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Photocatalytic inhibition of microbial fouling by anodized Ti6Al4V alloy

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Abstract Biofouling is one of the major concerns in the use of titanium, an excellent material with respect to corrosion resistance and mechanical properties, for seawater-cooled condensers of power plants. Earlier studies conducted in our laboratory have shown that anodized titanium with a thin film of anatase (TiO_2) inhibits attachment of *Pseudomonas* sp. of bacteria when illuminated with near-UV light (350–380 nm) from black light blue (BLB) florescent lamps. The following work compares the photocatalytic efficiencies of anodized commercially pure titanium (grade 2) and Ti6Al4V alloy, in order to understand the role of the alloying elements such as Al and V on the photocatalytic activity in relation to inhibition of microbial attachment. The study was carried out by employing both methylene blue (MB) dye degradation as well as microbial adhesion experiments under near-UV light illumination. The results have shown that the anodized Ti6Al4V surfaces showed an order of magnitude increase in photocatalytic activity, as shown by the decrease in microbial attachment compared to titanium grade-2. The oxide film on both the surfaces has been characterized using Glancing Incidence X-ray Diffraction (GIXRD) and Atomic Force Microscopy (AFM). The GIXRD and AFM results showed that the oxide formed on anodized Ti6Al4V surface has higher crystallinity and is composed of particles, which are smaller in size; both these attributes are reported to enhance the photocatalytic activity. Since, vanadium is reported to shift the photoresponse of the photoactive anatase thin film into visible range, the photocatalytic activity of anodized Ti6Al4V was also studied under visible light and it was observed that the surfaces showed significant photocatalytic activity even under visible light.

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Introduction

The use of photocatalysts to destroy organic compounds in contaminated air or water has been extensively studied for the last 25 years. Many organic compounds can be decomposed in aqueous solution in the presence of TiO_2 powders or coatings illuminated with near-UV light or sunlight [1–3]. TiO_2 in the anatase crystal form is a semiconductor with a band gap of 3.2 eV or more. Upon excitation by light whose wavelength is less than 385 nm, the photon energy generates an electron–hole pair on the TiO_2 surface. The hole in the valance band can react with H_2O or hydroxide ions adsorbed on the surface to produce hydroxyl radicals ($^{\cdot}OH^{-}$), and the electron in the conduction band can reduce O_2 to produce superoxide ions ($^{\cdot}O_2^{-}$). Both holes and $^{\cdot}OH^{-}$ are extremely reactive with contacting organic compounds and are able to completely oxidize these compounds [4].

Although a wealth of information on the efficacy of TiO_2 as a photocatalyst was available long back, it was Matsunaga and coworkers who first reported [5] that microbial cells in water could be killed by contact with TiO_2 upon illumination with near-UV light for 60–120 min. Later, the same group of workers successfully constructed a practical photochemical device in which

TiO_2 powder was immobilized on an acetylcellulose membrane and they reported that an *E. coli* suspension flowing through this device was completely killed [6].

Since titanium is considered an excellent material in terms of corrosion resistance, it is the choice heat exchanger material for seawater-cooled condensers in power plants. The only problem faced is that of extensive biofouling of the inert titanium surfaces, the present solutions offered are in the form of chemical treatment like chlorination of the cooling water. On the other hand, possible solutions aimed at modifying the titanium surfaces to make it hostile to microbial attachment seem more promising. Hence, based on the above research expertise available, the idea of growing a thin film of anatase type of TiO_2 on the titanium surface by anodization in order to inhibit fouling of titanium condensers was conceived.

Earlier studies conducted in our laboratory have shown that anodized titanium with a thin film of anatase (TiO_2) on its surface, can inhibit attachment of *Pseudomonas* sp. of bacteria when illuminated with near-UV light (350–380 nm) from black light blue (BLB) fluorescent lamps [7]. The optimum anodizing conditions for maximum photocatalytic activity in terms of anodizing voltage and anodizing time has been standardized as 30 V and 48 h [8]. The influence of heat treatment of anodized surfaces on enhancing the photocatalytic inhibition of microbial adhesion has also been investigated [9].

