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S. Sobieszczyk
Gdansk University of Technology, Faculty of Mechanical Engineering, Department of Mechanical Engineering and Materials Strength, 80-952 Gdańsk, Poland,

SELF-ORGANIZED NANOTUBULAR OXIDE LAYERS ON Ti AND Ti ALLOYS

Review Paper

ABSTRACT

To improve bioactivity of titanium and titanium, the implant surface modification by formation of self-organized TiO_2 nanotube arrays with electrochemical techniques is presented. The influence of electrolyte composition and deposition parameters during anodization is characterized. The enhancement of phosphates deposition by titanium nanotubular structure is discussed. The calcium phosphate ceramics is shown to be uniformly deposited on anodized titanium surface of a nanotubular oxide layer.

Key words: *electrochemical anodization, nanotubes, phosphate deposition, self-organization*

INTRODUCTION

Titanium implants exhibit good physic-chemical stability, mechanical integrity, good biocompatibility and excellent corrosion resistance in application as a osseointegrative implant material [1]. The osseointegration of the implant is *via* the natural oxide TiO_2 layer, but it is a long process [2]. In order to improve the bioactivity of titanium and enhance bone growth, the modification of surface layer can be applied, e.g. roughening by sand blasting [3], hydroxyapatite coating [4] or chemical methods like acid etching and alkali treatment [5]. One of the recently studied methods of surface modification is formation of self-organized TiO_2 nanotube layers, which can enhance calcium phosphate formation in comparison to dense TiO_2, by various techniques such as sol-gel method [6], electrophoretic deposition [7] and anodization [8]. Electrochemical method gets more attention due to its low cost and relative ease of fabrication that the other techniques [9].

Titanium and titanium alloys with anodic tubular oxide layer are very interesting materials for applying them as high performance biomedical materials, because they have excellent physical and chemical properties as well as biocompatibility [10-13]. In order to improve bioactivity and enhance bone growth, self-organized TiO_2 layers with controlled structure and morphology can be used [10,14-17]. Present research findings indicate that TiO_2 nanotubes could also be applied in gas sensors [18], bio-sensors [19,20], photocatalysis [21,22], photoelectrolysis [20,22], photoelectronics [23] as well as successfully used for surface treatment for biocompatibility purposes [24,25].

TiO_2 NANOTUBE FORMATION

Titanium oxide nanotubes can be prepared by various methods, like sol-gel [6], electrophoretic deposition (EPD) [7] and anodization [8]. The latest one is the best for nanotubular oxide layer creation for biological applications, as it enables to achieve the highest adhesion strength of the layer comparing to that of sol-gel and EPD.

There are some gaps between adjacent nanotubes of ~15 nm, which are important for minimizing the interfacial stresses between two dissimilar materials joined. Also, such gaps between nanotubes create pathways for body fluid supply with ions, nutrients, and proteins [14].

The typical nanotube arrays consist of single nanotubes of 80-150 nm in diameter and 0.5-25 μm in length [26,27]. The physical and chemical properties of nanotube layer can be controlled by changing the parameters of fabrication process [8,15,26,28-31].

The TiO_2 nanotube arrays formation in F^- - containing electrolytes is a result of two competing electric field-assisted processes: hydrolysis of Ti metal to form TiO_2 (1) and chemical dissolution of TiO_2 at the oxide/electrolyte interface (2) [32,33], which is presented in Fig. 1:

$$Ti^{4+} + 2H_2O \rightarrow TiO_2 + 4H^+ \qquad (1)$$

$$TiO_2 + 6HF \rightarrow [TiF_6]^2 + 2H_2O + 2H^+ \qquad (2)$$

The exact mechanism of TiO_2 nanotube formation and self-ordering kinetics is the subject of very intensive research but it remains still unclear [8,27-29,31,32,34-41].

