

## Visible-Light-Induced Bactericidal Activity of a Nitrogen-Doped Titanium Photocatalyst against Human Pathogens

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**The antibacterial activity of photocatalytic titanium dioxide (TiO<sub>2</sub>) substrates is induced primarily by UV light irradiation. Recently, nitrogen- and carbon-doped TiO<sub>2</sub> substrates were shown to exhibit photocatalytic activities under visible-light illumination. Their antibacterial activity, however, remains to be quantified. In this study, we demonstrated that nitrogen-doped TiO<sub>2</sub> substrates have superior visible-light-induced bactericidal activity against *Escherichia coli* compared to pure TiO<sub>2</sub> and carbon-doped TiO<sub>2</sub> substrates. We also found that protein- and light-absorbing contaminants partially reduce the bactericidal activity of nitrogen-doped TiO<sub>2</sub> substrates due to their light-shielding effects. In the pathogen-killing experiment, a significantly higher proportion of all tested pathogens, including *Shigella flexneri*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Acinetobacter baumannii*, were killed by visible-light-illuminated nitrogen-doped TiO<sub>2</sub> substrates than by pure TiO<sub>2</sub> substrates. These findings suggest that nitrogen-doped TiO<sub>2</sub> has potential application in the development of alternative disinfectants for environmental and medical usages.**

Disinfectants are antimicrobial agents that are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. They are essential for infection control and aid in the prevention of nosocomial infections (18). Compared to antibiotics, which provide comparatively selective activity against microorganisms, disinfectants typically have a broader biocidal spectrum (28) and are usually used with inanimate objects (33). A wide variety of active chemical agents exhibit bactericidal activities. Some of the most widely used, including alcohols, iodine, and chlorine, have been employed for a long time in disinfection and preservation (28). Compared to these widely used disinfectants, applications of photocatalyst-based antimicrobial disinfectant technologies are still in the developmental stage. Photocatalytic titanium dioxide (TiO<sub>2</sub>) substrates have been shown to eliminate organic compounds and to function as disinfectants (26). Upon UV light excitation, the photon energy excites valence electrons and generates pairs of electrons and holes (electron vacancy in valence band) that diffuse and become trapped on or near the TiO<sub>2</sub> surface. These excited electrons and holes have strong reducing and oxidizing activities and react with atmospheric water and oxygen to yield active oxygen species, such as hydroxyl radicals ( $\cdot\text{OH}$ ) and superoxide anions (O<sub>2</sub><sup>-</sup>) (12). Electron holes,  $\cdot\text{OH}$ , and O<sub>2</sub><sup>-</sup> are extremely reactive upon contact with organic compounds. Complete oxidation of organic compounds and *Escherichia coli* cells to

carbon dioxide could be achieved (16, 23). Reactive oxygen species (ROS), such as  $\cdot\text{OH}$ , O<sub>2</sub><sup>-</sup>, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) generated on irradiated TiO<sub>2</sub> surfaces, have been shown to operate in concert to attack polyunsaturated phospholipids in bacteria (26). In addition, it has been shown that photoirradiated TiO<sub>2</sub> catalyzed site-specific DNA damage via generation of H<sub>2</sub>O<sub>2</sub> (14). These findings suggested that TiO<sub>2</sub> might exert antimicrobial effects similar to those of the peroxide disinfectant H<sub>2</sub>O<sub>2</sub> (28). The oxidation of bacterial cell components, such as lipids and DNA, might therefore result in subsequent cell death (26).

Due to the widespread use of antibiotics and the emergence of more-resistant and -virulent strains of microorganisms (1, 32, 33), there is an urgent need to develop alternative sterilization technologies. The TiO<sub>2</sub> photocatalytic process is a conceptually feasible technology. The TiO<sub>2</sub> photocatalyst, however, is effective only upon irradiation by UV light at levels that would induce serious damage to human cells. This greatly restricts the potential applications of TiO<sub>2</sub> substrates for use in our living environments. Recently, the anion-doped anatase TiO<sub>2</sub>-based photocatalysts were identified, which work by irradiation with visible light (3, 15), offering the potential to overcome this problem. We previously developed several vapor deposition methods to prepare visible-light photocatalysts, such as films of nitrogen-doped TiO<sub>2</sub> [TiO<sub>2</sub> (N)] and carbon-doped TiO<sub>2</sub> [TiO<sub>2</sub> (C)], on various substrates, including silicon, glass, and quartz coupons (44, 45). The TiO<sub>2</sub> films absorbed only UV light (wavelength < 380 nm), while the TiO<sub>2-x</sub>N<sub>x</sub> and TiO<sub>2-x</sub>C<sub>x</sub> (where x represents the dopant [N or C] concentration in molar fraction in the host crystal [TiO<sub>2</sub>]) films showed visible-light absorption with the absorption edges red shifted

