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Surface modification of metallic implants with anodic oxide nanotubular arrays via electrochemical anodization techniques

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Introduction

Implantable biomaterials have been extensively applied to treat hard tissue disorders. It has been reported that 4.5 million Americans are living with artificial knees, including an estimated 500,000 who have had at least two replacement operations on the same knee [1, 2]. In 2011 alone, more than 600,000 knee replacements were performed in the U.S [1, 3]. The basic property requirements for materials used in orthopaedic and orthodontic application are mechanically strong and these materials must be possessing high resistance to corrosion and wear to prevent weakening of the mechanical properties and the release of toxicity species or debris in physiological culture. In addition, materials for implant must provide the ability to be machined with complex shapes. Specifically important, implantable materials must exhibit biocompatibility to avoid adverse biological response and to minimize allergic immune reactions [4]. Materials are also required to form a firm and lasting interface with bone in order to survive and function properly. Metals, ceramics and polymers are three major classes of materials adopted in biomaterials [5-8]. Among them, biologically compatible metals best satisfy the requirements for implants. The most widely used ones are surgical stainless steels (such as 316L and 317) [9, 10], Ti and its alloys (such as cp-Ti and Ti6Al4V) [11, 12], CoCr alloys (such as CoCrMo and CoCrNiMo) [13] and Ta [14]. Surgical stainless steels are mainly used for temporary implants since the immune system reaction to nickel is a potential complication [10]. The use of Ti and its alloys is owing to their high specific density and corrosion resistance. Additionally, Ti alloys with relatively low moduli than other metals have better match with the modulus of bone [11, 12]. Pure Ti and Ti6Al4V are the prevalent metals used for orthopedic and orthodontic applications. Because of the concern over the potential toxicity of V and mutagenicity of Al, new Ti alloys were also developed by substituting Al and V with other less toxic elements such as Nb, Ta and Zr (Ti-Nb-Ta-Zr) [15, 16]. CoCr alloys are encountered in the application of artificial knee and hip joint owing to the higher strength, excellent corrosion and wear resistance. However, their high stiffness causes the adjacent bone to be stress-shielded and results in disuse atrophy [13]. Ta is considered as the most corrosion resistant metal and is being exploited to create a highly porous form that favors bone ingrowth and achieve implant fixation [14].

When exposed to physiological culture after the surgical injury, metallic implants are able to form stable and compact oxide layers such as Cr_2O_3 (for stainless steels and CoCr alloys) [9, 13], TiO₂ (for Ti and its alloys) [11] and Ta_2O_5 (for tantalum) [14]. These layers insulate the reactive underlying metal from the surrounding environments and prevent the transmission of undesirable ions. Moreover, the existing of oxide layers also makes metal materials bioinert, resulting in fibrous capsules to surround implants [17]. These fibrous tissues forms due to the body's protection mechanism against any materials recognized as foreign. On the other hand, bone generation competes with the rejecting response [18]. Osteoblasts are differentiated from progenitor cells, migrating to the implant site, and secrete collagen to mineralize into new bone [8, 19]. The interaction between bone and implant is crucial to determine the performance and life span of the implant. An immediate bone-implant contact is highly desired to secure the mechanical stability. On the contrary, the formation of fibrous tissues retards the contact between bone and implants, resulting in a weak mechanical bonding, which can cause implant to loosen and is susceptible to a failure implantation [20].

Current implantable metals typically develop a thin layer of fibrous tissue at the interface with bone. The existing of this thin layer becomes a major challenge to decelerate the process of osseointegration and to extend the implant fixation time. Fast fixation is critical for the success rate of implantation and can reduce the micro-motion of implant and minimize the formation of fibrous

tissue, resulting in early physiologic loading and preventing the bone from disuse atrophy [20]. In addition, fast fixation of implant reduces the hospitalization time, cost, and improves the quality of life for patients. As a result, many attempts have been made to improve the interaction between bone and implants. One of the most actively pursued areas is the development of novel surfaces by modification techniques to improve the implants' surface properties and facilitate faster osseointegration and healing process [20].

Basic bone composition consists of mostly fibrous protein collagen, carbonated apatite $[Ca_5(PO_4, CO_3)_3(OH))$, CAP] and water [21, 22]. Some previous studies indicated that bone contains many different structures and is highly porous on the micrometer scale [23-25]. A current strategy is to consider that natural bone is a nanostructured material [26]. The type I collagen, which is the organic matrix of bone, has a triple helix structure with 300 nm in length, 0.5 nm in width and periodicity of 67 nm [26]. CAP (~70 wt% of the bone is CAP) is the inorganic mineral phase of bone with about 20-40 nm length and is uniquely patterned within the collagen network [27-29]. Considering the geometric factors of collagen and CAP, bony cell may be used to an environment in nanoscale rather than microscale. Thus proper nano-scale surface modification methods on metallic implant are highly desired to achieve better and rapid bonding to bone.

