APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2006, p. 6111–6116 Vol. 72, No. 9
0099-2240/06/$08.00+0 doi:10.1128/AEM.02580-05

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

Ming-Show Wong,[1] Wen-Chen Chu,[2] Der-Shan Sun,[3,4,5] Hsuan-Shun Huang,[3] Jiann-Hwa Chen,[6] Pei-Jane Tsai,[7] Nien-Tsung Lin,[8] Mei-Shiuan Yu,[8] Shang-Feng Hsu,[1] Shih-Lien Wang,[8] and Hsin-Hou Chang[3,4,9]*

Department of Materials Science and Engineering, National Dong-Hwa University, Hualien, Taiwan[1]; Institute of Molecular Biology, National Chung Hsing University, Taichung, Taiwan[6]; and Department of Life Science,[2] Institute of Medical Sciences,[3] Institute of Molecular and Cellular Biology,[4] Institute of Human Genetics,[5] Institute of Medical Biotechnology,[7] Institute of Microbiology, Immunology and Molecular Medicine,[8] and Tzu-Chi University Center for Vascular Medicine,[9] Tzu-Chi University, Hualien, Taiwan, Republic of China

Received 1 November 2005/Accepted 4 July 2006

The antibacterial activity of photocatalytic titanium dioxide (TiO_2) substrates is induced primarily by UV light irradiation. Recently, nitrogen- and carbon-doped TiO_2 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_2 substrates have superior visible-light-induced bactericidal activity against *Escherichia coli* compared to pure TiO_2 and carbon-doped TiO_2 substrates. We also found that protein- and light-absorbing contaminants partially reduce the bactericidal activity of nitrogen-doped TiO_2 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_2 substrates than by pure TiO_2 substrates. These findings suggest that nitrogen-doped TiO_2 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 biocidic 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_2) substrates have been shown to eliminate organic compounds and to function as disinfectants (26). Upon UV light excitation, the photon energy excites valance electrons and generates pairs of electrons and holes (electron vacancy in valence band) that diffuse and become trapped on or near the TiO_2 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 ($\cdot$ OH) and superoxide anions (O_2^-) (12). Electron holes, $\cdot$ OH, and O_2^- 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 $\cdot$ OH, O_2^-, and hydrogen peroxide (H_2O_2) generated on irradiated TiO_2 surfaces, have been shown to operate in concert to attack polyunsaturated phospholipids in bacteria (26). In addition, it has been shown that photoirradiated TiO_2 catalyzed site-specific DNA damage via generation of H_2O_2 (14). These findings suggested that TiO_2 might exert antimicrobial effects similar to those of the peroxygen disinfectant H_2O_2 (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_2 photocatalytic process is a conceptually feasible technology. The TiO_2 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_2 substrates for use in our living environments. Recently, the anion-doped anatase TiO_2-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_2 [TiO_2 (N)] and carbon-doped TiO_2 [TiO_2 (C)], on various substrates, including silicon, glass, and quartz coupons (44, 45). The TiO_2 films absorbed only UV light (wavelength < 380 nm), while the $TiO_{2-x}N_x$ and $TiO_{2-x}C_x$ (where x represents the dopant [N or C] concentration in molar fraction in the host crystal [TiO_2]) films showed visible-light absorption with the absorption edges red shifted

* Corresponding author. Mailing address: Room D407, Tzu-Chi University, No. 701, Section 3, Chung-Yang Road, Hualien 970, Taiwan. Phone: 886-3-8565301, ext. 7296. Fax: 886-3-8578386. E-mail: hhchang@mail.tcu.edu.tw.

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 (≥400 nm) irradiation compared to pure 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 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 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 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 TiO_2 (N) is an effective antibacterial photocatalyst which is user friendly compared to traditional UV-driven TiO_2 photocatalysts.