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by approximately 565 and 425 nm, respectively. The prepared nanosized carbon- and nitrogen-doped thin films showed an enhancement in the photodegradation efficiency of methylene blue under visible-light ( $\geq 400$  nm) irradiation compared to pure  $\text{TiO}_2$  thin film. The crystallinities and compositions of photocatalysts are correlated to their hydrophilic properties and photocatalytic activities during methylene blue degradation (44, 45). However, the antibacterial activity of these anion-doped  $\text{TiO}_2$  films has not been clearly demonstrated.

The aim of this study was to investigate the antibacterial activity of the visible-light-irradiated nitrogen- and carbon-doped  $\text{TiO}_2$ . We tested several human pathogens, including *Shigella flexneri*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Acinetobacter baumannii*. Among these microorganisms, *S. flexneri*, *L. monocytogenes* and *V. parahaemolyticus* were usually found in contaminating water, plants, and sewage (24, 27, 34, 42) and frequently lead to outbreaks in regions with poor sanitary conditions (9, 24). *S. pyogenes* and *S. aureus* are exotoxin-producing pathogens which can cause diseases such as soft tissue infections, food-borne disease, and toxic shock syndrome in humans (34). The emergence and rapid spread of multidrug-resistant *A. baumannii* isolates causing nosocomial infections are of great concern worldwide (30). Although the optimal antimicrobial conditions remain to be fully established, we found that the  $\text{TiO}_2$  (N)-coated substrates developed for this study possessed bactericidal activities that could reduce the bacterial population of all tested pathogens when illuminated by visible light. Our data suggest that  $\text{TiO}_2$  (N) is an effective antibacterial photocatalyst which is user friendly compared to traditional UV-driven  $\text{TiO}_2$  photocatalysts.

## MATERIALS AND METHODS

**Preparation of  $\text{TiO}_2$ -,  $\text{TiO}_2$  (C)-, and  $\text{TiO}_2$  (N)-coated substrates.** The  $\text{TiO}_2$ ,  $\text{TiO}_{2-x}\text{C}_x$ , and  $\text{TiO}_{2-x}\text{N}_x$  films were prepared in an ion-assisted electron beam evaporation system (Branchy Vacuum Technology Co., Ltd., Taoyuan, Taiwan). The distance between the rotating substrate holder and the electron beam evaporation source was 550 mm. The chamber was evacuated by a mechanical pump (ALCATEL-2033SD) and a cryopump (CTI-Cryo-Torr8) to a base pressure below  $2.7 \times 10^{-4}$  Pa. The substrates used were polished Si(100), quartz, and glass coupons, which were sputter etched with argon ions ( $\text{Ar}^+$ ) for 5 min prior to the deposition to remove any residual pollutants on the surface. The substrate temperature was maintained at 300°C by a quartz lamp. The  $\text{TiO}_2$  films were deposited in an oxygen atmosphere ( $6.7 \times 10^{-3}$  Pa) using rutile  $\text{TiO}_2$  (99.99%) as the source material. The nitrogen flow for  $\text{TiO}_{2-x}\text{N}_x$  films was 15 standard  $\text{cm}^3 \text{min}^{-1}$  through the ion gun at a constant pumping speed, and the chamber pressure was at  $4.4 \times 10^{-2}$  Pa. The carbon dioxide gas flow for  $\text{TiO}_{2-x}\text{C}_x$  films was 7 standard  $\text{cm}^3 \text{min}^{-1}$ , and the chamber pressure was  $2.6 \times 10^{-2}$  Pa. The ion gun beam current of 10 mA and voltage of  $-1,000$  V were maintained by a Commonwealth Scientific ion beam power supply controller. Sufficient energy and current of the ion beam are critical to incorporate significant dopant concentration in the film. Without ion bombardment, it is difficult for the dopant to compete with the oxygen for incorporation into anatase titania. The deposition rate was adjusted to  $0.2 \text{ nm s}^{-1}$ , using a quartz crystal monitor for all films deposited at a thickness of 1.2  $\mu\text{m}$ . The three films were prepared under the optimized conditions for their categories of anatase crystallinity and dopant concentration (44, 45).