Recently, a number of studies have been reported in which photocatalytically active anatase was doped with elements such as V, Cr, Ni, Al, Fe, Mn, etc. in order to enhance its photocatalytic efficiency. The following work focuses on comparing the photocatalytic efficiencies of anodized surfaces of commercially pure titanium (grade 2) and Ti6Al4V alloy, a widely used medical implant material, in order to understand the role of the alloying elements such as Al and V on the photocatalytic activity in relation to inhibition of microbial attachment. Doping with selective elements such as nitrogen [10], Cr, Ni [11] and V [12] is reported to shift the photoresponse of titania which was so far restricted within the near-UV range to visible range. Since the titanium alloy used in the study consisted of V as one of its alloying elements, investigations were conducted to confirm the photocatalytic activity of the anodized Ti6Al4V alloy under visible light too.

Experimental

Specimen preparation

Commercially pure titanium grade 2 coupons (3×2 cm^2) and Ti6Al4V (Ti–6%Al–4%V) alloy were pickled in an acid bath (nitric acid 400 g/L + hydrofluoric acid 40 g/L + water) and then ultrasonically cleaned using soap solution to remove all traces of acid from the surface, washed in running water and finally rinsed in distilled water and air dried. Anodization was carried out at 25 °C in orthophosphoric acid (30 g/L) for 48 h at two voltages namely 30 and 100 V. The acid pickled coupons were used as control.

Surface analysis of anodized surfaces

Surface analysis of oxide thin film grown on anodized commercially pure titanium and Ti6Al4V alloy surface was analyzed using GIXRD and AFM. GIXRD was done using a STOE (Germany) diffractometer using copper kα (λ = 1.5406 Å) radiation. The angle of incidence was maintained at 0.5°. The coupon was mounted on a silicon (911) plate to nullify the background.

AFM studies were conducted using a STOVER (Scanning probe microscope), NT-MDT (Russia). All the studies using AFM have been done using non-contact mode.

Evaluation of photocatalytic activity

The photocatalytic activity of the titanium surfaces anodized at 30 and 100 V for 48 h was evaluated by methylene blue (MB) degradation method [13] using acid pickled coupons as control surfaces. Grade-2 titanium and Ti6Al4V alloy coupons anodized at 30 and 100 V as well as acid pickled control coupons, each four in number, were immersed in separate petridishes containing 25 mL methylene blue (2 mg/L) solution. The photocatalytic activity of the anodized Grade-2 titanium and Ti6Al4V surfaces, under near-UV as well as visible light was evaluated. The photo response of these surfaces under visible light was studied using day light fluorescent lamps (400–700 nm) and near-UV response was evaluated using BLB lamps (<380 nm). A UV–vis spectrophotometer was used to estimate the concentration of the unreacted MB by measuring the attenuation at its absorption maximum of 660 nm initially after 10 h and thereon at 24 h intervals up to 100 h.

Test organism

The antibacterial properties of the anodized surfaces were evaluated using Gram negative *Pseudomonas* sp., as the test organism. The reason for the selection of the above genus is that it has been identified as the major colonizer of fresh water biofilms [14, 15]. Characterization and identification of the bacteria up to genus level was carried out based on morphological, physiological and biochemical tests as per Bergey's Manual of Systematic Bacteriology (Table 1).

Table 1 Results of morphological and biochemical tests of *Pseudomonas* spp.

Test	Response
Gram reaction	Gram negative
Morphology	Very small rods
Pigments	Green fluorescent colony, pigment diffuses into media
Catalase	+
Oxidase	+
Specific reactions	
Anaerobic glucose fermentation	–
Nitrate reduction	+
Citrate utilization	+
Growth on cetrimide agar	+
Indole production	+

