Biomimetic Calcium Phosphate Coating of Ti alloy in SBF and 1.5×SBF

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Abstract

In order to combine the excellent biocompatibility and bioactivity of hydroxyapatite with the optimal mechanical properties of Ti6Al4V, hydroxyapatite is commonly coated on the orthopedic and dental implants which are made up of this Ti alloy. As compared to the commercial methods such as plasma spraying, biomimetic method offers a promising alternative. In this study, Ti6Al4V plates were immersed into SBF for 20 days, however, complete coating could not be obtained. In order to accelerate the biomimetic deposition, the ionic concentration was increased to 1.5 times that of conventional SBF. In SEM micrographs, it was observed that the calcium phosphate precipitate forms small half-spheres and the entire surface was successfully coated with calcium phosphate after 2 weeks in 1.5×SBF. The coatings exhibited the standard hydroxyapatite XRD peaks.

Key words: Titanium alloy, Biomimetics, Hydroxyapatite Coating, SEM, EDS

1. Introduction

Ti6Al4V, which is a titanium alloy, is the most commonly used metallic material in the manufacture of orthopedic implants [1], owing to its unique properties, such as, good match with hard tissues in terms of mechanical properties, resistance to corrosion in body fluids and light weight. However, Ti6Al4V fails to show bioactivity, which is very important for the bone tissue integration of the implants. Therefore, it has no ability to bond chemically to the bone; rather a fibrous layer is formed between the implant and the natural bone tissue. On the other hand, in order to minimize the risks during the recovery of the patient after surgery, the surfaces of the implants are required to be bioactive for better osseointegration. Therefore, the Ti6Al4V implant surfaces are usually coated with hydroxyapatite, in order to take the advantage of its excellent biocompatibility and bioactivity.

Hydroxyapatite has very similar chemical composition and crystal structure to the apatite in the human skeletal system. As stated before, it is a bioactive, biocompatible and osteoconductive biomaterial inherently. However, despite these properties, hydroxyapatite cannot be used in load-bearing applications in bulk form, because of its limited mechanical properties, such as, low strength and brittleness. For this reason, hydroxyapatite is usually used as a coating material on metallic implant surfaces, such as titanium and its alloys [2].

There are various techniques for coating hydroxyapatite on metallic substrates. Some of them are plasma spraying, dipping, electrodeposition, pulsed-layer deposition, sputtering, and sol-gel-

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derived coating [3]. Among these techniques, plasma spraying is used widely due to its simplicity and economy. However, it has been reported that plasma spraying exhibited variation in bond strength, non-uniformity in thickness and density, changes in the phase composition and crystallinity of hydroxyapatite [4]. In addition, plasma spraying is a line-of-sight process and it is not very useful in coating complex implant surfaces. On the contrary, biomimetic method, which is based on dipping the metallic implants in simulated body fluid (SBF) at the physiological temperature and pH, is a better method especially for coating implants with complicated geometries.

In this study, Ti6Al4V plates are first pretreated with sodium hydroxide and heat, in order to increase the apatite forming ability of the surface. The plates were then immersed in SBF and 1.5×SBF, separately. The effect of pretreatment, Ca/P molar ratio and surface morphology of the coatings was concluded.

2. Materials and Method

Ti6Al4V (Grade-5, ELI, ASTM B265-10) alloy plates with dimensions of $20\times20\times2mm^3$ were first abraded with 400 grit SiC paper. The plates then ultrasonically cleaned in ethanol (70%), acetone and finally distilled water for 15 min in order to remove the post-production dirt remained on the Ti6Al4V surface.

2.1. Pretreatment of the Ti6Al4V Substrate

The ultrasonically cleaned Ti6Al4V plates were exposed to 5M sodium hydroxide (NaOH) at 80°C for 3 days. Following the alkali treatment, the specimens were gently washed with distilled water and dried at 40°C in a fanned convection oven overnight. The specimens were then heat-treated at 600°C with dry heat for 1 hour in a high temperature furnace and left in the oven overnight for gradual and slow cooling.

