Effect of Ca/P Ratio on Bioactivity of PEO Coatings

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Abstract: Ti and its alloys are used in biomedical applications because of their low density, high specific strength, and high biocompatibility. However, the poor bioactivity limits their wide application. Herein, ceramic coatings containing Ca and P were prepared by plasma electrolytic oxidation（PEO） on Ti6Al4V alloy, and the microstructure, surface morphology, and chemical composition of these coatings were evaluated. An effect of the different Ca/P ratios in the electrolyte on the $CaTiO_3$-forming ability was investigated; besides, the effect of different Ca/P ratios in these coatings on the hydroxyapatite-inducing potency was also investigated. In addition, the bioactivity of the coatings was compared between before and after the immersion in a simulated body fluid. It was found that the higher the Ca/P ratio in the electrolyte, the stronger the $CaTiO_3$-forming ability, and the higher the Ca/P ratio in the coating, the better the bioactivity of the coatings.

Keywords: Bioactivity, Ca/P ratio, Plasma electrolytic oxidation, Hydroxyapatite.

1. INTRODUCTION

Ti6Al4V alloy has been widely used in surgical, medical, orthopedic, and dental implants because of the high strength, high strength-to-weight ratio, low toxicity, chemical stability, superior mechanical properties, excellent corrosion resistance, and high biocompatibility [1–3]. However, Ti and its alloys exhibit the poor bioactivity [4]. The elements, Al and V, have been reported to be toxic and may potentially cause serious ailments [5, 6]. Especially, Ti and its alloys cannot chemically integrate with bone tissue (osseointegration) [7, 8]. Hydroxyapatite (HA), $Ca_5(PO_4)_3(OH)$, can provide chemical bonding between the bone and implants because of its biological and chemical similarity to bone [9, 10]. To manufacture an implant with excellent bioactivity, various surface modification methods have been developed to coat Ti and its alloys, such as plasma spray coating [11, 12], plasma electrolytic oxidation (PEO) [13], electrochemical deposition [11], electrophoretic deposition [14], and immersion in a simulated body fluid (SBF) [15]. The ceramic coatings produced by PEO on Ti and its alloys have been widely used and investigated in recent years [16]; these coatings can successfully resist the corrosion of substrates by body fluids and improve the biocompatibility and osteoinductivity of Ti alloys [17, 18]. In addition, PEO can introduce Ca and P ions into Ti surface by controlling the processing parameters such as the composition of the electrolyte, applied voltage, current density, and treatment times [19,20]. However, a HA-forming ability and bioactivity of the usual PEO coatings are not very good. The research results in the literatures [21, 22] show that Ca and P are able to promote the bioactivity of implants. SBF test is one of the important and common methods to observe the HA-inducing potency of medical materials and also evaluate the bioactivity of materials *in vitro* [23]. The Ti–OH groups have been found to be responsible for the rapid formation of HA during a SBF soaking test [24, 25].

Herein, we report a process to directly prepare HA coatings on Ti6Al4V alloy by PEO. The effect of the Ca/P ratio in electrolyte on the $CaTiO_3$-forming ability was investigated. Four samples with different Ca/P ratios in coatings were immersed in SBF for two weeks. Before and after the immersion in SBF, the structure, micromorphology and elemental content of the coatings were evaluated by X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). Based on the results, the effect of the Ca and P contents in the coatings on the HA-inducing potency was analyzed. The results indicate HA can be directly formed in the coatings on the certain condition of the Ca/P ratio in the electrolyte and significantly improve the bioactivity of implant materials. Therefore, this study provides important information for the clinical applications of Ti implant materials.

