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Photoinduced Bactericidal Activity of TiO_2 Films

S. N. Pleskova[a], I. S. Golubeva[a], Yu. K. Verevkin[b], E. A. Pershin[a], V. N. Burenina[b], and V. V. Korolichin[b]

[a] *Alexeev Nizhniy Novgorod State Technical University, Nizhniy Novgorod, 603155 Russia*
e-mail: pleskova@mail.ru

[b] *Institute of Applied Physics, Russian Academy of Sciences, Nizhniy Novgorod, 603150 Russia*

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Abstract—A decrease in CFU of gram-positive and gram-negative bacteria on the surface of UV illuminated TiO_2 films (wavelength of 380 nm) is shown. A 29, 45, and 47% decrease in bacterial viability of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*, respectively, was seen after 12-min exposition. It was first discovered that the reuse of TiO_2 films to test a bacterial suspension for viability removes UV-induced bactericidal activity. However, annealing of TiO_2 at a temperature above 400°C restores the photoinduced bactericidal activity to its initial state.

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An important objective of applied microbiology is the design and study of "self-sterilizing" surfaces; i.e., those inactivating and killing bacteria, breaking down organic pollutants, etc. [1]. The development of bactericidal surfaces actively involves photocatalysis where reactive oxygen species (ROS) such as superoxide anion-radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl-radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), released from nanofilm surfaces, are primary disinfection agents. Surfaces with such properties could be used to solve environmental problems (photocatalytic purification of air, drinking water, and industrial sewage), help to construct highly clean facilities (operating suites, laboratories), help to package and preserve food products [2]. To coat surfaces for imparting them with desired properties, TiO_2 thin films are best suited [3]. Sol-gel technology allows to coat nearly any surface with TiO_2 thin films with given structure and properties [4]. Armealo L. et al. [5] reported inhibition of bacterial growth after incubation on TiO_2 thin film surfaces that produced ROS after UV-illumination. However, in that study UV-induced bactericidal activity of TiO_2 thin films only occurred for one strain. A significant drawback of that study is the lack of data on the reproducibility of results in reuse of thin films.

The goal of this work is the evaluation of bactericidal activity of TiO_2 films under UV-irradiation (wavelength of 380 nm) on the gram-positive and gram-negative bacteria; and the determination of conditions to implement this property.

METHODS

Strains used were *Staphylococcus aureus* 956, *Staphylococcus epidermidis* 1061, and *Escherichia coli* 321-5. Pure cultures were isolated in the bacterial laboratory of Clinical Infectious Hospital no. 2 of Nizhniy Novgorod.

Production of TiO_2 thin films. TiO_2 coatings were chemically put on a glass plate using solutions of hydrolyzed compounds (sol-gel technology). 5% tetrabutyl oxytitan in isopropyl $Ti(OC_4H_9)_4$ was used as a film-forming agent. Chlorohydric acid was used as a catalyst and stabilizer. The coating solution was spin deposited on a Petri dish. A transparent layer homogeneous through the thickness developed on a glass surface, consisting of titanic acid polymers. Further temperature treatment (400°C, 5 h) resulted in the completion of decomposition reactions of intermediate hydrolysis products and complete removal of the solvent. Evaporation leaves a transparent TiO_2 thin film strongly attached to the glass surface [6, 7].

Bacterial suspension production. The strains were grown on beef-extract agar (ZAO NITS pharmakoterapii, Saint Petersburg) at 37°C for 20 h. Each agar slant was washed off with a sterile physiological saline solution (OAO Biochimik, Saransk) and twice washed with a physiological saline solution (pH 7.2); the transmission of the bacterial suspension was brought to 0.269 using a KFK-2MP photometer (Russia) (670 nm). It corresponded to 10 ME of turbidity standard. A series of dilutions was prepared from the suspension so that approximately 200 CFU per dish could grow. In a series of preliminary studies, the dilution of *S. aureus* 956, *S. epidermidis* 1061, and *E. coli* 321-5 was 1 : 200000, 1 : 100000, and 1 : 200000, respectively. Each dilution was evaluated on a TiO_2 thin film surface.