Exposure of specimens

Exposure studies were conducted in a cylindrical glass vessel containing the exposure medium. A glass rod positioned centrally in the glass vessel supported the glass pegs, which bore the coupons. The specimens were illuminated by six numbers of black light blue (BLB) lamps (4 W, Philips) arranged in a hexagonal configuration surrounding the test vessel. The light produced by BLB lamps has wavelength range of 350–380 nm and hence is referred to as near-UV light to distinguish it from the UV light used normally for disinfection. The near-UV light used in the study is not having any bactericidal property and it is transmitted through ordinary glass. Therefore, no quartz vessel was used for the study. A dilute nutrient culture was prepared by inoculating 1% (0.13 g/L) nutrient broth with 0.1 mL of 24 h culture of *Pseudomonas* sp. in 100% nutrient broth. This culture in dilute nutrient medium was recultured and used for exposure studies. This dilute nutrient culture was used to avoid pelagic growth of bacteria and to favor biofilm formation. The culture was mixed uniformly in an orbital shaker and incubated for 12 h at 32 °C before the coupons were introduced. The density of *Pseudomonas* sp. in the exposure medium (500 mL) was 6×10^8 cfu/mL. The exposure studies were conducted for a duration of 100 h.

Post exposure analysis

Three coupons of each experimental condition (triplicate experiments) were used for total viable count (TVC) estimation [16]. The coupons were removed from the medium and gently washed to remove loosely adhering cells and the bacterial cells on the coupons were dispersed into 15 mL sterile phosphate buffer (0.0425 g KH_2PO_4, 0.19 g $MgCl_2$ per litre) by ultrasonication for 5 min. The length of sonication for optimum recovery of cells was found to be 5 min. The ultrasonically cleaned surfaces were stained and observed to ensure complete recovery of cells. Serial dilutions of the bacterial cell suspension were prepared and 0.1 mL of each dilution was plated onto nutrient agar. The plates were incubated for 24–48 h at 32 °C and the number of colonies counted. Mean TVC values were calculated for each coupon and the results are expressed as colony forming units (cfu) per cm^2.

Two coupons of each experimental condition exposed in the nutrient culture were used for direct microscopic observation. The coupons were gently washed with sterile water and air-dried in a sterile chamber and the coupon surface was flooded using acridine orange (0.1% solution in distilled water). After 2 min, the excess stain was drained off and the coupons were washed in sterile water, dried and observed. Acridine orange, a fluorescent dye, differentially stains single stranded RNA and double stranded DNA, fluorescing orange when intercalated with the former and green while complexing with the latter [17] when observed under a Nikon Eclipse E600 epifluorescence microscope (excitation filter BP 490; barrier filter O 515). Thus, the number of orange fluorescing cells depict the actively metabolizing cells on the anodized and acid pickled titanium surfaces and the green fluorescing cell represent the photocatalytically inactivated microbial cells.

Results and discussion

Surface characterization

The anodic oxide films grown on titanium and Ti6Al4V alloy were characterized using GIXRD. The results showed that both the 30 and 100 V anodized titanium as well as alloy surfaces showed peaks corresponding to 25.2 indicating the presence of anatase type of TiO_2. However, the intensity of the hundred percent peaks of anatase was high in the case of the Ti6Al4V alloy surfaces anodized at 30 V for 48 h, depicting increased crystallinity of the oxide particles (Fig. 1). It was also observed that the peak intensity of the 100 V anodized titanium as well as alloy surfaces was relatively less.

According to Chen et al. [18] the sharper the peaks higher is the crystallinity of the oxide particles. High crystallinity minimizes the recombination of photoexcited electron–hole pairs thereby enhancing photocatalytic activity [19] this seems to be one of the reasons for the enhanced photocatalytic activity exhibited by the anodized alloy surfaces and the relatively lesser photocatalytic activity exhibited by the surfaces anodized at 100 V.

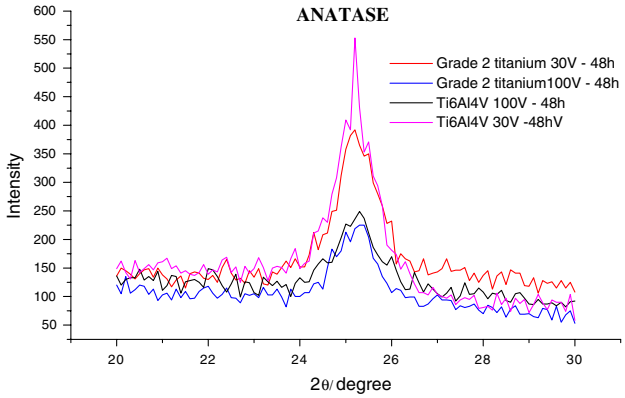

Fig. 1 GIXRD results of grade 2 titanium and Ti6Al4V alloy surfaces anodized at 30 and 100 V for 48 h