**Bacterial strains and culture.** *E. coli* (strain OP50) (31) was maintained and cultured in Luria-Bertani (LB) broth or LB agar (MDBio, Inc., Taipei, Taiwan) at 37°C using a standard laboratory *E. coli* culture method (2, 35). *L. monocytogenes* (laboratory strain 10430S) was provided by Eric Pamer (Sloan-Kettering Cancer Center) (46). A clinical isolated strain of *S. flexneri* was collected from central Taiwan in 1996 (9). Pandrug-resistant *A. baumannii* (strain M36788), *S. pyogenes* (strain M29588) (39), and *S. aureus* (strain SA02) were clinical isolates from Buddhist Tzu-Chi General Hospital in Hualien, Taiwan. All clinical isolates were initially differentiated into gram-positive and gram-negative strains, based

on the results of preliminary identification. Both gram-positive and -negative strains were directly cultured in tryptic soy broth supplemented with 0.5% yeast extract (TSBY) and LB at 37°C for 16 h and then identified by biochemical methods according to routine clinical laboratory procedures (29). *E. coli*, *S. flexneri*, and *A. baumannii* were maintained and grown in LB medium or LB agar at 37°C. *S. pyogenes* and *S. aureus* were grown in TSBY broth or TSBY broth agar (MDBio, Inc., Taipei, Taiwan) at 37°C. *V. parahaemolyticus* (strain 15427, serovar O3:K6) was a clinical isolate obtained from the Center for Disease Control in Taiwan (8). The strains were maintained and grown at 37°C in tryptic soy broth (Difco) supplemented with 3% NaCl. All bacteria were stored in 50% medium and 50% glycerol solution in freezers at  $-80^\circ\text{C}$  before use. To reactivate bacteria from frozen stocks, 25  $\mu\text{l}$  bacterial stock solution was transferred to a test tube containing 5 ml of freshly prepared culture medium and then incubated at 37°C under agitation overnight (16 to 18 h).

**Photocatalytic reaction and detection of viable bacteria.** In this study, bacterial concentrations were either determined by the standard plating method or inferred from optical density readings at 600 nm ( $\text{OD}_{600}$ ). For each bacterium, a factor for converting the  $\text{OD}_{600}$  values of the bacterial culture to concentration values (CFU/ml) was calculated as follows. A fresh bacterial culture was diluted by factors of  $10^{-1}$  to  $10^{-7}$ , and an  $\text{OD}_{600}$  of these dilutions was measured. Bacterial concentrations of these dilutions were determined by the standard plating method. The  $\text{OD}_{600}$  values were plotted against the bacterial concentration log values, and the conversion factors for particular bacteria were calculated. The conversion factor for *E. coli* OP50, for example, was calculated to be  $6 \times 10^8$  CFU/ml per  $\text{OD}_{600}$  by this method.

In order to determine the bactericidal effects of the  $\text{TiO}_2$ -related substrates, 200  $\mu\text{l}$  of bacterial overnight culture was transferred into 5 ml of culture medium and incubated at 37°C until an  $\text{OD}_{600}$  of 0.3 to 0.6 (log phase) was reached. The bacterial concentrations were calculated using the conversion factor for the bacteria, and the cultures were diluted to  $5 \times 10^7$  CFU/ml with culture medium. Fifty microliters ( $2.5 \times 10^6$  CFU) was then applied to an area of approximately 1  $\text{cm}^2$  of the  $\text{TiO}_2$ -related substrates by using a plastic yellow tip. The bacterium-containing substrates were placed under an incandescent lamp (Classicstone incandescent lamp, 60W, Philips; Taiwan) for photocatalytic reaction, and a light meter (model LX-102; Lutron Electronic Enterprises, Taiwan) was used to record the illumination density. In the dose dependence experiments, illuminations were carried out for 5 min at distances of 5, 10, and 15 cm from the lamp, corresponding to illumination densities of  $3 \times 10^4$ ,  $1.2 \times 10^3$ , and  $3 \times 10^2$  lux ( $\text{lumen/m}^2$ ), respectively. In the kinetic analysis experiments, illuminations were carried out for 1, 5, 10, 15, and 25 min at a distance of 5 cm, corresponding to an illumination density of  $3 \times 10^4$  lux. Unless specified, illuminations were carried out in a 4°C cold room. After illumination, the bacterial solutions were recovered from the  $\text{TiO}_2$ -related substrates, and an aliquot of fresh culture medium was used to collect the residual bacteria on the substrates. The two bacterial solutions were pooled to make a total of 100  $\mu\text{l}$ . The bacterial concentration was determined by the standard plating method immediately after the bacterial collection, and the percentage of surviving bacteria was calculated.