An electrochemical technique known as anodization or anodic oxidation is a well-established surface modification approach for metals to produce protective layers [30]. It has been successfully applied as a surface treatment for orthopedic implants in the past few decades and it has some new advances on fabrication of nanostructured surface in recent years [31-35]. Particularly, selforganized nanotubular oxide structure can be easily formed and controlled by varying the anodic conditions [33-37]. This type of self-aligned nanotubular structure has attracted more interests than others over the past 10 years. More than 3,000 papers related to this topic have been published over the past 5 years [38]. Since Ti-based metals have been paid more attention and represent an attractive model system for exploring this nanotechnology to create more effective implantable devices, self-assembled TiO₂ nanotubular layer can be easily fabricated on Ti implants to satisfy requirement for biomedical application [39, 40]. It is further remarkable that the selfordering anodization approach is not only limited to Ti and Ti-based alloys but can be applied to a large range of other transition metals or alloys to form highly ordered nanoporous or nanotubular oxide layers for potential biomedical application [41-45]. For these reasons, this review focuses on up-to-date research that describes the synthesis of these nanotubular structures and the factors that influence the degree of self-ordering, tubular geometry and crystal structure. We will also focus on the biocompatibility and physiological responses of these nanotubular layers on titanium and other valve metals, which are pertinent for orthopedic application. The final section summarizes the main points of this chapter and provides perspectives for future work in this field.

Fabrication of nanotubular arrays on metals via electrochemical anodization

Electrochemical anodization has been used to fabricate a thick and uniform oxide layers on metals (normally named valve metals) for almost several decades. Most recently, it has been established that self-organized nanoporous and nanotubular oxide layer can be grown on suitable metals [31, 32, 34, 46-50]. When most of valve metals (M as a representative symbol) expose to an anodic voltage in an electrochemical configuration as shown in Figure 12.1 [31, 32, 51], an oxidation reaction will be initiated at metal-oxide interface as $M \rightarrow M^{n+} + ne^{-}$ and the M^{n+} ions migrate

outwards under the applied field. At the same time, O^{2-} ions, provided by H_2O in the electrolyte, migrate towards the metal-oxide interface, react with M^{n+} and form a compact metal-oxide (MO) film. The anodization system is normally under a constant applied voltage. As the MO has higher resistivity than the electrolyte and the substrate, the applied filed within MOs is progressively reduced by the increasing oxide thickness. Although the oxide film will keep growing as long as the applied field is strong enough to drive the ion conduction through the oxide, the process is continuously slowing down resulting in a finite thickness of MO film. Under particular experimental conditions, a growing of porous MO layer takes place. Furthermore, under even more specific conditions, self-assembled nanoporous and nanotubular layers can be achieved.



FIGURE 12.1

(a) Mechanism of Oxide formation on valve metals. (b) Various morphologies obtained by electrochemical anodization of valve metals - a compact oxide film, a disordered porous oxide layer, a self-ordered nanoporous or a self-ordered nanotube layer (Redraw from Ghicov and Schmuki, Chem. Commun., 2009, 2791–2808). Copyright © 2009. Reproduced by permission of the Royal Society of Chemistry, from Ghicov A, Schmuki P. Self-ordering electrochemistry: a review on growth and functionality of TiO2 nanotubes and other self-aligned MOx structures. *Chem Commun (Camb)*. 2009;(20):2791–2808

Masuda et al. firstly demonstrated that a self-organized nanoporous oxide layer could be fabricated on aluminum in oxalic acid under specific voltage conditions [52]. This remarkable work has been considered a milestone on anodization of metal and triggered hundreds of papers dealing with the fabrication, modification and application of nanoporous alumina [53-55]. The as-formed nanoporous alumina was used as photonic crystals and template for nanomaterials synthesis [56-60]. Accordingly, several models have been put forward to explain the growth mechanism of the self-organized alumina nanoporous layers [31, 32]. A description can be explained by schematic steps showing in Figure 12.2 [31].



FIGURE 12.2

Schematic representation of alumina pore formation by electrochemical anodization: (a) formation of the anodic oxide on aluminium; (b) local field distribution correlated to the surface morphological fluctuations; (c) initiation of the pore growth due to the field-enhanced dissolution; (d) pore growth in steady-state conditions; (e) represents the current transient recorded during anodisation of AI; (f) and (g) show the influence of the volume expansion and the local acidity on the alumina pore growth, respectively (Redraw from Ghicov and Schmuki, Chem. Commun., 2009, 2791-2808). Copyright © 2009. Reproduced by permission of the Royal Society of Chemistry, from Ghicov A, Schmuki P. Self-ordering electrochemistry: a review on growth and functionality of TiO2 nanotubes and other self-aligned MOx structures. Chem Commun (Camb). 2009;(20):2791–2808. (a) Copyright © 2006 with permission from Elsevier. Reprinted from Bauer S, Kleber S, Schmuki P. TiO₂ nanotubes: Tailoring the geometry in H₃PO₄/HF electrolytes. *Electrochem Commun.* 2006;8(8): 1321–1325. (b) Copyright Wiley-VCH Verlag GmbH & Co. KGaA. © 2005. Reproduced with permission, from Macák JM, Tsuchiya H, Schmuki P. High-aspect-ratio TiO₂ nanotubes by anodization of titanium. Angew Chem Int Ed Engl. 2005; (14): 2100–2102. (c) Copyright © 2007 Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission, from Albu SP, Ghicov A, Macak JM, Schmuki P. 250 µm long anodic TiO₂ nanotubes with hexagonal self-ordering. Phys Status Solidi Rapid Res Lett. 2007;1(2):R65-R67. (c) Copyright © 2007, American Chemical Society. Reprinted with permission, from Paulose M, Prakasam HE, Varghese OK, et al. TiO₂ Nanotube Arrays of 1000 Length by Anodization of Titanium Foil: Phenol Red Diffusion. J Phys Chem C. 2007;111(41):14992–14997. (e) Copyright Wiley-VCH Verlag GmbH & Co. KGaA. © 2005. Adapted with permission, from Macák JM, Tsuchiya H, Schmuki P. High-aspect-ratio TiO2 nanotubes by anodization of titanium. Angew Chem Int Ed Engl. 2005;44(14): 2100–2102. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. © 2011. Adapted with permission, from Roy P, Berger S, Schmuki P. TiO₂ Nanotubes: Synthesis and Applications. Angew Chem Int Ed Engl. 2011;50 (13):2904–2939. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. © 2005. Adapted with permission, from Macak JM, Tsuchiya H, Taveira L, Aldabergerova S, Schmuki P. Smooth anodic TiO2 nanotubes. Angew Chem Int Ed. 2005;44:7463-7465.69 Copyright © 2008, with permission of Elsevier. Adapted from Macak JM, Hildebrand H, Marten-Jahns U, Schmuki P. Mechanistic aspects and growth of large diameter self-organized TiO₂ nanotubes. J Electroanal Chem. 2008;621:254–266.70

