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Photocatalytic TiO_2 particles confer superior antibacterial effects in a nutrition-rich environment: an *in vitro* study

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ABSTRACT

Titanium dioxide (TiO_2) is known to confer photocatalytic bactericidal effects under ultraviolet (UV) irradiation. Few reports are available, however, on the clinical applications of TiO_2 particle mixtures. Our objective in the present research was to evaluate the *in vitro* bactericidal effects of a TiO_2 particle mixture in a nutrition-rich biological environment. A bacterial suspension of *Staphylococcus aureus* and *epidermidis* 3×10^3 CFU/mL was added to a TiO_2 particle mixture (0.038 mg/mL) containing mainly sodium percarbonate and citric acid. To simulate a biological environment, 40 μL of 10% bovine serum albumin was added and the culture temperature was maintained at 37°C. The resulting product was irradiated by UV light and the bacterial survival rate was calculated for each time of UV irradiation. In the control sample treated with distilled water + UV, the bacteria survived at a high rate even after 180 min. In the TiO_2 mixture + UV sample, meanwhile, the bacterial survival rate dropped to 43.8% and 6.0% of the baseline values in *S. aureus* and *S. epidermidis*, respectively, after 60 min of UV irradiation. The photocatalytic antibacterial action of the TiO_2 particle mixture was high even in a protein-rich biological environment.

Even with careful preventative measures such as disinfection of the surgical field and surgical instruments, postoperative infection appears in 0.14% to 17.3% (9, 15, 18) of patients undergoing orthopedic surgery. Implant-related infections are also common occurrences and are often highly resistant to treatment. Two of the most common pathogenic bacteria responsible for postoperative implant-related infection are *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*), organisms with a thick cell walls that readily acquire multidrug resistance by mutation (2, 17). The methicillin-resistant strains of these organisms have an especially high resistance to antibiotic treatment (11). New techniques to prevent postoperative infection would clearly be of great value.

Our group has focused on the photocatalytic application of titanium dioxide (TiO_2) as a technique to reduce the incidence of postoperative infection in orthopedic surgery. On exposure to ultraviolet (UV) irradiation, TiO_2 releases free radicals such as −OH, O_2^-, and H_2O_2. This potent oxidizing power characteristically results in the lysis of bacteria and other organic substances (5, 6). In a previous paper we described the high photocatalytic antibacterial effects of a TiO_2 particle mixture against *S. aureus* (4). To explore the feasibility of application, we need to evaluate the advantages of TiO_2 in a clinical setting. Yet as of this writing, there have been very few

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studies on the antibacterial effects of TiO_2 on simulated postoperative infections in a biological environment. It will be important to conduct such studies, as the protein-rich and high-temperature conditions of biological environments are favorable for bacterial breeding. The objective of this study was to evaluate the photocatalytic antibacterial effects of the TiO_2 particle mixture against *S. aureus* and *S. epidermidis* in a biological environment.

TiO_2 particles (anatase 80%: rutile 20%) were prepared from titanium (IV) chloride gas by the vapor phase method and then annealed. The mean diameter and Brunauer-Emmett-Teller (BET) ratio surface area of the primary particles were 21 nm and 50 m^2/g, respectively. Next, a powder was prepared by mixing these TiO_2 particles with other substances, mainly sodium percarbonate and citric acid (Table 1). The sodium percarbonate, an oxidizer, accelerated the photocatalytic chemical reaction by providing a continuous supply of oxide. The citric acid adjusted the aqueous pH to neutral or low alkalinity (pH 8.0). The powder thus prepared was dispersed in distilled water to create a 1.0% mixture containing 38 ppm (0.038 mg/mL) of TiO_2 particles. All solutions and materials were sterilized by autoclaving at 120°C.