2. RESULTS AND DISCUSSION

2.1. Phase Compositions of the Coatings

The phase components of the coatings were analyzed by XRD using CuKα radiation at a scanning speed of 5°/mm, as shown in Figure **1.** The anatase and rutile phases on the surface were formed by the

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E-ISSN: 2409-3394/15

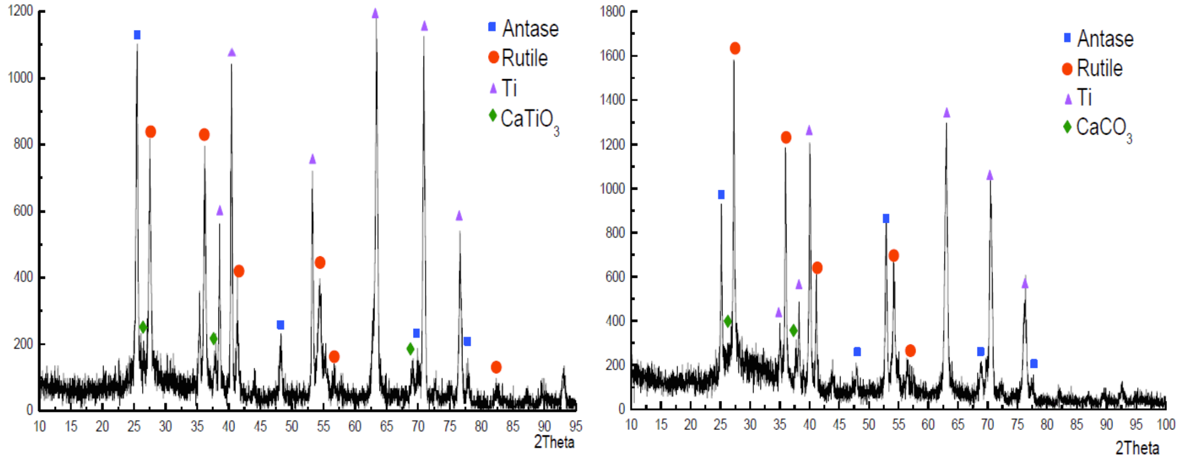
reaction of Ti^{4+} and OH^- ions under high-temperature and high-pressure conditions in microdischarge channels during the PEO. Figure **1** shows that the Ca/P ratio in the electrolyte significantly affected the contents of anatase and rutile TiO_2. The electrical conductivity of the electrolyte increased with increasing Ca/P ratio. A large amount of ions in the electrolyte were adsorbed in the coatings under a strong electric field. As a matter of fact, the transformation from anatase to rutile phase occurred from 700 K to 1300 K because the rutile phase of TiO_2 is stable at high temperatures [26]. It has been reported in the literatures [27,28] that the local temperature in microdischarge channels fluctuates from 800–3000 K to 2000–10,000 K during PEO. The high-temperature drives the phase transition from anatase to rutile TiO_2. As a result, the anatase content in the coatings increased under a high Ca/P ratio condition. At the high temperature and pressure, the surface (accumulation of Ca^{2+}) reacts with the phosphate anions to form the $Ca_3(PO_4)_2$ phase in the coatings [29]. The chemical reactions can be expressed as follows:

$NaH_2PO_4 \rightarrow Na^+ + H_2PO_4^-$; $Ca(CH_3COO)_2 \rightarrow Ca^{2+} + CH_3COO^-$;

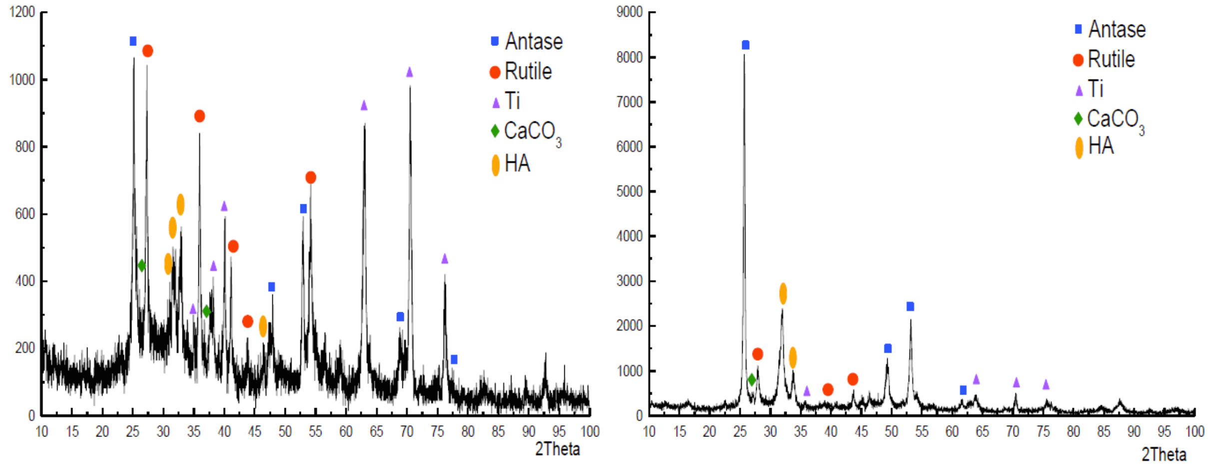
$H_2PO_4^- + OH^- \rightarrow HPO_4^{2-} + H_2O$; $HPO_4^{2-} + OH^- \rightarrow PO_4^{3-} + H_2O$;