The oxide morphology and structure was characterized using AFM. The AFM images showed that the oxide film formed on the 30 V-48 h anodized Ti6Al4V alloy surfaces were porous and showed a honeycomb-like network (see inset in Fig. 2) made up of aggregations of small particles which were further made of particles even more smaller in size (Fig. 2). It was observed that the titanium grade 2 surfaces, consisted of an oxide film of relatively bigger particles, compared to the anodized alloy surfaces (Fig. 3).

Yu et al. [20] have reported an increase in the photocatalytic activity of thin films with decreasing grain size. When the grain size is small there is an increase in the specific surface area available for the photocatalytic activity [21]. This also serves to explain the reason why the anodized alloy surfaces whose particle size was small showed better photocatalytic activity than the anodized titanium grade 2 surfaces.

Evaluation of photocatalytic activity

Methylene blue dye degradation method

The photocatalytic activity of the anodized and acid pickled commercially pure titanium and Ti6Al4V alloy surfaces was evaluated by methylene blue degradation method. The results showed that the photocatalytic activity as shown by the degradation of the methylene blue dye degradation under near-UV light illumination was

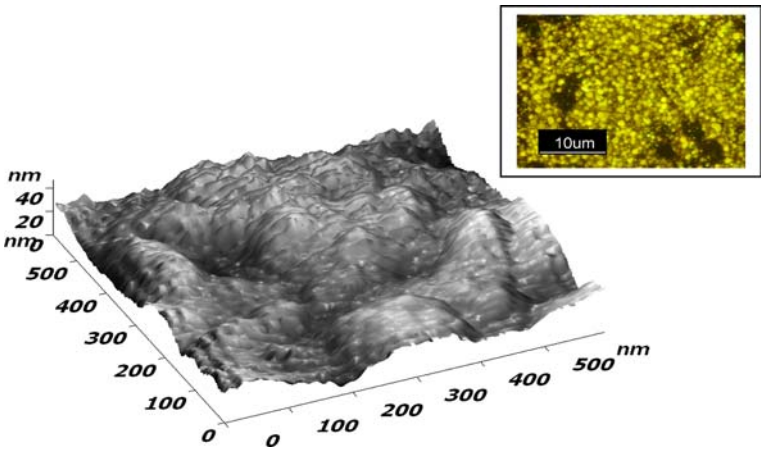

Fig. 2 AFM image of anodized Ti6Al4V alloy surface, inset showing epifluorescence micrograph of similar oxide film

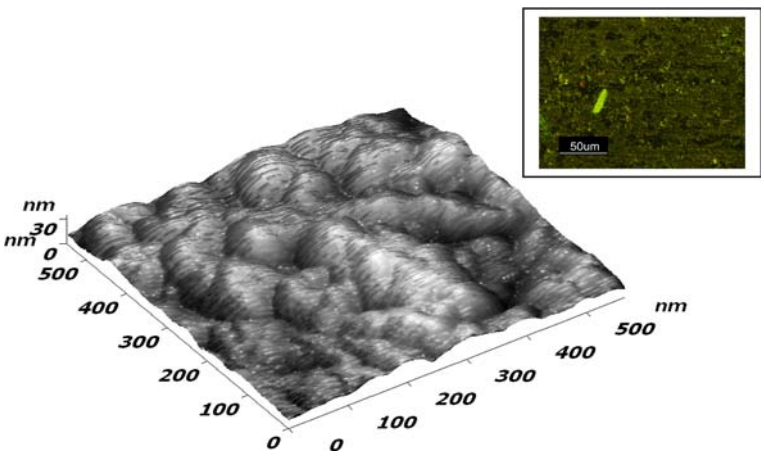

Fig. 3 AFM image of anodized pure titanium surface, inset showing epifluorescence micrograph of similar oxide film

