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Formation mechanism of biomedical apatite coatings on porous titania layer

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Abstract A titania containing calcium and phosphate with rough and porous structure was prepared by microarc oxidation. The in vitro bioactivity was examined by immersing the samples into the simulated body fluid (SBF). And the mechanism was also discussed. The results show that only 3 days of immersion in SBF, apatite was formed on the surface, and after 6 days, nearly all the surface covered by apatite. This indicates that the layer can induce the formation of apatite in simulated body fluid. It is analyzed that the key factors of the apatite formation are the hydrolysis of the $CaTiO_3$ and special structure.

Introduction

Successful clinical performance of commercially pure (c.p.) titanium implants has resulted in a wide spread usage of them. The biocompatibility of titanium is closely related to the properties of the surface oxide layer, in terms of its structure, morphology and composition. However, despite the excellent biocompatibility of thin native oxide films on titanium implants, it is generally known that native titanium oxide seldom forms a direct chemical bond to bone tissue and is often defined as inert ceramic biomaterial for bioactive materials have showed stronger bonding strength [1–6]. Various physical and chemical methods have been explored to coat materials such as calcium phosphate ceramics and hydroxyapatite onto metal implants with a view to obtaining the most biocompatible and bioactive implant surface [1–5]. On the other hand, in the bone of quantity and quality, the results have not always been so good, motivating the development of novel types of the osseointegrated implants [6]. The development of titanium implants has depends on new surface processing technologies.

Recently, developed clinical implants have been focus on topographical changes of implant surfaces rather than alterations of chemical properties [7–10]. Though the interfacial bonding strength of bioactive material is depends on the chemical composition of implants [11], a lot of animal and clinical studies have reported delamination of such coating material as well as biodegradation happened [12–16]. So, published in vivo investigations have shown significantly improved bone tissue reactions by modification of the surface oxide properties of titanium implants [6].

It is known that mechanical interlocking between tissue and implant materials relies on surface irregularities in the nanometer to micro level [6]. And it is also beneficial to bone tissue growth and enhanced anchorage of implant to bone; furthermore, a micrometer-sized porous surface has the function of an enhanced cell proliferation [17, 18]. On the other hand, nano-crystallized surface has been proved to be of toned down biological efficacy. For example, compared with conventional-crystallized titania, nano-crystallized titania can promote osteoblast adhesion and proliferation and osseointegration [19]. Therefore, the new aim for surface modification of titanium is to make titanium implant surface bioactive, porous, nano-crystalized and good mechanical properties.

A series of investigations have been carrying out modify the surface of titanium implant. A porous and rough titania layer containing calcium and phosphate can be obtained on titanium implants by micro-arc oxidation [20]. Though there are also some other researchers [21–24] having taken this method, most of them did not exhibit bioactivity because no apatite layer is formed on the films or it takes a long time

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(more than fifty days) to form the apatite layer after soaking in a Tris-buffered SBF solution at 37°C.

It is a criterion for an artificial material to have bioactivity that the material has ability to induce bone-like apatite on its surface in the body environment [25] or in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma [26, 27]. Several glasses and glass ceramics implanted into bone defects bonds directly to living bone without being encapsulated by fibrous tissue attributing to the formation of biologically active bone-like and carbonate-containing apatite [24]. Although such apatite layer is poorly adhesive, it is suggested that a specified structure of the layer has a potential of bioactivity.

In this study, micro-arc oxidation (MAO) was explored to modify the titanium surface at different voltages. In vitro bioactivity of the layer was examined by immersing the samples into the simulated body fluid (SBF). The dependence of bioactivity of the film was discussed in order to improve the bioactivity and the mechanism of the apatite formation was analyzed.

