Photocatalytic bactericidal property of an anodized Ti6Al4V alloy

P. Muraleedharan*, Judy Gopal, R. P. George and H. S. Khatak

Corrosion Science and Technology Division, Indira Gandhi Centre for Atomic Research, Kalpakkam 603 102, India

Anodization of titanium and its alloys produces a thin film of TiO_2, which is reported to exhibit photocatalytic activity when exposed to near-UV light (<380 nm). In this study, the influence of illumination by 'black light blue' fluorescent lamps on the adhesion of bacterial cells on acid-pickled as well as anodized Ti6Al4V specimens was carried out by exposure in a 0.1% nutrient culture of *Pseudomonas* sp. There was significant reduction in the attachment of the bacterial cells on the anodized surface in the presence of illumination. There was also reduction in the number of viable cells in the liquid medium in which the specimens were exposed. However, no reduction in the bacterial density of the nutrient culture was observed when it was illuminated without any titanium alloy specimens. This suggests that the anodized titanium alloy surface exposed to near-UV light exhibits strong photocatalytic bactericidal effect.

CERTAIN materials (oxides, sulphides, etc.) act as photoconductors on illumination with near-UV photons. If the two photoinduced charge carriers (electrons and holes) do not recombine, they can reach the surface and react with chemisorbed species giving reduction/oxidation reactions[1,2]. The naturally abundant titanium oxide (TiO_2) exists in three crystalline forms: brookite (orthorhombic), anatase (tetragonal) and rutile (tetragonal). Anatase has a higher degree of tetragonality than the rutile structure and is also less closely packed[3]. In recent years TiO_2, especially the anatase phase (band gap, 3.2 eV), is well known as a semiconductor with strong photocatalytic activity and has a great potential for applications such as environmental purification, decomposition of carbonic acid gas and generation of hydrogen gas[4–6]. This property of TiO_2 is being extensively used in the removal of organic chemicals in effluents[7]. Photocatalytic oxidation has been developed as an environmentally benign approach to waste-water remediation using natural sunlight. Commercial processes for waste-water treatment using TiO_2-based photocatalytic oxidation are now available[8]. In fact, this property of TiO_2 has been exploited in the development of total organic carbon analyser[9,10], in which the organic compound present in a liquid sample is oxidized photocatalytically and the CO_2 generated is estimated. Since the photocatalytic oxidation process occurs at near-UV wavelengths (300–400 nm), borosilicate glasses and inexpensive, long-lived 'black light blue' (BLB) fluorescent lamps can be used. There have also been attempts to coat stainless steel and other materials for domestic and medical application with a thin film of anatase so that disinfection can be achieved by illumination of the surfaces. Anodization of titanium and titanium alloys, which develops a thin film of predominantly anatase-type TiO_2, has been investigated in order to offer value-added titanium sheets with antibacterial, antifouling and deodourizing properties[11]. However, much work has not been reported in the open literature. Titanium and its alloys are extensively used in various industrial applications, especially in heat exchangers where sea water is the coolant, because of excellent corrosion resistance arising from stability over a wide potential-pH regime[12]. Since titanium and its alloys have good biocompatibility, they are highly prone to attachment of micro- and macro-organisms present in sea water. A study was carried out to understand the effect of near-UV light illumination of anodized titanium on the adhesion of *Pseudomonas* sp., a film-forming bacteria.

Ti6Al4V, a titanium alloy was used in the study. Specimens in the form of 30 mm diameter and 2 mm thick discs were acid-pickled by dipping in an acid bath (HNO_3 400 g/l + HF 40 g/l + water) for 10 min to remove oxide scales present on the surface. The thin amorphous oxide film of TiO_2 present on acid-pickled surface generally has a thickness of about 1.5 nm (ref. 3). The acid-pickled specimens were rinsed thoroughly in distilled water, ultrasonically cleaned in detergent solution followed by distilled water and dried. These specimens were anodized in 0.1% orthophosphoric acid solution at 50 V for 10 min at ambient temperature. The presence of anatase-type TiO_2 on the anodized surface was confirmed by glancing angle X-ray diffraction studies, which showed a peak at the 2θ value of 25.3°. The photocatalytic activity of the anodized specimens was evaluated by immersing the specimens in 0.1 M KI solution (with starch indicator added) for 2 h under illumination by BLB lamps. The solution turned blue, indicating photocatalytic decomposition of KI to iodine, giving a blue colour in the presence of starch.

