Fabrication of Titanium dioxide Nano tube layer by Rapid and Homogenous anodization in Perchloric acid medium

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Abstract

Because of its appreciable biocompatibility with living tissue use of titanium as biomaterial is possible. In biomedical applications titanium implant having titanium dioxide nanotubes on their surface show good fixation to bone. It is well known that anodic titanium nanotubes can be obtained in fluorine containing solution, but fabrication of titanium nanotube in chlorine based electrolyte provide ultrahigh aspect ratio. In this article, fabrication of similar titania nanotubes in perchloric acid + strontium chloride solution is reported. It was also demonstrated that the length and diameter of the nanotubes could be varied by changing the anodization conditions such as electrolyte and anodic potential. The dimensions of the nanotubes (length and diameter) were analysed by using Field emission gun scanning electron microscopy. The phase composition and crystallinity of the titanium nanotube layer was investigated by X-ray diffractometry. The cytotoxicity of the anodized titania sample was studied by Lymphotic culture.

Keywords: Titanium dioxide nanotubes, anodization, Perchloric acid, Fluorine free, Antibacterial.

1. Introduction

During past decades, scientists have developed technique for fabricating many new biomaterial focusing on the strong, increasing demand for implanting materials due to loss of body function caused by aging and muscoskeletal surgery arise because of rapid accidents. The implanting material synthesize are one dimensional, as the nanoscale, including nanoparticles, nanolayers and nanotubes. But fabrication of the nanoscaled implant materials with controlled properties is a remarkable and current challenge with in nanoscience and technology. Among these CdS nanowire arrays [1], carbon nanotubes [2] and titanium dioxide (titania) nanotube arrays [3] are a few examples. In past decades self organized, one dimensional highly ordered nanotubes are generated due to ease of fabrication of nanometrial by electrochemical anodization method in the absence of templates. Examples include titanium [4], zirconium [5], hafnium [6], niobium [7], tantalum [8] and tungsten [9]. But out of these Ti and its alloys have been employed as a versatile implant for biomedical applications because of its high corrosion resistance, biocompatibility, low Young's modulus and good mechanical strength [10]. Titanium and its alloys are generally used for load bearing implantation. But direct implantation of biomaterial in to the bone for a long period may lead to release of ion to an adjacent tissue to the implant and damaging inference for the organism [11,12], as it is bioinert metal. A thin and adherent oxide film, spontaneously formed on the titanium surface, which attribute to successful application of the titanium as a biomaterial. In biomedical application, porous, highly ordered, self organized, one dimensional nanotubes are desirable because of their large surface to volume ratio and high compatibility [13].In biomedical application, corrosion of a metal achieved due to the interaction of the metal surface with physiological environment, but the titanium dioxide layer with porosity and roughness has an important attribution in electrochemical measurement of corrosion. High surface area also encourage cell adhesion and deposition of components, which is similar to that of natural bone, encourage rapid osseointegration and implant stability inside the body[14,15,16].Besides biomedical implantation titania also used in all other applications in biomedical fields, such as molecular filtration, biosensor, drug delivery system and tissue engineering technology. One-dimensional titanium nanotube arrays also considered as an excellent material having outstanding properties, such as charge transport and carrier life time properties. Hence it also used in dye sensitized solar cells, sensors, generation of hydrogen by photo electrolysis method and super capacitors.