The process of TiO_2 tube growth consists of three stages, as explained in detail by Crawford *et al.* [32]:

1. Initial barrier layer formation – there is an exponential decrease in anodic current density until it reaches the steady state. A current drop is due to the formation of compact oxide film which increases the resistance reducing the current density [42].
2. Formation of uniformly distributed pores – chemical dissolution of barrier oxide layer and increase in current density. During this stage, nanopores are formed by random local dissolution of TiO_2 surface, as often indicated [36,41,43,44]. Beranek suggests that pore formation at the beginning takes place at random locations, and self-organization of pores is the effect of completion of simultaneous processes (1) and (2). Raja *et al.* [45] suggest that pore's ordering is the effect of local surface perturbations, where the strain energy increases causing the migration of F^- ions to regions with higher strain energy and of hydrogen ions in order to maintain electrical neutrality, and leading to the dissolution of Ti^{4+} ions.
3. Separation of interconnected pores into nanotubes – the current density again stabilizes. The nanotubes formation is the result of simultaneous growth of voids (regions between pores which are susceptible to field-assisted oxidation/dissolution) and pores as suggested by Mor *et al.* [44]. In contrast, Raja *et al.* [45] claims that separation of pores into individual nanotubes may be a result of repulsive force between cation vacancies. After 1 h nanotubes are grown, as confirmed also by Crawford [32].

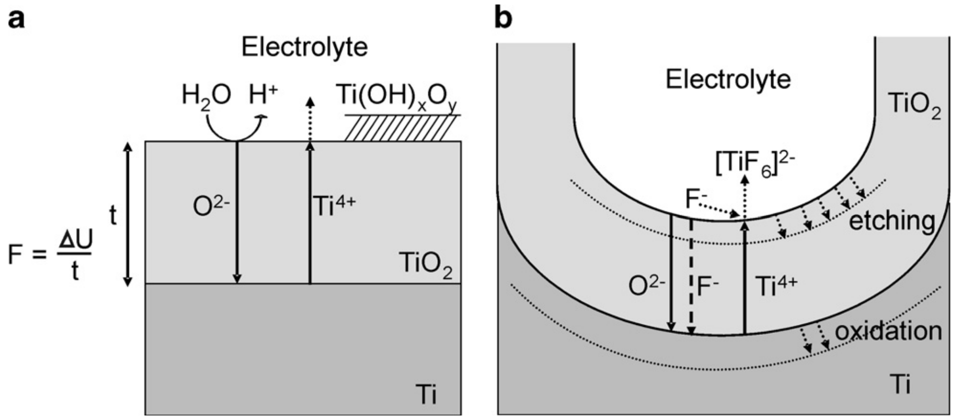

Fig. 1. Schematic representation of processes in TiO2 nanotube formation during anodization a) in absence of fluorides, and b) in presence of fluorides [36]

On the basis of the reports on growth mechanism of titania nanotube arrays, the nanotube formation can be schematically represented by Fig. 2 [46].