Briefly, as a result of the onset of electrochemical anodization in acidic condition, the surface of aluminum is covered entirely by a compact, uniform anodic alumina oxide layer (Figure 12.2a).

Since the surface of oxide layers fluctuates at the microscopic level, the distribution of electric field in the oxide layer is non-uniform, resulting in focused electric field at some certain place, as shown in Figure 12.2b. Consequently, field-enhanced dissolution in the anodic oxide takes place and the nanopores start to form (Figure 12.2c). Successively, the nanopore growth process reaches a steady-state and uniformly distributed pores are obtained (Figure 12.2d). Additionally, the selfordering of nanoporous alumina layers is also contributed by the stress at the metal-oxide interface owing to volume expansion or electrostriction, repulsion of electric fields, or stabling maximum current-flow conditions. Many of the mechanisms for self-organized nanoporous alumina layers can be transferred to the formation of self-ordering nanopores and nanotubular layers on other metals such as Ti, Zr, Ta, etc [31, 38, 46]. However, for these metals, in contrast to aluminum, an acidic condition (or a low pH condition) is not sufficient to create self-ordering porous metallic oxide layers but only to form a compact oxide layer [30-32, 34]. In order to form self-ordering nanopores and nanotubular oxide layer, the existing of fluoride ions (F) in electrolyte is strictly desired [30, 34, 37]. A key feature of the F- ions is that it is able to form water soluble metal-fluoride complexes. The complex formation aids the prevention of MO layer formation at the tubular bottom, but this also leads to mild but permanent chemical dissolution of the formed MO. Another important factor is that F^{-} ions are very small and compete with O^{2-} migration through the oxide layer [31, 32, 34]. It has been observed that F- ions may migrate at a rate twice as high as O²⁻ ions through oxide lattices [31, 32, 42]. As a result, a fluoride rice laver is formed at the metal-oxide interface. This laver is believed to be the origin of the nanotubular separation and formation. Several excellent reviews have well explained the formation mechanism of MO nanotubular arrays by means of electrochemical anodization. This section, therefore, will only give a brief summary of the formation of some MO nanotubular arrays under various conditions [31, 32, 34].

Self-Ordering TiO₂ nanotubular Arrays

The very first paper regarding the formation of porous TiO_2 oxide layer on Ti via electrochemical anodization in F- containing electrolyte was reported by Kelly in 1979 [61]. However, owing to the insufficient information of surface morphology by electronic microscopy, it was difficult to observe the self-ordering TiO_2 nanoporous arrays from their work, resulting relative low citation by other researchers. It is well accepted that the formation of self-ordering TiO_2 nanoporous structure by anodization in fluoride containing chromic acid was reported by Zwilling et al. in 1999 [62]. They pointed out that a small amount of fluoride ions in the electrolyte is the key form self-ordering TiO_2 nanoporous structure. Following this pioneer work, several research groups have carried out extensive work on optimization of the anodization conditions to develop self-ordering nanotubular arrays [31, 34].

Anodization to form tube layers is usually carried out by ramping a potential step at a constant voltage normally between 1-30 V in aqueous electrolytes or 5 - 150 V in non-aqueous electrolytes containing approximately 0.05 M - 0.5 M fluoride ions [35, 37, 41, 42]. Crucial factors on fabrication of TiO₂ nanotubular arrays are considered to be applied potential, fluoride concentration, pH value and anodization duration [31, 34]. In general, the nanotubular diameter is reported to be linearly dependent on the applied anodic potential during growth [31-34]. Yasuda et al. found out that the diameter of TiO₂ nanotubes correlate linearly with the growth factor, f_{growth}, of the Ti, where f_{growth} is growth factor and is 2.5 nm·V⁻¹ for TiO₂ (f_{growth} being f_{growth} = t_{film}/U, t_{film} being the compact oxide thickness that grows at a specific potential in Ti) [34]. By assuming that anodic oxide growth would take immediately in all directions leading to a hemispherical oxide structure with a certain radius R =