Table 1. CFU numbers after 15-min exposition to UV-light (wavelength of 380 nm) on a glass and a glass coated with a TiO_2 thin film (experiment)*

Strain	Glass surface (control)	TiO_2 thin film surface (experiment)
S. aureus 956	115.8 ± 10.7	82.5 ± 6.5
S. epidermidis 1061	153.2 ± 24.6	84.0 ± 17.0
E. coli 321-5	279.0 ± 34.5	148.3 ± 27.8

* Differences between the control and experiment are significantly different ($p < 0.05$).

Evaluation of the bactericidal activity of TiO_2 thin films. In an experiment, a bacterial suspension put on a TiO_2 thin film surface was irradiated with UV-light (380 nm) for 15 min. The density of UV-lamp light power (BIO-2, Ukraine), measured by a detector of radiation energy (IMO-2N, Russia), was 4.5 mW/cm^2. To remove short UV-wavelengths, that are bactericidal on their own ($\lambda < 365$ nm), a UFS-6 light trap was used. As a positive control, a bacterial suspension deposited on a sterile glass surface was UV-illuminated under the same experimental conditions. Further, a decrease in bacterial viability on a TiO_2 surface was compared against this control. As a negative control, a bacterial suspension was incubated both on TiO_2 surface and a glass surface (without TiO_2 film) in the dark.

Since no difference in CFU when bacteria were incubated both on TiO_2 surface and a glass surface was observed, the negative control was used in the first series of experiments and was omitted for later use. After 15-min UV-ullumination, a 0.05 ml of bacterial suspension was pipetted on an agar surface and evenly spread with a spatula. Cultures were incubated at 37°C for 20 h followed by CFU counting.

Statistical analysis was performed using Origin 7.0 Server package software. Mean values and standard deviations were determined. Significant differences between two sample sizes were evaluated by the Student's test.

RESULTS AND DISCUSSION

The study of the UV-induced bactericidal activity of TiO_2 thin films showed a significant decrease in viability of gram-positive and gram-negative bacteria (Table 1).

Particularly, a significant decrease in viability after exposure of TiO_2 thin film surfaces to UV-illumination was demonstrated for gram-negative bacteria (*E. coli* 321-5): CFU numbers decreased nearly twofold (Fig. 1).

According to published data, it is most likely that a decay in CFU numbers is attributable to the bacterial death caused by cell membrane disintegration. This occurs due to the oxidation of membrane proteins by ROS formed on TiO_2 film surface [5]. For the initial reaction to initiate the chain reaction of ROS formation, energy exceeding that of the TiO_2 band gap is required. In our studies, we used wavelengths of less 390 nm. According to L.M. Lynkov et al. [8], upon absorption of a photon by a TiO_2 thin film, interstitial holes Ti^{3+}, free hydroxyl radicals ($OH^\bullet$), and superoxide anion-radicals ($O_2^{\bullet -}$) are produced. Charge separation in TiO_2 thin films to produce electrons (e^-) and holes (h^+) occurs as follows:

$$TiO_2 + h\nu \longrightarrow TiO_2\,(e^- + h^+). \qquad (1)$$

The formation of ROS was confirmed by Lynkov et al. using electron spin resonance [8]. ROS oxidyze not only major structures of bacterial cell membranes and cell walls but also modify DNA nitrogen bases, causing breaks, and inhibition primary groups of bac-

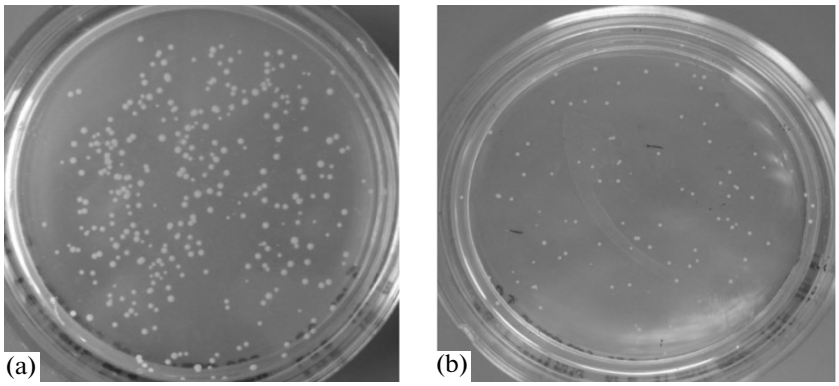

Fig. 1. Decrease in CFU of *E.coli* 321-5 after 15-min exposition to UV-light on a glass surface. (a) is Control (UV-illumination without a TiO_2 thin film). (b) is experiment (UV-illumination on a TiO_2 thin film).

terial enzymes [9, 10]. Combination of various mechanisms of ROS biocidicity resulted in a significant decrease in bacterial viability in our experiments. However, attempts to reproduce the results in reuse of TiO_2 thin films yielded unexpected results. After washing TiO_2 thin films off bacterial suspensions and further sterilization (180°C, 60 min), they exhibited no photocatalytic and bactericidal activities (Table 2). An increase in exposure time of bacterial suspensions to UV-illumination up to 30 min did not reverse the photoinduced bactericidal activity of TiO_2 thin films.