MATERIALS AND METHODS

Preparation of TiO_2-, TiO_2 (C)-, and TiO_2 (N)-coated substrates. The TiO_2, $TiO_{2-x}C_x$, and $TiO_{2-x}N_x$ 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 2.7×10^{-4} Pa. The substrates used were polished Si(100), quartz, and glass coupons, which were sputter etched with argon ions (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 TiO_2 films were deposited in an oxygen atmosphere (6.7×10^{-3} Pa) using rutile TiO_2 (99.99%) as the source material. The nitrogen flow for $TiO_{2-x}N_x$ films was 15 standard cm^3 min^{-1} through the ion gun at a constant pumping speed, and the chamber pressure was at 4.4×10^{-2} Pa. The carbon dioxide gas flow for $TiO_{2-x}C_x$ films was 7 standard cm^3 min^{-1}, and the chamber pressure was 2.6×10^{-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 nm s^{-1}, using a quartz crystal monitor for all films deposited at a thickness of 1.2 μm. 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°C before use. To reactivate bacteria from frozen stocks, 25 μl 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 (OD_{600}). For each bacterium, a factor for converting the 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 OD_{600} of these dilutions was measured. Bacterial concentrations of these dilutions were determined by the standard plating method. The 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 6×10^8 CFU/ml per OD_{600} by this method.

In order to determine the bactericidal effects of the TiO_2-related substrates, 200 μl of bacterial overnight culture was transferred into 5 ml of culture medium and incubated at 37°C until an 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 5×10^7 CFU/ml with culture medium. Fifty microliters (2.5×10^6 CFU) was then applied to an area of approximately 1 cm^2 of the TiO_2-related substrates by using a plastic yellow tip. The bacterium-containing substrates were placed under an incandescent lamp (Classictone 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 3×10^4, 1.2×10^3, and 3×10^2 lux (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 3×10^4 lux. Unless specified, illuminations were carried out in a 4°C cold room. After illumination, the bacterial solutions were recovered from the 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 μl. 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 (OD_{600} of 0.3 to 0.6) was diluted to 5×10^7 CFU/ml as described above, and 50 μl 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 OD_{600} of bromophenol blue. The mixtures were then applied to an approximately 1-cm^2 area of the 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 3×10^4 lux. Recovery of the surviving bacteria was performed as described above, with the exception of a total of 150 μl 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 (OD_{600} of 0.3 to 0.6) was adjusted to 0.5, 1, and 2 OD_{600} either by dilution with culture medium or by centrifugation and resuspension of the cell pellets into culture medium. Aliquots of 2.5×10^7 CFU bacteria were applied to the 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 3×10^4 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 (OD_{600} of 0.3 to 0.6) was adjusted to 2×10^{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 (6×10^8