 f_{erowth} U. Based on this estimation, TiO₂ nanotubular diameters from 5 to about 700 nm can be achieved in the anodization potential range that has been listed above [31]. Typically at the early stage of growth, the TiO_2 nanotubular length is controlled by the applied electric field and as a result, the nanotubular thickness is proportional to the applied voltage. Under the constant voltage U, the electric field is defined as F = U/d, where d is the nanotubular thickness. The electric field keeps dropping constantly as the d increases, thus lowering the driving force for solid-state ion (such as Ti^{4+}) migration [34]. The result is an exponential drop in the anodic current with time as shown in Figure 12.2e until the electric field effect is lost. At this point, a practically finite thickness is reached that mainly depends on the anodization voltage. The presence of fluorides ions strongly affects the anodization and self-ordering of TiO_2 nanotubular arrays. If the fluoride concentration is very low, normally \leq 0.05 wt.%, a stable compact TiO₂ layer is formed after anodization. Meanwhile, a high content of fluoride (\geq 1.5 wt.%) results in no oxide formation, as all the Ti⁴⁺ formed immediately reacts with the a drastic amount of fluoride to form soluble $[TiF_6]^{2^-}$, which is similar to an electropolishing process [35]. For fluoride content within the intermediate level, 0.05 -1.5 wt.%, a competition between TiO₂ formation and dissolution take place and nanotubular arrays formation can be observed [31, 32, 34]. Since the dissolution of TiO_2 highly depends on fluoride concentration, the elevation of fluoride content in the anodization electrolyte can lead to a long tube with large diameter.

When the anodization of titanium is carried out in aqueous electrolyte, most of the composition of fluoride species is in HF form. In acidic condition such as H_3PO_4 and H_2SO_4 , a maximum length of \sim 500 nm with about 140 nm diameter TiO₂ nanotubular arrays can be obtained under optimum conditions (Figure 12.3a) [31]. Longer nanotubular arrays (> 1 μ m) can be formed in buffered aqueous electrolyte $[(NH_4)_2SO_4 + NH_4F$ and $Na_2SO_4 + NaF]$ (Figure 12.3b). Such neutral or near neutral electrolyte has less acidity with less dissolution capability on TiO₂, TiO₂ nanotubes with diameter about 200 nm can grow up to 4 µm under some optimum conditions. Another strategy to carry out the anodization of Ti in non-aqueous electrolyte leads to a significant difference in morphology of as-formed TiO₂ nanotubes compared with nanotubes grown in aqueous electrolytes. Since organic electrolytes, such as ethylene glycol, glycerol, DMSO and ionic liquids, have a small amount of oxygen, the oxide chemical dissolution in these electrolytes highly depends on the water concentration. Owing to the low water content, very long (up to 1 mm) TiO_2 nanotubes with large diameters (up to 700 nm) can be obtained (Figure 12.3c and 3d) [34]. The fact that different morphology of TiO₂ nanotubes formed in aqueous and non-aqueous electrolytes can be ascribed to a large extent to the low conductivity of non-aqueous electrolytes and IR-drop effects, which will decrease the effective voltage of the electrode [31, 32]. The conductivity of the electrolyte changes as the reaction products are formed with the extension of the anodization time, resulting in nanotubes with larger diameters with longer thickness.

If the other electrochemical parameters are kept constant, the duration of anodization process, which can be also converted to the charge passed during the anodization, controls the nanotubular layer thickness. The thickness of the nanotubes linearly depends on the anodization time (Fig.3e). However, this only holds for a certain time. Due to etching of TiO_2 by the fluoride species in the electrolyte, an equilibrium state between the growth of the nanotubes at the bottom and chemical/electrochemical dissolution of nanotubes at top will be reached, which is commonly defined as a steady-state condition. At steady-state condition, no further increase in the nanotubular thickness is observed. If anodization is carried out for extended times, nanotubular walls are thinned out, perforated and the tube tops become decorated with tube wall remnants. Since the oxide growth and chemical dissolution of nanotubes highly depend on the water content in the anodization culture, the amount of water is another factor influencing the nanotube

formation [31, 32, 34]. A striking effect of the water content is that smooth nanotubular walls are obtained in low water containing electrolyte while side wall ripples are formed in higher water contents [34]. The reason for this effect is that for higher water contents, the fluoride rich layer between the nanotubes shows a faster chemical dissolution rate than the growth rate of the nanotubes into the underlying substrate; that is, ripples at the walls of the nanotubes can be ascribed to the continuous etching and passivation of the cell boundary regions.