$Ca^{2+} + HPO_4^{2-} \rightarrow CaHPO_4$; $3Ca^{2+} + 2PO_4^{3-} \rightarrow Ca_3(PO_4)_2$

Furthermore, this metastable $Ca_3(PO_4)_2$ phase can transform into a stable crystalline form of hydroxapatite [30]. HA was formed directly on the sample surface when the Ca/P ratio in the electrolyte was >4:1, as shown in Figure **1**. The formation mechanism of HA is similar to that of $Ca_3(PO_4)_2$; however, unlike the formation of $Ca_3(PO_4)_2$, the Ca^{2+} and PO_4^{3-} ions of the



(**a**) Ca:P=1.25:1 (**b**) Ca:P=2.5:1



(**c**) Ca:P= 4:1 (**d**) Ca:P= 5:1

Figure 1: XRD spectra of the samples with the different Ca/P ratios in electrolyte.

electrolyte reacted with H_2O molecules. The reaction can be expressed as follows:

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

It should be noted that there were the diffraction peaks of alkali titanate in the XRD profile in Figure **1**. It can be inferred as follows: the TiO_2 surface dissolved in the alkaline solution during PEO, which can be expressed as follows:

$$TiO_2 + OH^- \rightarrow HTiO_3^-$$

The surface with negatively charged reacts with the Ca^{2+} cations from the electrolyte, and the calcium titanate layer is obtained. Ca^{2+} ions reacted with $HTiO_3^-$ and OH^- ions under high-temperature, high-pressure conditions, the $CaTiO_3$ phase was formed in the ceramic coatings.

$$HTiO_3^- + Ca^{2+} + OH^- \rightarrow CaTiO_3 + H_2O$$

Besides, it can also be concluded that the higher the Ca/P ratio in the electrolyte, the stronger the $CaTiO_3$-forming ability, as shown in Figure **1**.

2.2. Test Results and Analysis for SBF Test

The four samples with different contents of Ca and P were immersed in SBF for two weeks. The elemental compositions of the coatings are listed in Table **1**. Both the Ca and P contents increased to some degrees. The reason is that OH^- ion, HPO_4^{2-} ion and Ti-OH groups are negatively charged. When the samples were soaked in SBF solution, these negatively charged selectively combined with positively charged Ca^{2+} ions to form Ca-rich calcium phosphate. However, with the accumulation of the calcium ions on the coating surfaces, the surfaces appeared overall positively charged, and then they preferred to combine with PO_4^{3-} ions.