Materials and methods

Pure titanium (99%) with a diameter of 15 mm was used as substrates. Sample surface was polished with abrasive paper, rinsed with distilled water then cleaned ultrasonically and washed with acetone and deioned water, and dried in a dessicator. For the MAO treatment, a pulsed DC power supply was employed, and a titanium disc was used as anode while a stainless steel plate was used as a cathode in an electrolytic cell. An aqueous solution containing calcium acetate, $(CH_3COO)_2Ca \cdot H_2O$ (0.2 mol/L) and natrium-β-glycero-phosphate $C_3H_7Na_2O_6P \cdot 5H_2O$ (0.2 mol/L) was used as the electrolyte. The voltage was 240, 350, 400 and 450 V respectively. The oxidation time was 5 min. After MAO, sample was immersed in simulated body fluid (SBF) at 37°C. The pH of the solution was 7.2 just after preparation. The solution was changed every two days. The ion concentrations of the solution are summarized in Table 1 [18]. The Scanning electron microscopy (SEM) was used to analyze the surface and profile morphology. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and electron diffraction x-ray (EDX) were used to analyze the phase and element composition.

Table 1 Ion concentrations of the SBF and human blood plasma (mM)

fx1	SBF	fx2
Na^-	142.0	142.0
K^-	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
$HCO_3{}^-$	4.2	27.0
$HPO_4{}^{2-}$	1.0	1.0
$SO_4{}^{2-}$	0.5	0.5

Results and discussion

Morphology and composition of the film on titanium

As shown in Fig. 1, the surface of titanium becomes rough after the treatment, and there are many pores of several micrometers in size which are formed during the discharge. Fused mass was around the pores like a volcano top. With the increasing of the voltage, the pore size increased.

X ray spectra (Fig. 2) indicates that the mass of the film is rutile TiO_2 and anatase TiO_2. With the increase of the applied voltage, the intensity of peak of rutile TiO_2 becomes stronger comparing with the intensity of peak of anatase TiO_2. With the increasing of applied voltage, the peak of $CaTiO_3$ appeared though it is very little. For the thermal effect during the plasma oxidation affords the energy for the transformation of rutile and anatase and the formation of $CaTiO_3$.

EDX shows that Ca and P in the electrolyte join in the plasma oxidation and enter into the film [20]. The subsequent XPS narrow scan spectra of Ca, P, O and Ti of the film shows that the chemical state of the each element (Fig. 3). The two peaks displayed in Ca 2p spectra confirm the presence of two chemical states of Ca at 348.8 and 349.5 eV, respectively. The first peak (also the main component of the spectra) exactly recovers the binding energy of Ca in calcium phosphate, revealing that most of Ca in the film is in form of the calcium phosphate. Two components at 134.8 and 136.7 eV are obtained in the peak fit of P 2p spectra (including system deviation 2.2 eV). The 134.8-eV peak is attributed to the 2p3/2 state of P whereas the 136.7-eV peak is ascribed to the spin-orbit splitting state 2p1/2 [28]. The P 2p spectra imply the presence of the phosphate phase in the film. Additional peak fitting performed on Ti 2p3/2 spectra yields two peaks at 460.2 and 461.1 eV, showing that titanium exists in the forms of TiO_2 or $CaTiO_3$ in the film.

Apatite on the porous layer

After immersion of 3 days in SBF, a significant change of the coating morphology was observed (Fig. 4) on the samples which was treated in high voltage (400 V, 450 V). Accumulational grains were covered the surface which is simulated to the apatite formed in SBF. After 6 days, nearly all the surface was covered by the white grains. And the magnification view of the white grains, it was very similar to the morphology of the apatite which was formed in SBF after

