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Research Article

GROWTH OF FOURTH-GENERATION ELONGATED TITANIUM OXIDE NANOTUBES IN MIXED ELECTROLYTES

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ABSTRACT

Objective: The effect of oxalic acid and phosphoric acid mixture in the formation of enlarged titanium oxide (TiO_2) nanotubes was investigated in the current study.

Methods: The anodization was carried out in an aqueous electrolyte containing oxalic acid, phosphoric acid, hydrogen peroxide in ethylene glycol in a simple two electrode system at room temperature using a potentiostat set-up.

Results: The coexistence of anatase and rutile phases was noticed where the ratio of anatase peaks to rutile peaks was found to be higher. The diameter of the TiO_2 nanotubes increased with elevated applied voltage. At low voltage, random cracks with no noticeable structures were seen on the Ti substrate. However, on the increasing value of the applied potential, determinable tubular structure of TiO_2 nanotubes emerged in the crack.

Conclusion: The presence of phosphoric acid in the electrolyte enhanced the growth of nanotube diameter and length. The ionic current increased with increase in phosphoric acid concentration that leads to the formation of the oxide layer and an increase in oxide forming efficiency. Having better mechanical properties, adhesion strength, surface roughness, hydrophilic properties, and the anodized titania nanotube surfaces are envisaged to have better cell activity, biocompatibility with antimicrobial property and also longevity. It can be applicable as the better implant surface in the human body environment.

Keywords: Titanium oxide nanotubes, Anodization, Oxalic acid, Fluorine free, Antibacterial.

INTRODUCTION

In the past 20 years, Ti and its alloys have been contemplated as gold standard biomaterials for orthopedic and dental implant applications. Due to their superior biocompatibility, high corrosion resistance and better mechanical properties, titanium (Ti)-based materials have been on increasingly strong demand for load bearing implant applications. However, to enhance its clinical success efficiency and to be compatible by human body environment, surface modification is required to counter its bioinert property [1-3]. By surface modification, a denser oxide film is developed that makes the implant stable in the physiological medium. However, the osseointegration, cell attachment on the implant can progress in a nanometer scale [4,5]. Hence, Ti oxide (TiO_2) anodic layer with nanoporous structure is preferable due to their large surface to volume ratio and higher biocompatibility. TiO_2 nanotubes can be fabricated by various techniques, but the electrochemical anodization method is frequently used by researchers due to its ease of fabrication and low cost. According to literature, fluoride-based electrolytes would be commonly described characteristics for the anodization process. In this case along with those additives, such as ethanol, ethylene glycol, glycerol or polyethylene glycol, and fluoride containing acids such as hydrofluoric acid, ammonium fluoride, and sodium fluoride are generally used. Each of these precursors is used in controlling the morphology and dimension of the TiO_2 nanotubes [6]. Since fluorine is hazardous for human body environment in the fabrication of TiO_2 nanotubes for biomedical applications, choosing of chlorine medium replacing fluorine is a foremost selection of the researchers. In 2007, Richter *et al.* [7] fabricated TiO_2 nanotubes in chloride containing a medium that consist of ammonium chloride, coupled with different types of acids such as trichloroacetic acid, oxalic acid, formic acid, sulfuric acid, and hydrochloric acid. Richter *et al.* reported that acid does not play a primary role for the synthesis of TiO_2 nanotubes. Later on, in the same year, Allam and Grimes investigated the importance of phosphoric acid within chloride containing acid medium and stated that low concentration of phosphoric acid leads to the fabrication of nanorods rather than nanotubes. In 2007, Chen *et al.* [8] also described the anodization of TiO_2 nanotubes using HCl as a major component in the electrolyte to obtain self-organized highly ordered nanotubes. To obtain fourth generation nanotubes with enhanced tube length, oxalic acid, phosphoric acid, and hydrogen peroxide-based mixed electrolytes have not been used. In the present study, TiO_2 nanotubes arrays were fabricated in oxalic acid, phosphoric acid, and hydrogen peroxide-based electrolyte mixture to obtain fourth-generation nanotubes with enhanced tube length.