In 1953, fabrication of hexagonally closed-packed alumina arrays by electrochemical method was first described by Keller et al., [17]. Later on Masuda et al., have dispensed the self-ordered porous alumina [18]. Actually, researchers have been used three generations of fluorine containing electrolytes to fabricate TiO_2 nanotubes with different aspect ratio and surface quality by electrochemical anodization method. In first generation, Zwilling et al., discovered ordered, nanoporous structure of titanium nanotubes by using hydrofluoric acid aqueous solution in 1997. The maximum thickness of TiO_2 nanotubes (TNTs) is limited to only specific hundred nanometers. Gong et al., have reported the well-organized aligned TiO_2 nanotube arrays of up to of 0.5 μm, because of etching and chemical dissolution of TiO_2 by fluorine ions in aqueous solution [19, 20, 21]. In second generation, the dissolution rate of titania was reduced by using fluoride salt, such as NaF, KF aqueous as an electrolyte. Cia et al., [22] reported that anodization in fluorine salt medium minimize the chemical dissolution of titania and obtained an increased length of nanotube arrays of 2-3 μm. In third generation, the increased length of nanotube arrays was investigated in organic electrolyte by anodization method. J. M Macak et al., have outlined fabrication of titania in organic electrolytes such as glycerol, ethylene glycol and polyethylene glycol and achieved the length about 7μm [23]. Later on Allam et al., reported the fourth generation of nanotube arrays by anodization method in fluorine-free electrolyte, particularly HCl [24,25]and mixture of HCl with H_2O_2 [26]. In 2005, Nakayamma et al., explored the formation of titanium dioxide nanotube arrays in perchloric acid solution and disclosed that fabrication of titanium nanotubes in chloride containing electrolyte is same to that of fluoride containing electrolyte . But in chlorine medium rapid synthesis of high-aspect-ratio titania nanotube arrays were investigated [27]. Further Hahn et al., supported this result in their experiment [28]. In this current work, fabrication of titania nanotube arrays were reported in chlorine containing mixture electrolyte of perchloric acid, strontium chloride and hydrogen peroxide. In our study, the formation of titanium nanotubes in chloride solutions is much faster and violent than in fluorine based similar solutions. In addition to this, the formation mechanism of titania nanotube arrays and influence of critical parameters on the growth of TiO_2 nanotubes were prospected.

2. Materials and Methods

Titanium sample with working area 1cm x 2 cm were used as an anode and stainless steel mesh served as the cathode in anodization method, carried out in chloride containing acid electrolytes. The mixture electrolyte contain 0.75 wt% of perchloric acid($HClO_4$), 0.5 wt% of Strontium chloride($SrCl_2$), 0.5 wt% hydrogen peroxide (H_2O_2)and 0.5 vol.% deionized water in ethylene glycol. At initial stage, titanium samples were mechanically polished using 600 and 1200 grit SiC papers. After polishing the substrates were thoroughly washed with deionized water and ultrasonicated in ethanol, aceton and deionized water about 10 mins separately. Then the titanium samples were dried in an air stream and etched in an acidic solution containing 1 part HF, 4 parts HNO_3 and 5 parts H_2O for 30 seconds. Then the substrates were again washed and dried at room temperature to undergo potentiostatic anodization of 45V in a bath for 20 minutes. The anodization process was carried out in an agitating bath by using magnetic stirrer. Then the anodized samples were again washed in deionized water and dried at room temperature [29].The amorphous phase of titanium nanotubes (TNTs) obtained was annealed at 500^0C for 2 hours to achieve a crystalline nanotube structure[30].

For growth of TNTs in chloride medium, the chemical reactions basically occurs are

Anodic oxidation:

$Ti + 2H_2O \rightarrow TiO_2 + 4H^+ + 4e^-$ ……………………………….(1)

Chemical dissolution:

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

Characterization

The structural characterization of titanium nanotubes was done by X-ray diffractometer (XRD; X' pert-APD, Philips, Netherlands) using Cu Kα radiation (λ = 0.154 nm, 40kV, 20 mA) and scanned in the 2Θ range of 15-90^0[31]. The surface morphology and thickness of the coating layer of anodized sample was studied by high resolution Field emission scanning electron microscopy (FE-SEM; JSM- 6700, JEOL, Tokyo, Japan). The roughness (Ra) of the coating was determined by surface measuring instrument (SURFTEST, Mitutoyo, Japan). By contact angle detection system (OCA15 Plus, Data physics, Germany) the coating wet ability and hydrophilicity was measured by capturing the image of ultrapure distilled water (0.6 µl) with a delivery span of 10 seconds. The tensile pull-off adhesion strength was evaluated by using a universal testing machine (Instron; 4202) under tension mode in conformance with ASTM (633-79). The cytocompatibility of the anodized sample in physiological environment was investigated by Lymphotic culture study by using human umbilical cord blood in microbiology laboratory.