2.2. Preparation of Simulated Body Fluid

In order to prepare the simulated body fluid (SBF), the method given by Kokubo et al. [5] was followed. For the preparation of 1000 ml of SBF, the reagents given in Table 1, was dissolved in 700 ml ion-exchanged distilled water, which is at 36.5±1.5°C, by adding in the order from the 1st to the 8th under constant stirring. Each weighing bottle was washed with several drops of ion-exchanged distilled water, which was added to the solution. After the first eight reagents were dissolved, TRIS and small amount of HCl were dissolved in the process of pH adjustment. Just before dissolving the TRIS, the pH of the solution should be between 2.0±1.0. 1M HCl and TRIS were added alternately into the solution, until the whole amount of TRIS was dissolved keeping the pH within the range of 7.42–7.45. Lastly, the pH of the solution was adjusted to 7.40 exactly at 36.5°C by adding 1M HCl dropwise. The resulting solution was used in biomimetic coating immediately after the preparation without delay or storing.

Table 1. The reagents for preparing 1000 ml of SBF [5].

Order	Reagent	Amount
1	NaCl	8.035 g
2	$NaHCO_3$	0.355 g
3	KCl	0.225 g
4	$K_2HPO_4.3H_2O$	0.231 g
5	$MgCl_2.6H_2O$	0.311 g
6	1.0M HCl	39 ml
7	$CaCl_2$	0.292 g
8	Na_2SO_4	0.072 g
9	TRIS	6.118 g
10	1.0MHCl	0–5 ml

2.3 Preparation of 1.5×SBF

The ionic concentrations of human blood plasma and 1.5×SBF are reported in Table 2. According to the given ionic concentrations, the amounts of reagents were raised. The same procedure for the preparation of SBF with conventional ionic concentration was followed while preparing the more concentrated solution. However, unlike SBF, 1.5×SBF was buffered at pH 7.2, in order to prevent spontaneous precipitation. The resulting solution was used immediately after the preparation without delay or storing.

Table 2. Ionic Concentrations of Blood Plasma and 1.5×SBF [6].

Ions (mM)	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HPO_4^{2-}	SO_4^{2-}	HCO_3^-
1.5×SBF	212.3	7.5	2.3	3.8	186.8	1.5	0.75	40.5
Blood	142.0	5.0	1.5	2.5	103.0	1.0	0.5	27.0

2.4 Biomimetic Hydroxyapatite Coating

The alkali and heat treated Ti6Al4V substrates were soaked in SBF and 1.5×SBF separately at 37°C for apatite depositing. The plates, after being placed into plastic beaker, were placed into the simulated body fluids of 50ml per $1cm^2$ plate. In every two days, new solutions were prepared and freshened. In each renewal of the solutions, by considering the possible precipitation on the walls, the plastic beakers were replaced with the clear ones. The solutions, in which the plates were soaked, were kept at 37°C by using a shaking water bath.

3. Results

The SEM images and EDS spectra of the surfaces of the Ti6Al4V plates which were abraded, pretreated and immersed in SBF for 5 and 20 days are given in Figure 1 with details at higher magnifications.

