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Short Communication

The Bactericidal Efficacy of a Photocatalytic TiO_2 Particle Mixture with Oxidizer against *Staphylococcus aureus*

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SUMMARY: By proving the bactericidal effects of a low-concentration titanium dioxide (TiO_2) particle mixture against *Staphylococcus aureus*, we hope to ultimately apply a mixture of this type as part of a clinical treatment regimen. A bacterial suspension of *S. aureus* 1×10^5 CFU/ml was added dropwise to a TiO_2 particle mixture (19 ppm TiO_2) and irradiated by ultraviolet (UV) light. The colony-forming units were counted and the bacterial survival rate was calculated. In the control sample, the bacterial survival rate was 83.3% even after 120 min. In the TiO_2 mixture + UV sample, the bacteria count dropped sharply, reaching 17.3% of the baseline value at 30 min and 0.4% at 60 min. TiO_2 particles dispersed in water mixtures are known to elicit highly efficient UV absorption and greater bonding to bacteria. A reaction of the TiO_2 with another oxidizer altered the aqueous pH and accelerated the photocatalytic chemical reaction. The TiO_2 particle mixture showed high antibacterial action against *S. aureus* even at a low concentration.

Even with careful preventative measures such as disinfection of the surgical field and surgical instruments, postoperative infection appears in 0.2 to 17.3% (1-3) of patients and is often highly resistant to treatment. One of the most common pathogenic bacteria responsible for postoperative infection is *Staphylococcus aureus*, an organism with a thick cell wall that readily acquires multidrug resistance by mutation (4,5). Methicillin-resistant strains are particularly resistant to antibiotic treatment (6,7).

Our group has focused on the photocatalytic application of titanium dioxide (TiO_2) as a potentially useful solution to the problems described above. On exposure to ultraviolet (UV) irradiation, TiO_2 releases free radicals such as ·OH, O_2^-, HO_2^-, and H_2O_2. This potent oxidizing power characteristically results in the lysis of bacteria and other organic substances (8-10). To explore the feasibility of these applications, we developed a fine particle mixture of TiO_2 in water. Several reports have been published on the bactericidal properties of TiO_2 against organisms such as *Escherichia coli* (11-13). Yet TiO_2 is a bioactive substance which may remain in trace amounts in the human body. Before TiO_2 can be used in clinical settings, steps must be taken to reduce its particle concentrations by improving its photocatalytic bactericidal activity.

Our objective in this study was to prepare a photocatalytic TiO_2 particle mixture with a low concentration of TiO_2 in order to evaluate the photocatalytic antibacterial effects of the mixture against *S. aureus* in vitro.

TiO_2 particles (anatase 80%: rutile 20%) were prepared by the vapor phase method from titanium (IV) chloride gas followed by annealing. The mean size of the primary particles was 21 nm, and the BET ratio surface area was 50 m^2/g. A powder was then prepared by mixing these TiO_2 particles with other substances, mainly sodium percarbonate and citric acid (Table 1). Sodium percarbonate, an oxidizer, accelerates the photocatalytic chemical reaction by providing a continuous supply of oxide. Citric acid was used to adjust the aqueous pH to neutral or low alkalinity (pH 8.0). Lastly, this powder was mixed in distilled water to create a 0.5% mixture containing 19 ppm (0.019 mg/mL) of TiO_2 particles.

Table 1. Sodium percarbonate added as an oxidizer accelerates the photocatalytic chemical reaction

Component	Content (%)
Percarbonate	37
Metasodium silicate	6
Citric acid	31
Sodium tripolyphosphate	25
Magnesium silicate	0.5
TiO_2	0.38

S. aureus (strain Seattle 1945) was cultured for 6 h at 37°C, then centrifuged to provide a bacterial sample with a concentration of 1×10^5 CFU/mL (pH 7.0). After adding 40 μL of the bacteria dropwise to 40 μL of the TiO_2 mixture in a transparent polypropylene conical tube, the resulting mixture was irradiated by UV black light (FL15BL-B; NEC, Tokyo, Japan) (illumination, 1.82 mW/cm^2; wavelength, 352 nm).

The bacterial sample in the TiO_2 mixture was diluted with phosphate-buffered saline (PBS). The collected bacterial samples were cultured for 24 h with a Compact Dry TC culture kit (Nissui Pharmaceutical, Tokyo, Japan) and then irradiated by UV. Colony-forming units (CFUs) were counted and the bacterial survival rate was calculated by the follow-

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ing formula:

Bacterial survival rate (α min) = CFU (α min) × 100/CFU (0 min)

The samples were divided into in five groups to evaluate the exact antibacterial effects of TiO_2, UV itself, and the soluble substances other than TiO_2. The results were examined statistically by one-way analysis of variance (ANOVA) in multiple comparisons.

Group 1: TiO_2 mixture + UV irradiation
Group 2: TiO_2 mixture + no UV irradiation
Group 3: Distilled water + UV irradiation
Group 4: Distilled water + no UV irradiation
Group 5: Mixture of soluble substances without TiO_2 + no UV irradiation

Five replicate experiments were performed for each sample.