These results could be explained by the phenomenon of superhydrophilicity: a hole, forming after removal of an electron, reacts with water to render TiO_2 thin film surface superhydrophilic. (Fig. 2) [3]. The occurrence of superhydrophilicity is highly likely in our experiments because TiO_2 thin films were overlaid with aqueous bacterial suspensions.

To confirm the finding that the surface hydrophobicity (initial state) turned into hydrophilicity (reused film) after UV-illumination in the presence of liquid, experiments were conducted with a droplet of distilled water. A droplet of water is distinctly hydrophobic on nonilluminated TiO_2 thin film surface (Fig. 3) with the contact angle being 134°. After UV-irradiation (380 nm) the droplet spread across the TiO_2 surface, which was accompanied by a decrease in the contact angle down to 42°.

Overall, two simultaneous reactions take place on nonilluminated TiO_2 thin film surface: ROS formation and surface hydrophilization occur. After reuse, a hydroxylated TiO_2 film loses its photocatalytic activity because it has become incapable of being an electron donor.

Attempts were made to reverse the photoinduced bactericidal activity of TiO_2 thin films. For this purpose, hydroxyl radicals had to be eliminated from the film surface. This could be achieved through repeated annealing of TiO_2 thin films for 5 h at 400°C. The experiments performed showed nearly the complete restoration of the initial properties of TiO_2 thin films (Table 3).

To rule out the potential influence of changes in medium pH on bacterial viability, pH values of bacterial suspensions were determined prior to the deposition of TiO_2 film surfaces and after 15-min UV-irradiation. The pH value remained constant and was 7.0 in both cases. Hence, the influence of pH on bacterial strains was not observed in these experiments.

Table 2. CFU numbers after 15-min exposition to UV-light (wavelength of 380 nm) on a glass and a glass coated with reused TiO_2 thin film (experiment)*

Strain	Glass surface (control)	Reused TiO_2 thin film surface (experiment)
S. aureus 956	459.2 ± 23.8	425.0 ± 46.7
S. epidermidis 1061	536.3 ± 37.1	521.0 ± 90.7
E. coli 321-5	162.2 ± 33.4	126.4 ± 18.5

* No evidence of statistical significance was established in any case ($p > 0.05$).

In summary, in the present paper, UV-induced bactericidal activity of a nanomaterial, TiO_2 films, on the gram-positive and gram-negative bacteria was observed. However, in studies of bacterial suspensions suspended in liquids (normal saline solution), the sterilizing activity was only observed in a single use because UV-illumination alters the surface itself. It acquires hydrophilicity evidenced by a threefold decrease in the contact angle. Hydrophilicity is generally considered as a positive feature since hydrophilic surfaces can be easily cleaned from various microorganisms and other organic pollutants by water. Even if this surface property meets the needs of construction and automobile industries, it is not enough for medicine, food, and ecological microbiology in the light of the fact that the primary objective of these sciences is not cleaning but the complete killing of bacteria, i.e., sterilization. To reverse the bactericidal activity of the surface, we attempted a reannealing of TiO_2 thin films. In this case, the UV-induced activity with respect to the stains studied restored. This approach is impossi-

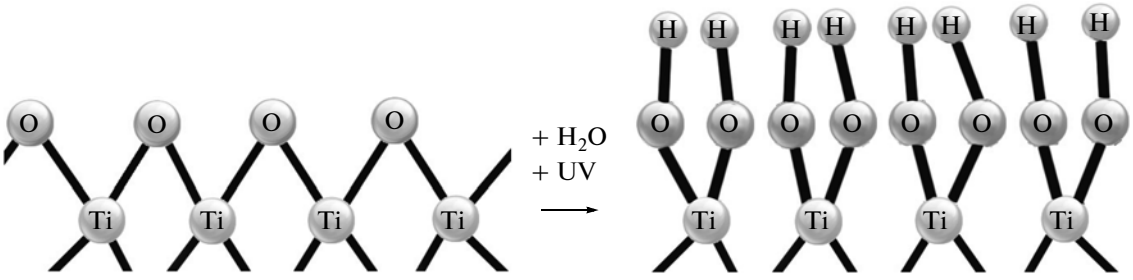

Fig. 2. Schematic of hydrophylization of TiO_2 thin film under UV-illumination: O is oxygen, Ti is titanium, and H is hydrogen.