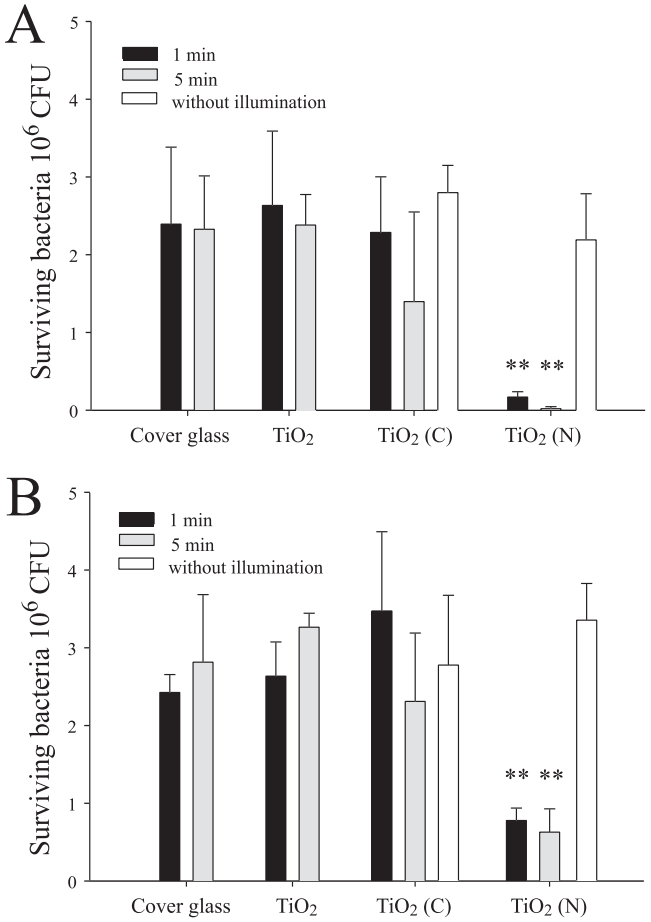

FIG. 1. Bactericidal activity analysis. Bactericidal activities of the TiO_2-related substrates after visible-light illumination at 25°C (A) or 4°C (B) were analyzed. Illumination was carried out at a light density of 3×10^4 lux for either 1 or 5 min. "Without illumination" indicates experiments conducted in a dark room without illumination. **, $P < 0.01$ compared to either the respective cover glass groups or the TiO_2 (N) groups without visible-light.

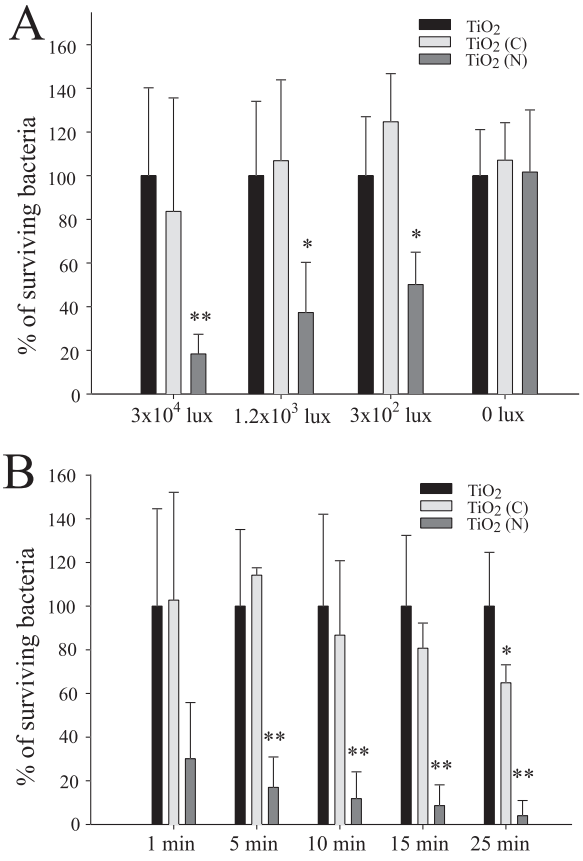

FIG. 2. Dose dependency and kinetics. Dose dependency (A) and kinetic analysis (B) of the bactericidal activity of the TiO_2-related substrates after visible-light illumination are shown. Illumination was carried out either at different light densities for 5 min (A) or at a light density of 3×10^4 lux for different times (B). Under each illumination condition, the percentages of the surviving bacteria on the TiO_2 (C) and TiO_2 (N) substrates were normalized to the percentage of the surviving bacteria on the TiO_2 substrates (100%). *, $P < 0.05$; **, $P < 0.01$ (compared to the respective TiO_2 groups).