Lymphocyte culture

The immune response of an anodized sample inside the human body environment can be studied by lymphocyte culture of the sample within the collected lymphocyte pellet. Umbilical cord blood (UCB) was collected in a sterile 15 or 50 ml size falcon tube (Tarson, Kolkata) with an aliquot of 100 or 250µl 1,000 IU heparin (HiMedia, Mumbai), immediately after delivery of an infant and the cord blood sample (less than 15 to 50 ml) was stored at 4^0C till use. Lymphocytes were isolated immediately or within at the best 24 hours after collection. For the isolation of lymphocytes, the collected UCB sample was diluted with an equal volume of phosphate buffered saline (PBS) solution. Further, the mixture was loaded carefully into a centrifuge tube for over-layering with lymphocyte separating medium (LSM, HiMedia), which was one-third the total volume of the mixture. The mixture was centrifuged at 1800 rpm for 25 minutes at 22-24^{0}C, as a result, four heavy to light layers, red blood cells (RBC), LSM, buffy coat and plasma were seen. The buffy coat layer with mononuclear cells was taken out carefully from the tube. To the separated cells of the buffy coat layer, after the addition of another aliquot of PBS for the 1:1 ratio, re-centrifugation was performed at 2,000 rpm for 5 minutes. The pellet of lymphocytes was taken for culturing and cell counts.

3. Results

3.1 Current – time curve

The evolution of electrochemical anodization can be clearly analyzed by current –time curve as shown in figure.1.

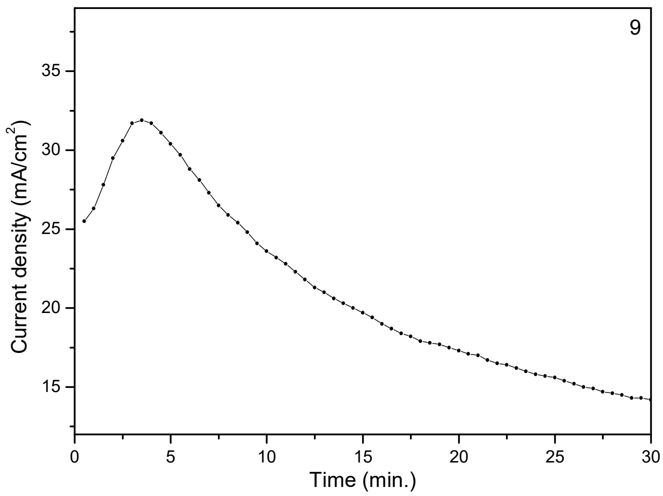


Figure. 1. Current- time curve in $HClO_4$ + H_3PO_4 mixture solution

According to the literature fluoride play primary act during electrochemical anodization for the formation of TNTs. In this current study, chloride provide thick oxide layer with ultra-high ratio replacing fluoride. Initially the Ti foil has greater value of conductivity due to applied potential. At this state current increases drastically within few seconds from 11mA to 33mA and continued for about few minutes. This was due to limiting value of current, depending up on our power supply. Then current started to decrease and slowly achieved steady state. The one set decrease of current may be assigned to the fabrication of the TiO_2 layer from the discharge of OH^- ions on the surface of Ti substrate, which leads to oxidation process. The obstructing effect of the devised oxide layer prompts the electronic configuration decreases in this region[32]. Here electronic field attracts chloride ions at the growth front to etch the surface causing pits and pores. The incessant in ward growth of pits was pursued to outline inner tubular spacing making the current to achieve steady state.

3.2 XRD

The XRD of the coating obtained on Ti substrate at 45V for 20 min is depicted in the figure. 1. The anodized TiO_2 layer, obtained in stirring condition of electrolyte showed amorphous phase. After annealing for 2 hours, at 500^0C, the titania nanotubes crystallized to anatase phase. The lattice parameter *a* and *c* of annealed coating were attained from their *d* spacing a = 3.788 A^0 and c =9.402 A^0. These evaluated value harmonized the standard pattern of anatase (JCDDS card 89-4921; a =3.777 A^0 and c = 9.501 A^0). But here some rutile phase also noticed. The X-ray diffraction (XRD) results procured is exactly mirror to that of fluoride- containing electrolyte. At 230^0C the fabricated TNT is amorphous, but on increasing temperature around 430^0C, the rutile phase emerges as reported in the fluoride-based electrolyte [33,34]. Shanmugam et al., [35] stated that the carbon content in the chloride based electrolyte is same as that of the “ core- shell” carbon-coated titania particles and reported that carbon-containing layer are too thin (≈ 2nm) to be detected in the XRD peak. Hence the rate of transition of anatase-to-rutile phase is due to carbon containing sample as compared to non-carbon-containing sample.

