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Photocatalytic bactericidal action of fluorescent light in a titanium dioxide particle mixture: an in vitro study

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Abstract

Traditional titanium dioxide (TiO_2) has photocatalytic bactericidal properties only under ultraviolet (UV) irradiation, which restricts its use in clinical treatment regimens. In this study, we evaluated the photocatalytic bactericidal effects of an aqueous system of TiO_2 particles irradiated by fluorescent light (FL) on *Staphylococcus aureus*. A TiO_2 particle mixture containing 19 ppm (0.019 mg/mL) of TiO_2 was prepared. A bacterial solution of 1×10^5 CFU/mL was added one drop at a time to the TiO_2 mixture. The resulting product was then irradiated with FL. The bacterial survival rate decreased steadily in the TiO_2 mixture group, reaching 76.7% after 30 min of FL irradiation and 10.9% after 60 min. After 60 to 180 min, the bacterial survival ratio of the TiO_2 mixture group was significantly lower than that of the control group ($P < 0.05$). The present study indicates that treating the surfaces of surgical devices and the surgical field with a TiO_2 particle mixture can create a nearly sterile environment that can be maintained throughout surgery, even at low luminous intensities.

When titanium dioxide (TiO_2) is exposed to ultraviolet (UV) light, various free radicals are released. These free radicals are a potent oxidant that can decompose bacteria and other organic substances (4, 7). In a previous study by the authors, a TiO_2 particle mixture was prepared in order to use its photocatalytic action to prevent postoperative infection. In comparison to the morphological characteristics of thin-film and large-particle TiO_2 formulations, the use of fine particles resulted in a greater surface area, improved UV exposure, and increased contact with the bacteria. This combination of factors provided potent antibacterial effects against *Staphylococcus aureus* (8). However, the physiological effects of the UV rays used to elicit photocatalysis must also be considered. This study evaluates the photocatalytic bactericidal effects on *Staphylococcus aureus* of an aqueous system of TiO_2 particles irradiated with fluorescent light (FL) containing no UV light whatsoever and having a lower luminous intensity than sunlight.

TiO_2 particles were prepared from titanium (IV) chloride gas using the vapor phase method followed by annealing at room temperature. The surfaces of the particles were rutile (20%) while the interiors were anatase (80%). A powder was prepared by mixing these TiO_2 particles with other substances, mainly sodium percarbonate and citric acid, in order to adjust the aqueous pH and generate a reaction with the TiO_2 (MEK-01; Nanowave, Aichi, Japan). The TiO_2 concentration was 0.38%. The mixture

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was dispersed in distilled water to create a 0.5% mixture containing 19 ppm TiO_2 particles (pH 8.0). The shape and dispersion of the TiO_2 particles in the aqueous mixture were monitored using a scanning electron microscope (SEM) (S-4300; Hitachi, Tokyo, Japan) with formbar support film (STEM150Cu grid; Okenshoji, Tokyo, Japan). The reflection absorption spectrum of the TiO_2 particles and the transmission absorption spectrum of the aqueous mixture were measured by means of UV-VIS-NIR spectrophotometry (V-660; JASCO, Tokyo, Japan).

The bactericidal properties of the TiO_2 particle mixture were evaluated using *Staphylococcus aureus* (strain: Seattle 1945). The bacterial sample was cultured for 6 h at 37°C in a trypticase soy broth medium and then centrifuged. After adjusting the concentration to 1×10^5 CFU/mL (pH 7.0), 40 µL of the bacterial solution was added one drop at a time to 40 µL of TiO_2 solution (TiO_2 concentration 0.019 mg/mL). The test tube was then irradiated with fluorescent light (LK-H344; Twinbird, Osaka, Japan) from directly overhead (Fig. 1). The maximum irradiation time was 180 min. The bacterial sample in the TiO_2 mixture was diluted with 1000 µL of phosphate-buffered saline (PBS). All of the diluted bacterial solution was collected and then cultured for 24 h using a Compact Dry TC culture kit (Nissui Pharmaceutical, Tokyo, Japan). Colony-forming units (CFUs) were counted 5 times for each sample, and the bacterial survival rate was calculated as shown below.

$$\text{Bacterial survival rate } (\alpha \text{ min}) = \text{CFU } (\alpha \text{ min}) \times 100 / \text{CFU } (0 \text{ min})$$

The bacterial survival rates for the TiO_2 mixture and distilled water (control) were compared and results were analyzed using the two-group Mann-Whitney U-test. $P < 0.05$ was considered significant.

