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Influence of tunable diameter on the electrochemical behavior and antibacterial activity of titania nanotube arrays for biomedical applications

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ABSTRACT

The conventional bone implants based on titanium metals requires long time treatment process. Since the osteoporosis become a common chronic disease, rapid osseointegration of bone implants will help to shorten the period of treatment and enhance the comfort of patients. The fabrication of nanostructured bone implants resembles like native bone promotes rapid osseointegration. Here we studied the influence of tunable diameter of titanium nanotube arrays for bio implant applications. A facile electrochemical anodization process was performed to fabricate array of nanotube structures on Ti surface using the mixture of glycerol and ammonium fluoride electrolyte containing water. The diameter of the nanotubes was controlled by varying water content and studied their surface morphology, corrosion behavior and antibacterial activity. The morphological studies revealed an increase in nanotube diameter and reduction in length with increasing addition of water. X-ray diffraction analysis showed that the intensity of anatase peak increases with the addition of water. Wettability measurements revealed that the formed titania nanotubes were hydrophilic in nature. The study examined the adhesion of *Staphylococcus aureus* on substrate and anodized specimens by bacterial viability test and the results showed that the anodized specimens showed higher percentage of inhibition compared to substrate. The decrease in i_{corr} value observed for TNTA specimens obtained from the potentiodynamic polarization studies was attributed to the variation in tube diameter and intertubular spaces with varying water content. The results of electrochemical impedance spectroscopy studies in Hanks' solution also have endorsed the structural changes occurred in the nanotube morphology by the variation of water content influenced the electrochemical properties.

1. Introduction

Titanium (Ti) and its alloys exhibit a unique combination of various properties such as low modulus of elasticity, desirable mechanical strength, light weight, remarkable corrosion resistance and biocompatibility. These attractive properties are due to the formation of protective TiO_2 oxide layer on the titanium surface which enables and accounts for their extensive use as implant materials over the past years [1–5]. The biologically inert nature of titanium implants when in used in human body environment restricts its use from long term application [6–8]. The long term normal functions of implants are related to early and rigid osseointegration. The lack of integration comes from the difference in structure and properties between bone/tissue and implant materials [9]. Due to the encapsulation of fibrous tissue around the implant, lack of initial bonding between Ti implants and bone occurs, which results in implant dislocation and premature loosening [10]. In order to increase the bioactivity and to improve the surface properties for direct cell surface interaction, surface modification becomes inevitable [11]. Various surface modification methods [12–16] have been employed for developing bioactive coating and promoting adhesion and proliferation of cells. Nevertheless, these modified coated surfaces are not reliable in the long span due to the relatively higher micrometer level thickness of the coated layer and poor adhesion to the substrate [17], fracture and delamination occurs at the interface between the bone and the implant [18]. Therefore, it is necessary to modify the surface of the implant material to improve the bioactivity.

It is necessary to understand the bone-material interface in order to design better implant materials. Nanoscale surface alterations and modifications are carried out to adjust or control the surface characteristics as the surface properties of implant materials intimately affect the initial protein adsorption from the biological fluid besides the selective recruitment as well as activation of favorable cell functions [19].A more sophisticated approach to surface structuring on the nanometer level is the formation of TiO_2 nanotubes in fluoride containing electrolyte [20]. Literature reports indicate that modifying Ti surfaces with TiO_2 nanotubes for orthopedic applications enhances bone bonding char-

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acteristics than conventional micro roughened surfaces [21]. Furthermore, the large surface area of TiO_2 nanotubes and their ability to precisely tune the tubular morphology render it a good platform for implant applications [10]. Ever since Zwilling et al. [22] first produced nanotubular titanium oxide, massive research is going on in this field. The nanotubes are usually formed in fluoride containing aqueous electrolyte [23] and non-aqueous electrolyte [24]. Due to the fact that anodization in organic electrolytes, particularly with glycerol or ethylene glycol (EG), leads to much higher aspect ratio and uniform growth, it is frequently used now days [25]. It is reported elsewhere that the tube growth is a diffusion control process and hence the formation of nanotubes in glycerol with high viscosity results in low growth rate of nanotubes. Addition of various water contents to decrease the viscosity of glycerol electrolyte solution plays an important role in determining the optimum growth rate, dimension and morphology of TNTA's [26]. Valota et al. [27] reported the influence of increased water content in the formation of ribs in the external walls of nanotubes.

Implant associated infections is a key issue impairing the normal function of commonly used titanium implants, which is usually difficult to treat, results in early orthopedic implant failure and repeated surgeries [28]. Reducing the adhesion of broad range of bacteria around the implants is desirable as the infections associated with implants are characterized by bacterial colonization, biofilm formation and spreading of infection to the adjacent tissues [10]. Hence in order to prevent the implant associated infections, surface with long term antibacterial activity is highly desirable. Prevention of bacterial adhesion by nanostructuring of the implant surface without the use of drugs could be an attractive method to reduce the infection. In addition corrosion resistance is also a very important factor determining the choice of metallic bio-implants. Electrochemically stable materials with effective long term corrosion resistance in the body is essential for implant applications [11].

