Hydroxyapatite Coating of Ti6A14V Alloys in Alanin-Alanin Sodium Salt Environment with Biomimic Method

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Abstract

Biomimetic is an expression used to define the substances, tools, mechanism, a systems created by humans through imitating the systems existing in the nature. With this method the surfaces of base materials activated by various chemicals are coated inside synthetic body fluid (SBF) that is created in the laboratory ground by using the method of defecation. As coating is conducted in an environment which has synthetic body fluid, biomaterials that are completely harmonious with the body can be obtained.In this study it is aimed to create hydroxyapatite (HA) coating that is completely harmonious with human blood plasma, in Alanin and Alanine Sodium Salt environment by using biomimetic technics for the first time in literature and to analyse it. In the process of HA coating, Ti6A14V alloyed implant materials have been used as base material.In the study, implant materials having Ti6Al4V alloy have been first sandpapered and then they were washed first with pure water and then with acetone. The materials that were also cleaned inside the ultrasonic bathroom, have been waited for 1 day in the 100 mL 5M NaOH + 0,5 mL H_2O_2 solution in drying-oven for being activated. Later on NaOH was transferred and the materials were washed with pure water. Afterwards it is left to dry at 60 ºC for 24 hours. After being dried, it was waited for 1 hour at 600 ºC and than cooled. With the completion of this process, the synthetic body fluid solution (2L) was obtained with the temperature at 37 ºC and pH value at ~7,4. Coating process was realised inside SBF with waiting periods of 24, 48, 72, and 96 hours. Relating to the coatings obtained by biomimetic method, surface smoothness and thickness specifications have been determined, their micro structure has been analysed by using Scanning Electron Microscope (SEM), the elementary analyses namely Energy Dispersive X-ray Spectroscopy (EDS) for the surfaces of coating have been determined and X-Ray Diffraction (XRD) analysis have been conducted for obtaining information regarding phases and the concentrations of the phases. The results obtained from the study have been evaluated and discussed.

Keywords — Alanin - alanine sodium salt, biomimetic coating, hydroxyapatite (HA), synthetic body fluid (SBF), Ti6Al4V.

1 Introduction

As the people are faced with various diseases and accidents all through their lives, some tissues or organs get injured and even some functions are lost in the human body. These tissues which are damaged or have lost their function are treated by translantation or implantation methods [1]. Implantation process is carried out with biomaterials which can adapt to the body as being tools or prothesis with specific designs. Implantation process is done by choosing the most suitable prothesis from among 4 main groups namely metallic, ceramic, polymer, or composite. In the production of orthopedic implants like hip prothesis, bone plates, and bone screws, a metallic biomaterial named Ti6A14V alloy is widely used. In order to increase biocompatibility of implant surface, coatings with ceramic basis named hydroxyapatite are applied

[2]. There are various methods relating to HA coating processes. But HA coating of metallic surface by using biomimetic method is a process which can be applied to all types of implants cause their prices are advantageous, they can be easily produced, they have a thin and durable bioactive plate, the structures have spores, and they do not change the morphology of the surface [3].

HA production process with biomimetic method is composed of stages as HA coating process in SBF by using chemical precipitation (in situ) method under biocompatibility conditions (human body temperature being 37 ºC and pH as 7,4) also including preparation of SBF, chemical processing, thermal processing, and keeping it waited in SBF. Synthetic body fluid known as metastable buffer solution which has a chemical structure equivalent to human blood plasma as shown in Table 1.relating to ionic concentration, is prepared by using chemicals as NaCl, $NaHCO_3$, KCl, $Na_2HPO_4 \cdot 2H_2O$,$MgCl_2 \cdot 6H_2O$, Na_2SO_4, $(CH_2OH)_3CNH_2$, HCl, and $CaCl_2 \cdot H_2O$ [3].

Table 1. Ionic concentration of SBF and human blood plasma

Ion	Kokubo (mM)	Taş (mM)	Sepahvandi et al. (Et al) (mM)	Faure et al. (mM)	Li (mM)	Xiaobo et al. (mM)	Pasinli et al. and Aydın et al. (mM)	Human Blood Plasma (mM)
Na	142.0	142.0	142.0	154.56	142.0	142	142.0	142.0
Cl^-	147.8	125.0	147.8	120.5	103.0	103.0	103.0	103.0
HCO^-_3	4.2	27.0	4.2	44.0	27.0	10	27.0	27.0
K^+	5.0	5.0	5.0	5.37	5.0	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5	0.8	1.5	1.5	1.5	1.5
Ca^{2+}	2.5	2.5	2.5	1.82	6	2.5	2.5	2.5
HPO_4^{2-}	1.0	1.0	1.0	1.0	2.4	1.0	1.0	1.0
SO_4^{2-}	0.5	0.5	0.5	0.8	0.5	0.5	0.5	0.5