FIGURE 12.3

Examples of TiO_2 nanotubular arrays obtained by electrochemical anodization in different electrolyte: (a) HF/H_2SO_4 solution (anodization voltage at 20 V), (b) (NH₄)₂SO₄ + NH₄F solution (anodization voltage at 20 V), (c) and (d) mixed NaF and glycerol solution (anodization voltage at 120 V and 60 V respectively);; (e) TiO_2 nanotube-layer thickness with anodization time for different electrolytes (anodization voltage for ethylene glycol electrolyte held at 60 V, and 40 V for other electrolytes). (a)from Bauer S, Kleber S, Schmuki P. TiO2 nanotubes: Tailoring the geometry in H₃PO₄/HF electrolytes. Electrochem Commun. 2006;8(8): 1321–1325.32; (b) from Macák JM, Tsuchiya H, Schmuki P. High-aspect-ratio TiO2 nanotubes by anodization of titanium. Angew Chem Int Ed Engl. 2005;44(14): 2100–2102.33; (c) Macak JM, Hildebrand H, Marten-Jahns U, Schmuki P. Mechanistic aspects and growth of large diameter self-organized TiO₂ nanotubes. J Electroanal Chem. 2008;621:254-266.70; (d) From Paulose M, Prakasam HE, Varghese OK, et al. TiO₂ Nanotube Arrays of 1000 µm Length by Anodization of Titanium Foil: Phenol Red Diffusion. J Phys Chem C. 2007;111(41):14992-14997.35 (E) Data from Macák JM, Tsuchiya H, Schmuki P. High-aspect-ratio TiO₂ nanotubes by anodization of titanium. Angew Chem Int Ed. 2005;44(14): 2100–2102; Roy P, Berger S, Schmuki P. TiO₂ Nanotubes: Synthesis and Applications. Angew Chem Int Ed. 2011; 50(13):2904-2939; Macak JM, Tsuchiya H, Taveira L, Aldabergerova S, Schmuki P. Smooth anodic TiO₂ nanotubes. Angew Chem Int Ed. 2005;44:7463–7465. Macak JM, Hildebrand H, Marten-Jahns U, Schmuki P. Mechanistic aspects and growth of large diameter selforganized TiO₂ nanotubes. J Electroanal Chem. 2008;621:254–266

Oxide nanotubular arrays on titanium alloys and other metals

The principle used to grow oxide nanotubular arrays on titanium by using electrochemical anodization technique in fluoride containing electrolyte can be transferred to biocompatible titanium alloys (shown in Figure 12.4). Self-organized oxide nanotubular layers have been reported

on binary alloys, such as Ti-Zr, Ti-Ta, Ti-Nb, and Ti-Mo], tenary alloys, such as Ti-6Al-7Nb, Ti-6Al-4Vand Ti-35Nb-5Zr, etc, and more complex alloys systems such as Ti-29Nb-13Ta-4.6Zr [34, 41, 45]. The addition of different elements in Ti alloys drastically affects the anodization process and the ultimate oxide nanotubular morphology and composition. In general, after alloy anodization, the composition of the oxide layer is consistent with the ratio in the alloy. For instance, the anodic oxide nanotubular layers on Ti-Al alloys are composed of TiO₂ and Al₂O₃ [41]. The fraction of two kinds of oxides is the respective fraction of Ti and Al in the base alloys. With the increasing of titanium content of Ti-Al alloy, TiO₂ and Al₂O₃ nanotubular separation has been observed. Similar phenomena have also been observed on Ti-Ta and Ti-Nb [45]. In some cases, minor amounts of mixed oxides may be present in the anodic nanotubular arrays. A mixed oxide nanotubular structure was reported on Ti-Zr alloys [42]. Zirconium titanate nanotubular arrays were formed on Ti-50Zr alloys via anodization [42].

Similar to the TiO_2 nanotubular arrays formed on pure Ti, the formation of oxide nanotubular arrays on Ti alloys depends on the anodization parameters, including anodic potential, anodization time, pH value and fluoride species concentration, that have been discussed in the previous section. However, owing to the difference in chemistry, including selective dissolution of the oxide in fluoride and solubility of the respective metal fluorides in different culture in anodization, the morphology and geometry of oxide nanotubular arrays formed on Ti alloys show some difference than the TiO_2 nanotubular arrays on pure Ti. In the case of Ti-Ta alloys, the anodization process resulted in the formation of nanoporous oxide layers first and dissolution followed by formation of nanotubular arrays. For a ternary alloy such as Ti-6Al-4V, both α and β phases of Ti were present since the addition of other element [41]. Ordered nanotubular arrays were observed on α phase and a mixture of nanotubular arrays and nanoporous structure was present on a complex $\alpha + \beta$ phases. Due to the easily dissolution of V_2O_5 (mainly in β phase) in fluoride containing culture, the entire β phase was easily to be attacked and dissolved until the etch reached an underlying α phase, where a nanotubular structure formed. A very interesting phenomenon was observed on the anodic nanotubular arrays on Ti-Zr-Nb alloys [43]. Two distinct tube diameters were formed with one large center tube surrounded by smaller tubes, repeated over the entire anodized area. The tubes had no difference on the length and showed the same degree of self-ordering, which was ascribed to availability of current at the different tips. However, the phenomenon is still not well understood and extensive work is required to explore the formation mechanism.

Depending on the exact electrochemical conditions, self-ordered nanotubular/nanoporous layers were reported for several other metals, such as Zr [38], Hf [48], Ta [47], Nb [34], W [34], Fe [31] and Mg [50] (Figure 12.4). For each case, some optimization of the electrochemical conditions specific to the element is desired to obtain organized high-aspect-ratio nanotubular structures. Lee et al. and Tsuchiya et al. were the first two groups of researchers to report on the fabrication of selfordered ZrO₂ nanotubular arrays in fluoride containing species by electrochemical anodization [46]. The formation mechanism of the nanotubular arrays has been described in detail with regard to the effect of changing the concentration of fluoride ions, pH value, the composition of the electrolyte and the applied potential. It was shown that by using organic electrolytes, significant thick and smooth ZrO_2 nanotubular arrays up to 200 μ m was obtained under a 40 V stimulated potential [38]. Irregular ZrO₂ nanotubular arrays were obtained by one-step anodization without any pretreatment owing to the existing of impurities such as carbide in Zr and the inhomogeneity of the surface. In order to obtain highly ordered nanotubes, pretreatments were applied on Zr to enhance the self-ordering. Dip-etching, two-step anodizing and electropolishing were applied on Zr substrate resulting in highly self-ordered ZrO₂ nanotubular arrays [38]. The removal of the impurities and the electropolishing, which reduced the surface roughness, had an influence on the