CFU/ml per OD_{600} unit) for the bacterial concentration calculation. The *E. coli* solution was divided into aliquots of 250 μl (5×10^9 CFU). Each mouse in the control groups received an intravenous injection of 5×10^9 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 TiO_2 or TiO_2 (N) substrates for 5 min at 4°C. The distance between the lamp and the bacterium-containing substrates was 5 cm, corresponding to an illumination density of 3×10^4 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 TiO_2 versus nitrogen- and carbon-doped TiO_2. To determine the bactericidal activities of TiO_2 (N) and TiO_2 (C), we first placed 2.5×10^6 CFU *E. coli* on various substrates, including cover glass (silica, without TiO_2 coating) and silica substrates coated with thin films of TiO_2, TiO_2 (N), and TiO_2 (C). These preparations were then irradiated with visible light, and the levels of surviving bacteria were quantified. The antibacterial activities of TiO_2 (N) and TiO_2 (C) were more pronounced when experiments were conducted at room temperature than at 4°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°C cold room but maintained the temperature of TiO_2 substrate surfaces at 4°C during irradiation. Although the antibacterial activity of TiO_2 substrates was reduced under these conditions, TiO_2 (N) still exhibited a significantly greater ability to reduce the number of *E. coli* than TiO_2 and 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°C cold room.

To obtain dose-dependent and kinetic data for *E. coli* on TiO_2 substrates, we further analyzed the effects of illumination by visible light at various time points or at various distances (5

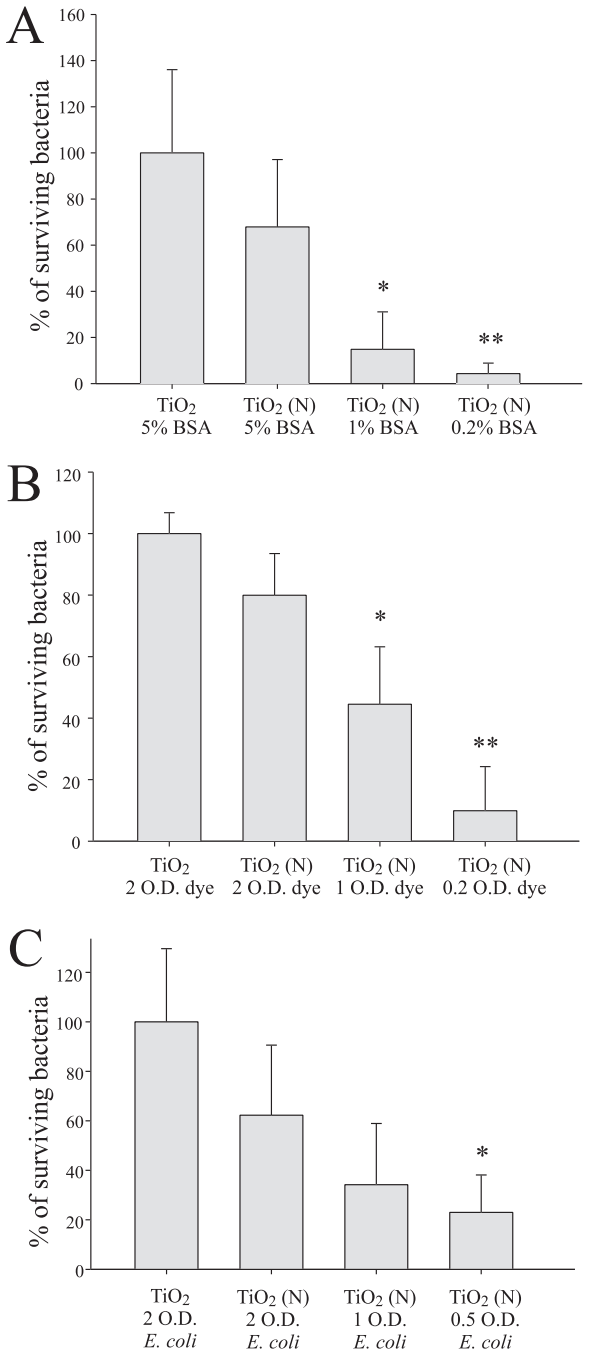

FIG. 3. Protein- and light-absorbing substances. The effects of BSA (A), bromophenol blue dye (B), and bacterial concentrations (C) on the bactericidal activity of the TiO_2-related substrates after visible-light illumination are shown. The percentages of surviving bacteria were normalized to the percentage of the surviving bacteria on the TiO_2 substrate plus 5% BSA (A), to that on the TiO_2 substrate plus 2 OD of dye (B), or to that from 2 OD of *E. coli* on the TiO_2 substrate (C). *, $P < 0.05$ compared to the group with TiO_2 plus 5% BSA (A), the group with TiO_2 plus 2 OD of dye (B), or the group with 2 OD of *E. coli* on TiO_2 (C); **, $P < 0.01$ compared to the group with TiO_2 plus 5% BSA (A) or the group with TiO_2 plus 2 OD of dye (B).