In the experiments for determining the mitigation effect of protein and dye in photocatalyst-mediated killing, a log-phase *E. coli* culture ( $\text{OD}_{600}$  of 0.3 to 0.6) was diluted to  $5 \times 10^7$  CFU/ml as described above, and 50  $\mu\text{l}$  was mixed with an equal volume of normal saline solutions containing either 5%, 1%, and 0.2% bovine serum albumin (BSA) (wt/vol), or 4, 2, and 0.4  $\text{OD}_{600}$  of bromophenol blue. The mixtures were then applied to an approximately 1- $\text{cm}^2$  area of the  $\text{TiO}_2$ -related substrates, and illumination was carried out under the incandescent lamp for 5 min at 4°C at a distance of 5 cm, corresponding to an illumination density of  $3 \times 10^4$  lux. Recovery of the surviving bacteria was performed as described above, with the exception of a total of 150  $\mu\text{l}$  solution being obtained. The bacterial concentrations were determined by the standard dilution and plating methods, and the percentage of surviving bacteria was calculated. In the experiments of photocatalyst-mediated killing of *E. coli* of different concentrations, a log-phase *E. coli* culture ( $\text{OD}_{600}$  of 0.3 to 0.6) was adjusted to 0.5, 1, and 2  $\text{OD}_{600}$  either by dilution with culture medium or by centrifugation and resuspension of the cell pellets into culture medium. Aliquots of  $2.5 \times 10^7$  CFU bacteria were applied to the  $\text{TiO}_2$ -related substrates. Illumination was carried out under the incandescent lamp for 5 min at 4°C at a distance of 5 cm, corresponding to an illumination density of  $3 \times 10^4$  lux. Recovery of the surviving bacteria and calculation of the percentage of surviving bacteria were carried out as in the dose dependence and kinetic analysis experiments.

**Septic shock mouse model.** Six- to 8-week-old C57BL/6J mice were purchased from the National Experimental Animal Center (Taipei, Taiwan) (20). A log-phase *E. coli* OP50 culture ( $\text{OD}_{600}$  of 0.3 to 0.6) was adjusted to  $2 \times 10^{10}$  CFU/ml saline by centrifugation, followed by resuspension of the cell pellet in sterile normal saline solution, using the conversion factor of *E. coli* OP50 ( $6 \times 10^8$

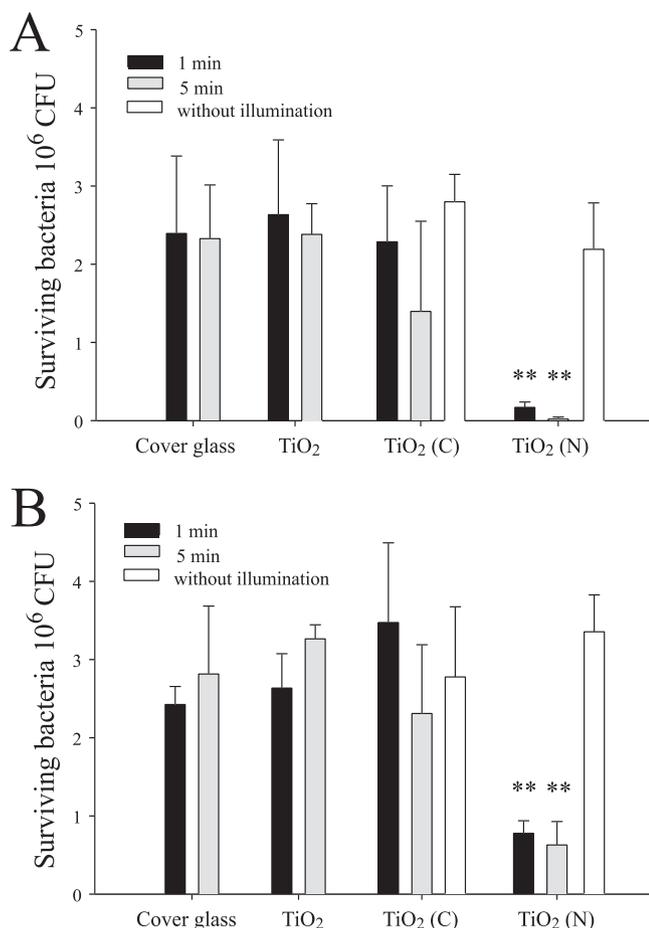


FIG. 1. Bactericidal activity analysis. Bactericidal activities of the  $\text{TiO}_2$ -related substrates after visible-light illumination at  $25^\circ\text{C}$  (A) or  $4^\circ\text{C}$  (B) were analyzed. Illumination was carried out at a light density of  $3 \times 10^4$  lux for either 1 or 5 min. “Without illumination” indicates experiments conducted in a dark room without illumination. \*\*,  $P < 0.01$  compared to either the respective cover glass groups or the  $\text{TiO}_2$  (N) groups without visible-light.