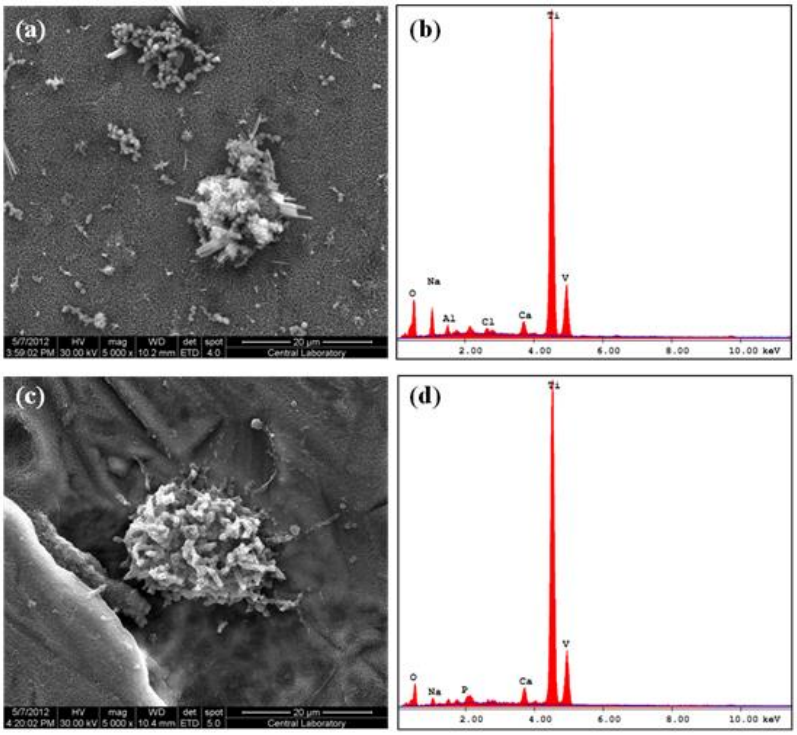

Figure 1. The surface of abraded, alkali and heat treated Ti6Al4V plate (a) SEM image after immersion in SBF for 5 days (5000×); (b) EDS spectrum of (a); (c) SEM image after immersion in SBF for 20 days (5000×); (d) EDS spectrum of (c).

The SEM images of the Ti6Al4V plates which were abraded, pretreated and immersed in 1.5×SBF for 7, 10 and 21 days are given in Figure 2.

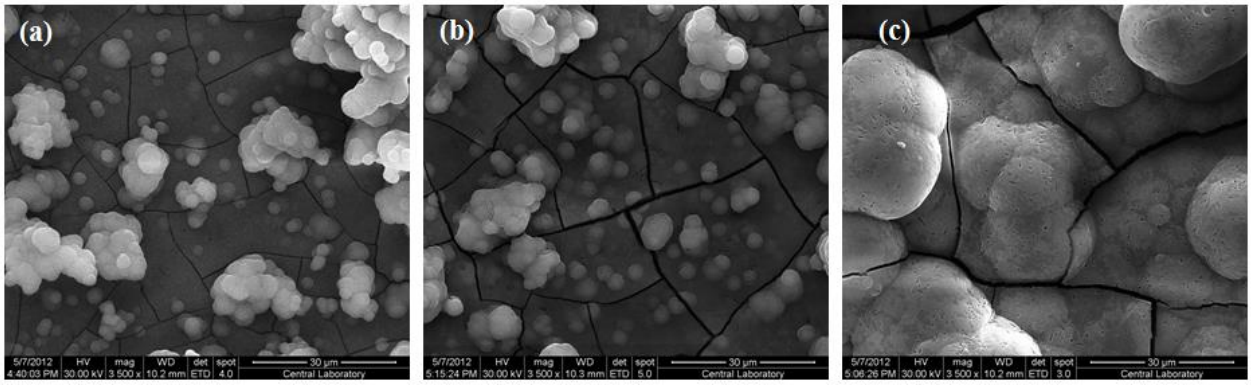

Figure 2. SEM images of the surfaces of # 400 SiC abraded Ti6Al4V plates after alkali and heat treatment and immersion in 1.5×SBF (3500×) for (a) 7 days, (b) 10 days, (c) 21 days.

From Figure 2, it was observed that the apatite deposited on the surface in the form of half spheres. The high magnification SEM image and the EDS spectrum of one half-sphere are given in Figure 3. The image belongs to a Ti6Al4V plate which was abraded, pretreated and immersed in 1.5×SBF for 21 days.