cm, 10 cm, and 20 cm and with respective illumination intensities of 3×10^4, 1.2×10^3, and 3×10^2 lux) (Fig. 2). The results showed that TiO_2 (N) substrates could kill *E. coli* in minutes when exposed to various degrees of illumination by

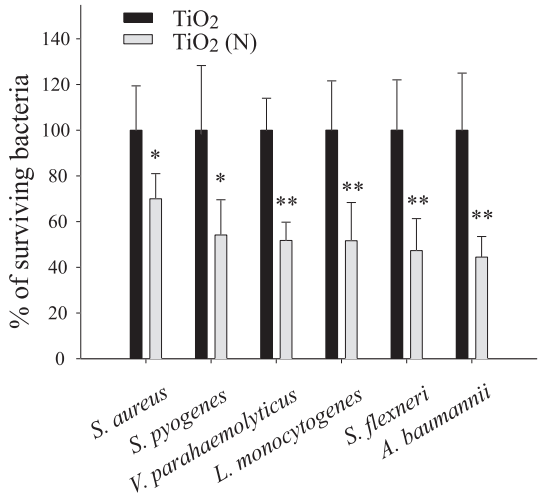

FIG. 4. Pathogen analysis. For each pathogen, the percentage of surviving bacteria on the TiO_2 (N) substrate was normalized to that on the TiO_2 substrate. *, $P < 0.05$; **, $P < 0.01$ (compared to the TiO_2 group).

visible light (Fig. 2A). The bacterium-killing efficiency in the TiO_2 (N) groups was significantly greater than that of the respective TiO_2 groups (Fig. 2A). On the other hand, the TiO_2 (C) substrates had less bactericidal effectiveness. Although prolonged illuminations seemed to increase the bacterium killing of TiO_2 (C) substrates (25 min) (Fig. 2B), the killing efficiency still did not match that of the TiO_2 (N) substrates (bacterial survival rate of 4% versus 70%) (Fig. 2B).

Bactericidal activity of TiO_2 (N) in solutions contaminated by protein- or light-absorbing substances. In order to investigate the potential for use of TiO_2 (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_2 (N). The results showed that the TiO_2 (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_2 (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_2 (N)-mediated killing experiments were further performed using different concentrations (OD) of *E. coli* cells in bacterial solution with equal amounts (2.5×10^7 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_2 (N) to eliminate pathogens. In pathogen experiments, TiO_2 (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_2 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

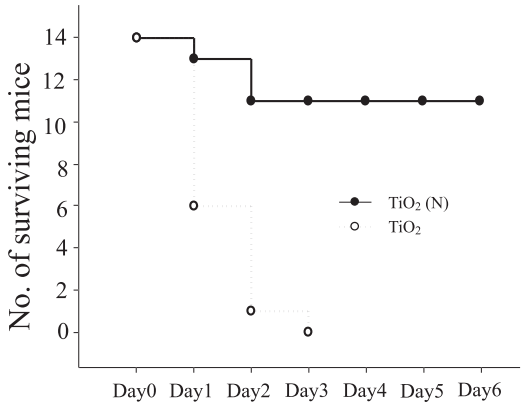

FIG. 5. Septic shock mouse model. Mortality of the C57BL/6J mice after intravenous injection of a lethal dose of *E. coli* (5×10^9 CFU) preexposed to TiO_2 or TiO_2 (N) substrates and visible-light illumination ($n = 14$).