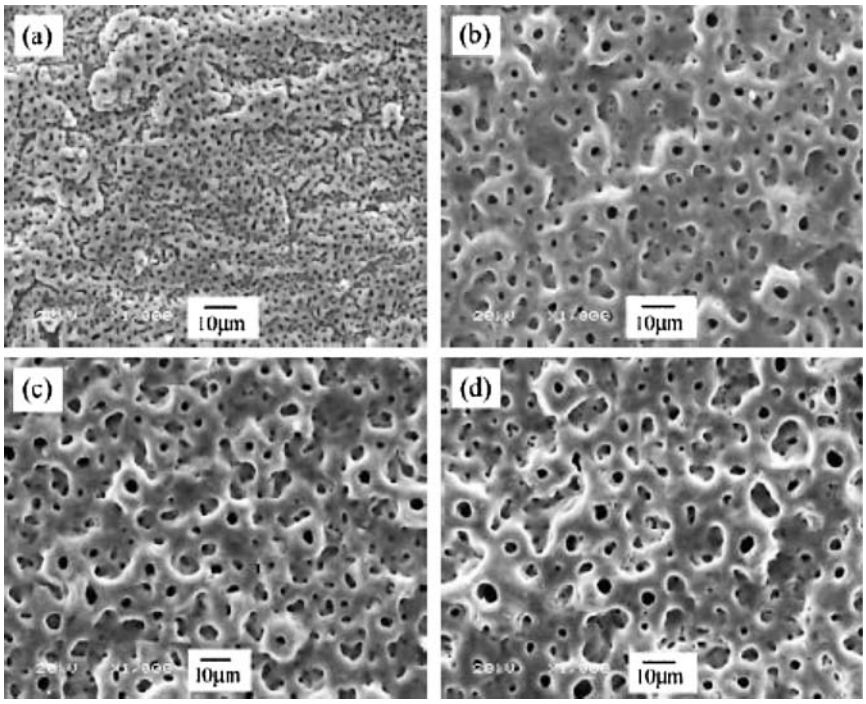

Fig. 1 Morphologies of MAO films synthesized at different voltage (a) 240 V (b) 350 V (c) 400 V (d) 450 V.

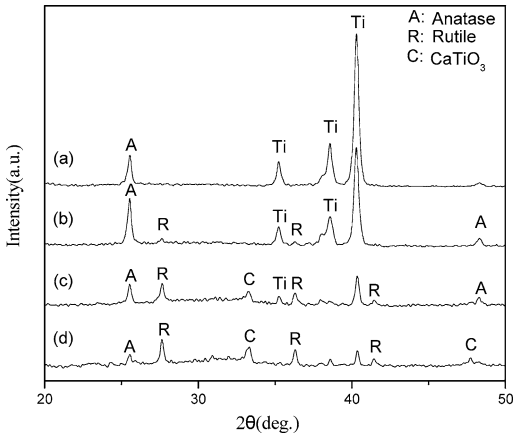

Fig. 2 XRD spectra of MAO films synthesized at different voltages (a) 240 V (b) 350 V (c) 400 V (d) 450 V.

chemical treatment [29]. The XRD patterns tested that it was apatite (Fig. 5). With the days increase, the intensity of apatite peaks become stronger, which implies apatite formed continuously. It is an important character of bioactivity for materials whether the material can induce the formation of apatite in SBF. While for the sample treated in low applied voltage (240 V, 350 V), no apatite was formed on the surface though fifty days pasted.

Mechanism of apatite formation

From above, it is can be inferred that TiO_2 with porous structure is not the key factor to the formation of the apatite. And the formation of $CaTiO_3$ in high voltage is the essential factor. $CaTiO_3$ is finely dispersed in the film, and it has a high point of zero potential (rutile: 4.6 ± 0.4, anatase: 5.9 ± 0.2, $CaTiO_3$: 8.1 ± 0.2)[30], so the film containing $CaTiO_3$ is easier to be polarizable than usual titania, that is to say the film can absorb OH^- and $HPO_4{}^{2-}$ in SBF under attractive force between electron charge. At the same time, OH^- and $HPO_4{}^{2-}$ can also absorb Ca^{2+} into the surface. These all induce over-saturation of OH^-, $HPO_4{}^{2-}$ and Ca^{2+} on the surface. The most important is $CaTiO_3$ is hydrolyzed in SBF, the reaction is following:

$$CaTiO_3 + 2H_2O \rightarrow Ca^{2+} + 2OH^- + TiO(OH)_2 \quad (1)$$

One of the hydrolysis products of $CaTiO_3$ on the surface is $TiO(OH)_2$ which is difficult to be soluble [31]. It is well known that the important reason of apatite formation in SBF of titania film produced by sol-gel is ascribed to the high concentration of Ti-OH group [32]. Li [33] also shows that the electronegative surface containing OH- can induce the nucleation of apatite, that is to say, there are some favorable conditions for apatite nucleation on such surface. The following is the calculation of critical nucleation work of apatite.