Bacterial adhesion studies were performed in a 0.1% nutrient culture of *Pseudomonas* sp., isolated from a freshwater open reservoir at Kalpakkam. The nutrient culture was prepared by inoculating 375 ml of autoclave-sterilized 0.1% nutrient broth (0.013 g/l) with 1 ml of 24-h-old, actively-dividing culture of the bacterial species in 100% nutrient broth and then homogenizing the culture by shaking for 30 min using an orbital shaker. Exposure studies were carried out in the 0.1% nutrient culture contained in a cylindrical glass vessel (60 mm inner diameter and 200 mm height) with a Teflon lid. The specimens were introduced into the glass vessel containing the bacterial culture 24 h after inoculation. Exposure tests were conducted both under illumination and under dark

*For correspondence. (e-mail: pmurali@igcar.ernet.in)

conditions. The specimens were suspended from glass pegs radiating from a glass rod which is held in position by the Teflon lid. The specimens were arranged in such a fashion that light falls uniformly over both the surfaces. Illumination of the specimens was done by arranging six BLB lamps (4 W, Philips) around the specimen chamber in a hexagonal configuration (at the corners of a regular hexagon with 150 mm length of each side). The nutrient culture was also illuminated without any specimens in order to find the effect of illumination on the suspended cells. Specimens were taken out after four days and 11 days and total viable count (TVC) of bacteria on the specimens was estimated by plate count method. For this, after gently rinsing with phosphate buffer, the specimens were ultrasonicated in 10 ml of the phosphate buffer. Various dilutions of this bacterial cell suspension was prepared and 0.1 ml of each of these dilutions was plated onto nutrient agar plates. The plates were incubated at a temperature of $32 \pm 2°C$ for 48 h and the number of colonies on the plates was counted. From these plate counts, the number of colony-forming units per unit area (cfu/cm^2) of the titanium specimens was calculated. The specimens were also stained with acridine orange dye and examined under epifluorescence microscope. The bacterial cell density in the liquid medium was also estimated at the beginning of the experiment and after four days.

The TVC of bacteria on various specimens, estimated by the plate count method is presented in Table 1. The TVC of bacteria on the anodized surface after four-days of exposure was three orders of magnitude less on the illuminated specimens (2.6×10 cfu/cm^2) compared to the specimens exposed under dark conditions (2.8×10^4 cfu/cm^2). The corresponding values for acid-pickled specimens were 2.9×10^3 cfu/cm^2 and 4.6×10^4 cfu/cm^2. The bacterial attachment was two orders of magnitude less on anodized specimen when compared to acid-pickled specimen under illumination. The bacterial density of the liquid medium also decreased from an initial value of 6.0×10^5 cfu/ml to 6.6×10 cfu/ml at the end of four days in the vessel containing anodized specimens under illumination. However, the bacterial density of the medium in which titanium alloy specimens were exposed in the dark condition was 1.5×10^3 cfu/ml. When the bacterial culture was illuminated without the titanium specimens, the bacterial density increased slightly from 1.0×10^5 to 1.4×10^5 cfu/cm^2 after four days. Under dark condition, the increase in bacterial density was much higher, to a value of 1.0×10^7 cfu/cm^2. The above results clearly indicate that there is a significant reduction in the bacterial numbers on anodized specimens under illumination. Illumination has little effect on the bacterial cells in the liquid medium if anodized titanium is not present in the medium. TVC on the anodized surface after exposure for 11 days was 8 cfu/cm^2 under illumination and 4.1×10^3 cfu/cm^2 under dark conditions. In the case of acid-pickled specimens, the corresponding values were 1.4×10^3 cfu/cm^2 and 4.5×10^3 cfu/cm^2.

The results of direct acridine orange counts gave a similar trend (Table 2), except that bacterial cell numbers were much higher than those estimated by the plate count method. This is expected since all the cells on a surface will not grow into colonies when transferred onto nutrient agar plate. It is also possible that cells with partial cell damage due to photocatalytic activity of the anodized surface may give fluorescence counts, but will not grow in nutrient agar. Attachment of *Pseudomonas* sp. on acid-pickled and anodized titanium alloy surfaces under near-UV illumination is shown in Figure 1.