exposure of bacteria to TiO_2 photocatalyst killing would result in less severe symptoms after host inoculation. The results showed that killing by TiO_2 (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 TiO2 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_2 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_2 (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_2 (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_2 (N) against human pathogens. Human pathogens were more resistant to TiO_2 (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 mechanisms are often associated with pathogen virulence (11, 17, 25). The greater resistance against TiO_2 (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_2-based photocatalysts, however, have several advantages compared to other disinfectants. First, because TiO_2 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_2, could enhance the photocatalysis activity of TiO_2-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_2 (N) has better visible-light photocatalytic bactericidal activity against human pathogens than TiO_2 or TiO_2 (C). Our results showed that the number of microorganisms was greatly reduced after treatment with a visible-light photocatalyst. These results suggest that TiO_2 (N) has the potential for use in the development of applications for environmental and medical decontamination.

ACKNOWLEDGMENTS

We would like to thank Tony Jer-Fu Lee, Dean of the College of Life Sciences, Tzu-Chi University, for his scientific suggestions and assistance in preparing the manuscript.

We also appreciate the financial support of the National Science Council of Taiwan, R.O.C., under grant no. NSC 93-2216-E-259-002 and NSC 93-2120-M-259-001.

REFERENCES

1. **Aiello, A. E., and E. Larson.** 2003. Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. Lancet Infect. Dis. **3:**501–506.
2. **Alterthum, F., and L. O. Ingram.** 1989. Efficient ethanol production from glucose, lactose, and xylose by recombinant *Escherichia coli*. Appl. Environ. Microbiol. **55:**1943–1948.
3. **Asahi, R., T. Morikawa, T. Ohwaki, K. Aoki, and Y. Taga.** 2001. Visible-light photocatalysis in nitrogen-doped titanium oxides. Science **293:**269–271.
4. **Bernard, B. K., M. R. Osheroff, A. Hofmann, and J. H. Mennear.** 1990. Toxicology and carcinogenesis studies of dietary titanium dioxide-coated mica in male and female Fischer 344 rats. J. Toxicol. Environ. Health **29:** 417–429.