homogeneous electric field distribution over the entire metal surface during the anodization, thus self-ordering nanotubular arrays grew regularly on the entire surface. Similarly, high-aspect ratio hafnium oxide nanotubular arrays can be achieved under a wide range of anodization parameters [36, 48]. Tantalum has extreme corrosion resistance in to acidic environments. Thus extreme conditions are required to obtain nanotubular structures. It was reported that Ta_2O_5 nanotubular arrays formed in a mixed H₂SO₄ and HF electrolyte with up to 1 wt% H₂O under the anodization voltage from 10-20 V with the anodization time between 5 sec to 120 sec [45]. Extension of anodization process resulted in the destruction of the nanotubular arrays and dimples on Ta substrate. The reason of the destruction of nanotubular arrays from surface was shown to be the formation of thin, fluoride-rich layer built up at the Ta/Ta_2O_5 interface [47]. Controversially, for other valve metals such as Nb and W, there were still no highly ordered and only comparably short nanotubular structures were reported [34]. For some non-valve metals such as Fe [47] and Mg [50], high-aspect-ratio oxide nanoporous structure and oxide-fluoride nanotubular structure were recently reported. The reasons for this different behavior may be ascribed to the solubility of a formed oxide structure in the anodizing electrolyte, the solubility of the fluoride species and the stress generated when the oxide is formed.

In general, all investigated self-ordering oxide structure fabricated by electrochemical anodization in fluoride-containing electrolytes on different metals and alloys seem to follow the same growth principles and key factors that are found out for fabrication of TiO_2 nanotubular arrays: the diameter of the tubes are determined by the anodization voltage; the tubular length depends on the chemical resistance of the oxide against fluoride etching, which relates to the anodization voltage and anodization time and the amount of oxygen, which is provided by water for tube growth.



FIGURE 12.4

Cross-sectional and top-view SEM images of ordered oxide nanotube or nanopore layers electrochemically grown on different valve metals and metal alloys (From Roy, Schmuki et al. *Angew. Chem. Int. Ed.* 2011, 50, 2904 – 2939). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. © 2011. Reproduced with permission from Roy P, Berger S, Schmuki P. TiO2 Nanotubes: Synthesis and Applications. *Angew Chem Int Ed Engl.* 2011;50(13):2904–2939

Biocompatibility approach of metals with nanotubular surfaces

Hydroxyapatite formation on nanotubular arrays

In view of a rapid ingrowth of biomedical implants in bone, a key factor is to quickly stimulate hydroxyapatite (HA) formation from body fluid because HA formation is important for osseointegration [24]. A number of surface treatments have been explored in order to enhance HA formation on metal implants, for example using different chemical and physical treatment. It is therefore of interest to study metallic oxide nanotubular surface in view of HA-induced effects for biocompatibility approach first.

The formation of HA on biomedical implant is based on heterogeneous nucleation phenomenon. For a nucleus, assuming spherical shape, to form in a supersaturated solution, the nucleation rate is [27]

$$J = A \times \exp\left(\frac{-B}{KT}\right) = A \times \exp\left[\frac{-16\pi\gamma^{3}\nu^{2}}{3K^{3}T^{3}(\ln S)^{2}}\right]$$
(1)

Where A is the rate coefficient, B is the activation energy, K is Boltzman's constant, T is the temperature, γ is the nucleus-solution interfacical energy, v is the molecular volume and S is the degree of supersaturation, defined as the concentration product/solubility product (Ksp). If a nucleus forms on a foreign substrate (such as implant materials), at a contact angle of θ , the nucleation rate becomes:

$$J' = A \times \exp\left(\frac{-\phi \times B}{KT}\right)$$
(2)

and,

$$\phi = (2 + \cos\theta)(1 - \cos\theta)^2/4 \tag{3}$$

Since $\phi < 1$, J' in eq. (2) is always higher than J in eq. (1). Meanwhile, J' increases with decreasing θ . When $\theta = 0$ (spread), the activation energy is also zero and J' reaches a maximum. Therefore, if the solution supersaturation (S) and the substrate condition (θ) are properly controlled, nucleation and crystallization of HA can preferentially occur on the substrate forming the coating. For example, TiO₂ and ZrO₂ nanotubular arrays have greater wetting behavior of simulated body fluid than flat Ti and Zr foils [34, 38]. In addition to surface chemistry, nanotubular arrays change the surface topography at micro-scale to enhance the nucleation site by large increasing the surface area. Thus it shows potential that HA formation can strongly accelerated on nanotubular surfaces compared with flat metal surfaces.

