Short Communication

The Bactericidal Efficacy of a Photocatalytic TiO₂ Particle Mixture with Oxidizer against Staphylococcus aureus

Tomohiko Asahara, Hironobu Koseki*, Toshiyuki Tsurumoto, Koutaro Shiraishi, Hiroyuki Shindo, Koumei Baba1, Hiroshi Taoda2, and Nao Terasaki1

Department of Orthopedic Surgery, Graduate School of Biomedical Science, Nagasaki University, Nagasaki 852-8501; 1Industrial Technology Center of Nagasaki, Nagasaki 856-0026; and 2Materials Research Institute for Sustainable Development, National Institute of Advanced Industrial Science and Technology, Aichi 489-0884, and 3Measurement Solution Research Center, National Institute of Advanced Industrial Science and Technology, Saga 841-0052, Japan

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SUMMARY: By proving the bactericidal effects of a low-concentration titanium dioxide (TiO₂) 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 1×10⁵ CFU/ml was added dropwise to a TiO₂ particle mixture (19 ppm TiO₂) 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 TiO₂ mixture + UV sample, the bacteria count dropped sharply, reaching 17.3% of the baseline value at 30 min and 0.4% at 60 min. TiO₂ particles dispersed in water mixtures are known to elicit highly efficient UV absorption and greater bonding to bacteria. A reaction of the TiO₂ with another oxidizer altered the aqueous pH and accelerated the photocatalytic chemical reaction. The TiO₂ 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 (TiO₂) as a potentially useful solution to the problems described above. On exposure to ultraviolet (UV) irradiation, TiO₂ releases free radicals such as •OH, O₂⁻, HO₂⁻, and H₂O₂. 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 TiO₂ in water. Several reports have been published on the bactericidal properties of TiO₂ against organisms such as Escherichia coli (11-13). Yet TiO₂ is a bioactive substance which may remain in trace amounts in the human body. Before TiO₂ 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 TiO₂ particle mixture with a low concentration of TiO₂ in order to evaluate the photocatalytic antibacterial effects of the mixture against S. aureus in vitro.

TiO₂ 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 m²/g. A powder was then prepared by mixing these TiO₂ 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 TiO₂ particles.

S. aureus (strain Seattle 1945) was cultured for 6 h at 37°C, then centrifuged to provide a bacterial sample with a concentration of 1×10⁵ CFU/mL (pH 7.0). After adding 40 μL of the bacteria dropwise to 40 μL of the TiO₂ 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/cm²; wavelength, 352 nm). The bacterial sample in the TiO₂ 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-

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Content (%)</th>
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<tbody>
<tr>
<td>Percarbonate</td>
<td>37</td>
</tr>
<tr>
<td>Metasilicate sodium</td>
<td>6</td>
</tr>
<tr>
<td>Citric acid</td>
<td>31</td>
</tr>
<tr>
<td>Sodium tripolyphosphate</td>
<td>25</td>
</tr>
<tr>
<td>Magnesium silicate</td>
<td>0.5</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.38</td>
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</tbody>
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*Corresponding author: Mailing address: Department of Orthopedic Surgery, Graduate School of Biomedical Science, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Tel: +81-95-819-7321, Fax: +81-95-849-7325, E-mail: f2101@cc.nagasaki-u.ac.jp
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.

**REFERENCES**


