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Characterization on titanium surfaces and its effect on photocatalytic bactericidal activity

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ABSTRACT

Many studies have been published on the use of TiO_2 as a photocatalyst, which decomposes various organic compounds under UV illumination by generating various radicals. The purpose of the present study was to evaluate the photocatalytic bactericidal effects of variously treated titanium surfaces on *Escherichia coli* K-12. The specimens were fabricated from grade 4 commercially pure titanium, 12 mm in diameter and 1 mm in thickness. Five different surfaces were prepared (MA: machined surface; AO: anodized at 300 V; NO: NaOH-treated; NW: NaOH- and water-treated; and HT: heat-treated). Surface analysis was performed using scanning electron microscopy, optical interferometer, and thin-film X-ray diffractometry. Photocatalytic activity of each group was confirmed by degradation of methylene blue (MB). The antibacterial activity was assessed by calculating the survival ratio in a drop of a culture of *E. coli* placed on the surface under UV illumination. Significant photocatalytic activity and bactericidal effects were observed on the titanium surfaces of AO and NW, regardless of the surface roughness ($P < 0.01$). The group with anatase was the most susceptible to the photocatalytic effect, while the surface without anatase showed the least susceptibility. Based on this in vitro study, the crystallography of the oxide layer on its titanium surfaces is an important factor affecting the photocatalytic bactericidal activity.

1. Introduction

Different characteristics on the Ti surfaces will be obtained in the different conditions of the surface treatment. Surface roughness provides a favorable condition for the biological response in bone–implant interface [1]. However, a high risk of bacterial infection is one of the concerns in the rough implant surfaces. Rough surfaces harbor significantly higher amounts of microorganisms than smooth surfaces [2,3], although there is no correlation between the type of surface and the selection of aggressive colonizing bacterial species [3,4].

The excellent corrosion resistance of titanium is due to the spontaneous formation of the highly inert, tenacious oxide film which is formed even when exposed to a mere trace of oxygen or moisture [5]. Much research has been carried out to identify the crystallography of titanium oxides formed on titanium surfaces [6,7]. Among modifications of titanium surfaces, anodic oxidation of titanium metal and its alloys in simple electrolytes would produce anatase on their surfaces by conditioning the anodic oxidation process [8]. Recently, it was found that titanium metal and its alloys subjected to NaOH and heat treatments show apatite-forming abilities. The sodium titanate gel on the metal formed from the NaOH treatment is shown to transform into anatase by a simple immersion in pure water. On the factors that influence the crystallization of TiO_2 into anatase, Yanagisawa et al. [9] reported that the presence of H_2O was a significant boost. Anatase formation can be greatly enhanced by conjoining simple water and subsequent heat treatments [10].

The crystalline titania, such as anatase and rutile, behave like n-type photoconductors [8]. When such materials are illuminated with a light of an appropriate wavelength, electron–hole pairs are produced in the oxide by the transfer of a valence band electron to conduction band.

Anatase titanium dioxide (TiO_2) displays relatively high photocatalytic activity when illuminated with near-UV light (350–380 nm) [11]. Upon being excited by UV light of energy greater than the band gap (3.2 eV), the photon energy generates an electron–hole pair on the TiO_2 surface. The hole in the valence band can react with H_2O or hydroxide ions adsorbed on the surface to produce hydroxyl radicals ($OH^{\bullet}$), and the electron in the conduction band can reduce O_2 to produce superoxide ions (O_2^-) [12]. Both holes and ($OH^{\bullet}$) are extremely reactive upon contact with organic compounds. In

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an organic containing aqueous solution, hydroxyl radicals prefer oxidizing the organic substance [13,14]. This property can be applied for killing bacteria. Photocatalytic activity in TiO_2 has been extensively studied because of its wide range of functions and the detailed mechanism has been well-documented [15–17].

The authors hypothesized that there would be differences in UV induced photocatalytic bactericidal effects due to various surface treatments of titanium specimens. In order to evaluate this relation, this study is focused and designed on the relation between surface characteristics, especially morphology, roughness, and crystalline structure of the oxide layer, and photocatalytic bactericidal effects. The prepared titanium specimens were assessed by calculating the survival ratio in a drop of a culture of *E. coli* placed on the surface using both control without UV illumination and a Petri dish model.

The purpose of the present study was to evaluate the photocatalytic bactericidal effects of variously treated titanium surfaces on *Escherichia coli* K-12.