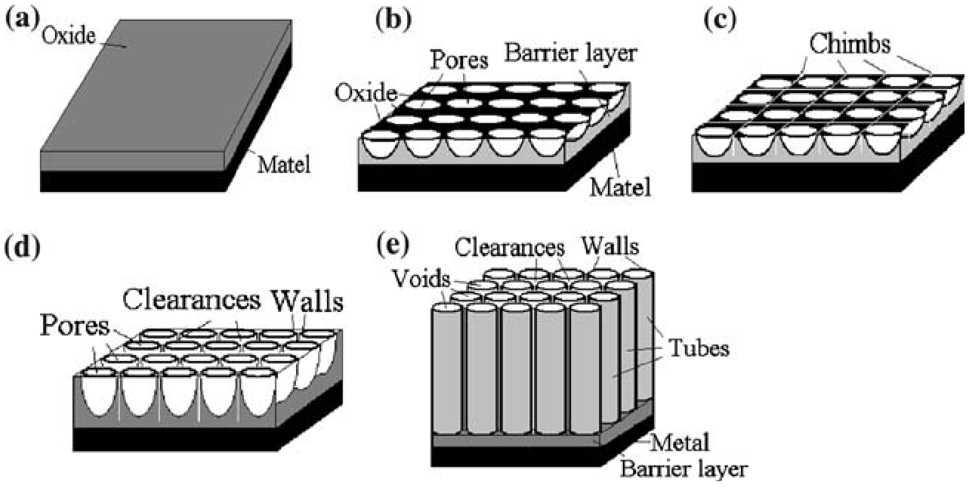

Fig. 2. Schematic diagram of the evolution of titania nanotubes in anodization: (a) oxide layer formation; (b) pore formation on the oxide layer; (c) chimbs formation between pores; (d) growth of the pores and the chimbs; (e) fully developed titania nanotube arrays [46]

Effect of electrolyte

A vertically aligned nanotube array of titanium oxide can be fabricated on the surface of titanium substrate by anodization in slightly dissolving fluoride-containing electrotyles [14,17,29,46-48]. Electrolytes without fluoride, e.g. sulfuric acid [9] have been widely used to fabricate a nonporous compact titanium oxide layer at low potential, and porous oxides at high potentials due to the electrical breakdown of the oxide [49]. Anodization in fluoride-containing electrolytes results in ordered nanotube arrays, rather than porous or nonporous structures of the oxides. Chemical composition and nanotube architecture can be adjusted by the type of electrolytes, voltages and anodic oxidation times.

Table. 1. *Aqueous electrolytes for anodic oxidation*

Electrolyte	Voltage [V]	Duration [h]	Outer diameter of nanotube [nm]	Nanotube length [nm]	Wall thickness of nanotube [nm]	Reference
0,5 wt.% HF + H_2O	20	0.33	100		15	[56]
	20	0.5	100	0.25÷250 μm	15	[14]
	20	0.5	100			[57]
	40	24	170	12 μm	30	[17]
		0.5	100	250	15	[58]
		0.33	80	400		[59]
1 m H_2SO4 + 0.3÷0.5 wt.% HF	20	2	100	500	15	[60]
				500		[41]
	20	0.5	20÷30	500		[61]
	12	4	40	0.12μm		[55]
1 m H_2SO_4 + 0.1 m NaF	20	0.25÷4	45÷50	234÷625	12÷15	[32]
	20	2	20÷150	0.25÷720 μm	7÷35	[62]
0.5÷1 m H_3PO_4 + 0.3÷0.5 wt.% HF	20	0.5÷12		250 nm÷1 μm		[63]
	20	2	60÷100	400÷500		[7]
	20	2	100	1÷2 μm		[10]
	10	2	50			[30]
	1÷25		15÷120	500-600		[36]
		2	110	1.1 μm		[64]
	20	2	100	1 μm	10	[65]
	10	2		500		[66]
0,5 m H_3PO_4 + 0.138 m NaF	20	2	60÷100	400÷500	~20	[7]
	20	28 min	100			[50]
1 m NaH_2PO_4 + 0.5 wt.% HF	20	2		1 μm		[66]
	20	2				[67]
1 m $(NH_4)_2SO_4$ + 0,5 wt.% HF		2	100	2.5 μm		[68]
1÷2 m $(NH_4)_2SO_4$ + 0.1÷0.5 wt.% NH_4F	20		50; 100			[69]
		3	50			[52]
		2	90	10.7 μm		[70]
	20	4	60÷80	300		[71]
0÷2 m $(NH_4)_2SO_4$ + 0.5 wt.% HF		6	20÷110	110÷700		[72]
1 m Na_2SO_4 + 0.5 wt.% NaF	20	3	100	1.66 μm	10÷20	[26]
	20	2				[62]
1.75 m H_2SO_4 + 0.1÷0.5 m NH_4F		1	120	300		[46]
			120	300		[73]
0.3÷1 m H_3PO_4 + 0.135 m NH_4F	20		50÷100			[74]
		2	90÷180	400	10÷20	[75]

There are different kinds of solutions, among them are several aqueous electrolytes [9,50] which have been used to create TiO_2 tubular structures. Li [17] has noticed that when using aqueous electrolytes, nanotubes are short in length and contain ridges and circumferential serrations (*Table 1)*. The smooth, long and ridges-free nanotubes can be achieved in highly viscous organic electrolytes like glycerol and ethylene glycerol electrolytes as well as fluorinated dimethyl sulfoxide and formamide [17,27,51-54]. It has been confirmed that if the anodization treatment is carried out in less aggressive electrolytes like ethylene glycol solutions containing HF, higher thickness of layer can be achieved, up to 250 μm [40,55].