CFU/ml per  $\text{OD}_{600}$  unit) for the bacterial concentration calculation. The *E. coli* solution was divided into aliquots of  $250 \mu\text{l}$  ( $5 \times 10^9$  CFU). Each mouse in the control groups received an intravenous injection of  $5 \times 10^9$  CFU *E. coli*, a lethal dose for mice. In the experimental groups, mice were injected with the same batch of bacterial aliquots, but the *E. coli* solution was pretreated with visible-light illuminations on  $\text{TiO}_2$  or  $\text{TiO}_2$  (N) substrates for 5 min at  $4^\circ\text{C}$ . The distance between the lamp and the bacterium-containing substrates was 5 cm, corresponding to an illumination density of  $3 \times 10^4$  lux. The mortality of mice in this septic shock model, which was affected by the viability of treated bacteria, was then recorded. The Animal Care and Use Committee of Tzu-Chi University approved the protocol of the mouse experiments.

**Statistical analysis.** All results were calculated from the data of three independent experiments. A *t* test was used to assess the statistical significance of differences in results of antimicrobial effects. A *P* value of less than 0.05 was considered significant. The statistical tests were carried out and output to graphs using Microsoft Excel (Microsoft Taiwan, Taipei, Taiwan) and SigmaPlot (Systat Software, Point Richmond, CA) softwares.

## RESULTS

**Bactericidal activities of  $\text{TiO}_2$  versus nitrogen- and carbon-doped  $\text{TiO}_2$ .** To determine the bactericidal activities of  $\text{TiO}_2$  (N) and  $\text{TiO}_2$  (C), we first placed  $2.5 \times 10^6$  CFU *E. coli* on

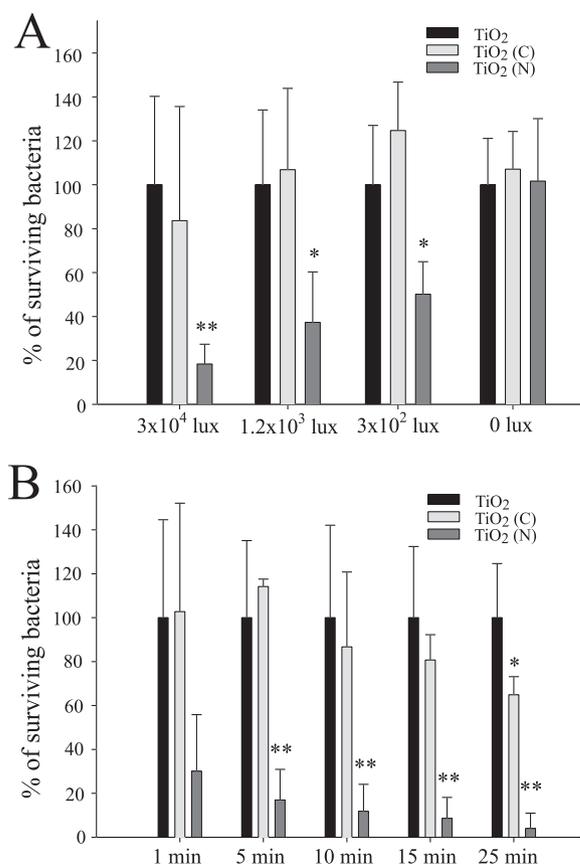


FIG. 2. Dose dependency and kinetics. Dose dependency (A) and kinetic analysis (B) of the bactericidal activity of the  $\text{TiO}_2$ -related substrates after visible-light illumination are shown. Illumination was carried out either at different light densities for 5 min (A) or at a light density of  $3 \times 10^4$  lux for different times (B). Under each illumination condition, the percentages of the surviving bacteria on the  $\text{TiO}_2$  (C) and  $\text{TiO}_2$  (N) substrates were normalized to the percentage of the surviving bacteria on the  $\text{TiO}_2$  substrates (100%). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (compared to the respective  $\text{TiO}_2$  groups).