2. Experimental

2.1. Specimen preparation

The disks were fabricated from grade 4 commercially pure titanium (Dynamet, Carpenter Technology Co., Washington, PA, USA), 12 mm in diameter and 1 mm in thickness. Five different surfaces were prepared as follows:

MA: machined surface.
HT: heat-treated surface.
NO: NaOH-treated surface.
NW: NaOH- and water-treated surface.
AO: anodized surface under 300 V.

The disks of MA were ultrasonically cleaned using a soap solution for 4 h, washed in running water and finally rinsed in distilled water and air dried. HT were heat-treated at 600 °C for 2 h. NO disks were treated with 10 M NaOH at room temperature for 24 h. The disks of NW were treated with 10 M NaOH at room temperature for 24 h and subsequently subjected to water-treatment at room temperature for 96 h. And finally those in AO disks were anodized in 0.25 M H_2SO_4 and H_3PO_4 at 300 V. All specimens were packed, sealed and sterilized with ethylene oxide gas (130 °C, 10 psi, 3 h).

2.2. Surface analyses

Micrographs of the disks were observed by using a scanning electron microscope (JSM-840A, JEOL, Tokyo, Japan). The SEM micrographs were taken at several randomly chosen areas on each specimen.

The surface roughness was measured by an optical interferometer (Accura 2000, Intekplus Co., Seoul, Korea). This system provides the following numerical values for the different surface roughness parameters: Ra, which is defined as the arithmetical mean deviation of the assessed profile, in micrometers. Rq, which is the root mean square deviation of the assessed profile, in micrometers. Rt, which is the total height of the profile, in micrometers. The area of measurement was 50 μm × 50 μm. Five readings were made for each surface randomly on five samples and the results were averaged. And phase components were analyzed using thin-film X-ray diffraction (TF-XRD: X'Pert PRO, PNAalytical, Almelo, The Netherlands).

2.3. Evaluation of photocatalytic activity

The photocatalytic activity of the specimen was evaluated by the degradation of methylene blue [18]. The concentration of the standard methylene blue solution used was set to 10 ppm. To eliminate reduction in the concentration of methylene blue owing to absorption by the specimens, the specimens were soaked in 100 ml of the standard methylene blue solution for 24 h prior to the test. Each disk was immersed in 1000 μl of the standard methylene blue solution. The specimens were then illuminated with a UV light, a type F15T8BLB black light blue lamp (Sankyo Denki, Kanagawa, Japan), for 100 min. The light intensity was 2.0 mW/cm^2 at a peak wavelength of 352 nm. The light source was placed 10 cm above the samples.

UV illumination was performed according to the following protocol:

(1) Experimental group: 5 different specimens with UV illumination.
(2) Control group: 5 different specimens without UV illumination.

For the control group, the light was covered with an aluminum foil during the experiment. After UV illumination the solution was retrieved, and the methylene blue concentration was calculated with the optical density (OD) value. A UV–vis spectrophotometer (Lambda 25, PerkinElmer, Waltham, MA, USA) was used to estimate the concentration of the unreacted methylene blue by measuring the attenuation at its absorption maximum of 665 nm at 20 min intervals up to 100 min. Each of the five disks was evaluated, and the mean methylene blue concentration value was recorded.

2.4. Evaluation of bactericidal effect

The ability of the titanium surfaces to destroy bacteria under UV illumination was monitored in vitro. The specimen surfaces were exposed to liquid cultures of *E. coli* cells and incubated for 24 h at 37 °C in 10 ml Todd–Hewitt (TH) broth and then suspended in sterilized distilled water to a concentration of 8×10^6 cells/ml. A total of 1 ml of the suspension was added onto the disk. The disks were illuminated with UV, according to the same protocol as that applied for the methylene blue degradation test.

UV illumination was taken at 40 min and 80 min. The bactericidal effect in each group was represented by the bacterial survival ratio. The viable cells in each group were counted with FACS (BD Biosciences, San Jose, USA). The bacterial survival ratio after UV illumination was compared with that of the control (a Petri dish). The following equation was used to represent antibacterial activity:

$$\text{survival ratio (\%)} = \left(\frac{\text{the viable cells count after irradiation}}{\text{the viable cells count before irradiation}}\right) \times 100$$

Three replicate experiments were performed.

2.5. Statistical analysis

The mean and standard deviation of the topographic parameters of the disks ($n = 5$) and of the survival ratio of the bacterium cells ($n = 3$) were calculated. Statistics were derived by an analysis of variances (ANOVA), followed by post Duncan's test for a post hoc comparison, with the value of statistical significance set at 0.01.