S. aureus (strain Seattle 1945) and *S. epidermidis* (ATCC35984) were cultured for 6 h at 37°C, then centrifuged to provide bacteria samples at a concentration of 3×10^3 CFU/mL (pH 7.0). Forty μL of the bacteria solution was combined with 40 μL of the TiO_2 mixture in a transparent polypropylene conical tube. To simulate a biological environment, 40 μL of 10% bovine serum albumin (Gibco, Invitrogen Japan K.K., Tokyo, Japan) was added and the culture temperature was kept at 37°C. The resulting mixture was irradiated by UV black light (FL15BL-B; NEC, Tokyo, Japan) (illumination, 1.82mW/cm^2; wavelength, 352 nm). The bacterial samples in the TiO_2 mixture were diluted with phosphate-buffered saline (PBS), cultured for 24 h with a Compact Dry TC culture kit (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), and irradiated by UV. Colony-forming units (CFUs) were counted and the bacterial survival rate was calculated (4). The samples were divided into three groups: Group 1, distilled water + no UV irradiation; Group 2, distilled water + UV irradiation; and Group 3, TiO_2 mixture + UV irradiation. Six replicate experiments were performed for each sample. The results were examined statistically by one-way analysis of variance (ANOVA) in multiple comparisons.

Table 1 *Components of TiO_2 powder*

Components	Content (%)
Sodium Percarbonate	37
Metasodium Silicate	6
Citric Acid	31
Sodium Tripolyphosphate	25
Magnesium Silicate	0.5
TiO_2	0.38

Sodium percarbonate added as an oxidizer accelerates the photocatalytic chemical reaction.

Fig. 1A and 1B show the bacterial survival rates at different irradiation times. The bacteria added to the Group 1 (distilled water + no UV irradiation) samples survived at high rates (mean 117.8% in *S. aureus* and 87.8% in *S. epidermidis*) even after 180 min. This confirmed that the bovine serum albumin and culture temperature (37°C) conferred biological conditions favorable for bacteria breeding. The bacterial survival rates of Group 2 (distilled water + UV irradiation) decreased gradually over time, reaching mean values of 98.5% in *S. aureus* and 66.7% in *S. epidermidis* at 60 min, and 94.2% in *S. aureus* and 50.6% in *S. epidermidis* at 150 min. This decline in bacterial survival in Group 2 was presumably the result of the bactericidal capabilities of the UV itself (16). Many of the sterilization systems now in use for surgical instruments and operating rooms rely on UV irradiation. In Group 3 (TiO_2 mixture + UV irradiation), meanwhile, the bacteria count dropped sharply, reaching 43.8% in *S. aureus* and 6.0% in *S. epidermidis* at 60 min, and 4.0% in *S. aureus* and 1.5% in *S. epidermidis* at 120 min. The inhibition of bacterial survival was significantly greater in the Group 3 samples than in the Group 2 samples after 60 min of irradiation in *S. aureus* and after 30 min of irradiation in *S. epidermidis* (ANOVA: $P < 0.05$). These findings indicate that the photocatalytic action of the TiO_2 particles against *S. aureus* and *S. epidermidis* remained potent even in a nutrition-rich environment advantageous for bacteria.

TiO_2 crystals appear in three forms (rutile, anatase, and brookite), all of which characteristically become semiconductors under UV irradiation. The electrons and positive holes created on the crystal surface react with water and oxygen to form various superoxides. The oxidizing action of TiO_2 is more potent than that of chlorine, hypochlorous acid, or hydrogen peroxide, and is capable of degrading organic substances such as bacteria (5, 6). Several reports have been published on the bactericidal effects of TiO_2 against organisms such as *Escherichia coli*