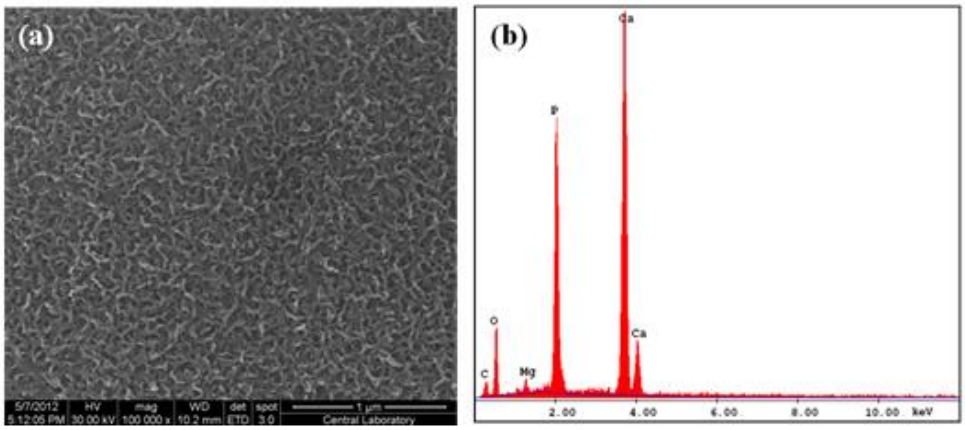

Figure 3. The surface of one globule (a) High magnification SEM image of abraded, alkali and heat treated Ti6Al4V plate after immersion in SBF for 21 days (100000×); (b) EDS spectrum of (a).

The XRD patterns of the surfaces of pretreated Ti6Al4V plates after soaking in 1.5×SBF for a period of 4, 10 and 21 days are given with standard hydroxyapatite (ICDD card No. 1-1008) in Figure 4.

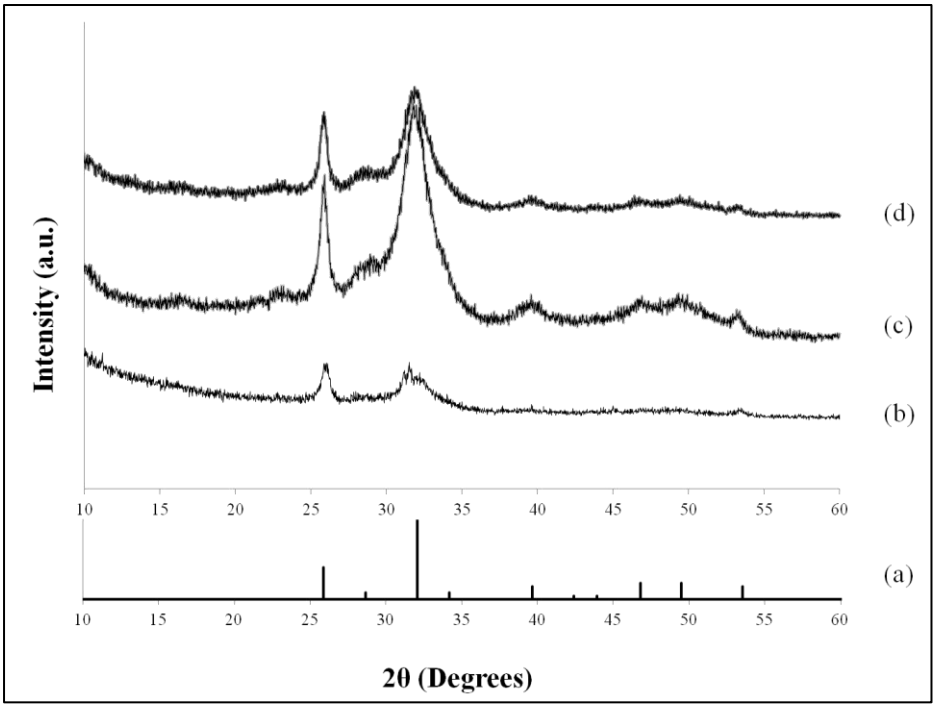

Figure 4. XRD patterns of (a) standard hydroxyapatite (ICDD card No. 1-1008) and Ti6Al4V plates after soaking in 1.5×SBF with normal ionic composition for a period of (b) 4; (c) 10; (d) 21 days.

4. Discussion

The alkali and heat treatment can provide bioactive surface and enhance the bone bonding ability of the implant [6]. The purpose of the alkali treatment is to obtain a porous network layer of sodium titanate, on which apatite formation can be induced. In addition, in order to increase the stability of titanate layer, the plates can be subjected to heat after alkali treatment. This would transform the amorphous titania gel to crystalline phase. However, during the heat treatment, if the temperature exceeds 600°C, this might have caused a decrease in Na^+ release thus formation of less TiOH groups [7], which would negatively affect the apatite forming ability of the surface. Therefore, higher sintering temperatures (over 600°C) were not applied.