METHODS

Sample preparation and TiO_2 nanotube formation

To synthesize TiO_2 nanotubes, electrochemical anodization was performed in a simple two-electrode cell at room temperature using potentiostat (Amel 7050, Italy) set up. A Ti substrate with a working area of 1 cm×2 cm served as anode and platinum was served as the cathode. Here anodization was carried out in a stirred bath in constant potential. Before the process, the samples were made mechanically glossy using 600 and 1200 grit sized SiC papers. After polishing, the specimens were cleaned with deionized water and ultrasonicated for 10 min with ethanol, acetone, and deionized water consecutively. Then, the samples were dried and etched in an acidic solution containing 1 part HF, 4 parts HNO_3, and 5 parts deionized water for 30 s. Then, the samples were again thoroughly washed and dried at room temperature to undergo anodization at constant DC voltage of 35 V for 30 min in chloride ions containing acidic solutions. The electrolyte used in this work contained oxalic acid $C_2H_2O_4$ (0.75 wt%), phosphoric acid H_3PO_4 (0.5 wt%), and hydrogen peroxide H_2O_2 (0.5 wt%) in an aqueous electrolyte of ethylene glycol (95/5: V/V). After anodization, the samples were again washed in deionized water and dried at room temperature. The amorphous anodized TiO_2 nanotubes after annealing at 500°C for 2 h were converted to crystalline mixed phase [9].

Surface characterization

After anodization, the structural properties of the samples were obtained by high resolution X-ray diffractometer (XRD) (XRD; X'pert- APD, Philips, Netherland) using Cu Kα radiation (λ=0.154 nm, 40 kV, 20 mA) and scanned in the 2θ range of 15°–90° [10]. The nanotubular structure of TiO$_2$ nanotubes was characterized by field emission scanning electron microscopy (SEM; JSM-6700, JEOL, Tokyo, Japan). The elemental analysis of TiO$_2$ nanotubes was studied using energy dispersive X-ray (EDX) spectroscopy. The roughness of the coating was determined by surface measuring instrument (SURFTEST, Mitutoyo, Japan). By contact angle detection system (OCA15 Plus, Dataphysics, Germany) coating wettability or hydrophilicity was measured. The tensile pull-off adhesion strength of the anodized sample was determined by Universal Testing Machine (Instron; 4202) under tension mode [11].

Antibacterial activity

Antimicrobial activity of the synthesized TiO$_2$ nanotubes surfaces was inspected by a diffusion method against bacterial strain *Escherichia coli* (ATCC 8739). The procured bacterial cultures were sustained on nutrient agar slants that contained peptone 5.0, meat extract 1.0, yeast extract 2.0, sodium chloride 5.0, and agar 15.0g/L of distilled water. The test bacterial suspensions (50 μl) containing 10^4 cells ml^{-1} were spread on nutrient agar plates. Then, anodized TiO$_2$ nanotubes substrates were placed within the agar medium. Initially, the samples were incubated for 15 min at 4°C (allow diffusion) and later on at 37°C for 24 h for the bacterial culture. Antibacterial activity was studied by analyzing the cell viability of the sample in the agar medium after incubation.

RESULTS

The tube lengths of TiO$_2$ nanotubes achieved are of micron-sized in short reaction time. Moreover, we have perceived remarkable dependence between the size and crystallinity of the TiO$_2$ layers and the enhancement of adhesion/proliferation of osteoblasts due to ion immobilization is envisaged. Antibacterial properties of the anodized samples have been studied.

i-t characteristics obtained in oxalic acid – phosphoric acid mixture solution

According to literature, the "anodization current plateau" increases drastically by the applied voltage for every chlorine containing acids used, but except oxalic acid to meet a smooth curve, which was obtained in this work, as shown in Fig. 1.