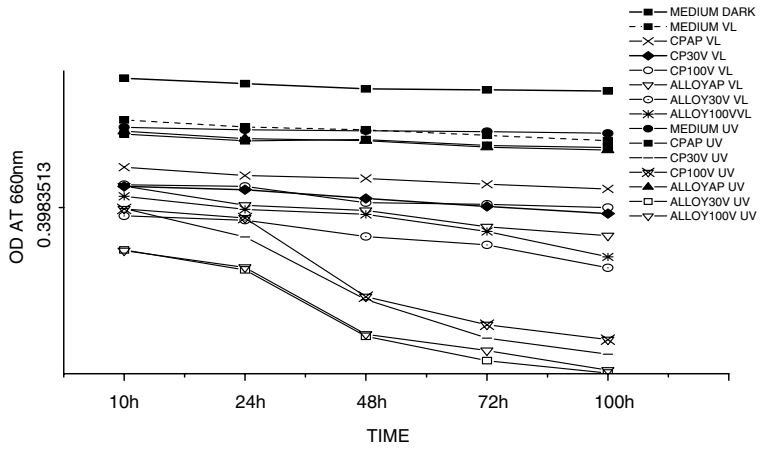

Fig. 4 Photocatalytic activity of the anodized titanium and alloy surfaces under near-UV and visible light illumination by methylene blue dye degradation method. VL—visible light, UV—near-UV light illumination

significantly high on the 30 V-48 h anodized alloy surfaces compared to the 30 V-48 h anodized commercially pure titanium surfaces. The 100 V-48 h anodized alloy as well as pure metal surfaces showed relatively lesser photocatalytic activity. The results of the experiments conducted under visible light showed that the Ti6Al4V alloy surfaces showed excellent photocatalytic activity even under visible light as shown by the significant degradation of the dye as a function of time (Fig. 4). Whereas, the grade 2 titanium surfaces showed negligible photocatalytic activity under visible light.

Microbial adhesion experiments

The photocatalytic efficiency of the anodized alloy and pure titanium surfaces was assessed under near-UV as well as visible light illumination, both by determining the total viable counts (TVC) of bacteria surviving the photocatalytic activity, enumerated by plating in a solid medium as well as direct acridine orange counts (DAOC) of live fluorescing bacteria adhering to the anodized surfaces by observing under an epifluorescence microscope. The results showed that compared to the 100 V-48 h anodized alloy as well as titanium grade 2 surfaces the 30 V-48 h anodized alloy as well as titanium grade 2 showed significant photocatalytic activity under near-UV light illumination (Fig. 5). Besides, it was also observed that the 30 V-48 h anodized alloy further exhibited an order magnitude decrease in microbial attachment, thereby showing better photocatalytic activity than the anodized titanium (grade 2).

The antibacterial properties of the anodized grade 2 titanium as well as Ti6Al4V alloy surfaces under visible light illumination was also studied. The results showed that the anodized alloy surfaces exhibited significant photocatalytic activity even under visible light (Fig. 6).

Finally, it was observed that the anodized Ti6Al4V alloy surfaces exhibited significantly high photocatalytic activity compared to the anodized pure titanium surfaces. Table 2

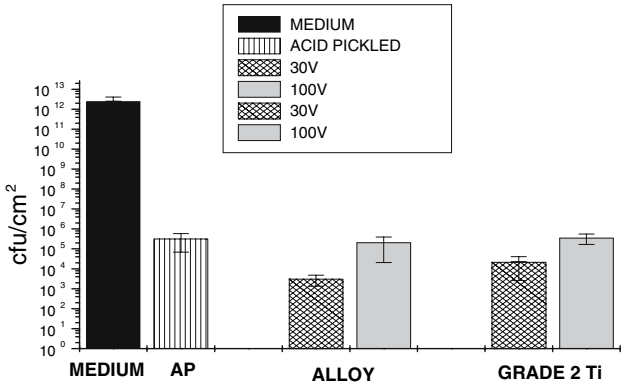

Fig. 5 Total viable counts (TVC) of *Pseudomonas* spp. on anodized pure titanium and alloy surfaces under near-UV light illumination