The white granular powder containing TiO_2 particles was dispersed quickly in distilled water at room temperature. SEM images showed the primary TiO_2 particles (approximately 20 nm in diameter) to be condensed and well dispersed in the mixture, with aggregates almost 100 nm in diameter (Fig. 2). From this, the BET surface area was calculated to be approximately 50 m^2 g^{-1}. The reflection absorption spectrum, measured by means of UV-VIS-NIR spectrophotometry, extended into the visible light range at more than 400 nm, and particularly into the longer wavelengths at 500 nm and above. The absorption bands for the transmission absorption spectrum of the aqueous mixture also extended into the visible range (Fig. 3A and 3B). Fig. 4 shows the bacterial survival rates for various irradiation times. Although the bacterial survival rate decreased gradually in the control group, the mean value after 180 min was still relatively high at 64.8%. In contrast, this value decreased steadily in the TiO_2 mixture group after FL irradiation, reaching 76.7% after 30 min and 10.9% after 60 min. The bacterial survival rate was significantly lower after 60 min of irradiation, $P < 0.05$. The *Staphylococcus aureus* was completely deactivated after 150 min of irradiation.

Surgeons consider postoperative infection to be an extremely significant complication and an issue that must be resolved (14, 16, 17). The pathogenic bacteria *Staphylococcus aureus* is one of the most common causes of postoperative infection (2, 15). Nosocomial infections by methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are a serious

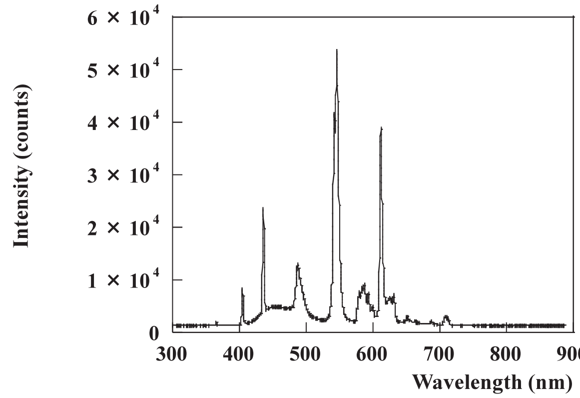

Fig. 1 Fluorescent light wavelength. Wavelength is measured at a distance of 5 cm; the same distance used in the experiment. The luminous intensity is almost 300 lux. The light itself contains no UV rays.

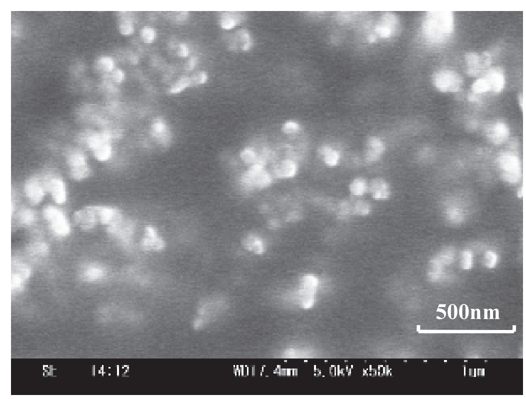

Fig. 2 SEM images of TiO_2 particles in the aqueous mixture. Aggregates approximately 100 nm in diameter are well dispersed among the primary particles. Scale bar = 500 nm