various substrates, including cover glass (silica, without  $\text{TiO}_2$  coating) and silica substrates coated with thin films of  $\text{TiO}_2$ ,  $\text{TiO}_2$  (N), and  $\text{TiO}_2$  (C). These preparations were then irradiated with visible light, and the levels of surviving bacteria were quantified. The antibacterial activities of  $\text{TiO}_2$  (N) and  $\text{TiO}_2$  (C) were more pronounced when experiments were conducted at room temperature than at  $4^\circ\text{C}$  (Fig. 1A and B). The irradiation produced heat after absorption by the photocatalyst, and this greatly influenced bacterial survival. To avoid the effects of heat, we performed the same experiments in a  $4^\circ\text{C}$  cold room but maintained the temperature of  $\text{TiO}_2$  substrate surfaces at  $4^\circ\text{C}$  during irradiation. Although the antibacterial activity of  $\text{TiO}_2$  substrates was reduced under these conditions,  $\text{TiO}_2$  (N) still exhibited a significantly greater ability to reduce the number of *E. coli* than  $\text{TiO}_2$  and  $\text{TiO}_2$  (C) ( $P < 0.01$ ) (Fig. 1B). To control for the effects of heat and determine the pure “ROS-mediated killing” effect, all subsequent bacterium-killing experiments were performed in a  $4^\circ\text{C}$  cold room.

To obtain dose-dependent and kinetic data for *E. coli* on  $\text{TiO}_2$  substrates, we further analyzed the effects of illumination by visible light at various time points or at various distances (5

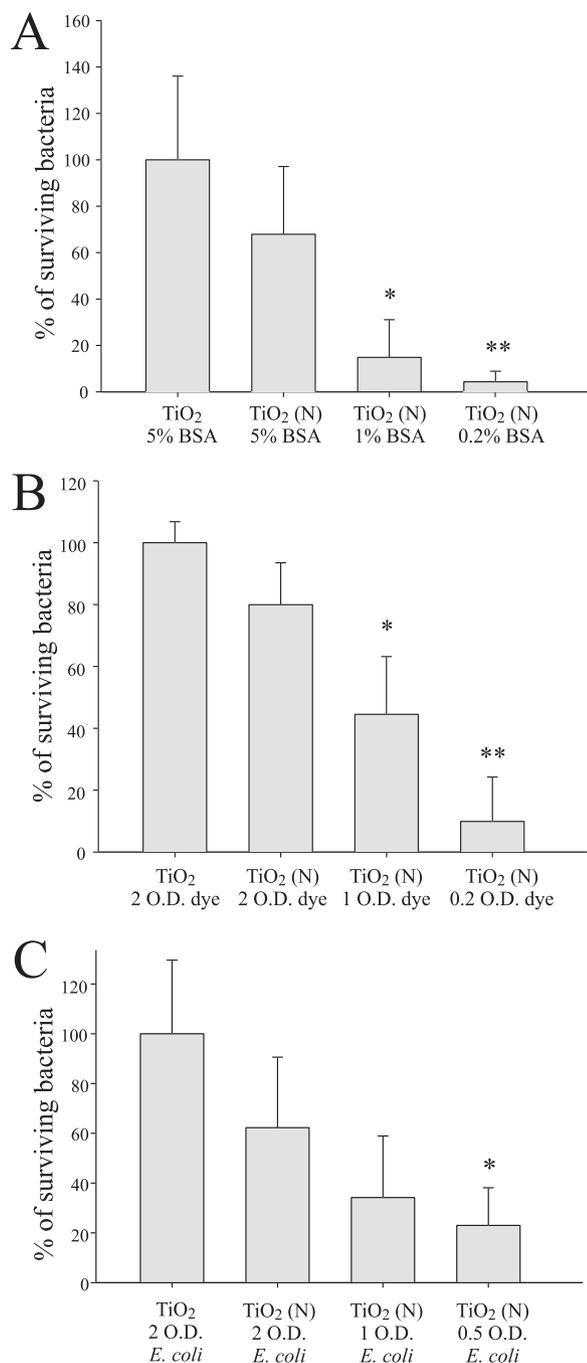


FIG. 3. Protein- and light-absorbing substances. The effects of BSA (A), bromophenol blue dye (B), and bacterial concentrations (C) on the bactericidal activity of the TiO<sub>2</sub>-related substrates after visible-light illumination are shown. The percentages of surviving bacteria were normalized to the percentage of the surviving bacteria on the TiO<sub>2</sub> substrate plus 5% BSA (A), to that on the TiO<sub>2</sub> substrate plus 2 OD of dye (B), or to that from 2 OD of *E. coli* on the TiO<sub>2</sub> substrate (C). \*,  $P < 0.05$  compared to the group with TiO<sub>2</sub> plus 5% BSA (A), the group with TiO<sub>2</sub> plus 2 OD of dye (B), or the group with 2 OD of *E. coli* on TiO<sub>2</sub> (C); \*\*,  $P < 0.01$  compared to the group with TiO<sub>2</sub> plus 5% BSA (A) or the group with TiO<sub>2</sub> plus 2 OD of dye (B).