Hydroxyapatite is formed according to the following reaction:

$$10Ca^{2+} + 6PO_4{}^{3-} + 2OH^- \Leftrightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (2)$$

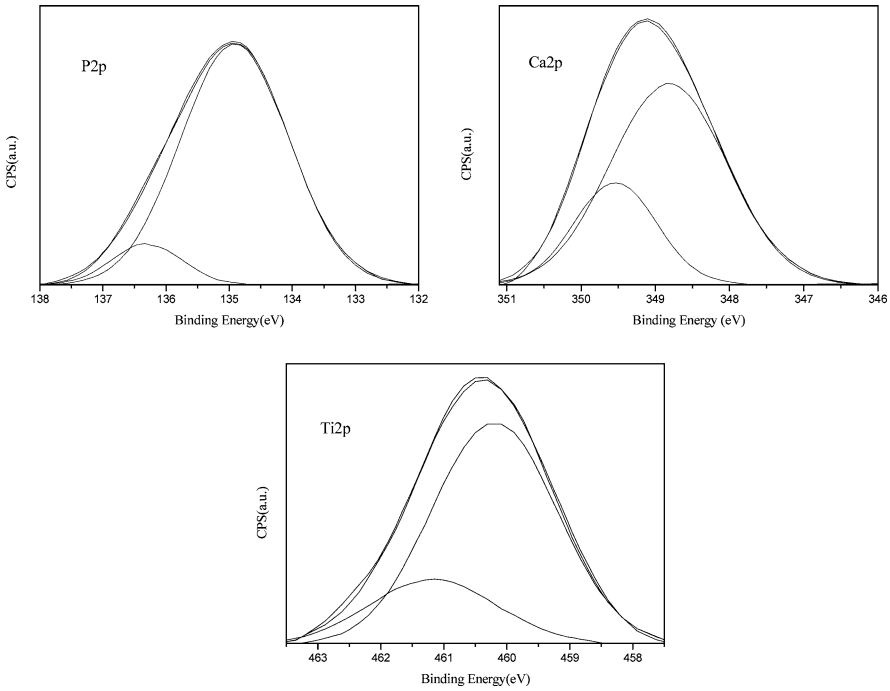

Fig. 3 XPS spectra of (a) Ca2p, (b) P2p and (c) Ti2p of sample treated at 450 V.

