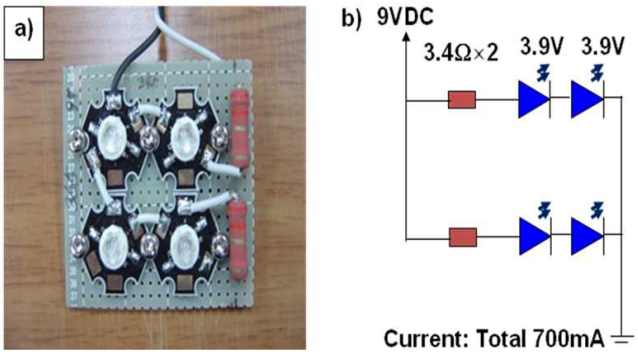

Fig. 1. LED module (a) and circuit diagram of the LED module (b) used in this work.

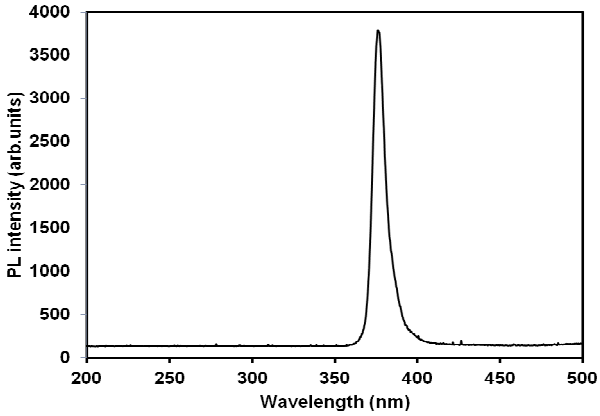

Fig. 2. Emission spectrum for the LED used in this work.

3.4 Ω resistors were wired in series with LEDs to create 3.9 V across LED at 700 mA with a 9 V input voltage from a power supply, as shown in Figs. 1 (b). Emission spectrum for the LED light source was measured with a fiber coupled luminescence meter (Fiber Optics, KMAC, Korea), as shown in Fig. 2.

E. coli strain (DH5α) was purchased from Korean Culture Center of Microorganism (KCCM 11234) for disinfecting index [9]. *E. coli* is used extensively as an index of contamination levels of water. *E. coli* cells cultured overnight in Luria-Bertani agar (LB) plate were collected by centrifugation for 3 min at 10,000 rpm, washed twice with phosphate buffered saline (PBS, pH 7.4), and adjusted to an optical density (O. D.) of 1.0 at 600 nm for a bacterial concentration of 2×10^8 colony forming units (CFUs) per mL for batch irradiation testing. Then 600 μL of saline solution with bacteria was added dropwise onto the surface of each Ag-doped TiO_2/Al substrates. After irradiation at 375 nm for various times, the specimens were washed using 10 mL buffer solution which was subsequently spread onto a nutrient agar plate. After spreading, all Petri dishes were incubated overnight at 37°C, and the number of colonies were counted and averaged. All tests were completed within 2 hours. The distance between LED modules and bacterial suspension was 20 mm.

Parameters	Optimal condition
Applied Voltage	25 kV
Flow rate	0.3 mL/h
Heating Temperature	80°C
Distance between nozzle and substrate	20cm

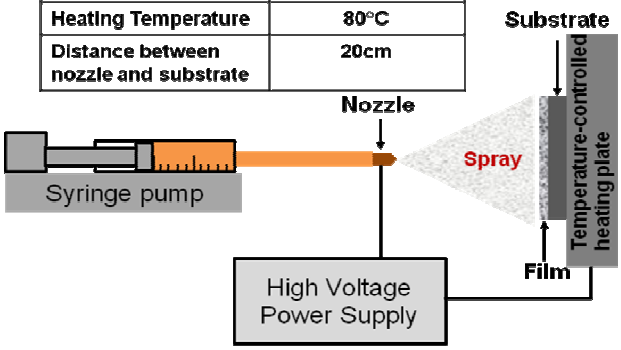

Fig. 3. Schematic diagram of ESD setup and list of the coating parameters.

The *E. coli* reduction rate according to colony-forming ability assay is calculated by the equation, as below,

$$\boldsymbol{R}\ (\%) = [(N\text{-}N_0) / N] \times 100 \qquad (1)$$

Where, $\boldsymbol{R}$, N and N_0 are *E. coli* reduction rate, number of bacteria colonies before irradiation and number of bacteria colonies after irradiation, respectively.