From Figure 1, it can be seen that the surface of the pretreated Ti6Al4V plate gained a micro-porous and loose structure after alkali and heat treatment. It can be said that the calcium phosphate nuclei formed as acicular crystals on the surfaces of the plates which was immersed in SBF for 5 days. However, as expected, there was not a real coating even though 20 days had passed.

1.5×SBF, which is more concentrated than SBF, was more successful in coating the plates and accelerating the biomimetic precipitation. As shown in Figure 2, nucleation of calcium phosphate crystals took place first, and on the 7^{th} day, it was seen that the crystals grew on top of the surface. In addition, the morphology of calcium phosphate deposits changed with the duration of immersion in SBF. Accumulation of calcium phosphate on the surface started in the form of small particles then took the form of clusters in the following days. On the 21^{st} day, it can be said that the calcium phosphate deposits transformed into the shape of bigger globules side by side. In Figure 3, a close look to one of these globules was given. It was seen that the biomimetic coating was in the form of nanosized plates that were forming spherical aggregates.

From the EDS analysis given in Figure 3, it can be inferred that the entire surface was coated with calcium phosphate on day 21 in 1.5×SBF. After 3 weeks, EDS spectrum lacked peaks coming from the substrate, on the contrary, in the EDS spectra of the plates coated in SBF (Figure 1 (b) and (d)), titanium, aluminum and vanadium peaks were present. In this coating obtained from 1.5×SBF, calcium (Ca) and phosphorous (P) peaks arising from the presence of calcium phosphate coating were detected. It was interesting to see that the coating contains also very small amount of magnesium impurity, which is a favorable since magnesium plays an important role in the formation and growth of bone tissue [8].

All coatings give the standard amorphous hydroxyapatite (ICDD card No. 1-1008) XRD peaks (Figure 4). The coatings exhibited main peaks at 2 theta angles of about 25.8 and 32.1 degrees. With the increasing waiting time, XRD peaks were expected to strengthen due to the increase in the thickness of the coating. The XRD peak intensity of the coating on the 21^{st} day was less than the XRD intensity of the peaks of the coating on the 10^{th} day. This was thought to be due to the detachment of some of the thick coating on the surface. From the XRD analyses, the coatings obtained by soaking the Ti6Al4V plates into the 1.5×SBF with normal ionic concentration for 10 days yielded highest intensities.

Conclusions

When compared to SBF with conventional ionic composition, 1.5×SBF yielded calcium phosphate coatings on alkali and heat treated Ti6Al4V plates more quickly and successfully. The resulting calcium phosphate coating was amorphous hydroxyapatite. The SBF composition can further be modified to include various ions in order to give different characteristics to the coatings.

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References

[1] Mavis B, Tas AC. Dip coating of calcium hydroxyapatite on Ti-6Al-4V substrates, J Am Ceram Soc 2000;83:4:989–91.
[2] Wang D, Chen C, He T, Lei T. Hydroxyapatite coating on Ti6Al4V alloy by a sol-gel method, J Mater Sci: Mater Med 2008;19:2281–86.
[3] You C, Oh S, Kim S. Influences of heating condition and substrate-surface roughness on the characteristics of sol-gel-derived hydroxyapatite coatings, J Sol-Gel Sci Technol 2001;21:49–54.
[4] Yang Y, Kim KH Ong JL. A review on calcium phosphate coating produced using a sputtering process-an alternative to plasma spraying, Biomaterials 2005;26:3:327–337.
[5] Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 2006;27:15:2907–15.
[6] Bigi A, Boanini E, Panzavolta S, Roveri N. Biomimetic growth of hydroxyapatite on gelatin films doped with sodium polyacrylate, Biomacromolecules 2000;1:752–56.
[7] Kim H-M, Miyaji F, Kokubo T. Effect of heat treatment on apatite forming ability of Ti metal induced by alkali treatment, J Mater Sci: Mater Med 1997;8:341– 47.
[8] Rabadjieva D, Tepavitcharova S, Gergulova R, Sezanova K, Titorenkova R, Petrov O, et al. Mg-and Zn-modified calcium phosphates prepared by biomimetic precipitation and subsequent treatment at high temperature, J Mater Sci: Mater Med 2011; 22:10:2187–96.