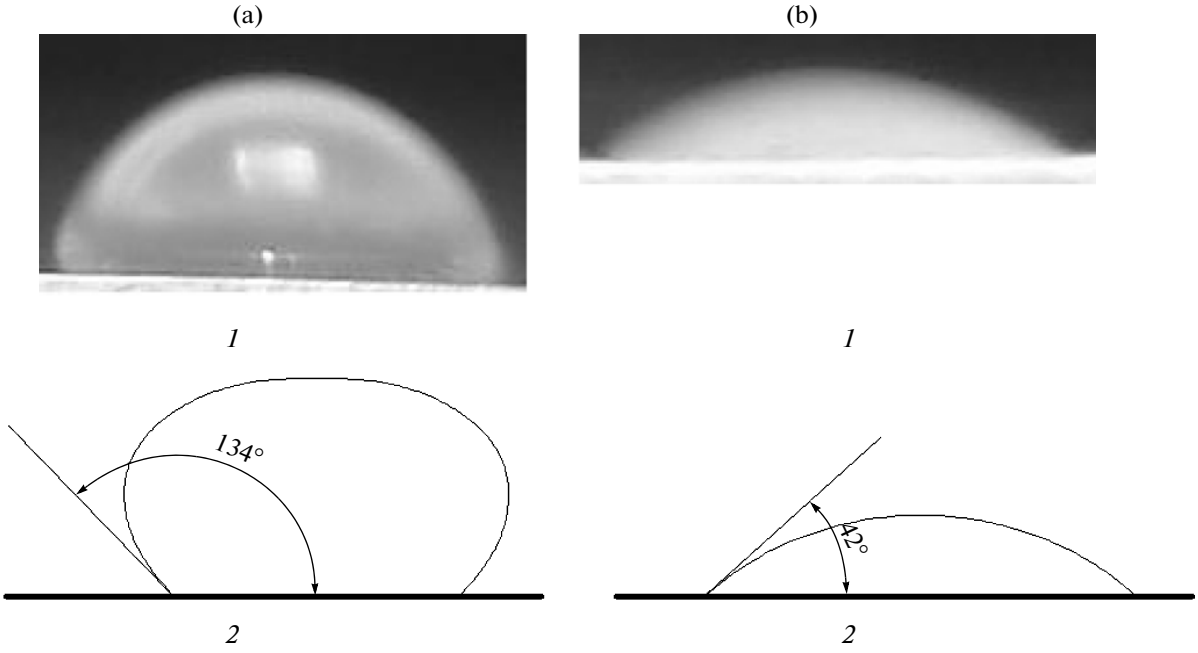

Fig. 3. Conversion of the surface (a) hydrophobicity into (b) hydrophylicity after UV-illumination (380 nm) for 5 min: (*1*) is photomicrograph of a droplet on a TiO_2 thin film surface, (*2*) is schematic of a droplet with the contact angle being measured.

ble to call satisfactory because the annealing conditions are quite stringent (high temperature of 400°C and long exposition of 5 h). This is the reason why it cannot be put into practice in health care facilities. This difficulty being solved, TiO_2 thin films would hold promise as a material to coat medical instruments, containers in food industry, etc. because it would become possible to sterilize surfaces under UV-light for a short time.

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Table 3. CFU numbers after 30-min exposition to UV-light (wavelength of 380 nm) on a glass and a glass coated with reannealed TiO_2 thin film after repeated annealing (400°C, 5h) (experiment)*

Strain	Glass surface (control)	Reannealed TiO_2 thin film surface (experiment)
S. aureus 956	142.8 ± 15.8	79.2 ± 9.1
S. epidermidis 1061	44.8 ± 9.9	12.8 ± 7.7
E. coli 321-5	87.0 ± 14.0	36.0 ± 15.9

* Differences between the control and experiment are significantly different ($p < 0.05$).

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