The investigations indicated that pH of the electrolyte influences the thickness of the nanotubular TiO_2 layer [26,53]:

pH up to 1 – the thickness of TiO_2 ~1 µm
pH neutral – the thickness ~2 µm (in solution of $(NH_4)_2SO_4$
pH (ethylene glycol solution) – the thickness $\geq$ 250 µm

The concentration of HF has significant influence on creation of tubular structure of oxide film. In electrolytes whose acidity is too high or too low, no titania nanotube arrays can be formed. According to Schmuki [52], anodization in 0.15-0.4 wt% HF concentration enables nanotubular formation. Concentration less than 0.15 wt% results in disordered and spongy-like porous titanium oxide films. Also, when increasing HF concentration (from 0.15 to 0.4 wt%), the average pore diameter and pore spacing decreases, from 89 to 56 nm and 156 to 106 nm, respectively [42].

The anodization process resulting in TiO_2 nanotube formation in aqueous electrolytes (not only) is often performed by using a 20 V anodization voltage for 20-30 minutes at room temperature [7,14,50,58].

Effect of anodization voltage

The structure of nanotubular TiO_2 arrays depend on the voltage applied during anodic oxidation due to the equilibrium between electrochemical formation of titania and the chemical dissolution of titania in fluoride-containing electrolytes [37,46]. The tube diameter and thickness can be adjusted by changing the applied voltage, anodization time and pH [10,29,75]. The investigations have indicated that tube diameters and layer thickness linearly depend on the applied potential (*Table 1*). This is in line with the experimental findings [75]. Kodama *et al.* [10] also have noticed that change in the pH of electrolytes affects the layer thickness while keeping the diameter constant.

It is important to note that the higher anodization potential is, the larger elements of the arrays are formed [30.46,76]. The results have been confirmed by Bestetti *et al.* [42] during the anodization in 1 M H_2SO_4 and 0.15 wt.% HF at 10-35 V. An increase in cell voltage from 10 to 25 V results in increasing average pore diameter from 30 nm to 150 nm. At 30 V of voltage and above this value the nanotubular structure disappears.

Effect of current density

In the first few seconds of anodization process there is an exponential current density decrease due to the formation of compact oxide film, then the current density starts to grow as the solubility of the Ti oxide increases [42]. The investigations have shown that there is a characteristic peak current before it starts to decrease and stabilize [61,77] which is related to transition from an initial irregular sponge-like porous structure to an ordered tubular structure. The decrease in current density is related to the diffusion distance that increases following the growth of nanotubes [70,77].

The current values during anodization process show regular periodical fluctuations due to the growth and dissolution of the oxide film, passivation and depassivation reactions related to TiO_2 nanotube formation [40,42]. The experiments carried out by Macak *et al.* [40] revealed also that periodical of current oscillation have influenced the structural features of the nanotube causing ripples on their walls. The exact mechanism of the interaction between current oscillations and structural features is still unknown [29,40,78].

The fluctuations of the current density may be attributed to hydrogen bubbles produced during anodization which remain on cathode surface until they have sufficient buoyancy to break free from the surface, as have been indicated by experiments of Kaneco *et al.* [28]. In order to reduce hydrogen bubble size, methanol can be added to the solution to enhance the hydrogen removal from the cathode surface. Methanol reduces the hydrogen bubble size due to the reduction in surface tension. Methanol also enables to control the current density precisely as shown in Kaneco experiments [28].