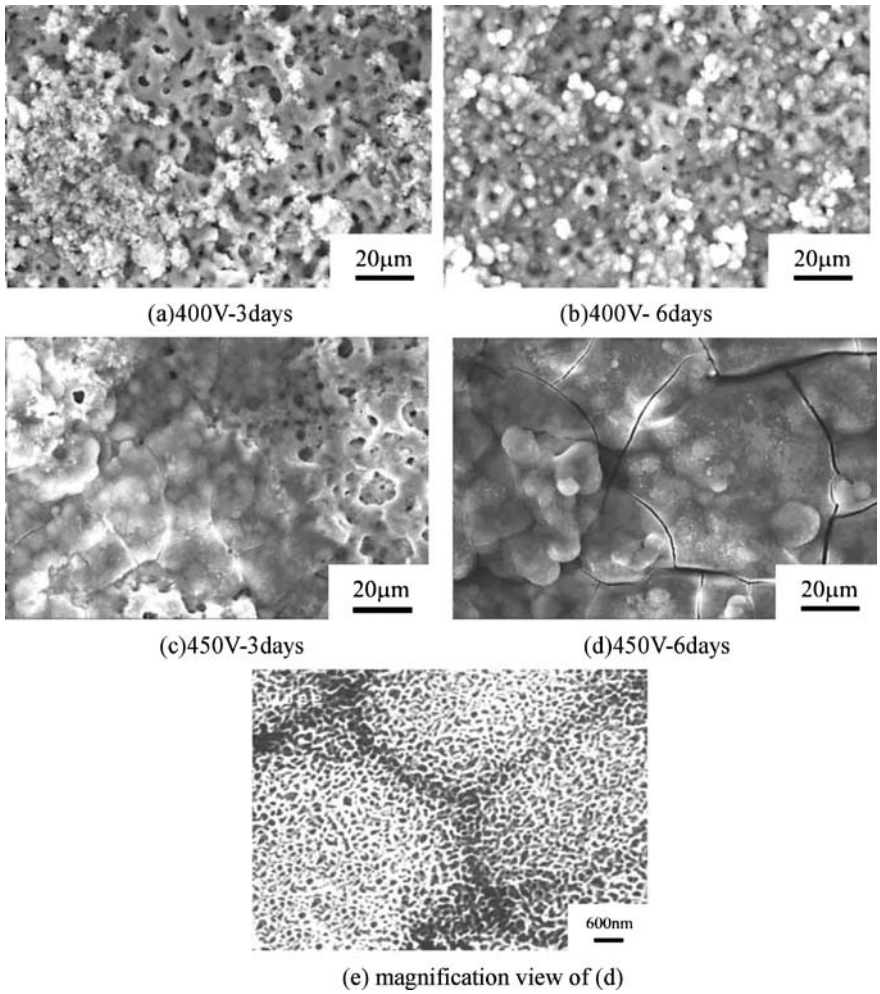

Fig. 4 Surface morphologies of the samples synthesized at 400 V and 450 V after immersed in SBF solution for different days (a) 400 V-3 days, (b) 400 V-6 days (c) 450 V-3 days (d) 450 V-6 days and (e) magnification view of (d).

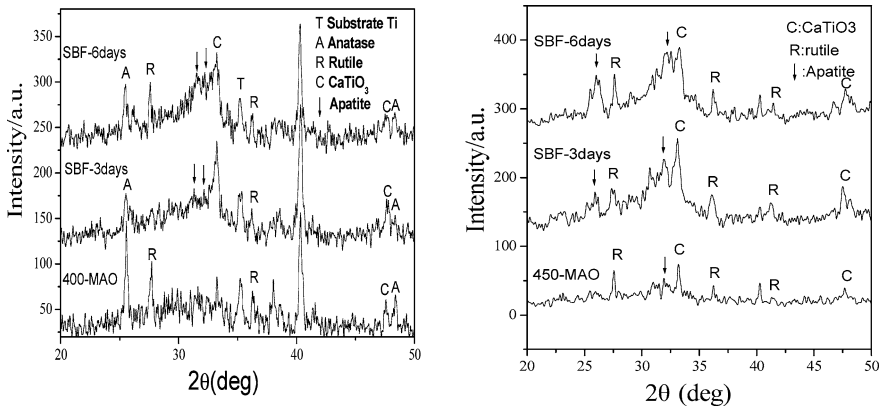

Fig. 5 XRD spectra of the samples synthesized at 400 V and 450 V after immersed in SBF solution solution.