The surface morphologies of the samples were analyzed by SEM using a CAMBRIDGE S-360 scanning electron microscope, and the results were compared before and after the immersion in SBF, as shown in Figure **2**. The surface of the samples also remained a porous structure after SBF immersion. However, these surface morphologies were quite different from those after PEO treatment. Clearly, the micropores on the coatings became small or were almost covered after the immersion in SBF. This can be explained as follows: first, the Ca^{2+}, OH^-, and $TiO(OH)_2$ species are obtained by the $CaTiO_3$ hydrolysis ($CaTiO_3 + 2\ H_2O \rightarrow Ca^{2+} + 2\ OH^- + TiO(OH)_2$.) [31]. However, $TiO(OH)_2$ hydrates are difficult to dissolve; therefore, they deposited on the surface of the coatings; Second, the formed Ti-OH groups could also play a deceive role in the formation of a bonelike apatite layer (HA) during the subsequence SBF immersion. The formation of HA depends on the time that the sample is immersed in SBF solution. Consequently, the soaked sample surfaces form a HA layer. Therefore, it can be concluded that the coverings of the coatings mainly consist of $TiO(OH)_2$ hydrates and HA.

The phase components of the coatings were analyzed by XRD, as shown in Figure 3. The XRD results show that HA was formed on the coatings; however, the composition of the anatase and rutile phases on the coating did not change clearly after the immersion in the SBF. Therefore, it can be concluded that the hydrolysis of $CaTiO_3$ is the main reason for forming HA on the coating surface in SBF. Based on the HA content in the coatings, it can be concluded qualitatively that the higher the Ca/P ratio in the electrolytes and coatings, the better the bioactivity. Ca and P element introduced during PEO could increase the opportunity to form hydroxapatite in SBF immersion. This finding has important significance for

Table 1: EDS Results of the PEO Coatings Before and After the Immersion

Test No.	Before the Immersion			After the Immersion		
	Ca Content	P Content	Ca:P	Ca Content	P Content	Ca:P
1	1.21	0.62	1.95:1	1.58	0.79	2:1
2	1.81	0.68	2.66:1	2.22	0.73	3.04:1
3	1.95	0.65	3.00:1	5.52	1.67	3.30:1
4	3.94	1.21	3.26:1	7.98	2.38	3.35:1