From current transient curve, it was noticed that the nanotubes were formed by a sharp increase in anodization current. However, according to previous viewpoints, during anodization of a standard solution, for each acid, only within a fixed range of voltage, i.e., above 8 V and below 20 V nanotubes are formed. At threshold voltage for each acid, nanotubes are formed. An interesting observation about nanotube formation was noticed for tube length variation over time that is based on the characteristic properties of the current transient where the discontinuation of anodization with time occurred [12]. According to literature, the different stages of the tube formation entirely depend on the progression of current [13,14]. Initially, the current increases due to the emergence of the compact oxide layer and then it drops exponentially. During this decrease of the current, to maintain the electrolysis process, the electrolyte continues to supply Cl$^-$ ions and these ions borrow the inner part of the cavity forming pits and pores and grew deeper by enhancing diffusion species and thus by increasing the current. This process continues until a maximum current reached and TiO$_2$ nanotubes sustained to elongate until the electrolyte decreases to supply Cl$^-$ ions. The fabrication of the TiO$_2$ nanotubes arrays under the effect of Cl$^-$ ions is given by equation (1).

$$TiO_2+4H^++6Cl^-\rightarrow TiCl_6^{2-}+2H_2O \qquad (1)$$

Structural properties

From the XRD pattern, structural properties such as crystal phase and orientation of the nanoporous TiO$_2$ nanotube arrays were analyzed. XRD of the TiO$_2$ nanotubes is shown in Fig. 2. The XRD peaks of TiO$_2$ nanotubes were observed to be in anatase phase along with few rutile phases.

Cai *et al.* investigated the effect of annealing temperature on the structure of TiO$_2$ nanotubes by annealing the TiO$_2$ nanotube substrate up to 580°C. They perceived that up to 230°C of annealing temperature, the nanotube preserved its amorphous phase, but at a temperature near 280°C, crystallization appeared in anatase phase. On increasing the temperature, crystallinity increased as amorphous regions became crystalline as observed at 480°C. In the present work, the coexistence of anatase and rutile phases was noticed to be from a higher ratio of anatase peaks to rutile peaks [15]. It has been found that the anatase phase of TiO$_2$ nanotubes provides better nucleation and cell growth in bio-environment than in rutile phase [16]. The more numbers of crystallized anatase peaks enforce the bioactive nature of the TiO$_2$ nanotube sample. XRD patterns not only provided detailed confirmation about the crystal phase and orientation of TiO$_2$ nanotubes coatings but also calculated crystallite size indirectly using Scherrer equation [17], $D = (k\lambda)/(\beta\cos\theta)$, where D is the mean for crystallite size, k is the mean for shape factor, that is, reported in literature to be 0.9 [18,19], λ is the wavelength of k-α1 having value of 0.15406 nm; β refers to full width at half maximum and θ is the diffraction angle of the superior diffraction peak. By calculating θ, highest crystallite size was noticed for the anatase phase, which is larger

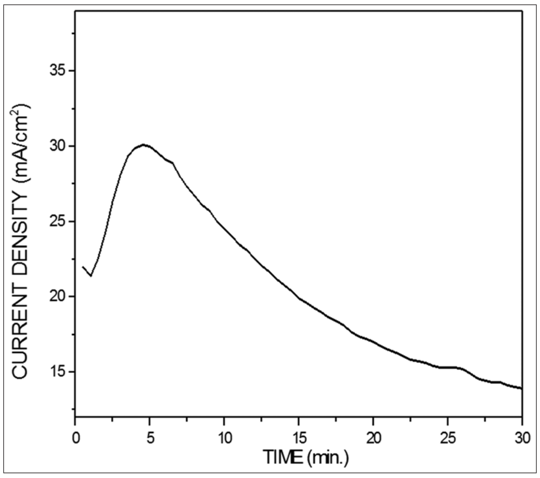

Fig. 1: Current transient curve in oxalic acid-phosphoric acid mixture solution

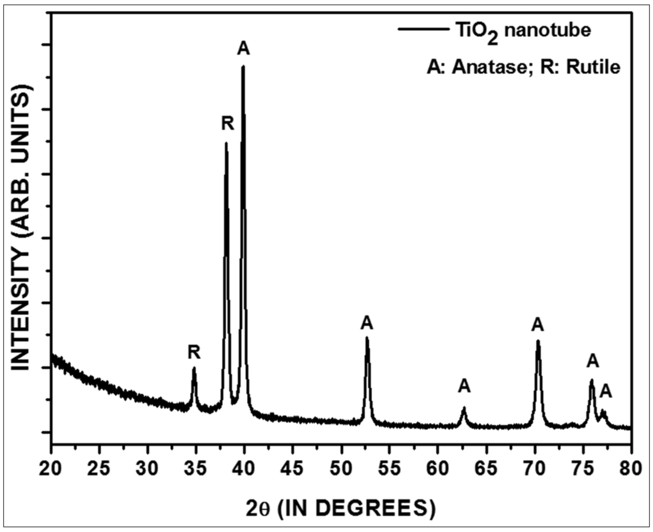

Fig. 2: X-ray diffractometer pattern showing anatase and rutile peaks due to of titanium oxide nanotubes