To the best of our knowledge, studies on the impact of different size nanotube diameter synthesized from glycerol fluoride electrolyte on the electrochemical stability of titanium implant along with its antibacterial property, still remains to be explored. The present work focuses in the elaboration of nanotubes fabricated via electrochemical anodization of titanium by changing the viscous nature of the electrolyte medium with the addition of water in order to obtain different diameter size nanotubes. Whether and to what extend the various water content influence the stability of nanotubes was studied. The phase composition, morphology, surface roughness and wettability of the nanotubes were studied. The paper mainly extends the investigation to correlate the influence of addition of different amount of water in the formation of tunable nanotubes with the electrochemical corrosion behavior. Moreover the antibacterial inhibition efficiency of the nanotubes were evaluated and compared.

2. Materials and Methods

2.1. Fabrication of Titania Nanotube Arrays (TNTA)

Titanium specimens used for the growth of titania nanotubes were procured from Ti Anode Fabricators, Chennai. Prior to anodization, Ti specimens of dimensions (1.5 × 2 × 0.2 cm) were first mechanically polished with silicon carbide abrasive paper up to 1200 grit size in order to obtain smooth surface and then ultrasonically cleaned in acetone and double distilled water respectively. Subsequently the titanium specimens were etched in a mixture of HF, HNO_3 and H_2O in the volume ratio of 2.5:6:1.5 for 10 s, rinsed with double distilled water and air dried. Titanium nanotube arrays (TNTA) were fabricated using electrochemical anodization with Titanium as anode and platinum as cathode. A direct current (dc) voltage source (M/S Aplab Model L1285) was used to control the voltage at 20 V for 1 h. The distance between the two electrodes was kept at 2 cm for all the experiments. A mixed electrolyte solution of glycerol containing 1 wt% of NH_4F with water contents 10, 20, 40 vol% were used as the electrolyte solution. The electrolyte volume was held constant at 30 ml and was continuously stirred using a magnetic stirrer to enhance the mobility of the ions inside the solution so as to get the nanotubes with uniform size. Hereafter the titania nanotube arrays (TNTA) synthesized in this work with 10, 20 and 40 vol% H_2O contents were designated as TNTA-10, TNTA-20 and TNTA-40 respectively. After anodization, the specimens were thoroughly washed with water and subsequently dried under air atmosphere. The as formed nanotubes were annealed at 500 °C for 2 h in the furnace to convert the amorphous phase to anatase phase.

2.2. Morphology and Composition Characterization

To evaluate the presence of various phases of TNTA, X-ray diffraction (XRD-Xpert Pan Pro Analytical X-ray diffractometer fitted with CuKα source, λ = 0.15418 nm) analysis was used. The specimens were scanned from 20° to 80° at a scanning rate of 0.02°/minute. The ATR-FTIR spectra of substrate and anodized specimens were recorded in the 400–4000 cm^{-1} range on a Perkin Elmer FT-IR spectrometer spectrum Two with UATR Two Accessory and KBR window to find out the functional groups present on the surface. The Raman spectra of the specimens were obtained from FT-RamA006E, Bruker RFS 27 instrument at room temperature. The excitation source used was Nd; YAG operating at 1064 nm. In order to characterize the surface morphology of the TNTA specimens, high resolution scanning electron microscope (HR-SEM, Model FEI Quanta FEG 200) was employed. The 3D images of the anodized specimens were obtained by using scanning probe image processor WSxM 4.0 βeta software [29]. Cross sectional measurements of the formed nanotubes were carried out on mechanically cracked samples.

2.3. Roughness and Wettability Studies

Surface roughness was measured from the 3D images. Image analysis and processing were performed using WSxM 4.0 βeta software. The water contact angle (CA) of the anodized specimens was measured using contact angle analyzer model Phoenix 300 Plus instrument. A small droplet of water with a drop volume of 8 μl was dropped on the sample surface with a syringe and the CA's were obtained by measuring at different positions on each sample and the average value was reported.

2.4. Antibacterial Studies

The bacterial viability test [30] was done to assess the survival of bacteria on substrate and anodized specimens. A total of 200 ml LB agar (5 g LB broth and 1.6 g agar with 193 ml glass-distilled deionized water) (Himedia laboratories Pvt Ltd) mixed with 0.2 ml of *S. aureus* (*Staphylococcus aureus*) (1×10^6 cfu/ml) was prepared. The substrate and anodized specimens were placed on sterile plates. The plates were subsequently overlaid and immersed into 8 ml LB agar containing *S. aureus*. After air drying for 30 min at room temperature, the plates were incubated at 37 °C for 16 h and visible bacterial colonies on the LB agar plates were counted. The number of bacteria growths was then determined by counting the colony-forming units (cfu). The adherent number was expressed by the ratio of the total bacteria growths on the measured sample to the area of the measured sample.

2.5. Electrochemical Measurements

Electrochemical measurements were carried out at room temperature using potentiostat (Model PGSTAT 12, Autolab, The Netherlands B.V) controlled by a personnel computer with dedicated software (GPES version 6.0). A conventional three electrode cell was used for the electrochemical studies. The cell assembly consisted of the test speci-