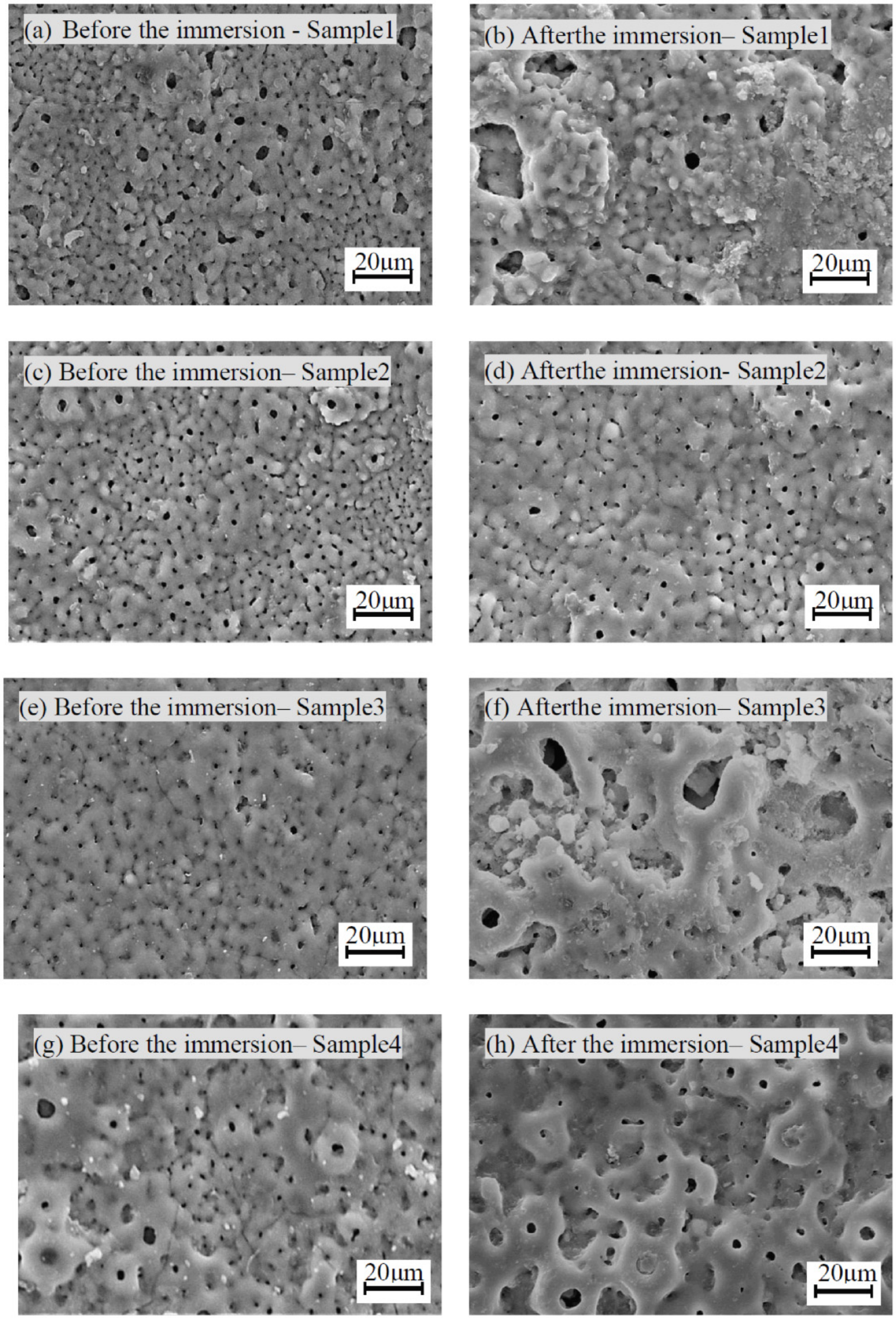


Figure.2: SEM surface morphology changes before and after the immersion.

developing the bioactivity of Ti implant materials.

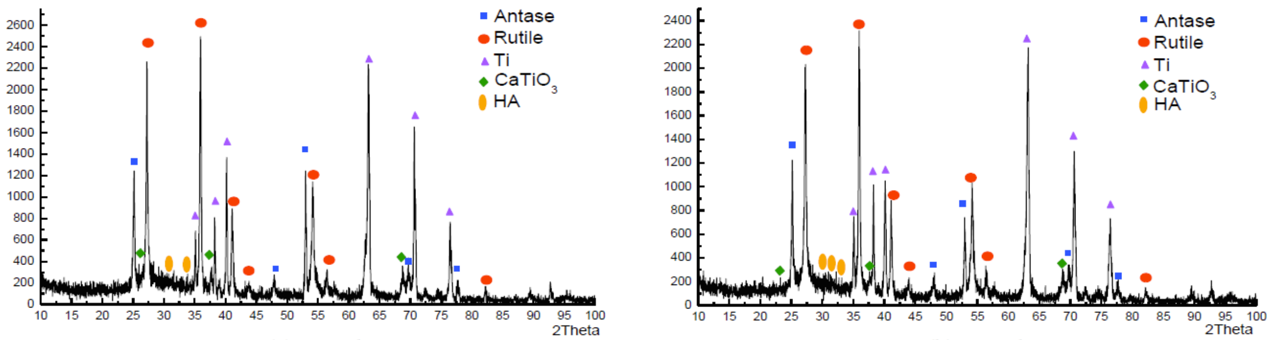
3. EXPERIMENTAL SECTION

3.1. Material

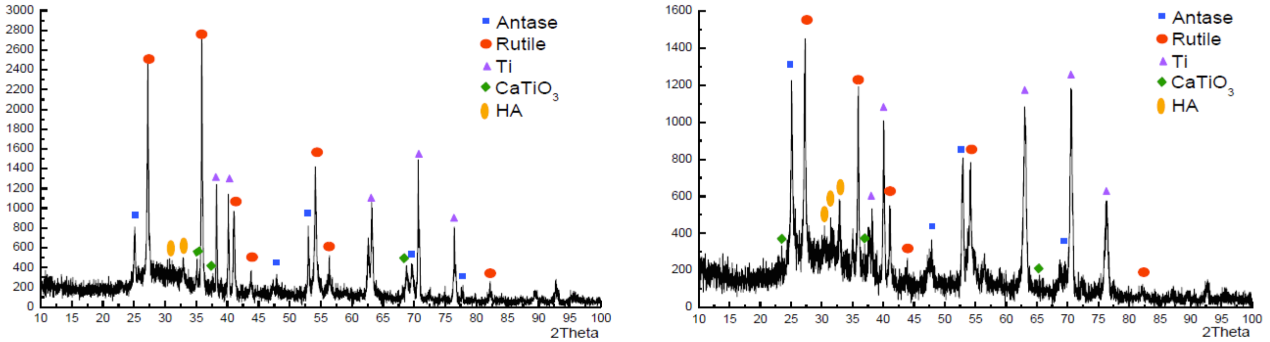
Commercial Ti6Al4V plates (sample size of 30 × 20 × 2 mm3), whose chemical composition is shown in Table **2**, were used in this study.