5. **Bogdan, C., M. Rollinghoff, and A. Diefenbach.** 2000. Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Curr. Opin. Immunol. **12:**64–76.
6. **Booth, T. F., B. Kournikakis, N. Bastien, J. Ho, D. Kobasa, L. Stadnyk, Y. Li, M. Spence, S. Paton, B. Henry, B. Mederski, D. White, D. E. Low, A. McGeer, A. Simor, M. Vearncombe, J. Downey, F. B. Jamieson, P. Tang, and F. Plummer.** 2005. Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units. J. Infect. Dis. **191:**1472–1477.
7. **Chen, K. T., P. Y. Chen, R. B. Tang, Y. F. Huang, P. I. Lee, J. Y. Yang, H. Y. Chen, J. Bresee, E. Hummelman, and R. Glass.** 2005. Sentinel hospital surveillance for rotavirus diarrhea in Taiwan, 2001–2003. J. Infect. Dis. **192**(Suppl. 1)**:**S44–S48.
8. **Chiou, C.-S., S.-Y. Hsu, S.-I. Chiu, T.-K. Wang, and C.-S. Chao.** 2000. *Vibrio parahaemolyticus* serovar O3:K6 as cause of unusually high incidence of food-borne disease outbreaks in Taiwan from 1996 to 1999. J. Clin. Microbiol. **38:**4621–4625.
9. **Chiou, C.-S., W.-B. Hsu, H.-L. Wei, and J.-H. Chen.** 2001. Molecular epidemiology of a *Shigella flexneri* outbreak in a mountainous township in Taiwan, Republic of China. J. Clin. Microbiol. **39:**1048–1056.
10. **Egerton, T. A., S. A. Kosa, and P. A. Christensen.** 2006. Photoelectrocatalytic disinfection of E. coli suspensions by iron doped TiO2. Phys. Chem. Chem. Phys. **8:**398–406.
11. **Franzon, V. L., J. Arondel, and P. J. Sansonetti.** 1990. Contribution of superoxide dismutase and catalase activities to *Shigella flexneri* pathogenesis. Infect. Immun. **58:**529–535.
12. **Fujishima, A., and K. Honda.** 1972. Electrochemical photolysis of water at a semiconductor electrode. Nature **238:**37–38.
13. **Hearing, V. J.** 2005. Biogenesis of pigment granules: a sensitive way to regulate melanocyte function. J. Dermatol. Sci. **37:**3–14.
14. **Hirakawa, K., M. Mori, M. Yoshida, S. Oikawa, and S. Kawanishi.** 2004. Photo-irradiated titanium dioxide catalyzes site specific DNA damage via generation of hydrogen peroxide. Free Radic. Res. **38:**439–447.
15. **Iwasaki, M., M. Hara, H. Kawada, H. Tada, and S. Ito.** 2000. Cobalt ion-doped TiO(2) photocatalyst response to visible light. J. Colloid Interface Sci. **224:**202–204.
16. **Jacoby, W. A., P. C. Maness, E. J. Wolfrum, D. M. Blake, and J. A. Fennel.** 1998. Mineralization of bacterial cell mass on a photocatalytic surface in air. Environ. Sci. Technol. **32:**2650–2653.
17. **Janulczyk, R., S. Ricci, and L. Björck.** 2003. MtsABC is important for manganese and iron transport, oxidative stress resistance, and virulence of *Streptococcus pyogenes*. Infect. Immun. **71:**2656–2664.
18. **Kampf, G., and A. Kramer.** 2004. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. Clin. Microbiol. Rev. **17:**863–893.
19. **Kaneko, M., and I. Okura (ed.).** 2002. Photocatalysis. Springer-Verlag, New York, N.Y.
20. **Kau, J. H., D. S. Sun, W. J. Tsai, H. F. Shyu, H. H. Huang, H. C. Lin, and H. H. Chang.** 2005. Antiplatelet activities of anthrax lethal toxin are associated with suppressed p42/44 and p38 mitogen-activated protein kinase pathways in the platelets. J. Infect. Dis. **192:**1465–1474.
21. **La Montagne, J. R., L. Simonsen, R. J. Taylor, and J. Turnbull.** 2004. Severe acute respiratory syndrome: developing a research response. J. Infect. Dis. **189:**634–641.
22. **Lan, Y. C., T. T. Liu, J. Y. Yang, C. M. Lee, Y. J. Chen, Y. J. Chan, J. J. Lu, H. F. Liu, C. A. Hsiung, M. S. Ho, K. J. Hsiao, H. Y. Chen, and Y. M. Chen.** 2005. Molecular epidemiology of severe acute respiratory syndrome-associated coronavirus infections in Taiwan. J. Infect. Dis. **191:**1478–1489.
23. **Legrini, O., E. Oliveros, and A. M. Braun.** 1993. Photochemical processes for water treatment. Chem. Rev. **93:**671–698.
24. **Lima, A. A.** 2001. Tropical diarrhoea: new developments in traveller's diarrhoea. Curr. Opin. Infect. Dis. **14:**547–552.