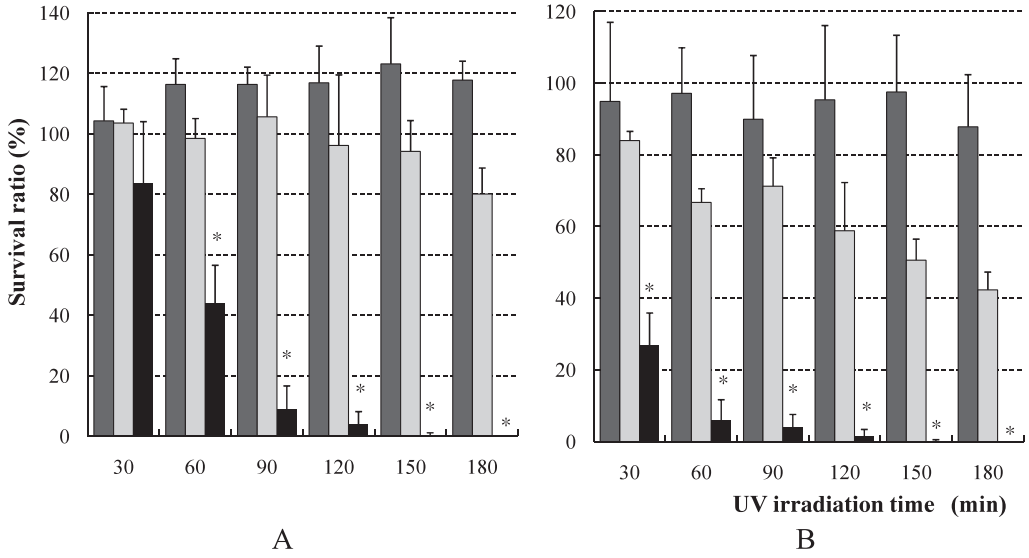

Fig. 1 Bacterial survival rates of *S. aureus* (A) and *S. epidermidis* (B). The inhibition of bacterial survival was significantly greater in Group 3 than in Groups 1 and 2 at irradiation times after 60 min in *S. aureus* and after 30 min in *S. epidermidis* (*$P < 0.05$). Group 1, Group 2, Group 3

(5, 8, 10, 12). Little has been done, however, to evaluate the antibacterial and bactericidal effects of TiO_2 particle mixtures for preventing postoperative infections in clinical settings. In the present study we have confirmed that our aqueous mixture of TiO_2 particles exerts photocatalytic antibacterial affects against *S. aureus* and *S. epidermidis*. We expect that it may be possible to eradicate bacteria on contact and inhibit further bacterial proliferation by adding a TiO_2 mixture to washing solutions or by spraying such a mixture onto implant surfaces before implantation in the body. Mixtures of this type may also be useful for the prevention and treatment of strong bacterial biofilms bridged by extracellular polysaccharides.

In clinical application, however, there are concerns about the negative effects of TiO_2 on the human body. Though biocompatible (7, 19), TiO_2 belongs to a class of bioactive materials which can also be potently biotoxic. By mixing TiO_2 with soluble substances not reported in the previous investigations, our group developed a TiO_2 particle mixture with improved photocatalytic activity at even lower TiO_2 concentrations (0.038 mg/mL). Sodium percarbonate accelerates the photocatalytic chemical reaction by providing a continuous supply of oxide. A more alkaline mixture would permit a higher photocatalytic reaction with TiO_2, but high alkalinity would seriously harm the human body, especially the eyes and skin. For added safety, citric acid is added to adjust the aqueous pH to a neutral or low alkalinity (pH 8.0).

The negative effects of UV rays on the human body also pose potential problems in clinical application. A good deal of research is underway to resolve this problem using materials with photocatalytic actions triggered by visible light (1, 3, 13, 14). By adjusting the TiO_2 concentration and reacting the TiO_2 with other components, our TiO_2 particles form a chelator which might feasibly shift the absorption spectrum towards visible light spectrums (4). Further research will be needed to evaluate the antibacterial effects of visible light alone.

Our present experiments have revealed that when TiO_2 particles react with oxidizer, they confer superior photocatalytic antibacterial affects against *S. aureus* and *S. epidermidis* even in a nutrition-rich biological environment. Further laboratory or *in vivo* studies under more sophisticated conditions will be required for comprehensive evaluation. In the meantime, these simple configurations with the TiO_2 particle mixture are particularly encouraging for use in the early stages of assessment. Our simple study allowed for greater control over experimental variables and produced fewer artifacts in the results.

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