cm, 10 cm, and 20 cm and with respective illumination intensities of  $3 \times 10^4$ ,  $1.2 \times 10^3$ , and  $3 \times 10^2$  lux (Fig. 2). The results showed that TiO<sub>2</sub> (N) substrates could kill *E. coli* in minutes when exposed to various degrees of illumination by

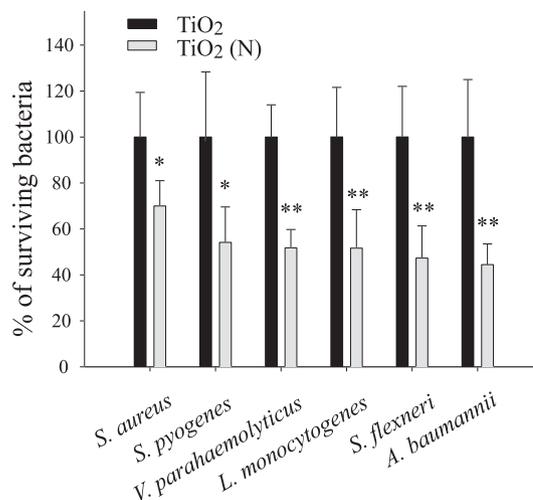


FIG. 4. Pathogen analysis. For each pathogen, the percentage of surviving bacteria on the TiO<sub>2</sub> (N) substrate was normalized to that on the TiO<sub>2</sub> substrate. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (compared to the TiO<sub>2</sub> group).

visible light (Fig. 2A). The bacterium-killing efficiency in the TiO<sub>2</sub> (N) groups was significantly greater than that of the respective TiO<sub>2</sub> groups (Fig. 2A). On the other hand, the TiO<sub>2</sub> (C) substrates had less bactericidal effectiveness. Although prolonged illuminations seemed to increase the bacterium killing of TiO<sub>2</sub> (C) substrates (25 min) (Fig. 2B), the killing efficiency still did not match that of the TiO<sub>2</sub> (N) substrates (bacterial survival rate of 4% versus 70%) (Fig. 2B).

**Bactericidal activity of TiO<sub>2</sub> (N) in solutions contaminated by protein- or light-absorbing substances.** In order to investigate the potential for use of TiO<sub>2</sub> (N) in environmental or medical materials which may become contaminated with protein- or light-absorbing substances, we introduced various concentrations of BSA or the dye bromophenol blue into *E. coli* incubation medium and then measured the bactericidal activity of TiO<sub>2</sub> (N). The results showed that the TiO<sub>2</sub> (N) substrates became less effective only when the contaminants reached a high level (Fig. 3A and B). When the protein concentration was 1% or the dye contaminant was present at 1 OD, the TiO<sub>2</sub> (N) substrate exhibited significant antibacterial ability (bacterial inhibition of 82% or 58%, respectively) (Fig. 3).

To control for the OD and light-shielding effects of bacterial concentration, the TiO<sub>2</sub> (N)-mediated killing experiments were further performed using different concentrations (OD) of *E. coli* cells in bacterial solution with equal amounts ( $2.5 \times 10^7$  CFU) of *E. coli*. We found that the bactericidal activity was not significant when the concentration of *E. coli* solution was adjusted to 1 OD or greater (Fig. 3C).

**Bactericidal activities of TiO<sub>2</sub> (N) to eliminate pathogens.** In pathogen experiments, TiO<sub>2</sub> (N) was significantly more effective at killing all of the tested pathogens, including *S. flexneri*, *L. monocytogenes*, *V. parahaemolyticus*, *S. pyogenes*, *S. aureus*, and *A. baumannii*, than TiO<sub>2</sub> substrates. This effectiveness was not influenced by whether the target was gram-positive or gram-negative bacteria (Fig. 4).

**Experimental sepsis mouse model.** Septic shock experiments in a mouse model were used to investigate whether previous

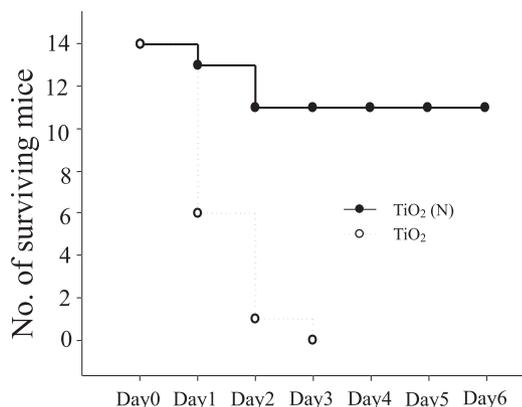


FIG. 5. Septic shock mouse model. Mortality of the C57BL/6J mice after intravenous injection of a lethal dose of *E. coli* ( $5 \times 10^9$  CFU) preexposed to TiO<sub>2</sub> or TiO<sub>2</sub> (N) substrates and visible-light illumination ( $n = 14$ ).

exposure of bacteria to TiO<sub>2</sub> photocatalyst killing would result in less severe symptoms after host inoculation. The results showed that killing by TiO<sub>2</sub> (N) substrates reduced the number of viable cells of *E. coli* and significantly reduced their ability to cause host mortality on inoculation (Fig. 5).