Kokuba et al. have first successed with the biomimetric method by HA coating on biomaterials inside the synthetic body fluid [4]. Taş, has made changes in the quantities of HCO_3^- and Cl^- under the biomimetric conditions with pH value as 7,4 and temperature as 37 ºC inside synthetic body fluid that was prepared by Kokuba, and in this way an SBF that is closer to the ionic concentration inside the blood plasma was obtained. He has stated that as a result of his study, HA in the form of ceramic powder having high chemical homogeneity and purity was obtained [5]. In the studies conducted by Sepahvandi, Faure, Li and Xiaobo together with the collegues, blood plasma values could be derived in specifications inside SBF environment [6 - 9]. But the values that were exactly the same as blood plasma values could be realised for the first time by Pasinli et al. [10]. Aydın et al. have used citric acid- sodium citrate tampon system for the first time in the world and they have prepared a synthetic body fluid solution that is equivalent to ionic values in blood plasma and more successful results were obtained [11].

In this study, Hydroxyapatite (HA) coating that is completely harmonious with the body blood plasma has been produced in Alanin and Alanine Sodium Salt environment by using biomimetric technics for the first time in literature and the related evaluations have been made.

2 Materials and Method

2.1. Choosing The Implant Materials

In this study, Ti6A14V alloy that is the most biocompatibility one in long term implementation as being decisive in chemical reactions, also being structurally and surface wise biocompatibility as regards to its nontoxic and hypoallergic characteristics has been prefered as the base material. The chemical composition of materials used as shown in Table 2.and their mechanical features are provided in Table 3.

Table 2. Chemical composition of Ti6Al4V material (ASTM, F-67-89, 1992)

Element	Ti	N	C	H	Fe	O	Al	V	Other
%	Remaining	0,05	0,08	0,0125	0,25	0,13	5,5-6,5	3,5-4,5	0,1-0,4

Table 3. Mechanical features of Ti6Al4V material (ASTM, F-136-84, 1992)

Yield Stress (MPa)	Tensile Stress (MPa)	Elongation Ratio (%)	Shrink Ratio (%)
883	960	13	50

2.2. Preparation of Coating

In the study, implant materials having Ti6Al4V alloy have been first sandpapered and then they were washed first with pure water and then with acetone. The materials that were also cleaned inside the ultra-sonic bathroom, have been waited for 1 day in the 100 mL 5M NaOH + 0,5 mL H_2O_2 solution in drying-oven for being activated. Later on NaOH was transferred and the materials were washed with pure water. Afterwards it is left to dry at 60 ºC for 24 hours. After being dried, it was waited for 1 hour at 600 ºC and than cooled. With the completion of this process, the synthetic body fluid solution (2L) shown in Table 4.was obtained with the temperature at 37 ºC and pH value at ~7,4. Then the materials went under the process of rinsing with waiting periods of 24, 48, 72, and 96 hours at 37 ºC and the coating process with biomimetric method was realised. After the completion of process, the materials were washed with pure water and dried at 60 ºC for 24 hours. These coating processes done at different were all realised separately.

Table 4. Inorganic salts in the synthetic body fluid (SBF) (Total Volume = 2 L)

Chemical Substance	Quantity (mg)
KCl	746,0
NaCl	10519,2
$Na_2HPO_4 \cdot 2H_2O$	356,0
Na_2SO_4	142,0
$NaHCO_3$	4536,6
β-Alanin	5327,6
$CaCl_2 \cdot 2H_2O$	735,2
$MgCl_2 \cdot 6H_2O$	610,0
β-Alanin (89,99 g/L)1M	

3 Results and Discussions

3.1. Mechanic Test Results

Surface roughness values of the coatings have been measured in terms of µm with the MitutoyoSurftest SJ–301 device that is at Machine Engineering Division in Celal Bayar University. Measurement interval and speed were defined as 12,5 mm and 0,5 mm/s respectively. The measurement of each sample realised as per waiting periods of 24, 48, 72 and 96 hours in SBF were repeated five times and the average of results obtained was taken. As Table 5.is reviewed, it is seen that roughness values of coated surfaces have increased depending on the waiting periods in synthetic body fluid.