The absorption spectrum of the TiO_2 particles in UV/visible absorption spectrophotometry (V-660; JASCO, Tokyo, Japan) extended into visible light (Fig. 1) (14). Fig. 2 shows the bacterial survival rates at different irradiation times. The bacteria added to the Group 4 samples survived at high rates (mean 83.3%) even after 120 min. In Group 1, however, the bacteria count dropped sharply, reaching 17.3% at 30 min and 0.4% at 60 min. The inhibition of bacterial survival was significantly greater in the Group 1 samples than in the other group samples at irradiation times between 30 and 60 min (ANOVA: $P < 0.05$). The rate of bacterial survival gradually fell in the 0.5% mixture of the other soluble substances without TiO_2 (Group 5), but it remained as high as 93.9% at 30 min and 45.1% at 60 min.

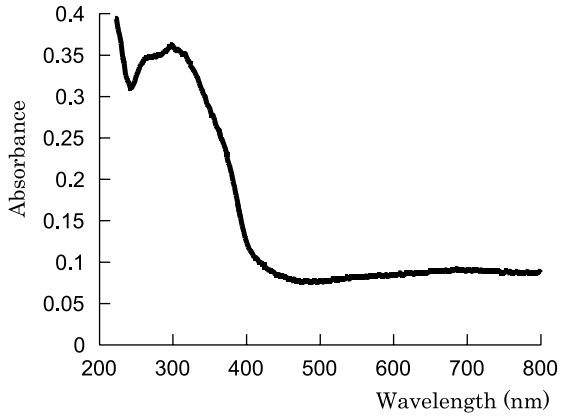

Fig. 1. Absorption spectrometry. A portion of the absorption band extended into visible light regions (>400 nm).

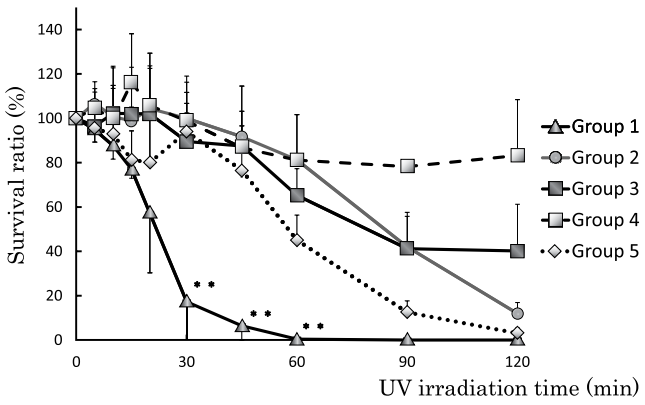

Fig. 2. Bacterial survival rate of *S. aureus*. The inhibition of bacterial survival was significantly greater in Group 1 than in the other groups at irradiation times between 30 and 60 min ($P < 0.05$).

The mixture of TiO_2 particles in water elicited highly efficient light absorption and enabled greater and more frequent adhesion with bacteria. These effects are conducive to a strong photocatalytic antibacterial action. Yet bioactive materials such as TiO_2 can be potently biotoxic. Before formulations of this type can be considered for medical applications, the physiological effects of the TiO_2 must be precisely understood. By mixing TiO_2 with soluble substances not reported in previous investigations, our group developed a TiO_2 particle mixture which exhibits improved photocatalytic activity at even lower TiO_2 concentrations (0.019 mg/mL) (11-13). 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. Therefore, citric acid is added to adjust the aqueous pH to a neutral or low alkalinity (pH 8.0).

The bactericidal capabilities of UV are widely recognized. Our present study adds further evidence of these bactericidal capabilities by revealing a gradual deactivation of *S. aureus* with increased irradiation time in Group 3. Yet from 30 to 60 min of UV irradiation, the samples treated with the TiO_2 mixture + UV (Group 1) showed significantly greater inhibition of bacterial survival than the other sample types, including Group 3 and Group 5. Although sodium percarbonate becomes (hydrogen) peroxide in disinfectant solutions, its antibacterial effect was inferior to that of Group 1 in our experiments. These findings indicate that the photocatalytic action of the TiO_2 particles was effectively expressed against *S. aureus*.

We note that the negative effects of UV rays on the human body pose potential problems in clinical applications. A good deal of research is underway to resolve these problems using materials with photocatalytic actions triggered by visible light (15,16). 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 (Fig. 1) (14). The gradual reduction in the bacterial survival rate seen in Group 2 may have been a response to the effects of sunlight or fluorescent light within the laboratory. Further research will be needed to evaluate the antibacterial effects of visible light only.

Our present experiments have revealed that when TiO_2 particles are reacted with an oxidizer, they have superior photocatalytic antibacterial effects against *S. aureus* even at low particle concentrations. Further laboratory studies under more sophisticated conditions will clearly be required for comprehensive evaluation. In the meantime, these simple configurations with the TiO_2 particle mixture are particularly encouraging as tests for use in the first stages of assessment. Our simple study allowed for greater control over experimental variables and produced fewer artifacts in the results.

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