Table 2: Chemical Composition of Ti6Al4V Plate

Ti	Al	V	Fe	C	N	H	O	Other
Rest	6.1	3.95	0.11	0.07	0.02	0.003	0.13	<0.40



(a) Sample 1 (b) Sample2



(c) Sample 3 (d) Sample 4

Figure.3: XRD spectra of the samples after immersion.

3.2. PEO experiments

The electrolytes with different Ca/P ratios were prepared by dissolving different amounts of reagent-grade chemicals ($Ca(CH_3COO)_2$ and NaH_2PO_4) in deionized water, and the pH of the solutions were adjusted to pH 11 with NaOH, as shown in Table **3**.

Table 3: Ca/P Ratio of the Electrolytes

Test No.	1	2	3	4
Ca:P	1.25:1	2.5:1	4:1	5:1

An adjustable voltage and frequency power supply unit was used to support the PEO experiments. In this study, the applied voltage was fixed at 450 V; the treatment time was fixed at 20 min; the pulse frequency and duty ratio were kept at a constant value of 350 Hz and 50%, respectively, and the temperature of the electrolyte was maintained below 45°C.

3.3. SBF Test

The SBF solution was prepared according to the procedure reported in reference 14. The ionic composition of the solution is 142 Na^+, 5 K^+, 1.5 Mg^{2+}, 2.5 Ca^{2+}, 147.8 Cl^+, 4.2 HCO_3^-, 1 HPO_4^{2-}, and 0.5 SO_4^{2-}, almost equal to that of human blood plasma, as shown in Table **4**. The SBF was refreshed every 48 h. After immersing in SBF for two weeks, the four samples with different Ca/P ratios in the coatings were removed from the SBF, washed with distilled water and air-dried. The crystal phase compositions were analyzed by XRD; besides, the energy spectrum analyses and surface morphologies of the coatings after the immersion in SBF were measured by EDX and SEM, respectively.

Table 4: Formula of the SBF Solution

NaCl	$NaHCO_3$	KCl	$K_2HPO_4 \cdot H_2O$	$MgCl_2 \cdot 6H_2O$	HCl	$CaCl_2$	Na_2SO_4	$NH_3(CH_2OH)_2$
3.1984g	0.14g	0.0896g	0.0912g	0.122g	18ml	0.1112g	0.03g	2.4228g

4. CONCLUSIONS

(1) The Ca/P ratio in the electrolyte played an important role in forming $CaTiO_3$ in the coatings. The higher the Ca/P ratio in the electrolyte, the stronger the $CaTiO_3$-forming ability.

(2) The coverings of the coatings after the immersion in SBF mainly consisted of $TiO(OH)_2$ hydrates and HA.

(3) When the Ca/P ratio in the electrolyte was >4:1, HA could be directly formed in the coatings.

(4) The higher the Ca/P ratio in the coating, the better the bioactivity.

In addition, the interacting mechanisms of Ca and P elements should be researched and addressed in future research.

ACKNOWLEDGMENTS

We appreciate the National Natural Science Foundation of China (Grant number: 51371022) for financially supporting this work.

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Received on 17-04-2015 Accepted on 21-04-2015 Published on 29-04-2015

http://dx.doi.org/10.15379/2409-3394.2015.02.01.2