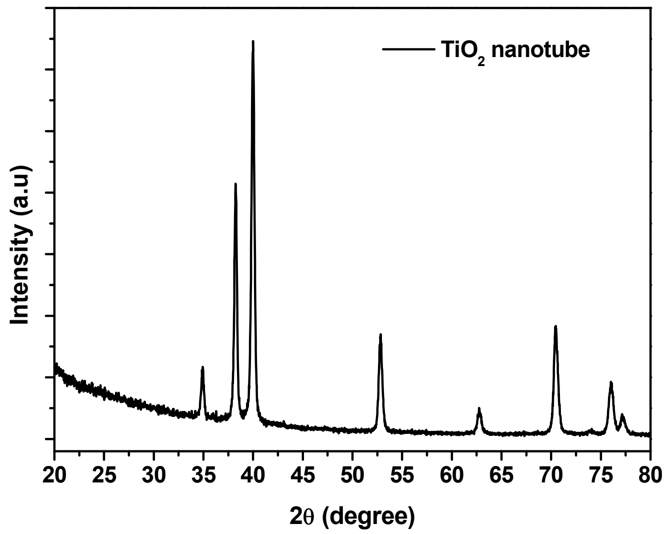
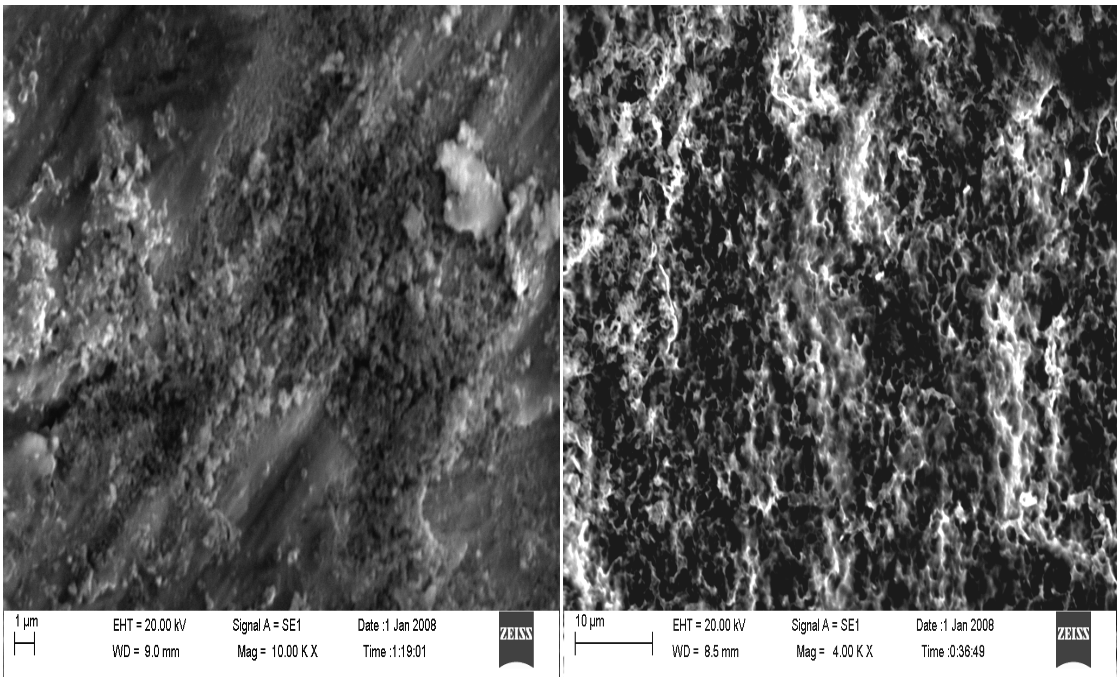


Figure.2. XRD patters for anodic titanium oxide nanotubes after annealing at 500^0 C.