A very thin layer (~25 nm) of nanoscale HA phase was introduced on TiO₂ nanotubular arrays after immersion in simulated body fluid (SBF) for a week [63]. However, a pretreatment of TiO₂ nanotubular arrays in alkaline solution was required according to the researchers [64]. Thus, the case might not directly show the benefit of nanotubular arrays for HA formation. Systematic studies on the formation of HA coating on TiO₂ nanotubular arrays were firstly reported by Schmuki group [41] (Figure 12.5). In order to obtain a uniform and thick HA coating, it is highly desired to fabricate nanotubes with larger opening diameters and longer depth for calcium and phosphorous species nucleation and growth. In order to achieve the HA coating by immersion of TiO₂ nanotubular arrays from SBF, a minimum opening diameter of 15 nm is required [41]. Several studies have confirmed that at least 14 days were required to obtain HA coating with over 1 micrometer thick on TiO₂ nanotubular arrays, as compared no coating formed on flat Ti [41]. Additionally, the role of TiO₂ crystallinity (anatase/rutile) also influenced the HA coating formation. A mixture of anatase and rutile TiO₂ nanotubular arrays showed an enhancement of HA formation rate at least 2 folds [41]. Moreover, the advantageous 3D structure of nanotubular arrays is optimal for embedding precursors for HA formation that additionally promote HA nucleation and accelerate its formation. Several attempts have been carried out to induce amorphous calcium phosphorous particles or nanocrystalline HA in the nanotubular structure by wet chemical methods before the formation of HA in SBF [38]. These methods highly relied on the size of the nanotubular arrays rather than tube crystal structure. By applying these methods, the HA formation rate can be enhanced about 10 folds. Another attempt to incorporate anion to enhance the HA formation on nanotubular arrays has been made by Wang et al., who created ZrO₂ nanotubular layers, which was incorporated with a vast amount of phosphorous anions by anodization in phosphorous species containing electrolyte [64]. By immersion such nanotubular arrays in SBF, the existing of phosphorous anions can adsorb Ca²⁺ to precipitate and form HA coatings only within 4 days. The incorporation of anions in electrolytes shows a potential to modify the nanotubular arrays with designated species for a particular application [65].



FIGURE 12.5

SEM images of the as-prepared amorphous TiO_2 layers after soaking in SBF for different periods. Compared are the 2 μ m long-nanotubes, the 500 nm nanotubes, and the compact (50 nm thick) TiO_2 layer (From Tsuchiya and Schumuki, J. Biomed Mater. Res. A 2006, 77A 534-541). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. © 2006. Reproduced with permission from Tsuchiya H, Macak JM, Taveira L, Ghicov A, Schmuki P. Hydroxyapatite growth on anodic TiO2 nanotubes. *J Biomed Mater Res.* 2006;77(3):534–541

In vitro and in vivo studies of nanotubular arrays

Cytocompatibility leads to promoted bone integration and growth on implant. It is therefore worth to study the responses of living matter and biological relevant species, such as bone cells, to nanotubular layers on metallic implants. The most widely used cell types for studies of bioactivity of nanotubular arrays are osteoblasts (bone cells), fibroblasts (connective tissue cells), bone marrow cells and stem cells (pluripotent undifferentiated cells) [30]. It is therefore more spectacular of the interaction of living cells with nanotubular layers. A pioneer work on cell interactions with TiO_2 nanotubular arrays reported by Schmuki group in 2007 showed that

mesenchymal stem cells react in a very pronounced way to the diameter of nanotubes [66]. The vitality of the cells was significantly increased for nanotubes as compared with flat metals. Diameters of ~ 15 nm TiO₂ and ZrO₂ strongly promote cells adhesion, proliferation and differentiation, and nanotubes with diameter greater than 50 nm were found to be detrimental on cells vitality, inducing programmed cell death [67] (shown in Figure 12.6). This effect may be related to the effective size-scale of the integrin-based focal contact formation between cells and nanotubular surfaces and the optimum nanotube diameter seems to enhance cellular activities compared to smooth surfaces. In several conflicting cases, however, osteoblast cells respond to and proliferate on TiO₂ nanotubes greater than 100 nm [67]. Other than the size effect, crystallinity of nanotubular arrays, remaining fluoride concentration and surface pretreatment were also concerns of the cell activity. It was shown that anatase/rutile TiO₂ slightly enhanced the proliferation of cell activities in short term (1 day) in vitro cell culture test. Immersion of as-formed nanotubular arrays in alkaline solution highly decreases the remaining fluoride species and results in an appropriate chemical culture for cell proliferation. Additionally, deposition of nanoscale Au particle in TiO₂ and ZrO₂ nanotubular arrays enhanced the mesenchymal stem cells attachment [67]. However, most of work has clearly demonstrated that the effect of size of nanotubular arrays dominates over tubular crystal structure, fluoride content and other surface pretreatment. Moreover, some researchers studied the mechanism of enhancement of bone cell function on TiO₂ nanotubular structure [34]. Two kinds of proteins, fibronectin and vitronectin, are major proteins that involved in osteoblast adhesion. Results showed significantly increased both fibronectin (15%) and vitronectin (18%) adsorption on nanotubular structures compared to flat titanium samples. Since the cells adhered to the metal surface via pre-adsorbed proteins, increased fibronectin and vitronectin adsorpotion on TiO₂ nanotubular structure could explain the observed enhanced osteoblast functions.