Kaneco *et al.* [28] reported the results obtained during anodic oxidation in methanol and water mixture electrolytes in presence of 1 wt.% HF under constant-current conditions, by adjusting the voltage. The results show that by controlling the current density during the electrolysis the tubes of different diameter could be produced. The current density plays a significant role in nanotube formation with respect to pore size and morphology. As the current density increases, the electrochemical etching rate, power and electric field intensity increase.

Effect of anodization duration

Crawford *et al.* [32] reported that the tube diameter and wall thickness do not vary significantly with anodization time. Their foundings are well in line with the observations made by other researchers under similar conditions of processing [37,79].

Effect of temperature

Prida *et al.* [42] reported the temperature influence on the growth of self-ordered TiO_2 arrays. Especially, a low temperature (about 2-3^{0}C) inhibits the nanotubular structure formation resulting in sponge-like randomly porous structure. Typically, anodic oxidation is conducted at room temperature [14,17,29,47].

Wettability of TiO_2 nanotubes

The hydrophobic and hydration forces play a significant role for biological systems in protein adsorption and cell adhesion [42]. The nanotube oxide layer can alter surface properties like wettability modifying titanium surface from hydrophobic to hydrophilic because the nanotubular structure allows the liquid to penetrate deeper into the film and then decreasing a contact angle [33,68]. As a result, higher hydrophilicity and surface energy improve the ability to apatite forming in simulated body fluid (SBF) [33].

The wettability of the tube surface also can be adjusted by an attachment of monolayer of some compounds (octadecylsilane or octadecylphosphonic acid) on the surface by UV-light treatment. The modification of tubular TiO_2 was found to be extremely stable over time in contrast to UV modified dense TiO_2 surface [68].

Effect of water content and viscosity

The water content in electrolytes affects strongly the tube geometry as shown in ethylene glycol and glycerol water mixtures [30,40,52]. The tubes have significantly higher length and smooth morphology in less aggressive electrolytes, like organic non-acidic electrolytes [55,68].

Alkali treatment

NaOH is added to the electrolyte in order to adjust the pH to different values enhancing the bioactivity of titanium oxide nanotubes [10,14,56].

HAp formation can be accelerated by creation of sodium titanate layer when titanium surface is subjected to NaOH treatment followed by heat treatment [14,58]. The nano-fiber structure appears at the top of nanotubes possibly due to the surface tension related to NaOH which has difficulty in getting into and between TiO_2 nanotubes [14,58]. The sodium titanate composed of mixture of $Na_2Ti_5O_{11}$ or $Na_2Ti_6O_{13}$ about 8 nm in diameter and 50-100 nm in length [58].

The metallic titanium with titania nanotube arrays significantly shortens the time required for the alkali treatment to initiate the apatite formation after only 30 min. For titanium without titania arrays the apatite appears after at least 24 h [56]. It is noteworthy to say that there is no apatite formation on titania nanotube arrays without NaOH treatment after soaking in 1.5 x SBF [56].

Acid etching

The etching process removes the native oxide layer and is applied in preparation process prior to anodization process [27,50].

The etching process of the titanium surface leads to surface roughness, which is important for increasing wettability of HAp precursor solution on the substrate and enhancing mechanical interlocking between HAp layer and substrate [9,14,50].

Annealing treatment

As-formed nanotube layer is amorphous [10]. Annealing of the amorphous nanotube layer transforming this form to anatase or a mixture of anatase and rutile can enhance the apatite formation [10,58]. It is well established that anatase phase is more efficient in nucleation and growth of apatite than the rutile phase of TiO_2 because of the better crystal lattice match with HAp phase [58].

