Productivity of Reactive Oxygen Species by Low-Intensity Focused Ultrasound Irradiation to Titanium Dioxide Particles

1. Introduction

Titanium dioxide (TiO₂), known as photocatalyst, produces reactive oxygen species (ROS) such as hydroxyl radical (OH⁻) by ultrasound irradiation1). Recently, cancer therapy using citotoxicity of ROS produced by ultrasound irradiation to TiO₂ particles (sonodynamic therapy) has been studied2). In addition, TiO₂ particles can be applied for infection prevention. In our group, anti-infective catheter system for the exit site of catheter retained in the skin was proposed3, 4). For producing ROS less-invasively, using focused ultrasound is considered to be effective. However, optimal conditions of ROS generation by low-intensity focused ultrasound irradiation have not been investigated. To decide the optimal irradiation conditions, more understanding of mechanism of ROS generation by ultrasound irradiation was required. We hypothesized two mechanisms of ROS generation. One is that TiO₂ (anatase type) works as photocatalyst. When rupture of cavitation bubbles occurs, sonoluminescent light including ultraviolet range is emitted5). Anatase type TiO₂ is activated by light at the wavelength of 387 nm and OH⁻ is generated. The other is that TiO₂ works as a reflector of ultrasound. It is reported that cavitation of water can generate ROS such as OH⁻ and singlet oxygen (¹O₂6, 7). (¹O₂ is generated under the presence of dissolved oxygen.) When TiO₂ particles exist in water, negative sound pressure increases by ultrasound reflection and cavitation threshold became smaller. Therefore, ROS is considered to be generated at less intensity than without particles.

In this study, we compared the productivity of ROS by low-intensity focused ultrasound irradiation to TiO₂ (anatase type), TiO₂ (rutile type), and aluminum oxide (Al₂O₃) particles by fluorescence measurement, and investigated which mechanism is mainly contributed to ROS generation.

2. Materials and Method

4 mg of anatase type TiO₂ (particle size d = 0.15 µm, Ishihara Sangyo Kaisha A-100), rutile type TiO₂ (d = 0.25 µm, Ishihara Sangyo Kaisha CR-EL) and Al₂O₃ (d = 0.3 µm, Denka ASFP-20 d50) were respectively added to 2 mL of ultrapure water (2 g/L). As a control condition, 2 mL of ultrapure water without any particles was also prepared. As a fluorescence reagent of ROS, 2 µL of aminophenyl fluorescein (APF, 5 mmol/L, Sekisui medical 423673) were added in each suspension and water. Suspensions and water were respectively put into sample tanks with diameter of 15.8 mm. The bottom of sample tanks was consist of natural rubber with the thickness of approximately 0.1 mm.

A transducer of focused ultrasound (frequency: 500 kHz) with an aperture element diameter of 40 mm and focal length of 30 mm were set at the bottom of water bath. The sample tank was set into the water bath as shown in Fig. 1. The distance between the bottom of sample tank and the transducer was 30 mm. The transducer driven by burst mode of sinusoidal wave current generated by a function generator (Tektronix AFG 3021B), and amplified by a power amplifier (NF HSA4101) at an applied voltage of 50 V. Therefore, the transducer produced pulsed ultrasound. Pulse repetition rate was 1 kHz and the pulse width were 100 µs (Duty ratio: 10%). Then, focused ultrasound were irradiated to each sample tank for 10 minutes. During this experiment, samples were covered with aluminum foil to avoid surrounding ultraviolet light.

After ultrasound irradiations, sample suspensions were centrifuged for 5 min at 5000 rpm, and 100 µL of supernatant solution were put into 96-well microplate. A microplate reader (Biotek Synergy HTX) were used for fluorescence measurement that shows the presence of ROS (OH⁻). Excitation and emission wavelengths were 485±20 and 528±20 nm.

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References

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3. Results and discussion

In degassed ultrapure water bath, sound pressure at the focal point was measured at this condition by hydrophone (Onda HNP-0200), which was 1.56±0.05 MPa. The intensity at spatial peak and time average (I_{SPTA}) and the mechanical index (MI) were calculated to be 1.286±0.007 W/cm² and 2.10±0.07.

Fig. 2 shows the fluorescence intensity of anatase type TiO₂, rutile type TiO₂ and Al₂O₃ suspensions and water with/without ultrasound irradiation. In all particles, fluorescence intensity was increased by ultrasound irradiation. Therefore, all particles produced ROS (OH⁻) by focused ultrasound irradiation. If anatase type TiO₂ worked as photocatalyst, fluorescence intensity of anatase type TiO₂ suspension should be greater than the suspensions of other particles. However, in this experiment, that was not significantly higher. Therefore, this result suggests that the effect of reflection at the surface of particles contributed ROS generation. To verify this mechanism, the difference of sound pressure or cavitation threshold with and without ultrasound should be investigated.

To enhance ROS generation, smaller particle is considered to be effective because it has larger reflection area at the same amount (mass). In addition, lower frequency ultrasound that can cause cavitation more easily than high frequency will be effective. However, directivity become lower and the size of transducer increases at lower frequency. Therefore, as a next step, the relationship between the frequency and ROS productivity should be investigated, and the suitable frequency for practical use should be decided.

In this experiment, fluorescence intensity without ultrasound irradiation was different between all particles and no particles. Some reasons are considered; the adsorption of fluorescence reagent at the surface of particles, insufficient light shielding or centrifuge.

According to this result, not only TiO₂, but also other particles that well reflects ultrasound might be useful. Therefore, further investigation of suitable materials for practical use is required.

4. Conclusion

The productivity of OH⁻ by low-intensity focused irradiation of TiO₂ (anatase and rutile types) and Al₂O₃ were evaluated. OH⁻ generation was confirmed in all suspensions with ultrasound irradiation. Therefore, ultrasound reflection at the surface of particles are suggested to be the main cause of ROS generation.

References