Table 5. Changes in roughness values of coated surfaces as per waiting periods in synthetic body fluid

Surface Roughness [Ra] (µm)	
24 Hours	1,40 ± 0,216
48 Hours	2,22 ± 0,017
72 Hours	2,94 ± 0,341
96 Hours	3,27 ± 0,479

Hayakawa et al. have measured the average surface roughness of HA coating applied on the titanium material surface which they have used as base material in their studies was equal to 1,3µm [12]. Yoshinari et al. have also applied HA coating process on the titanium material surface and found that the average surface roughness of coating was 1,1µm [13]. Pasinli et al. have reported on their study that they have measured surface roughness values for 1 SBF, 1,5 SBF, 3 SBF as approximately between 1,8-2,0 µm, 2,0-2,4 µm and 2,0-

2,8 µm depending on the concentration [10]. Citeau et al. also used titanium and HA in their studies and they have found the average surface roughness of coating as 1,57µm [14]. Xiaobo et al. have stated that they have measured surface roughness values of HA coatings in between 0,23 – 1,21 µm [9]. By using a new tampon system in literature for the first time, Aydın etal. have measured surface roughness values of coatings as ~1,20, ~1,90, ~2,60 ve ~3,85 µm respectively for waiting periods of 24, 48, 72, and 96 hours in synthetic body fluid respectively [11].

Application with hydroxyapatite has been made to each coating surface for five times by using the ElectrophysicsMinitest 730/Sensor FN 1,5 HD branded device at Ege University Ege Occupational High School Laboratory and the averages have been taken and shown in Table 6. As the values on the table are reviewed, it is seen that the coating thicknesses increase depending on the waiting period at synthetic body fluid.

Table 6. Changes in values of surface coating thickness depending on the waiting periods in synthetic body fluid

Coating Thicknesses (µm)	
24 Hours	8,25 ± 0,863
48 Hours	8,85 ± 0,168
72 Hours	9,10 ± 0,203
96 Hours	10,35 ± 0,514

Şimşek et al. have reported in their studies that they have defined thickness values in SBF at temperature 37 ºC to be between 10 and 100 µm [15]. Li et al. have stated that a coating with thickness of approximately 40 µm on the surface of sample had occurred within 24 hour waiting period in synthetic body fluid with the system established as based on biomimetic method [16]. Pasinli et al. have obtained HA coatings with thicknesses of approximately 6,78µm, 8,93 µm and 19,13 µm for 1 SBF, 1,5 SBF and 3 SBF respectively by using biomimetic technics [10]. Aydın et al. have stated that they have measured the coating thicknesses within the intervals 7-8 µm, 9-11 µm, 13-14 µmve 18-20 µm for the waiting perios of 24, 48, 72, and 96 hours in SBF [11].

3.2. Results of Metalographic Analysis

Microscopic analysis of sample surfaces to which HA coating is applied for different time periods has been realised by using Philips XL 30S FEG model electron microscope with scan (SEM) which is placed in İzmir High Technology Institute Material Research Center. SEM 100X and 2500X images of coatings obtained during waiting periods of 24, 48, 72, and 96 hours at SBF have been shown in Figure 1.and Figure 2. As SEM images obtained are analysed, it is seen that coating thickness increases depending on the waiting periods at SBF. The cracks on the coating are seen as a natural outcome of the process depending on the density of solution and thickness of the coating.

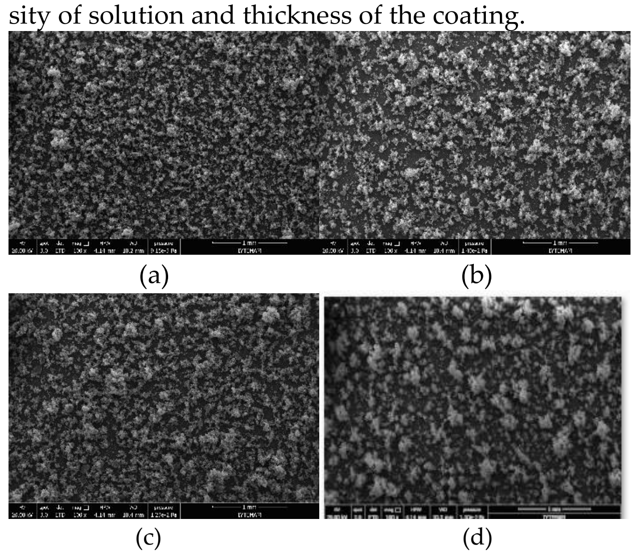

Figure 1.SEM images (100X) of coatings obtained during waiting periods of a) 24 hoursb) 48 hours c) 72 hours d) 96 hours in SBF