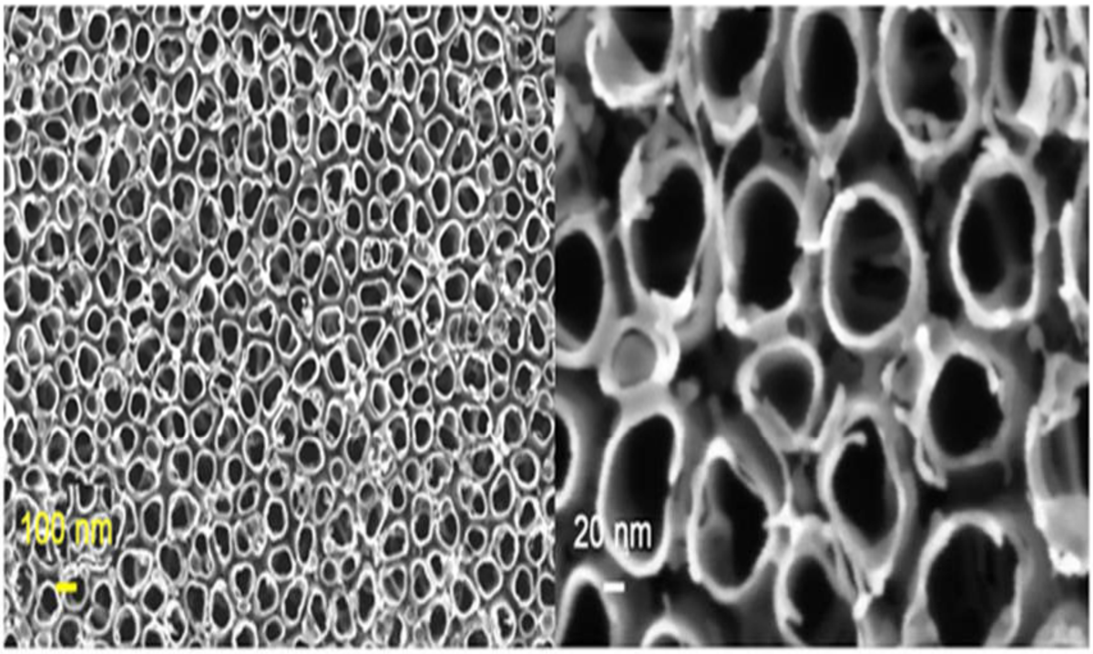
3.3 SEM

The surface morphology of all the samples was illustrated in figure.2. At low voltage random cracks were noticed but no any considerable structures were visualized on the Ti substrate (as shown in sample s_1). But on increasing the voltage to 20V (sample S_2) a well defined tubular structure appears slowly with increasing value of voltage. The nanotube diameter is detected as less than 150 nm. On increasing the anodization voltage well defined tubular nanotube structure observed and the resultant length of nanotube come in to view to be of 14µm with suitably formed individual tube walls as shown in figure S_3. The average diameters of the nanotubes were noticed to be of 120nm. Here it was detected that the chloride based electrolyte obey the same hypothesis as that of fluoride based electrolyte, that on increasing anodization voltage diameter increases appreciably [36, 37,38].



(S_2)

(S_1)



(s_3)

Figure.3. SEM images of TiO_2 nanotubes (S_1) below 20 V, (S_2) at 40V(S_3) at 45V.