than nanotube diameter. However, in case of fluoride-based electrolyte distribution of the nanotube arrays was uniform but the crystallite size cannot exceed the size of nanotube wall [20] (due to the obstruction of nanotube wall on crystallite size). Due to the presence of more anatase phase which provides a porous crystalline surface, enhanced bioactive nature of the implant is envisaged.

Morphological properties

From SEM, the morphological properties of the anodized TiO_2 nanotubes arrays were identified. SEM micrographs explained that chloride based electrolyte is the best substitute for fluoride based electrolyte for fabrication of the TiO_2 nanotubes by electrochemical anodization method. This outcome is well perceived by Hahn *et al.* in their experiments [21]. It is evident that, in fluoride containing electrolytes, the nanotube diameter (or pores) increased with increase in value of the anodization voltage [22,23] and the same result was also observed in chloride medium and in the present work where diameter of the TiO_2 nanotubes increased with elevated applied voltage [24,25]. In chloride medium containing phosphoric acid, relatively higher film stability was noticed with increasing value of potential, during 1st min of anodization. Moreover within a fixed potential range, with an increase in the concentration of H_3PO_4 and anodization time, the elevated value of current density was observed [26]. For the substrate in Fig. 3a at low voltage, random cracks with no noticeable structure were seen on the Ti substrate. However, on the increasing value of the applied potential, determinable tubular structure of TiO_2 nanotubes emerges in the crack. At around 15V, nanotube structure of TiO_2 was observed as shown in Fig. 3b.

When anodization potential was additionally increased to 20 V and 35 V (samples in Fig. 3c and d, respectively) the nanotube diameter extended appreciably with length at high-aspect-ratio. The length of the nanotube achieved using this electrolyte is 17 µm that showed a higher value of electrochemical etching rate, which depends on applied potential. The increased length of nanotubes in chloride containing mixed electrolyte is attributed to the presence of H_2O_2 in the electrolyte [6].

In a comparative study, fabrication of TiO_2 nanotubes in fluoride-based electrolyte yielded orthogonally elevated nanotubes on Ti substrate [6] whereas, in chloride-based electrolytes, the anodized TiO_2 nanotubes were aligned toward an invisible concentric point of a cavity. According to literature, the diameter of the nanotube in chloride-based electrolyte is <50 nm [8]. In the current study, the average diameter of the nanotube was found to be 88 nm, which is equivalent to that formed in fluorine-based electrolytes. This may be due to the presence of phosphoric acid in this medium. It is evident in Fig. 3d that the elevated nanotubes on the Ti substrate coextensively point toward the same direction.

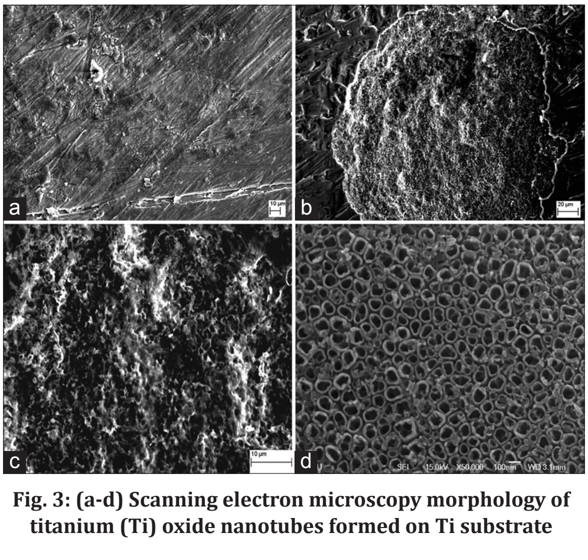

Fig. 3: (a-d) Scanning electron microscopy morphology of titanium (Ti) oxide nanotubes formed on Ti substrate

It was noticed that the anodized implant surface having a higher value of surface roughness (Ra) shows better bond strength to the coating produced on it. The surface roughness of the anodized sample was 0.59 µm. The contact angle on TiO_2 nanotubes surface was found to be 44°.