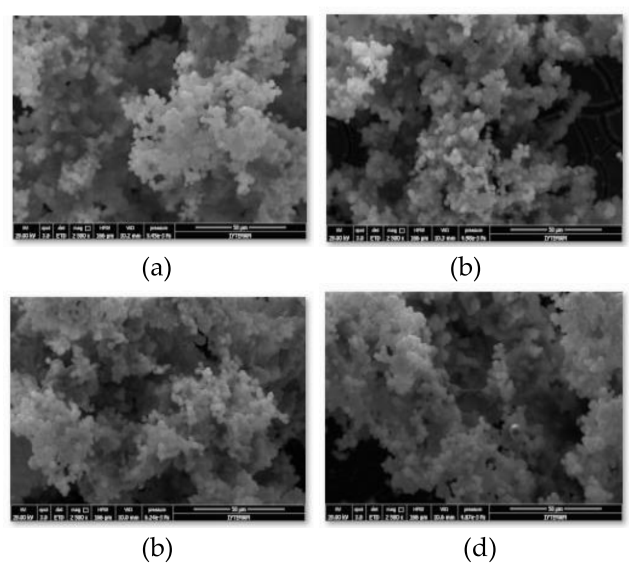

Figure 2.SEM images (2500X) of coatings obtained during waiting periods of a) 24 hours b) 48 hours c) 72 hours d) 96 hours in SBF

For conducting elemental anaylsis of coating surfaces, Philips XL 30S FEG model electron microscope with scan has been used. As this microscope has EDX detector, it is also used for determining elemental content of structures. EDS results of coatings obtained during waiting periods of 24, 48, 72 and 96 hours in SBF solution are shown in Figure 3. – Figure 6. When EDS results are reviewed, it is seen that there are Ca and P structures on HA coated surfaces.

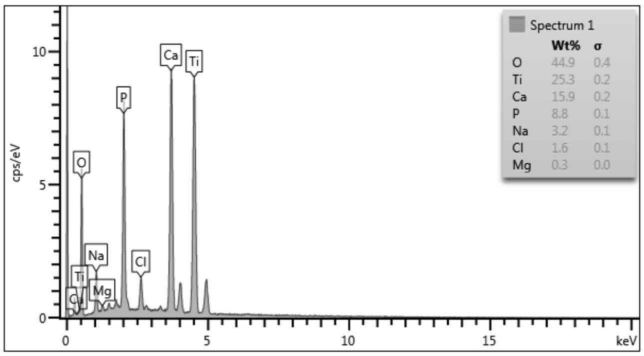

Figure 3.EDS analysis results of coating surfaces obtained by waiting for 24 hours in SBF

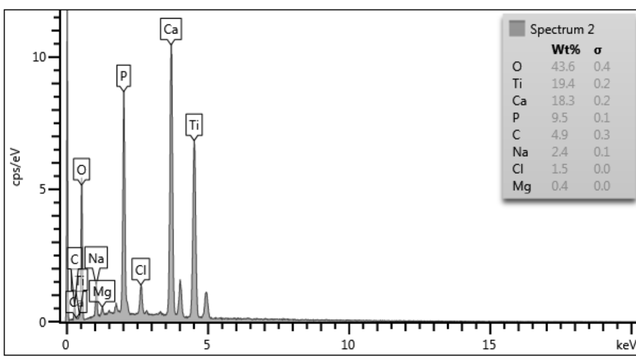

Figure 4.EDS analysis results of coating surfaces obtained by waiting for 48 hours in SBF

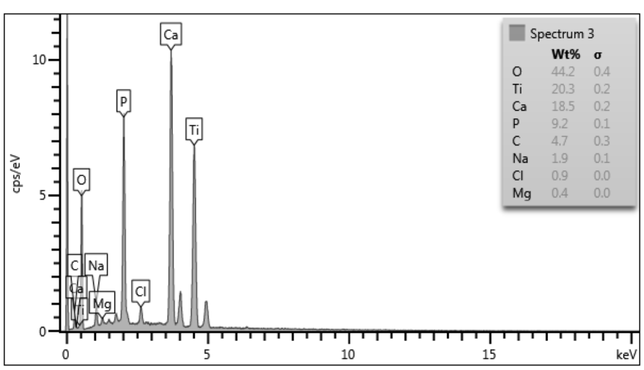

Figure 5.EDS analysis results for coating surfaces obtained by waiting for 72 hours in SBF