To increase antibacterial efficiency at 370~400 nm, photoreactive Ag-doped TiO_2 films were coated on Al by electrostatic spraying deposition (ESD). Titanium tri-chloride and silver nitrate were used as reactants for the synthesis of Ag-doped TiO_2 solution (Doping mol% = 1, 3 and 5). The sol was diluted with methanol to adjust the viscosity for preparing films by ESD.

The ESD offers many advantages over some conventional deposition method, such as simple and low cost set-up, high deposition efficiency, low temperature synthesis, and easy control of the composition and surface morphology of the deposited coatings. The working principles of the ESD have been described in the literature [9]. The ESD set-up consists of three parts; an electrostatic spray unit, a liquid-precursor feed unit, and a temperature control unit. The electrostatic spray unit comprises a high DC voltage power supply (SHV120-30K-RD, Convertech Co., Ltd., Korea), a stainless steel needle (0.1 mm inner diameter), and a grounded and a heated substrate holder. The liquid precursor feed unit consists of a flexible silicon tube and a syringe pump (KD 200, KD Scientific Inc., U. S. A.). The temperature unit for the coating temperature includes a heating element and a temperature controller. To obtain stable con-jet mode of electrostatic atomization, the high voltage, 25 kV, was applied between the needle tip and ground electrode. Al substrates on the ground electrode were heated at 80°C for 30 min to vaporize organic compound. A transparent precursor solution was pumped for 30 min (flow rate: 0.3 mL / 60 min) through the nozzle

which was placed 20 cm above the substrates. The as-deposited film was prefired at 500°C for 10 min in air. Then the final heat treatment was performed in air at 500°C for 60 min. Fig. 3 shows a schematic diagram of the ESD system used and collects the ideal parameters to obtain dense films.

3. Results and Discussion

The surface morphology of the Al substrate and as-prepared 3 mol% Ag-doped TiO_2/Al was examined by field emission - scanning electron microscope (FE-SEM). Fig. 4 (a) shows the FE-SEM photograph of the Al substrates used in this work. Well-grown 50 nm-sized crystallites were obtained. 3 mol% Ag-doped TiO_2 films present smooth and dense surface, no visible pores and defects over the films, as shown in Fig. 4 (b). The fractured cross-section of the 3 mol% Ag-doped TiO_2 layer with approximately 1 μm-thickness appears dense and uniform, not shown here.

To evaluate crystallinity of the 3 mol% Ag-doped TiO_2 films coated on Al substrates, X-ray diffraction (XRD) was performed. Fig. 5 shows the X-ray pattern in 2θ from 20° to 50° of the films after annealing. The diffraction peaks are observed at 2θ = 25.3°, 37.9° and 48.1° being assigned to (101), (004) and (200) reflections. A good match of peaks from the TiO_2 spectra with a library spectrum of anatase TiO_2 was published in JCPDS File 78-2486. It further confirms that TiO_2 films are composed by an anatase crystalline structure without a significant amount of any other TiO_2 phase, such as rutile and pyrochlore. Furthermore, there are no precipitation peaks owing to the metallic silver. From the diffraction patterns, the anatase TiO_2 are in the form of small crystalline, since the XRD peaks are broad and weak. As clearly shown in Figs. 4 (b) and 5, the 3 mol% Ag-doped TiO_2 layer with nano-sized crystals will be responsible for its photo-catalytic ability.

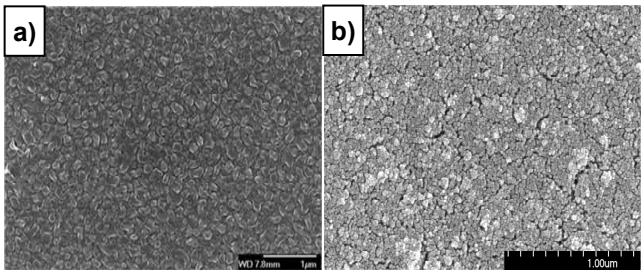

Fig. 4. FE-SEM images of the bare-Al substrates (a) and 3 mol% Ag-doped TiO_2 films on Al substrates (b).