## DISCUSSION

Pure TiO<sub>2</sub> photocatalyst is effective against bacteria upon UV irradiation. Exposure of humans to UV light at the necessary levels, however, would cause great damage to the skin and eyes (13, 37), thus limiting the potential for the use of TiO<sub>2</sub> substrates in environments where humans would be exposed. Public environments are ideal places for the transmission of pathogens (7, 41). The visible-light-induced antibacterial activity of TiO<sub>2</sub> (N) offers the potential for use as a disinfectant in public areas, specifically those indoor environments without adequate air circulation, such as public toilets, schools, hospitals, stations, airports, hotels, and public transportation. The surfaces of objects such as door handles and push buttons are constantly contacted by people, and a method which provides a constant disinfection process may be able to limit pathogen spread (6, 38). Since these objects would also be exposed to natural and/or artificial light sources, a TiO<sub>2</sub> (N)-coated surface offers the potential for developing such a solution. Many techniques have been developed to coat surfaces with photocatalysts, including wet methods, such as sol-gel and spraying, to achieve the fixation of powder as a film as well as dry processes, such as evaporation, ion-assisted deposition, sputtering, and metal-organic chemical vapor deposition (19).

In this study, we investigated the antimicrobial properties of visible-light photocatalyst TiO<sub>2</sub> (N) against human pathogens. Human pathogens were more resistant to TiO<sub>2</sub> (N)-mediated killing than the laboratory *E. coli* strain OP50, with a killing efficiency of approximately 50% versus 80 to 95%. Several pathogens were shown to evolve resistance mechanisms against ROS. For example, specific enzyme systems for the elimination of ROS were found in *S. aureus*, *S. flexneri*, and *S. pyogenes* (11, 17, 25). Because ROS production by phagocytes is part of the innate immune system of hosts (5, 36), these anti-ROS mech-

anisms are often associated with pathogen virulence (11, 17, 25). The greater resistance against TiO<sub>2</sub> (N)-mediated killing of these pathogens than that of *E. coli* might be attributable to the presence of these enzyme systems, although this possibility remains to be investigated. Even though exposure of inoculates to visible-light photocatalysts significantly reduced the mortality in our septic shock mouse model, this bactericidal efficiency was not comparable to that of commonly used disinfectants, which can almost completely eliminate the target microorganisms (28). The TiO<sub>2</sub>-based photocatalysts, however, have several advantages compared to other disinfectants. First, because TiO<sub>2</sub> is a chemically stable and inert material, it could continuously exert antimicrobial action when illuminated by light. Second, because it is inert, a previous study showed that it is not harmful when ingested by animals (4). Third, the bactericidal activity can be switched on and off or modulated by controlling the light intensity. These advantages might be complementary to existing disinfectants and provide the potential for developing a variety of alternative antimicrobial applications. In addition, several recent technical advancements, such as metal (silver dopant), the addition of the electric field, and the creation of mixed-phase crystals of TiO<sub>2</sub>, could enhance the photocatalysis activity of TiO<sub>2</sub>-based photocatalysts (10, 40; Chou et al., unpublished), furthering their potential for use in the design of disinfection technology.

Due to urbanization, population growth, and heavy traveling, infectious diseases can quickly spread worldwide from one local area; the epidemic of severe acute respiratory syndrome during 2003 is an example (21, 22, 43). Visible-light photocatalysts have the potential for use in a variety of settings to reduce the transmission of pathogens in public environments. The emergence of increasingly virulent and antibiotic-resistant pathogens in hospital settings (1, 32) provides another motivation for the development of alternative disinfection approaches using visible-light photocatalysts.

This study demonstrated that TiO<sub>2</sub> (N) has better visible-light photocatalytic bactericidal activity against human pathogens than TiO<sub>2</sub> or TiO<sub>2</sub> (C). Our results showed that the number of microorganisms was greatly reduced after treatment with a visible-light photocatalyst. These results suggest that TiO<sub>2</sub> (N) has the potential for use in the development of applications for environmental and medical decontamination.

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