Antibacterial test

The metals and metal oxides have been extensively studied for their antibacterial activity. Ti and Ti dioxide nanoparticles reveal antimicrobial properties due to their reactive oxygen species reproduction and effective for their physical structure and metal ions release. In the current study, the antibacterial effect of TiO_2 nanotube substrate was investigated against *Escherichia coli* bacteria in agar medium because this bacterial species commonly are culpable for post-surgical infection in orthopedic surgery. In the antibacterial study, by recording the change in the number of *E. coli* bacteria in agar medium, antibacterial effects were visualized. More number of *E. coli* bacteria was noticed on the agar medium without TiO_2 than with TiO_2.

DISCUSSION

Richter *et al.* [7] successfully investigated the fabrication of TiO_2 nanotubes in chloride based electrolyte and reported that chlorine is not the only replacement of more harmful fluorine but also it offered a longer nanotube with shorter anodization time. The nanotubes up to 50 µm were fabricated in <5 min, whereas to achieve similar length fluorine-based electrolyte, it takes up to 70 h. According to theoretical aspects, high adjustability of the nanotube geometry and longer nanotubes in chlorine-based phosphorous containing electrolyte is because phosphoric acid acts as a buffer that controls local acidity during the nanotube growth.

In the anodization mechanism, phosphate ions reveal various advantages over acid and base electrolytes. During anodization, phosphoric acid shows a less corrosive attack on the Ti substrate as compared to the other acidic media which manifest the strong adsorption of phosphate anions on the surface. The non-dissociated acid molecules and $H_2PO_4^-$ of phosphate ions coexist in the solution and they express strong attractive force toward most metal cations [27,28]. Hence, anodizing in phosphate solution assists to the consolidation of phosphate ions onto the TiO_2 oxide layer, affecting their bioactivity and biocompatibility of the anodized substrate. Hence, it provides excellent characteristic properties of the Ti implant for medical purpose. Moreover, the diameter of the TiO_2 nanotubes increases with the suitable concentration of H_3PO_4 is given chloride medium. Due to the presence of $H_3PO_4^-$, ionic current increases to form an oxide layer and increase the oxide forming efficiency. In the present work, phosphoric acid attributes for longer nanotube formation and enhances the bioactive properties of the implant. H_2O_2 also attributes for longer nanotubes which are revealed in this experiment. H_2O_2 is a strong oxidizing agent and could increase the oxidation rate on the substrate during anodization to produce thicker oxide layers in the specific period [29]. Using EDX spectroscopy, the elemental concentration of the nanotubes was analyzed. According to literature, a significant amount of carbon was found in every one of the organic acids. The carbon content of the tubes grown in the oxalic acid medium is nearly same around 20%. The XRD data revealed the presence of Ti oxycarbide for the organic acid sample. This conclusion coincides with the outcomes obtained by Shanmugam *et al.* [30] who reported that the carbon content in the organic acid samples is roughly equal to that of the "core-shell" carbon coated Ti particles. The carbon-containing layer is thin (~2 nm) to have the long-range order essential to give noticeable XRD peaks. In carbon-containing samples, the transition of anatase to rutile phase seems to conquer as compared to the non-carbon containing the sample. Hence, in the present work anatase phase of XRD peaks at 500°C may be due to the presence of carbon contained in the sample. Furthermore, from literature, in the non-electrochemical system, the combination of chlorine with TiO_2 is nonreactive. Fracassi and D'Agostino [31] reported the pure chemical reaction between TiO_2 and Cl_2 that needs a high temperature (350°C) and the presence of a reducing agent, such as carbon to occur. Yang and Hlavacek [32] investigated that the only acceptable mechanism for low-temperature chlorination of TiO_2 involves a ternary activated complex of TiO_2- C-Cl. Hence, it was found that a suitable amount of carbon content is necessary to fabricate TiO_2 nanotubes in an electrolyte containing organic acid along with chlorine salt. Hahn *et al.* [33] reported that the dissolution rate of the chlorine species under voltage bias is much more sensitive to slight variation in the electric field or the sample uniformly. The high reactivity in chloride medium is due to the penetration and break down of TiO_2 nanotubes layer under a sufficiently high bias voltage. D'Agostino *et al.