Heat treatment can be conducted at different temperatures (300-600^oC) resulting in the phase change of tubular oxide. After anodization, the π-TiO_2 layer is amorphous as has been observed by Li *et al.* [17], The tests show that after heat treatment at 300^oC there is anatase π-TiO_2 phase while heat treatment in higher temperature of 500^oC- 600^oC gives some rutile TiO_2 phase (a mixture of anatase and rutile) [17]. There is a gradual transition from anatase to rutile phase when increasing a temperature. Oh *et al.* [58] also noticed that heat treatment ~500^oC produces anatase crystal structure of TiO_2 but heating in a higher temperature, exceeding 600^oC results in the undesirable rutile structure and even some collapse of TiO_2 nanotubes. It is well in line with the observation made by Macak *et al.* [40].

It is noteworthy that as formed nanotubes have some components of hydroxides built-in on the surface of walls and quite significant amounts of fluoride ions are present in TiO_2 structure [81]. After annealing, an almost complete loss of the fluorides is noticed at around 300^oC and surface of hydroxides is also reduced [40].

Kunze *et al.* [26] have noticed that the nucleation rate of apatite is high also for non-annealed TiO_2 nanotubes, but no homogeneous growth of apatite occurs due to the amorphous structure of the oxide. The crystallographic structure does not play an important role in the nucleation of apatite on tubular films at the initial stage of apatite growth, as similar content of Ca and P are measured, no matter if the nanotubes are annealed or non-annealed. However, at later growth stages, the crystallographic structure of the substrate is substantial for homogeneous apatite formation as only on crystalline substrate the stable growth of apatite is possible [26].

The time exposure to SBF is very important, as the amount of apatite at different time exposure remains low as a function of time for non-annealed TiO_2, but the significant increase is observed for annealed TiO_2 nanotubes [26].

Moreover, the investigations carried out by Kodama *et al*. [10] have indicated that heat treatment do not alter the tubular morphology of layers. As shown on crystalline tubes the precipitate apatite is formed from a flower-like initial layer passing then to a compact thick apatite film with the typical fiber-like structure of biomimetic apatite [43]. As a comparison, on amorphous titanium layer the initial flower-like layer does not develop further into the compact apatite film.

In case of pure nanotubular TiO_2 at annealing temperature 450^0C the amorphous phase changes to anatase form. There is a difference in case of $Ti_xNb_yO_z$ tubes as niobium atoms are inhibiting the anatase nucleation and growth processes [47]. Self-organized amorphous $Ti_xNb_yO_z$ tubes transform into anatase TiO_2 after annealing at 550-650^0C.

FABRICATION OF NANOTUBE OXIDE LAYERS ON Ti ALLOYS

The nanostructures on Ti alloys are different from those obtained on Ti substrate [27,78,80] with micro and nanoscale hierarchical surface obtained [27]. Tsuchiya *et al.* [69] have reported self-organization of nanotube layers of two distinct sizes and arrangements on Ti-Nb-Ta-Zr. Yasuda and *et al.* [82] presented the self-organized zirconium titanate nanotube multilayers that were grown by a repeated anodization sequence under different conditions. The bimodal morphology of nanotubes was reported for Ti45Nb [77] and Ti28Zr8Nb alloys [78], related to cooperation between the two alloying elements and their oxides. After 60 min of anodization in 1M $(NH_4)_2SO_4$ solution with an addition of 0.25 M HF at ambient temperature, two-scale self-organization processes have been established with ordered arrangements of nanotubes of 157 nm and 280 nm in diameter. It is noteworthy that the diameters of both smaller tubes and larger nanotubes reveal a linear dependence on the applied potential when applying potential from 15 to 50 V. After the potential reaches 55 V the nanotubes are loosing self-organization starting to have irregular arrangement [77].

HAp DEPOSITION INTO TiO_2 NANOTUBES

The formation of bone-growth-related material such as calcium phosphate is an important issue for biomedical implants as a bone is a calcium phosphate based material containing 70% HAp. In order to enhance the formation of such calcium phosphate, the morphology and kinetic of hydroxyapatite growth have been studied [14,26].

The investigations on the preparation of HAp coating on the TiO_2 nanotubes were seldom reported [10,50,58].