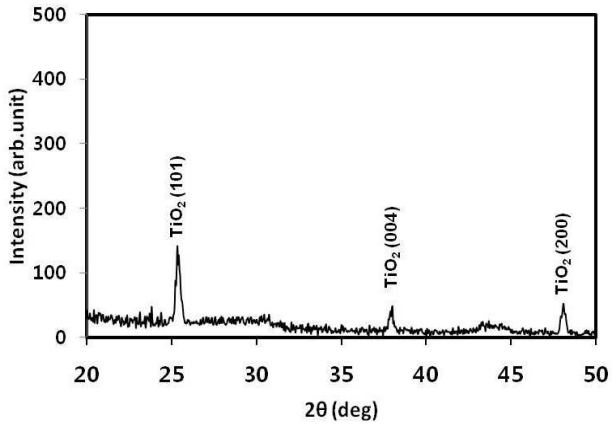

Fig. 5. XRD patterns of 3 mol% Ag-doped TiO_2 films on Al substrates.

The antibacterial performances of the specimens were evaluated using the Gram-negative bacterium *E. coli* at 375 nm. The effects of irradiation time on the inactivation of *E. coli* were evaluated as one of experimental parameters in assessing the antimicrobial activity. Fig. 6 shows reduction rate of *E. coli* on bare Al substrate and Ag-doped TiO_2 thin films on Al substrates as a function of irradiation time. As shown in Fig. 6, the reduction rate of *E. coli* on bare Al substrates and 1 mol% Ag doped TiO_2 layer at 375 nm-radiation was 7% and 59%, respectively, after being exposed for 2 hrs. However, the reduction rate of *E. coli* on photo-reactive 3 or 5 mol% Ag-doped TiO_2 coatings was 99% after being exposed for 2 hrs at 375 nm. The photographs of *E. coli* bacteria colony growth on agar plates with different surfaces clearly indicate that after irradiation for 2 hrs at 375 nm on 3 or 5 mol% Ag-doped TiO_2/Al substrate, a 99% inhibition of bacterial growth was achieved, as shown in Fig. 7.

The semiconductor photo-catalyst is excited by the appropriate electromagnetic wave to produce electron and hole pairs. The electron combines with O_2 to produce superoxide free radicals, and the hole captures the electron from H_2O in air to generate hydroxyl free radicals. Both free radicals have powerful bonding ability with bacteria and fungi. Therefore, bacteria and fungi will be suppressed with DNA damaged by superoxide and hydroxyl free radicals [11]. The oxidizing activity of hydroxyl free radicals

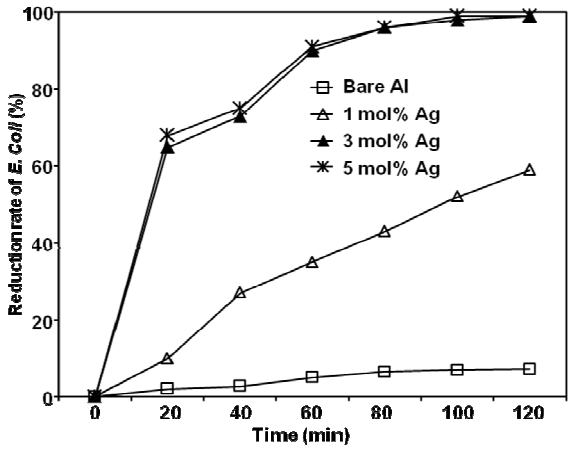

Fig. 6. Reduction rate of *E. coli* on bare Al and Ag-doped TiO_2/Al as a function of irradiation time.

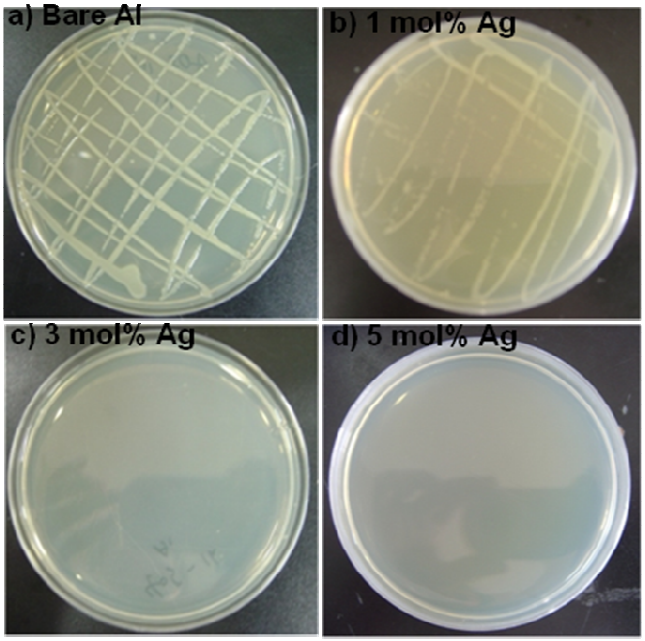

Fig. 7. Photographs of *E. coli* colony growth after irradiation for 2 hrs at 375 nm as the function of Ag doping mol%.