* [34] reported that chloride ions are a lot less mobile and able to penetrate the TiO_2 layer as compared to ions such as fluorine or oxygen. Hence, it can be concluded that high bias voltage may favor the supply of oxygen species over chlorine species and attribute the stabilization of oxide formation layer in this medium. The rapid increase in the oxide layer thickness along with the evolution of O_2 is accountable for a large initial spike as noticed in anodization i versus t curve. For biomedical stability, to avoid sliding of the implant surface on natural bone and to anchor with bone, roughened surface is more preferable [35]. The surface roughness of the anodized sample was 0.59 µm, which is nearly equal to the control TiO_2 surface. This elevated value of surface roughness shows better osteoblast activities and enhance the biological property in the human body environment. The contact angle on TiO_2 nanotubes surface was found to be 44°. This lower value of contact angle signifies more hydrophilic nature of the implant surface that has the potential to inflate the protein formation and osseointegration. Adhesion strength is an important property since it expresses weight loss when eroded against bone. To provide better cell attachment, an implant should have an adhesion strength of at least 18 MPa [36]. In the present study, the adhesion strength of the anodized surface was found to be 13 MPa, which is suitable for cell attachment as an implant in a biological medium. According to literature, the antibacterial activity of TiO_2 nanoparticles is due to its germicidal mechanisms in which the positively charged metal oxide undergoes electromagnetic attraction with the negatively charged microorganism to promote oxidation and yield for cellular death of microorganisms [8]. The negative charge is due to the thiol group (-SH) of protein on the cytoplasmic membrane [37]. According to literature self-organized, highly ordered nanotubes fabricated with the ultra-high-aspect ratio in chloride-containing the electrolyte. Richter *et al.* reported [7] ordered nanotubes of length around 60 µm and diameter of about 25 nm in oxalic acid (0.4 M) and ammonium chloride (0.5 M) mixture. Ishibashi *et al.* stated [38] the tubular nanotube arrays of variable length and diameter in different electrolytes. In a mixture electrolyte of 60% of perchloric acid and 99.5% ethanol, the length and diameter of TiO_2 nanotubes were achieved about 10 µm and 70 nm, respectively, whereas in an electrolyte containing 0.1 M of perchloric acid solution same length is achieved but the diameter is reduced to 30 nm. According to literature, the fabrication of the TiO_2 nanotubes in oxalic acid medium shows that average diameter of about 25 nm and length was either relatively ordered in bundle up to 60 µm long or sometimes irregular agglomeration [39]. In the present work self-organized, ordered nanotube arrays fabricated were 17 µm long and diameter about 88 nm, which is better than other reported work in chloride containing electrolytes.

CONCLUSION

The high-aspect-ratio TiO_2 nanotubes obtained in the present work was synthesized by anodization process in chloride-based electrolyte with H_2O_2, phosphoric acid, oxalic acid, and EG as additives. The fabrication mechanism was ascertained to be comparatively similar to that of nanotubes fabricated in fluoride-based electrolytes. It was found that carbon-enriched TiO_2 nanotubes can be fabricated using oxalic acid as the electrolyte with carbon content, approximately around 20%. Here additional chlorine atoms increased the reactivity of the acid anions. The certain threshold voltage was recommended to initiate the formation of the nanotube and overall, the nanotube diameter and length depend on applied potential. The presence of phosphoric acid in the electrolyte enhanced the growth of nanotube diameter and length. The ionic current increased with increase in H_3PO_4 concentration that leads to the formation of the oxide layer and an increase in oxide forming efficiency. The suitable concentration of H_3PO_4 within the chloride medium also enhances the biocompatibility of the anodized sample. In this work, ordered, self-organized nanotube arrays fabricated in an oxalic acid medium with enhanced length and better diameter were found as compared to other chlorine-containing the electrolyte. Having better mechanical properties, adhesion strength, surface roughness, hydrophilic properties, and the anodized TiO_2 nanotube surfaces are envisaged to have better cell activity, biocompatibility with antimicrobial property and also longevity. It can be applicable as the better implant surface in the human body environment.

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