Mechanism of nanotube formation

On application of the applied voltage current abruptly rose to maximum value to create a TiO_2 layer on Ti substrate, which instantly provide resistive medium to the substrate. To maintain chemical dissolution within the electrolyte, Cl^- ions drifted towards anode and arbitrary attacked TiO_2 surface to form pits and cracks [39].On increasing the voltage above "threshold voltage" nanotube grown and elongated inside the pits. Until the electrolyte ceases to supply Cl^- ions at a steady state of current. During anodization negatively charged ions i.e OH^- and Cl^- drifted towards Ti at constant rate. OH^- were plays major role to form TiO_2 layer and Cl^- successively etched TiO_2 layer to obtain nanotubes. The etching characteristics of Cl^- could be expressed by equation (2). The order of case obtaining during the nanotube formation is similar to the suggestion given by Taveira et al.,[40]. During initial stage a compact oxide layer of TiO_2 formed and that layer expected for a compact high-field oxide formed at 40V. At initial stage of polarization, during the formation of TiO_2 layer, suddenly current drop consonance with the high –field-law. But due to the drift of Cl^- ions from electrolyte towards oxide substrate interface, dissolution and breakdown of barrier oxide film occur and simultaneously current again start to increase. Due to this breakdown stage, disordered warm like structure noticed along with ordered nanotube structure. Here a low pH region established towards the bottom of the pore, which leads to enhance dissolution rate of TiO_2 and pore penetration occure in to the substrate [41]. The lower value of pH at the bottom of the pore prolonged it further in to the substrate, causing a tubular structure having well defined diameter at the top. But due to agitation of electrolyte the wall of the nanotube attacks to have a crinked structure. According to Kar et al., the adhesion strength of nanotube titanium dioxide layer developed during anodization in the fluoride bath to be in the range of 16 to 21 MPa[42]. But for body implantation the adhesion strength of the implant material demand to be of 18MPa to suitable with matching value of human bone. In this current experiment, the adhesion strength of the anodized nanoporous titanium dioxide layer is obtained as 15 MPa, which is nearly to the matching value of adhesion strength of the implant with the human bone.

3.4 Lymphocyte culture

Lymphocyte system plays an important act in the immune response to infectious agents. In living body bone marrow regularly generates cells that will become lymphocytes. In few cases the produced cells will directly enter to our blood stream but in many cases it passes through the lymphatic system. In living body system lymphatic system is nothing but a group of tissues and organs like spleen, tonsils, lymph nodes, which provide immune response to the infectious agents such as, bacteria, virus and other toxins that make us sick. Also white blood cells (WBC) in living body are a supreme part of the immune system and lymphocytes are one of the various different types of white blood. Lymphocyte culture of the anodized titanium sample in perchloric acid and phosphoric acid mixture electrolyte reveals the immune response of the sample being used as an biomedical implant in human body environment.

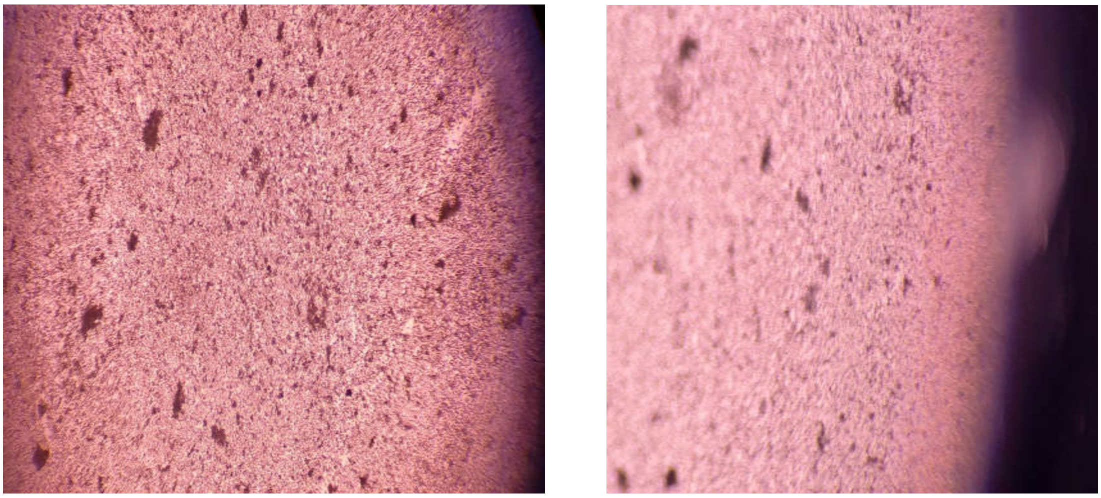


Figure.4. Shows the images of lymphocyte culture in $HClO_4$ + H_3PO_4 mixture solution

4. Discussion

Surface roughness is an important factor for bone-anchoring and biomechanical stability. The surface rough nesses of the anodized titanium surface are greater than that of controlled anodized TiO_2 i.e 0.61µm. Ra of anodized sample is 0.67µm**.** With increasing value of surface roughness, the adhesion strength of the substrate increases and in contrast smooth surface provide less adhesion strength. Roughed surface furnish better mechanical anchoring of the biomaterial surface by means of bone in-growth. Also the rate of osseointegration is directly proportional to their composition and surface roughness. Surface roughness of an implant is more compatible for bone anchoring and biomechanical stability. The surface property, like topography enhances the mechanical stability of the implant/ bone tissue interface. If the surface roughness is more, more is the osseointegration and tensile pull of strength of the coating [43]. The direct interaction of bone matrix and osteoblasts with the implant biomaterial encouraged by bone implant interfaces.