Kodama *et al.* [10] have suggested that TiO_2 nanotube layer can significantly stimulate forming of apatite layers by soaking in SBF comparing with 'solid' oxide layer on titanium. Also, the TiO_2 nanotubes promote the formation of very thick apatite films, whereas on solid (compact) TiO_2 surfaces the apatite layer becomes much thinner [26].

Calcium phosphate can be deposited onto nanotubular TiO_2 layer by electrodeposition in an electrolyte containing salts (*table 3*) [7,50]. The coating morphology can be controlled in the process of electrochemical deposition by varying the electrochemical potential, current density, electrolyte concentration and temperature [83].

Table 3. *Electrolytes for calcium phosphate electrodeposition*

Electrolyte	Temp. [°C]	pH electrolite	Reference
0.04 M $Ca(NO_3)_2$+0.027 M $NH_4H_2PO_4$ + 0.1 M $NaNO_3$	25	4,5	[50]
$Ca(OH)_2$ + 0.02 M $(NH_4)_2HPO_4$	25		[10]
0.04 M $Ca(NO_3)_2$ + 0.027 M $NH_4H_2PO_4$ + 0.5M NaCl	65	4 and 6	[7]

In order to deposit CaP starting from the bottom of nanotubes, the potential pulsing was applied in two step process composed of first pulsing the potential between -1200 mV and 200 mV for 60 cycles and then applying a constant potential of -1200 mV [7]. In contrast, Wang *et al.* [50] used cathodic deposition of calcium phosphate onto nanotubular oxide at a constant potential of 3V, where the HAp nanocrystals were anchored inside and between the nanotubes.

Kodama *et al.* [10] used alternative immersion method (AIM) treatment which preloads nanotubes with synthetic HAp, achieving flake-like deposits on the nanotube layer as well as inside each single tube. The experiments reveal that there is a linear dependence of the amount of synthetic HAp with increasing tube diameter, giving the best results for the 100 nm round tubes, which means that capillary forces and diffusion length are less dominant than the available area of the tube wall and the available empty tube volume. That means that the tube diameter and tube length are the most important factors. They also noticed that without AIM treatment, no significant apatite formation could be found [10]. The nanotubular surfaces may be about 46 times as large as the respective compact TiO_2 surfaces [26], so that nucleation of apatite is accelerated because a larger specific surface area is available for nucleation. As a result, nanotubular surface can carry more OH^- groups and leads to a very dense formation of apatite nuclei. It is well known, that OH groups adsorbed on the oxide layer, play an important role in nucleation process since the adsorption of Ca is supposed to initiate the nucleation of apatite [10].

After alkali treatment (in NaOH), which generally results in amorphous sodium titanate, the formation of HAp in SBF is accelerated [58]. In presence of sodium titanate the HAp can be formed by ion exchange between Na^+ on the host structure and Ca^+ in the SBF solution [15].

BIOACTIVITY STUDIES

The bioactivity assessment of bone-implant interface can be evaluated by *in vitro* apatite deposition during exposure in SBF, with ionic concentration nearly equal to human blood plasma [26]. HAp formation in SBF is influenced by the surface roughness, electrical charge of host structure, concentration and pH of SBF [58]. Also, according to Webster [84], the nanostructure, as it is in case of nanotubular oxide layer, significantly improves osteoblast adhesion.

The titanium specimens with tubular oxide layer have been soaked in SBF solution [10,17] for 2-14 days under static conditions at 37°C. After soaking for only 2 days spheroidal (typical shape of apatite crystallized from SBF) apatite has been formed, 10 μm in diameter, and after 2 weeks the layer achieved the thickness of 13 μm.

Kodama *et al.* [10] proposed an alternative immersion method in which the tubular coatings are preloaded with synthetic hydroxyapatite by cyclic exposure to saturated $Ca(OH)_2$ and 0.02M $(NH_4)HPO_4$. As observed this treatment significantly promotes natural apatite formation. Additionally, AIM treatment enhances to the greatest extent the apatite deposition for amorphous nanotubes.