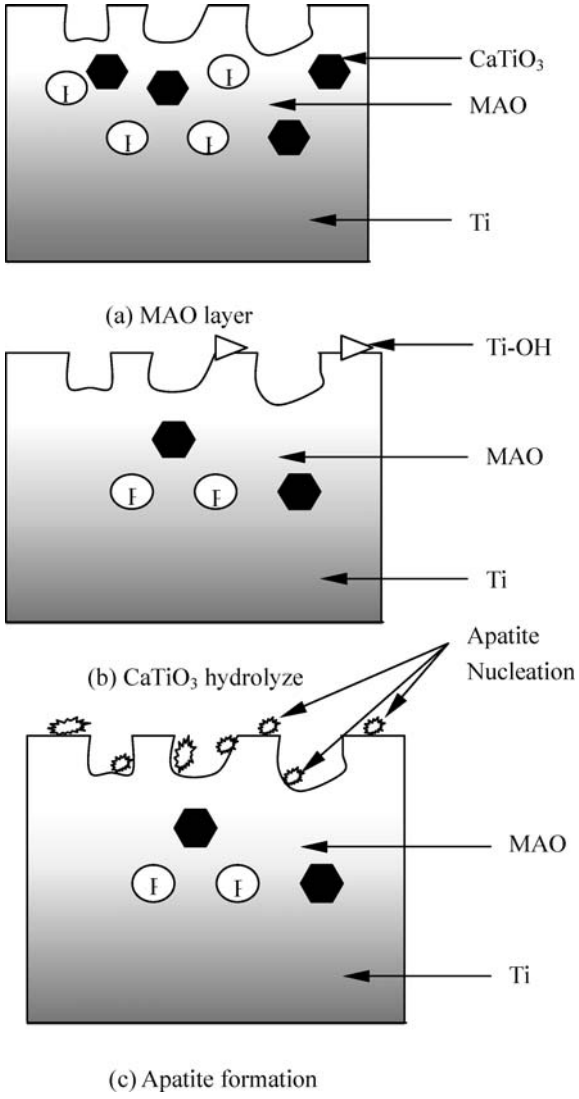

Fig. 6 schematic of apatite formation in SBF.

So heterogeneous nucleation work is:

$$\Delta G^* = \frac{16\sigma^3 f(\theta)}{3(\Delta G_V)^2} = \frac{16\sigma^3 f(\theta)}{3(kT/V_\beta(IP/K_0))^2} \tag{3}$$

where σ is the interface energy between nucleus and solution; IP is ion activity of the crystal in the solution; K_0 is IP value under equilibrium state; $f(\theta)$ is the function of contact angle between the substrate and crystal; V_β is molecular volume of crystal.

For $f(\theta)$ and IP depend on the properties of the substrate, they are also expressed as following:

$$f(\theta) = \frac{(2+\cos\theta)(1-\cos\theta)^2}{4} \tag{4}$$

$$IP = (a_{Ca^{2+}})^{10}(a_{PO_4{}^{3-}})^6(a_{OH^-})^2 = (\gamma_{Ca^{2+}})^{10}(\gamma_{PO_4{}^{3-}})^6(\gamma_{OH^-})^2 \times [Ca^{2+}]^{10}[PO_4{}^{3-}]^6[OH^-]^2 \tag{5}$$

where α is activity, γ is activity coefficient, $\gamma_{Ca^{2+}}$, $\gamma_{PO_4{}^{3-}}$, γ_{OH^-} is 0.36, 0.06, 0.72 respectively [32].

Usually, nucleus is easily formed at the concave, where there has low nucleation work. In our test, not only it has enough Ti-OH groups on the surface, but also the surface is porous. Even more, calcium-containing titania can release calcium ions on immersion in SBF to increase the ionic activity product, and according to function (5), the nucleation work can be decreased, thereby provide much more favorable conditions for apatite nucleation [34]. Once the apatite nuclei are formed, they can grow spontaneously by consuming the calcium and phosphate ions in the surrounding fluid because the body fluid is highly supersaturated with respect to apatite [34] (Fig. 6). So apatite can be formed in a shorter time. That is to say, the film has bioactivity.

Conclusion

Porous and rough film has been formed by microarc oxidation. The film contains the new phase $CaTiO_3$ at high voltage 450 V and 400 V. After immersion in SBF only 3 days, bone-like apatite appears. After 6 days, nearly all the surface is covered by apatite. The key factor is the hydrolysis of the $CaTiO_3$, which makes the surface contain more OH^-,

decreasing the nucleation work of apatite. At the same time, it also attributes to the rough and porous structure.

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