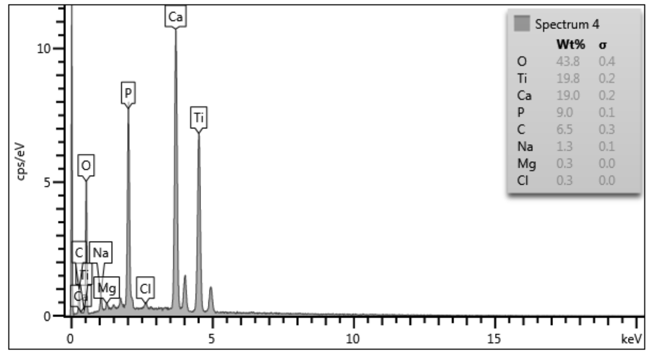

Figure 6.EDS analysis results for coating surfaces obtained by waiting for 96 hours in SBF

Ideal Ca/P ratio and the calculated density of hydroxyapatite with calcium phosphate basis forming the inorganic structure of bone tissue are 10/6 (~1,66) and 3,219 gr/cm^3 [11]. With EDS analysis, weight percentages of atomic structures on the coating surfaces obtained during different periods of waiting in SBF have been obtained. The ratios of % Ca values to % P values are calculated and shown in Table 7. Reviewing the data obtained, it is seen that the value which is closest to the ideal value of ~1.66 was 1,81 that was obtained by waiting for 24 hours.

Table 7. Ca/P values that change depending on waiting periods in SBF

Waiting periods in SBF	Ca/P ratio
24 Hours	1,81
48 Hours	1,93
72 Hours	2,01
96 Hours	2,10

Despina et al. have defined Ca/P molar change value for HA coating as 1,65 [17]. Xiaobo et al. have applied HA coating processes on the material surfaces of Ti1200, Ti600, Ti240 and Ti120 in SBF and Ca/P ratios were calculated as 1,70, 1,69, 1,72 and 1,73 respectively [9]. Han et al. have determined calcium phosphate components ($Ca(OH)_2$, $CaHPO_4$ve HA) inside synthesised powder with a ratio of 1,57 Ca/P using the hyrothermal method (for 30 minutes with 600 bar and 300 ºC) [18]. Pasinli et al. have also determined Ca/P ratio of HA coating on Ti6Al4V as 1,26 in their studies [10]. Aydın et al. have found the value of 1,66 in coatings of

48 hours during their studies [11].

The required analysis for obtaining information about the phases and the concentration of phases contained in Ca-P coatings were conducted by using PANalytical Empyrean model device which is at Celal Bayar University, Experimental Scientific Applications and Research Center and the results were shown in Figure 7. As per the test results, HA crystals were found at: (002) 26,1010º peak, (121) 31,884º peak, (112) 31,965º peak, (030) 32,59º peak, (203) 45,580º peak, (222) 46,97º peak, (123) 49,71º peak, and (004) 53,43º peak points.

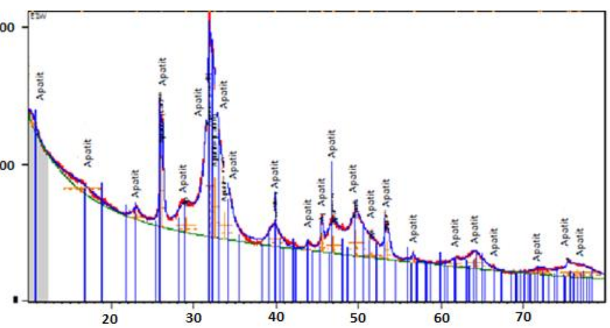

Figure 7.XRD analysis results

Takadama et al. have interpreted peaks of 2Theta=23,310 and 480 in addition to Ti peaks in link to the sodium titanata ($Na_2Ti_5O_{11}$) and rutile (TiO_2) crystals as per XRD analysis results obtained from biomimetric studies [19]. Barrere et al. have reported that (211), (112), (300) and (202) HA crystals formed at the peak point of 2Theta=32,060 of the coating after waiting for 24 hours in highly concentrated solution (5SBF) [20]. Pasinli et al. have reported that HA crystals of titanium crystals have formed at peak points of (002) 25,700, (210) 29,320, (211) 32,140, (310) 40,340 and (113) 43,300 [10].

4Conclusions

As a result, synthetic body fluid solution has been prepared in Alanin-Alanine Sodium salt environment which does not show any toxic effect on human body for the first time in literature by using biomimetric method and Ca-P coating processes have been realised. All of the values in blood plasma obtained by Pasinli et al. and Aydın and al. for the first time in literature have also been realised by us in the new tampon system. As a result of the study, a successful HA coating process was done which would cover the material surfaces densely.

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5 References

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