For bone-biomaterial interaction, osteoblast adhesion is highly required. By lymphatic culture the immune response and the extended filopodia of the coating surface is noticed, which provide strong evidence of the spreading of the osteoblast cells and forming of the lamella on the coating surface. According to the literature, cell culture provides adsorption of proteins, mainly extracellular matrix (ECM) on to the implanted substrate, which enhance the cell attachment on to the metal surface. The two prime cell attachment advancing proteins, present in serum for osteoblast culture are fibronectin (FN) and Vitronectin (VN). Both FN and VN smoothly adsorb onto positively charged surface of materials and effect over all cell attachment and spreading. Here strontium ions present on the titanium surface form the location of positive charge and favorablr for the adhesion of FN and VN.The contact angle of the anodized Ti sample is less than that of controlled TiO_2 sample i.e 66^0. Hence the low value of contact angle 56^0 shows hydrophilic surface. Hence hydrophilic surface of TNTs anodized sample enhance the formation of proteins and osseointegration. The adhesion strength has greater attribute for implantation in biological medium, which reveal weight loss when wear against bone. The low value of adhesion strength i.e 13.2 MPa of our coating characterize better matching of the implant with human bone. Lymphotic culture shows the immune response of the implant in the osteoblast-like cells. Hence anodized Ti sample shows remarkably high cell detection than control. For bone implant interaction the direct interlinking of bone matrix and osteoblast with biomaterial is an essential factor. Hence osteoblast adhesion is an important aspect for bone-implant interaction. Hence anodized titanium implant material shows higher Ra, hydrophilicity and mechanical strength with good proliferation within the human body environment, which show better protein aggregation and osseointegration in the implanted condition.

In chlorine containing electrolyte, the titanium oxide nanotube were fabricated at high-aspect-ratio as compared to fluorine-containing electrolyte. Here anodization obtained at higher growth rate (13 µm/min)[44]. Hence rapid growth occur, due to which the achieving nanotube diameter would be independent of the applied potential[45,46]. But in this case due to the presence of the carbon ions in the electrolyte the rapid break down anodization (RBA) converted to plastic flow mechanism in chloride containing electrolyte. Because of which one-dimentional self-organized growth of NTs were achieved. At slow reaction rate the mechanism of nanotube formation shifted from RBA to plastic flow, at which the diameter of anodized NTs depend up on applied potential, which is noticed in our current work [47].

