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Hydroxyapatite Coating on Ti6Al4V Alloy Surface Through Biomimetic Method Using Glycolic Acid - Sodium Gluconate Buffer System and Examination of Properties of the Coating

Araştırma Makalesi / Research Article

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ÖZ

İskelet – kas sistemimizde doğuştan ya da edinsel yollarla karşılaşılan rahatsızlıkların tedavisinde implant malzeme kullanımı önemli cerrahi müdahaleler arasındadır. Kullanılan bu implant malzemeler içerisinde kemiğin mekanik değerlerine yakın olması sebebiyle metalik biyomalzemeler sıklıkla tercih edilmektedir. İmplantasyon işleminde bu alaşımların yüzeyleri seramik esaslı biyomalzemeler ile kaplanarak biyoaktivite ve biyouyumluluk özellikleri artırılmaktadır.

Bu çalışmada, literatürde ilk defa biyomimetik teknik ile glikolik asit – sodyum glukonat tampon sistemi kullanılarak insan kan plazmasına tam uyumlu ortamda Ti6Al4V alaşımı üzerine hidroksiapatit (HA) kaplama üretimi yapılıp incelenmesi amaçlanmıştır. Kaplama işlemi yapay beden sıvısı (YBS) içerisinde 24, 48, 72 ve 96 saatlik bekletme sürelerinde gerçekleştirilmiştir. Elde edilen kaplamaların, yüzey pürüzlülük ve kalınlık özellikleri saptanmış, taramalı elektron mikroskobu (SEM) kullanılarak mikro yapıları incelenmiş, kaplama yüzeylerinin elementsel analizleri (EDS) belirlenmiş ve kaplamaların içerdiği fazların konsantrasyonu hakkında bilgi almak amaçlı XRD analizleri yapılmıştır. Testler sonucunda başarılı yüzey pürüzlülük değerleri ve ideal değere yakın Ca/P oranı yakalanmıştır. Ayrıca altlık malzeme üzerinde sıkı ve homojen bir dağılıma sahip, yoğun faz yapısındaki HA kristallerinin varlığı gözlenmiştir.

Anahtar Kelimeler: Biyomimetik kaplama, glikolik asit – sodyum glukonat, hidroksiapatit (HA), Ti6Al4V, Yapay Beden Sıvısı (YBS).

Ti6Al4V Alaşımı Üzerine Biyomimetik Yöntemle Glikolik Asit - Sodyum Glukonat Tampon Sistemi Kullanılarak Hidroksiapatit Kaplanması ve Kaplama Özelliklerinin İncelenmesi

ABSTRACT

The use of implant materials is one of the important surgical interventions in the treatment of disorders that are congenital and acquired deformities in musculoskeletal system. Metallic biomaterials within these implant materials are often preferred because of their proximity to the mechanical properties of the bone. In the implantation process, the surfaces of these alloys are coated with ceramic based biomaterials to increase the bioactivity and biocompatibility properties.

In this study, it is aimed to create hydroxyapatite (HA) coating that is completely harmonious with human blood plasma environment, on Ti6Al4V alloy, using glycolic acid - sodium gluconate buffer system with biomimetic method for the first time in literature. Coating was realised inside synthetic body fluid (SBF) with waiting periods of 24, 48, 72 and 96 hours. Relating to the coatings, surface smoothness and thickness specifications have been determined, their micro structure has been analysed by 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 the concentrations of the phases. As a result of the experiments, successful surface smoothness values and ratio of Ca/P close to optimal value have been obtained. Additionally, the presence of HA crystals with intense phase structure, with compact and homogeneous distribution on the substrate have been observed.

Keywords: Biomimetic coating, glycolic acid - sodium gluconate, Hydroxyapatite (HA), Ti6Al4V, Synthetic Body Fluid (SBF).

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1. INTRODUCTION

Broken bones, degenerative and inflammatory diseases of bones and joints, congenital and acquired deformities, spinal deformities, tumoral diseases of musculoskeletal system affects millions of people every year. These injuries and loss of function often indicate the need for surgical intervention [1]. Implantation method is used instead of damaged tissue and organs, either by the individual or by another individual, or where animal tissue or organ replacement is not possible. The materials produced in the laboratory and called as biomaterial can be used to perform the function of the damaged region within the body and outside the body [2].