can decompose the cell wall and the cell membrane of *E. coli* attached on TiO_2 thin films. The leakage of intracellular molecules will result in a change in the cell viability. But the percent of UV light from natural light is only 5 % which results in the lower antibacterial activity. To effectively detoxify noxious organic pollutants the semiconductor photo-catalyst generally requires UV light as the excitant source; the reason is that UV energy is greater than the band gap of the semiconductor, and it will deduce the electron hole pairs generated when the semiconductor is illuminated by UV.

M. D. Blake et al reported [12] direct evidence of cell membrane damage by the irradiation of a thin transparent TiO_2 film to examine the photo-catalytic degradation of endotoxin from *E. coli*. The endotoxin is a component of the outer membrane of Gram-negative bacteria and is released only when the cellular structure is destroyed. The result indicated that the TiO_2 photo-catalyst destroys the outer membrane of the *E. coli* cell and causes the death of the bacteria. Recently, the mechanism of cell killing of *E. coli* on TiO_2 thin films has been investigated by atomic force microscope (AFM) [13]. The damage process of the cell wall and the cell membrane was observed by AFM imaging. Also, the permeability of the cell membrane was examined by K^+ leakage from *E. coli*. Results showed that the intracellular K^+ leaked out from *E. coli* very quickly after TiO_2 thin film was irradiated by UV. The researchers believe the cell death was caused by the decomposition of the cell wall first, and then subsequent decomposition of cell membrane [14]. Damage of the cell membrane directly leads to leakage of minerals, proteins, and genetic materials, causing cell death.

In fact, *E. coli* cells can completely disappear on TiO_2 after about one week under a UV irradiation of 1 mW/cm^2. The cell deactivation can be achieved in a much shorter irradiation time, but still it takes nearly 1 h under outdoor UV light intensity [14]. In contrast, the typical indoor UV light intensity is about several hundred nW/cm^2, which is almost about three orders of magnitude weaker than the outdoor one. Therefore, the photo-catalytic deactivation of microorganism requires a much longer time under indoor conditions than under outdoor ones; thus, it cannot become a real practical technology. However, the anti-bacterial function of a TiO_2 photo-catalyst is markedly enhanced even with weak UV light, using a fluorescent lamp and the aid of either silver or copper, which is harmless to the human body. Antibacterial effects of transition metal silver ions have been well known in previous work [15]. It is generally believed that heavy metals react with proteins by combining the –SH groups of enzymes, which leads to the inactivation of the proteins.

UVA-LEDs are less efficient at disinfection than light in the germicidal UVC-LEDs since light in the UVA range is poorly absorbed by DNA [2]. UVA radiation inactivates microorganisms by damaging proteins and producing hydroxyl and oxygen radials that can destroy cell membranes and other cellular components. This process takes much more time than the damage produced by UVC, which directly affects the DNA of microorganisms by producing cyclobutane thymine dimmers, among other products, inactivating them without intermediate steps. [16].

Many studies have utilized and applied the strong oxidizing power of TiO_2 photo-catalysts in environmental systems such as purification, water disinfection and hazardous waste remediation [17, 18]. In an effort to commercialize TiO_2 photo-catalysts, 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 [17, 18]. Although a wealth of TiO_2 photo-catalyst coated products containing antimicrobial activity have already been developed and commercialized, as far as we know, there have been a few report suggested for automobile air conditioning system as a combination of an Ag-doped TiO_2 coating layer on Al evaporator and UVA-LEDs.

4. Conclusions

This work suggests that the Ag-doped TiO_2 films on Al substrates for an automobile evaporator can successfully evaluate the antimicrobial activity of UVA-LEDs at 375 nm. Well-grown nano-sized crystallites and homogeneous surface were confirmed by FE-SEM. XRD revealed that films were composed by an anatase structure without a significant amount of any other TiO_2 phase. The antibacterial performance rate of 3 or 5 mol% Ag-doped TiO_2 films/Al

at 375 nm was much higher than that of the specimen without photoreactive coating. A sterilization system integrating Ag-doped TiO_2 thin film with UVA-LED has the potential for air-purification in automobile and would find wide use in the field of antibacterial.

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