Macak et al.,[48]investigated that high-aspect-ratio nanotube can be fabricated in fluoride based electrolyte by maintaining the field assisted dissolution rate at a high level and reducing the chemical dissolution of the developing titanium dioxide nanotubes. To achieve this they utilized an anodization method in which pH value of the fluoride based electrolyte is kept high (pH 5) in the top region of the developing titanium dioxide nanotubes and low (pH 2) at the bottom region of thegrowing titanium dioxide nanotubes. Paulose et al.,[49] reported the mechanism of the fabrication of the high-aspect- ratio titanium dioxide nanotube in organic polar electrolyte with higher value of dielectric constant. Here such electrolyte (formamid-based electrolyte) promotes the extraction of Ti ions from the bottom levels of the growing titanium dioxide nanotubes, yielding to a steep growth rate of the nanotubes. In this current study, the fabrications of the titanium dioxide nanotubes in perchloric acid medium ao lower value of pH (pH $\approx$3) exert similar effect of anodization as performed by electrolyte used by paulose et al. In this current study much higher field- assisted dissolution and oxidation rate observed as compared to fluoride based electrolyte. The achievement of long, ordered nanotube in this cuuent study is because of lower chemical dissolution rate and higher field- assisted dissolution rates and oxidation rates. According to the literature the high-aspect-ratio nanotube formation in the new chlorine-based electrolyte is may be also due to the role of carbon. It is well-noted that chlorine and TiO_2 are un reactive with each other. Fracassi and D' Agostino [50] reported that, " the pure chemical reaction between TiO_2 and Cl_2 needs high temperature (350^0 C) and presence of reducing agent, such as carbon to occure". Later on Yang and Hlavacek,[51] reported a mechanism supporting to the Fracassi et al., using gas-phase kinetic data that at " low temperature" chlorination of TiO_2 include a ternary activated complex of $TiO_2 – C – Cl$ and stated that incorporation of carbon as an essential reducing agent play major role in the fabrication of the highly-ordered, high-aspect-ratio nanotubes in an chlorine based medium. The presence of H_2O_2 also attribute to enhance the reducing capacity in anodization medium during nanotubes formation, because H_2O_2 is a good reducing agent. Here it was suggested by Yang et al., that chlorine playa a catalytic role, during anodization to form a ternary $TiO_2 – C – Cl$ complex and high carbon content may yield of the very quick fabrication of these nanotubes. The mechanism of the formation of the nanotubes is that the thermodynamically less stable atoms or molecules like negatively charged organic ions and carbon derived from their reduction diffusion away and restore by thermo dynamical more stable molecules or atoms yielding larger nanotube in an instant. Hence rapid fabrication resulted by normally more impurity, in other word existence of organic acid, will mean carbon capture. It was recorded that strontium can substitute calcium in hydroxyappatite lattice. Sr plays vital role in the bone formation and exerts an anabolic effect on osteoporotic bone enhancing osteoblastic cells activity on implant. Also it was studied that sr can promote osteogenic differentiation of human C3H10T1/2 cells. The presence of Sr in this current electrolyte advantageous to promote bioactive nature of the anodized sample by increasing osteogenic potential of human bone marrow stromal cells (BMSCs) at the bone implant interface. Hence presences of Sr extremely enhance the bioactivity of the anodized titanium in perchloric acid medium. This current study provide less hazardous way in fabricating TiO_2 and investigating the effect of ratio DI:EG (perchloric acid based) electrolyte towards synthesizing highly ordered, self organized, biocompatible titanium dioxide nanotube for biomedical applications.By analyzing all its characteristics the current anodized sample is demanded as better biomedical implant, exhibiting better osteogenic and biocompatibility in human body environment.

Sr^{2+} ions within the electrolyte act as high reactive precursor, bring about high nucleation and growth rate under anodized condition, to obtained a self organized, highly ordered titania nanotube. Also, also strontium has been recorded to influence the ability to promote mew bone regeneration and restrict the resorption of osteoblast cells [52,53]. According to the literatutre [54,55]low concentration Sr ions encourage the new bone formation and proliferation. By lymphatic culture, the cell formation and proliferation on the anodized implant material is analysed clearly in this current work, which may be due to the suitable amount of the strontium ions present in the electrolyte. From the literature it has reported that the surface energy and hydrophilicity of the surface directly infect the biocompatibility by changing the absorption ability of the implanted surface. The highest surface energy of Sr/TNTs obtained due to the peculiar surface chemistry of $SrTiO_3$- TiO and the notable topography of nanoparticle furnished on the nanotube. Hence presence of strontium ions in the electrolyte enhances nanotube length also enhance proliferation, osteoblast activity and biocompatibility of the current anodized implant.

5. Conclusion

In this present work, fourth-generation's titanium nanotube arrays fabricated by anodization method in perchloric acid, phosphoric acid mixture electrolyte have demonstrated. Ultra-high density single nanometer-scale titania nanotubes readily grow during anodization and structural features of the nanotubes can be controlled by electrochemical parameters, such as voltages, time, pH of electrolyte, temperature and concentration of acid solution used. The perchloric acid anodizing leads to ultrahigh super hydrophilic properties with appreciable surface roughness and contact angle on the titania substrate. Also provide advantageous properties such as cell viability, antimicrobial activity, osteogenic and cell proliferation, super biocompatibility along with better immune response to the infectious agents. So perchloric acid anodizing titania nanotube implant can be used as a next generation bioimplant in bone tissue engineering.

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