FIGURE 12.6

Cell densities of adherent cells on ZrO₂ nanotubes with different diameters count under fluorescence microscope after 24 h adhesion (a) and measured using colorimetric WST-assay after 3 d proliferation (b). Fluorescence images of GFP-labeled mesenchymal stem cells after 24 h adhesion and 3 d proliferation (c) (From Bauer et al. Integr. Biol., 2009, 1, 525-532). Copyright © 2009. Reproduced by permission of the Royal Society of Chemistry, from Bauer S, Park J, Faltenbacher J, Berger S, von der Mark K, Schmuki P. Size selective behavior of mesenchymal stem cells on ZrO2 and TiO2 nanotube arrays. *Integr Biol (Camb)*. 2009;1(8–9):525–532.81

Schmuki et al reported the first and the only work on evaluation of the bioactivity of nanotubular arrays in vivo [39] (Figure 12.7a and 12.7b). Tests from adult pigs showed that titanium implant with nanotubular structure surface does influence bone formation and bone development by enhancing osteoblast function and that higher implant bone contacts can be established if implant are coated with a nanotubular arrays. Additionally, this nanotubular coatings also resisted shearing forces that evoked by implant insertion, which were an unexpected advantage for nanotubular arrays on implant surfaces. However, in terms of a complex culture in vivo, some authors pointed out that negative effect of nanotubular arrays may also be exploited on surfaces when cell proliferation is not desired [32].

Nanotubular arrays for drug delivery and other preloads application

The 3-D geometry of the metallic oxide nanotubular arrays indicates that the materials are appropriate carriers as drug-delivery capsules and drug-eluding coatings on biomedical implant materials [31]. A potential in vivo capsule in terms of TiO₂ nanotubular arrays has been designed by Schmuki group [34] (Figure 12.7c). The drug with long molecules can be attached to TiO_2 surface by wet chemical methods. The nanotubes are filled by magnetic Fe₃O₄ particles before the attachment of drugs and these tubes can be magnetically guided to a designated location. Drugs can be released photocatalytically via ultraviolet reactions [34]. Additionally, drugs can also be released by electronic stimulated catalysis and more importantly by X-rays, which allows in vivo treatment through living tissue. Such TiO_2 can be used directly for photocatalytic reactions with cells or tissues, including for the site-selective killing of cancer cells. Metal oxide/aqueous interfaces play an important role in the adsorption/desorption of organic payloads. Thus the wettability of nanotubular arrays was adjusted for different payload filling and release. An amphiphilic TiO₂ nanotubular arrays was created composed by hydrophilic drugs and hydrophobic monolayer caps as an in vivo capsule. The cap does not allow body fluids to enter into the tubes after implantation in body unless opened by a photocatalytic interaction. Once the hydrophobic layer was removed, body fluids could enter into the tubes and wash out hydrophilic drugs loaded within the tubes. In order to achieve an appropriate elution time for loadings, some researchers suggested that capping of drug-loaded tubular or mesoporous nanotubular layer with biopolymer might represent efficient and promising drug-release system [34].

Conclusion and other aspects

As a surface modification method, electrochemical anodization can lead to desired chemistry and/or topography changes and could be used with other treatments (e.g., hydrothermal) together. First, anodization provides a controlled way to create nano-roughness or even nanofeatures. Generally, there are two mechanisms that are responsible for osseointegration of bone: biomechanical interlocking and biological interactions. For biomechanical interlocking, it depends on the roughness, and surface irregularity. Current femoral stems made of valve metals or alloys (e.g. Ti and Ti alloys) are usually macro-textured to provide such surface features for bone to mechanically interlock. For biological interactions, it involves complex systems. Considering roughness in different scales, it is reported that increased micro/submicron roughness could enhance bone cell function, such as ALP activity, while some other studies have revealed the enhanced cell-implant interactions on nanoporous or nanophase materials. It is thus proposed that the future titanium implant should possess roughness in a mixed microscale and nanoscale. One possible approach to accomplish this is by subjecting implants to techniques like polishing and mechanical grinding that promote micro-roughness, and then to induce nanotubular structures by a quick anodization process. Second, HA films produced using nanotubular metallic oxides show some advantages over conventional ones. Moreover, HA deposited onto the nanotubular metallic oxides could be nano-scale in dimension. One problem that still needs to be more fully investigated is how to optimize the bond strength between apatite crystals and the anodic oxide. Furthermore, electrochemical anodization to form nanotubular structure can be used to incorporate drug delivery into metallic implants to enhance new bone formation. The nanotubular structures could serve as reservoirs of chemical mediators, such as bone morphogenetic protein-2 (BMP-2) and osteogenic protein-1 (OP-1, BMP-7). In a word, electrochemical anodization as a quick and efficient modification method of metallic implants shows significant potential for enhancing their 10 to 15 year lifetime. This work was prepared in conjunction with project under contract No. YETP0419 with the Beijing Higher Education Young Elite Teacher Project and the start up fund from University of Science and Technology Beijing.



FIGURE 12.7

SEM pictures of the histological specimen. (a) The interface between the anodic TiO_2 nanotube implant and the bone can be seen. A partially breakage of the interface is due to the histological preparation. (b) Magnification reveals that the anodic TiO_2 nanotubes keep their structure and do not get damaged by shearing forces due to the implantation process; (c) TiO_2 nanotube for guided drug release: Representation of magnetically loaded TiO_2 nanotubes with attached drug (F). Release is triggered by photocatalytic chain scission upon UV irradiation. Inset: an example where a blue fluorescent molecule is released from magnetically actuated nanotubes [(a) and (b) from Schmuki et al. J. Biomed. Mater. B Appl. Biomater. 2009, 89B, 165-171; (c) from Schmuki et al., Angew. Chem. Int. Ed. 2009, 48, 969]

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