In SBF, OH^- groups are absorbed on oxide surface resulting in Ti-OH and the solid-liquid interface. Ti-OH groups are acidic or neutral depending on the pH of the surrounding solution [26]. The surface shows zero net charge at the pH=5-6 for titanium oxide. The physiological pH=7.4 makes the surface slightly negatively charged due to the presence of deprotonated acidic hydroxides. Such a surface attracts Ca^+ cations which in turn react with HPO_4^{2-} or $H_2PO_4^-$ to form calcium phosphate [26,56].

Adhesion of cells such as osteoblasts is an important prerequisite to subsequent cell functions such as synthesis of extracellular matrix proteins and formation of mineral deposits. The investigation made by Webster [84] indicates that a nanostructured ceramics significantly improves osteoblast adhesion.

The osteoblastic cells can be attached well on rough surfaces in contrast to fibroblast cells which are prone to attach on smooth surface layers (unfortunately forming the fibrous tissues at the boundary interface between implant and the growing bone, inhibiting the osteoblast adherence to the implant surface and resulting in implant loosening).

For cell adhesion studies *in vitro* cell culture experiments, the different cells are used, e.g. MC3T3-E1 osteoblast cells (mice cells of the type CRL-2593, sub-clone 4) [14] which are seeded onto the TiO_2 layer. The experiments revealed that filopodia of propagating osteoblast cells go into the vertically aligned nanopores of the TiO_2 nanotubes (giving lock-in cell configuration) [14]. The number of adhered cells on the TiO_2 nanotubes increases by over 400% as compared to the Ti metal.

MECHANICAL PROPERTIES

The stability and adhesion strength properties are among the most important features of implant tissue materials. It is significant to improve adhesion between the bioactive layer and implant materials as well as between bioactive layer and bone [27].

Tubular oxide layer enhance adhesion (bond strength) by means of 'cup and cone' structure at the interface metal-oxide which facilitates good mechanical interlocking [17,32]. It is known that micrometer-sized bioactive materials like HAp on Ti substrate exhibit quite poor interfacial locking. The nano-scale HAp or TiO_2 nanotubes form much stronger bonds and stable nanoporous layer which enhances bonding strength and reduce interfacial failure [14].

The adhesion strength between the nanotubular oxide layer and Ti substrate can be evaluated by standard methods of adhesion test for coating layer like bonding pull-of method [27,32] and scratching test [27]. According to [27], the adhesion strength for crystalline nanotubes is relatively low, about 7 N when comparing to that of amorphous nanotubes arrays, about 40 N. Although the annealing process enhances transformation to favorable anatase phase, it simultaneously reduces the adhesion strength about 5 times. Nevertheless, the adhesion strength is higher for nanotube oxide layer than for solid oxide layer on titanium substrate.

Crawford *et al.* [32] described nanomechanical characteristics of TiO_2 nanotube structures. They have indicated that the coating thickness influences the delamination which occurs during nanoindentation experiments; delamination starts on unloading for thin coatings 230-250 nm. The results suggest that in order to improve adhesion strength, the coating thickness should be increased to at least 600-650 nm.

Raja *et al.* [7] measured the bond strength of nanotubular oxide layer with nanoscale calcium phosphate crystals deposited into the TiO_2 arrays as of 16-19 MPa (ISO 13779-2:2008 requires for bond strength the value 15 MPa [85]).

CONCLUSIONS

The electrochemical anodization of titanium and titanium alloys in fluorinated electrolytes is a relatively simple method to synthesize tubular structures with well defined pore size, wall thickness and tube length. Up-to-date research results indicate that by choosing specific kind of electrolyte, controlling the applied potential, pH value of electrolyte, and anodization time, the geometry of nanotubes determined by their diameter, nanotube wall thickness and length can be controlled. Such nanoarchitectural oxide film has a particular advantage as a biomaterial due to the large surface area and the defined geometry, and can act as an intermediate layer for calcium phosphate deposition, thus improving the bond strength between apatite and titanium substrate, and the bioactivity of biomedical implant materials.

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