The results of this study have shown that there is a three order of magnitude decrease in the TVC of bacteria on the anodized surface under near-UV light illumination. There is no significant difference in the TVC values of anodized and acid-pickled specimens exposed under dark conditions. Between the dark and illuminated specimens of the acid-pickled alloy, TVC on the illuminated specimen was an order of magnitude less compared to dark specimen. This shows that the photocatalytic effect is more pronounced on the anodized surface when compared to the acid-pickled surface. As mentioned earlier, anodization of a titanium alloy produces anatase-type of oxide on the surface. The thickness of the oxide increases with the voltage used for anodizing and for the 50 V employed in the present study, the thickness of the anatase film could be approximately 100 nm (ref. 3). When illuminated with photons of wavelength less than 380 nm, anatase-type of TiO_2 produces electron–hole

Table 1. Total viable count of bacteria on titanium alloy surface under various conditions of exposure

Specimen (exposure condition)	Total viable count (mean value) cfu/cm^2	
	Four days	Eleven days
Anodized (dark)	2.8×10^4 (8200)*	4.1×10^3 (770)
Anodized (illuminated)	2.6×10 (15)	8 (4)
Acid-pickled (dark)	4.6×10^4 (4800)	4.5×10^3 (1400)
Acid-pickled (illuminated)	2.9×10^3 (840)	1.4×10^3 (10)

*Standard deviation for three specimens is given in parentheses.

Table 2. Direct acridine orange count of bacterial cells on titanium alloy surface exposed to 0.1% nutrient culture of *Pseudomonas* sp.

Specimen (exposure condition)	Total bacterial count (mean value) cfu/cm^2	
	Four days	Eleven days
Anodized (dark)	6.1×10^4 (11000)*	9.9×10^3 (2700)
Anodized (illuminated)	2.1×10^2 (115)	2.0×10^3 (800)
Acid-pickled (dark)	9.8×10^4 (24000)	3.0×10^4 (8000)
Acid-pickled (illuminated)	5.5×10^4 (15000)	3.7×10^3 (2500)

*Standard deviation for three specimens is given in parentheses.

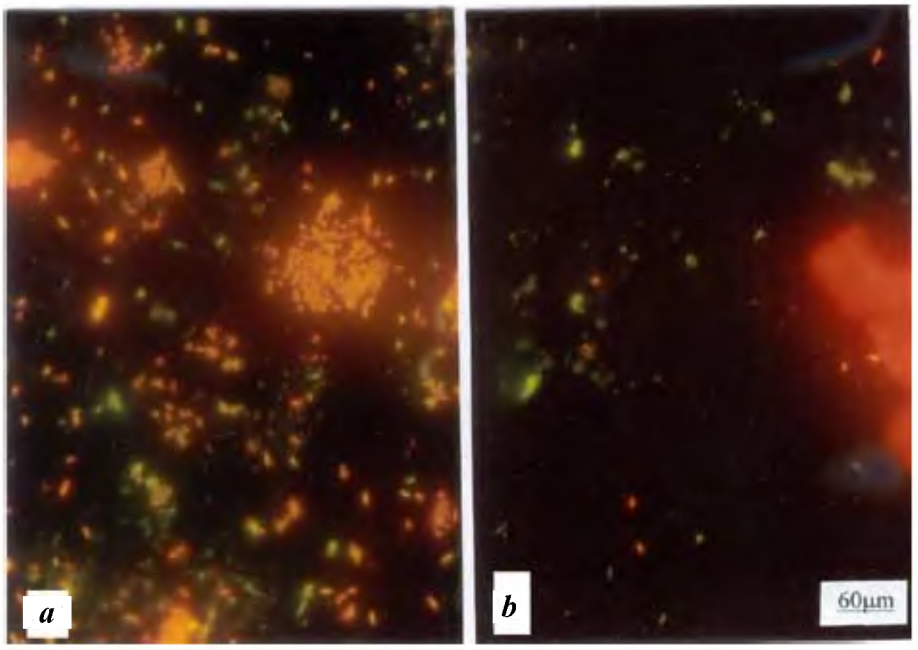

Figure 1. Epifluorescence micrograph of *Pseudomonas* sp. attached to titanium alloy surface under illumination: ***a***, Acid-pickled, and ***b***, Anodized.

pairs in the oxide. This has a very strong oxidizing force and produces highly reactive free radicals like $^{\cdot}OH$ and $^{\cdot}O_2^-$. These free radicals will sterilize microbial cells attached to the surface, explaining the reduced TVC on the anodized surface. Since bacterial attachment on a substratum is a dynamic process involving adhesion of bacterial cells, growth and detachment, it is possible that cells damaged due to photocatalytic activity of the surface get detached, and actively dividing cells adhere to the surface. By this continuing process of surface attachment, photocatalytic oxidation and detachment, most of the cells in the liquid medium also are sterilized as evident from the very low bacterial counts in the liquid medium in which anodized titanium specimens were exposed. Direct sterilization of bacterial cells suspended in the medium is not considered feasible for the UV wavelength produced by the BLB lamps. This is evident from the observation that illumination had little effect on the suspended bacterial cells in the absence of anodized titanium specimens. Thus the present investigation has clearly shown photocatalytic bactericidal activity of anodized titanium alloy surface under near-UV illumination.

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