depicts the relative differences in photocatalytic activity between these surfaces under near-UV as well as visible light illumination. As observed there was an order of magnitude decrease in microbial attachment on the 30 V anodized commercially pure titanium surfaces compared to the unanodized surfaces, whereas a two order magnitude decrease in attachment was recorded by the anodized alloy surfaces. Hence, compared to the anodized titanium surfaces the Ti6Al4V surfaces showed an order magnitude increase in photocatalytic activity under near-UV light illumination. The anodized grade 2 titanium surfaces showed no or negligible photocatalytic activity under visible light while the anodized Ti6Al4V surfaces showed highly significant activity as shown by the two order decrease in microbial attachment on these surfaces.

Yu et al. have reported [20] that specific photocatalytic activity decreases with increasing thickness and that photocatalytic activity depended on many factors such as the distance to which the reactant should reach to capture the electrons/holes generated in TiO_2 thin films, the amount of hydroxyl ions per unit weight TiO_2, film thickness, average grain size and so on. Earlier studies conducted in our

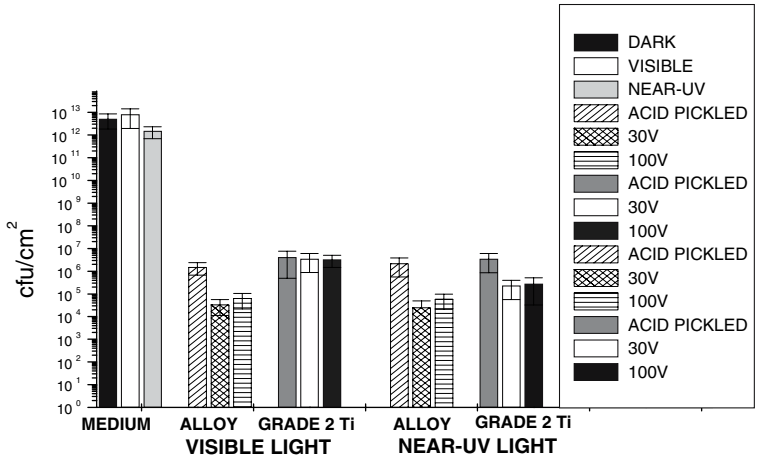

Fig. 6 Comparison of bactericidal activity of anodized titanium and Ti6Al4V alloy surfaces under visible and near-UV light illumination

Table 2 Consolidation of results showed by the test surfaces in terms of order of magnitude of increased photocatalytic activity

Comparison of test surfaces	Light source	TVC values	Photocatalytic activity (magnitude of difference)
AP vs. 30V (Ti grade 2)	Near-UV light	AP (4.2×10^6) 30 V (2.3×10^5)	One order magnitude
AP vs. 30V (Ti6Al4V)	Near-UV light	AP (4.2×10^6) 30 V (2.5×10^4)	Two order magnitude
30 V (Ti grade 2) vs. 30 V (Ti6Al4V)	Near-UV light	Ti G2 (2.3×10^5) Alloy (3.4×10^4)	One order magnitude
AP vs. 30 V (Ti grade 2)	Visible light	AP (6.5×10^6) 30 V (3.5×10^6)	No difference
AP vs. 30 V (Ti6Al4V)	Visible light	AP (6.5×10^6) 30 V (3.4×10^4)	Two order magnitude
30 V (Ti grade 2) vs. 30 V (Ti6Al4V)	Visible light	Ti G2 (3.5×10^6) Alloy (3.4×10^4)	Two order magnitude

laboratory have confirmed that the thickness of the oxide film increased with increasing anodizing voltage, whereas the photocatalytic activity as shown by the decrease in microbial attachment did not increase with increasing film thickness nor anodizing voltage, but was maximum on surfaces anodized at 30 V-48 h [22]. This explains the decreased photocatalytic activity shown by the 100 V-48 h anodized alloy as well titanium grade 2 used in the present study.