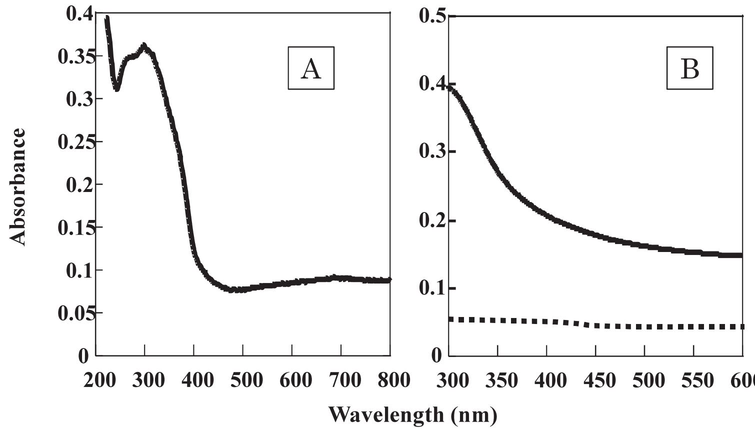

Fig. 3 Absorption spectrum analysis. A: Reflection absorption spectrum (TiO_2 particle powder). Light absorption bands are present in the visible light range at 400 nm and above. B: Transmission absorption spectrum (TiO_2 particle mixture). The TiO_2 particle mixture (solid line, 5 mg/mL concentration) shows higher active wavelengths, extending into the visible range, than pure water (dotted line).

problem, and there is a real risk that overuse of the few antibiotics currently effective against MRSA will result in the development of even more highly resistant bacterial strains (9, 10). In the present study, we evaluated the antibacterial and bactericidal effects on *Staphylococcus aureus* with the objective of preventing postoperative infection.

When TiO_2 is exposed to UV rays, the photon energy excites the valence band electrons and generates pairs of electrons and positive holes (electron-vacancy in the valence band), which, through reactions with water and oxygen, produce a variety of superoxides. This oxidizing action is more potent than that of chlorine or hypochlorous acid and is capable of degrading organic substances such as virulent strains of bacteria. Because TiO_2 has a stable chemical structure and is not biotoxic (5, 18), it is used in a wide range of environmental fields such as water quality and the removal of atmospheric pollutants (4). Traditional TiO_2 photocatalyst, however, is effective only when irradiated with UV light, which would seriously harm the human body, including the eyes and skin. The present study shows that effective bactericidal activity can be obtained from irradiation that is limited to FL, which contains no UV. Recent research in the area of visible-light reactive TiO_2 has explored Sn doping and electric field intensification (1, 3, 12, 13). Despite this advantage, photocatalyst-based technologies are still in the development stage. Their antibacterial properties cannot match those of commonly available disinfectants in postoperative infections. The TiO_2 used in this research can react with soluble constituents such as sodium percarbonate and citric acid, so it was possible for the absorption spectrum to extend into the visible light range.

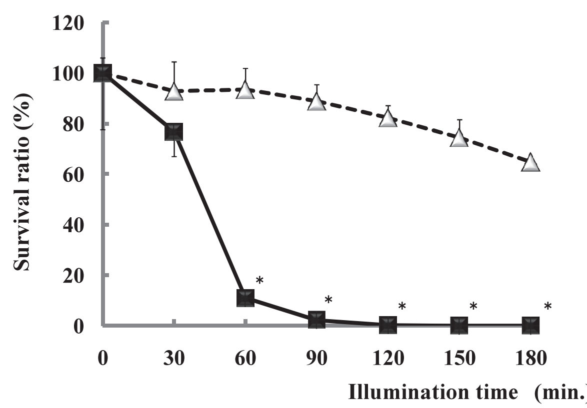

Fig. 4 Bacterial survival rate for *Staphylococcus aureus*. The TiO_2 mixture group shows significantly lower bacterial survival rates after 60 min or more of irradiation with FL (*$P < 0.05$). Control group (△), TiO_2 mixture group (■)

In the present study, a TiO_2 particle mixture exposed to FL for at least 60 min was significantly better than the control at reducing the bacterial survival rate. Even low FL luminous intensities provided effective bactericidal action against *Staphylococcus aureus*. Postoperative infections are caused by bacterial contamination of the surgical field during surgery. Because ordinary surgeries are commonly equipped with fluorescent lighting, treating surgical device surfaces with this TiO_2 particle mixture can ensure a nearly sterile surgical environment and effectively prevent postoperative infections. TiO_2 must be exposed to light for it to have a photocatalytic effect, so the substance's antibacterial properties would be activated only during surgery, when bacterial contamination is most likely to occur. After the surgical wound is closed, any TiO_2 within the body becomes chemically inactive and has no negative effects on the physiological environment. In this respect, TiO_2 differs greatly from devices containing silver ions or antibiotics (6, 11). In addition, the photocatalytic action of TiO_2 is nonselective with regard to the bacterial strain, which means that it can effectively prevent postoperative infections and that its use will not induce bacterial resistance.

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