25. **Mandell, G. L.** 1975. Catalase, superoxide dismutase, and virulence of Staphylococcus aureus. In vitro and in vivo studies with emphasis on staphylococcal–leukocyte interaction. J. Clin. Investig. **55:**561–566.
26. **Maness, P.-C., S. Smolinski, D. M. Blake, Z. Huang, E. J. Wolfrum, and W. A. Jacoby.** 1999. Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. Appl. Environ. Microbiol. **65:**4094–4098.
27. **Martino, M. C., G. Rossi, I. Martini, I. Tattoli, D. Chiavolini, A. Phalipon, P. J. Sansonetti, and M. L. Bernardini.** 2005. Mucosal lymphoid infiltrate dominates colonic pathological changes in murine experimental shigellosis. J. Infect. Dis. **192:**136–148.
28. **McDonnell, G., and A. D. Russell.** 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. **12:**147–179.
29. **Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken.** 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
30. **Navon-Venezia, S., R. Ben-Ami, and Y. Carmeli.** 2005. Update on Pseudomonas aeruginosa and Acinetobacter baumannii infections in the healthcare setting. Curr. Opin. Infect. Dis. **18:**306–313.
31. **Rodger, S., B. S. Griffiths, J. W. McNicol, R. W. Wheatley, and I. M. Young.** 2004. The impact of bacterial diet on the migration and navigation of Caenorhabditis elegans. Microb. Ecol. **48:**358–365.
32. **Russell, A. D.** 2004. Bacterial adaptation and resistance to antiseptics, disinfectants and preservatives is not a new phenomenon. J. Hosp. Infect. **57:**97–104.
33. **Russell, A. D.** 2003. Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. Lancet Infect. Dis. **3:**794–803.
34. **Salyers, A. A., and D. D. Whitt.** 1994. Bacterial pathogenesis: a molecular approach. ASM Press, Washington, D.C.
35. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
36. **Segal, A. W.** 2005. How neutrophils kill microbes. Annu. Rev. Immunol. **23:**197–223.
37. **Slominski, A., and J. Pawelek.** 1998. Animals under the sun: effects of ultraviolet radiation on mammalian skin. Clin. Dermatol. **16:**503–515.
38. **Tong, T. R.** 2005. Airborne severe acute respiratory syndrome coronavirus and its implications. J. Infect. Dis. **191:**1401–1402.
39. **Tsai, P. J., Y. H. Chen, C. H. Hsueh, C. H. Hsieh, Y. H. Liu, J. J. Wu, and C. C. Tsou.** 2006. Streptococcus pyogenes induces epithelial inflammatory responses through NF-kappaB/MAPK signaling pathways. Microbes Infect. **8:**1440–1449.
40. **Vohra, A., D. Y. Goswami, D. A. Deshpande, and S. S. Block.** 2005. Enhanced photocatalytic inactivation of bacterial spores on surfaces in air. J. Ind. Microbiol. Biotechnol. **32:**364–370.
41. **Walther, B. A., and P. W. Ewald.** 2004. Pathogen survival in the external environment and the evolution of virulence. Biol. Rev. Camb. Philos. Soc. **79:**849–869.
42. **Wong, H.-C., S.-H. Liu, T.-K. Wang, C.-L. Lee, C.-S. Chiou, D.-P. Liu, M. Nishibuchi, and B.-K. Lee.** 2000. Characteristics of *Vibrio parahaemolyticus* O3:K6 from Asia. Appl. Environ. Microbiol. **66:**3981–3986.
43. **Wu, H. S., Y. C. Hsieh, I. J. Su, T. H. Lin, S. C. Chiu, Y. F. Hsu, J. H. Lin, M. C. Wang, J. Y. Chen, P. W. Hsiao, G. D. Chang, A. H. Wang, H. W. Ting, C. M. Chou, and C. J. Huang.** 2004. Early detection of antibodies against various structural proteins of the SARS-associated coronavirus in SARS patients. J. Biomed. Sci. **11:**117–126.
44. **Yang, M. C., T. S. Yang, and M. S. Wong.** 2004. Nitrogen-doped titanium oxide films as visible light photocatalyst by vapor deposition. Thin Solid Films **469–470:**1–5.
45. **Yang, T. S., C. B. Shiu, and M. S. Wong.** 2004. Structure and hydrophilicity of titanium oxide films prepared by electron beam evaporation. Surface Sci. **548:**75–82.
46. **Zhong, M. X., W. A. Kuziel, E. G. Pamer, and N. V. Serbina.** 2004. Chemokine receptor 5 is dispensable for innate and adaptive immune responses to *Listeria monocytogenes* infection. Infect. Immun. **72:**1057–1064.