The Ti6Al4V alloy whose photocatalytic activity was compared with titanium grade 2 has alloying elements such as Al and V incorporated in a proportion of 6% and 4%, respectively. The results of our experiments as described above support the fact that anodized Ti6Al4V showed better photocatalytic activity than anodized titanium grade 2 surfaces. The results also show that unlike the anodized titanium grade 2, which shows photoresponse only in the near-UV region, the anodized Ti6Al4V alloy showed significant photocatalytic activity even under visible light.

The answer to the question why the Ti6Al4V exhibits better photocatalytic activity is not only because of the differences in the structure and morphology of anatase as described under the GIXRD and AFM results but also seems to be due to the presence of alloying elements such as Al and V. A large amount of research is on to improve the photocatalytic activity by adding dopants to TiO_2. The idea of doping can be divided into two categories: one is associated with shifting the absorption band gap edge to the

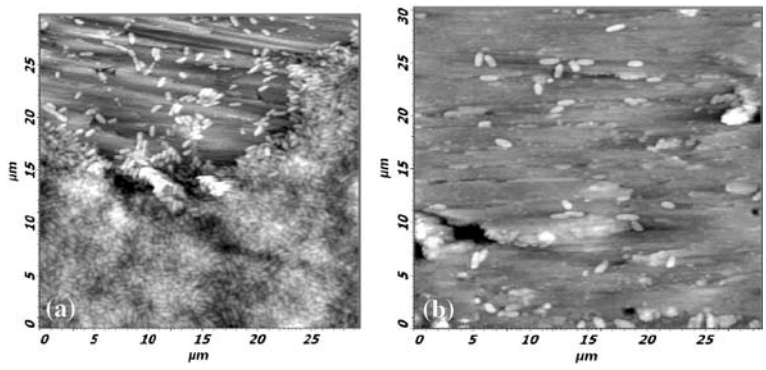

Fig. 7 AFM images of bacterial attachment on acid pickled and 30 V-48 h anodized titanium surfaces

red in order to enhance the activity in visible portion of the spectra. The second category of research has been to increase the photocatalytic activity of TiO_2 in the near-UV light by doping TiO_2 with elements such as Nd, Pd, Pt, Fe, Ni, Li, Zn, Cd, Co, Cr, Al, Au, Ag, Rh, Mn, Si and V [23]. It is generally agreed that a lower rate of recombination will translate into an increase in photoefficiency. The most common method used to achieve this is doping of transition metals ions to TiO_2. Metal ion dopants may act as electron–hole traps and consequently alter electron–hole pair recombination rates [24]. Jeffrey et al., Saila et al. have confirmed that Vanadium and Aluminum doped titania is a highly efficient photocatalyst operative even under visible light [25]. The increased photocatalytic activity shown by the anodized Ti6Al4V alloy surfaces possibly suggests the role of the alloying elements such as Al and V in the making of a photocatalytically better surface, although validative proofs of their existence has not been provided for in the present investigation.

AFM is presently developing into a powerful tool in visualizing bacteria adherent to material surfaces. The main advantage is that one can view the morphology of the bacteria without any sample preparation. A preliminary effort has been made by us to view the attachment of bacterial cells to the acid pickled (control) as well as anodized titanium surfaces. The AFM images as seen in Fig. 7 show the significant reduction in bacterial attachment on the anodized titanium surfaces (b) compared to the control surfaces (a).

Conclusions

1. Ti6Al4V alloy surfaces anodized at 30 V exhibited an order of magnitude increase in photocatalytic activity compared to the 30 V anodized titanium grade 2 surfaces.
2. It was observed that the anodized Ti6Al4V surfaces possessed bactericidal activity (two order of magnitude) even under visible light whereas the anodized grade 2 titanium surfaces showed no photoresponse under visible light.
3. GIXRD and AFM studies showed that there was structural and morphological differences in the anatase formed on Ti6al4V and titanium grade 2. The oxide formed on Ti6Al4V showed higher crystallinity and was composed of smaller particles; both these factors enhance photocatalytic activity.
4. The enhanced photocatalytic activity of Ti6Al4V alloy both under near-UV as well as visible light may be possibly due to the incorporation of Al or V ions or both in the TiO_2 lattice.

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