The most suitable biomaterials for loads to which the musculoskeletal system is exposed and the mechanical properties of this system are metal based materials. Pure or alloyed versions of metallic biomaterials are frequently used in surgical applications because of their high stability and fatigue resistance, their modulus of elasticity close to the system values and their easy sterilization. Titanium and its alloys are often used among other metal based biomaterials due to features thereof such having the best biocompatibility in the long-term implantation process, not causing allergic and toxic effects in the body and having low possibility of entering into chemical reactions by virtue of the passive layer on the surface thereof [1, 3, 4].

The surface of the material selected for implantation is coated with ceramic based biomaterials in order to providing surface biocompatibility by increasing the corrosion resistance and increase the surface activity at the grade of bonding with contact tissues. Especially in orthopedic practices, hydroxyapatite ceramics which contain calcium and phosphate atoms in the structure are often preferred for coating [3]. At our study is the biomimetic method preferred because of its price and manufacture advantage, the thin and resistant bioactive layer doesn't change the surface morphology of the implant, be used on all implants including porous structures and screws [5].

HA coating on various biomaterials in SBF with biomimetic method have realized by Kokubo et al. for the first time in this field [6]. Taş, by virtue of the synthetic body fluid prepared thereby, achieved both the body temperature (37 ºC) and the blood pH value (pH 7.4) in addition to working with ion values closer to ion values constituting the blood compared to the ion values of SBF prepared through Kokubo et al. [7]. In the studies conducted by Sepahvandi, Faure, Li and Xiaobo together with the colleagues, blood plasma values could be derived in some ions inside SBF environment [8 - 11]. But the values that were exactly the same as blood plasma values have performed by a new improved method for the first time by Pasinli et al. [12]. Aydın has used citric acid-sodium citrate tampon system for the first time in the literature and he has prepared a SBF solution that is equivalent to ionic values in blood plasma and more successful results were obtained [3]. Çağlayan and Kırman have realized successful results in their studies by accepting these pioneering studies as guides [13, 14]. The SBF ion values of these studies have shown in Table 1.

In this study, HA coating that is completely harmonious with the human blood plasma has been produced in glycolic acid - sodium gluconate environment by using biomimetic technic for the first time in literature and the related evaluations have been made.

2. MATERIALS AND METHOD

2.1. Choosing the Specimens

Ti6Al4V alloy is used in this study, which is frequently preferred on orthopedic practices as base specimen. The dimensions of the base specimens are 10x10x1.2 mm and

Table 1. Ion concentrations of synthetic body fluids and human blood plasma (mM)

(mM)	Na^+	Cl^-	HCO_3^-	K^+	Mg^{2+}	Ca^{2+}	HPO_4^{2-}	SO_4^{2-}
Kokubo et al.	142.0	147.8	4.2	5.0	1.5	2.5	1.0	0.5
Taş	142.0	125.0	27.0	5.0	1.5	2.5	1.0	0.5
Sepahvandi et al.	142.0	147.8	4.2	5.0	1.5	2.5	1.0	0.5
Faure et al.	154.56	120.5	44.0	5.37	0.8	1.82	1.0	0.8
Li et al.	142.0	103.0	27.0	5.0	1.5	6.0	2.4	0.5
Xiaobo et al.	142.0	103.0	10.0	5.0	1.5	2.5	1.0	0.5
Pasinli et al.	142.0	103.0	27.0	5.0	1.5	2.5	1.0	0.5
Aydın	142.0	103.0	27.0	5.0	1.5	2.5	1.0	0.5
Çağlayan	142.0	103.0	27.0	5.0	1.5	2.5	1.0	0.5
Kırman	142.0	103.0	27.0	5.0	1.5	2.5	1.0	0.5
Human Blood Plasma	142.0	103.0	27.0	5.0	1.5	2.5	1.0	0.5

of Ø19x25.4 and Ø28x25.4 mm in accordance with the ASTM (American Society for Testing and Materials) F 1044-99 and F 1658-95 standards. The chemical composition of the alloy is indicated in Table 2, the mechanical features in Table 3.

Table 2. The chemical composition of the Ti6Al4V material (ASTM F 1044-99)

Element	Ti	N	C	H	Fe	O	Al	V	Other
Wt.- %	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 the Ti6Al4V material (ASTM F 1658-95)

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

2.2. Preparation of Coating

In the study, specimens having Ti6Al4V alloy have been first sandpapered and then they were washed with pure water and then with acetone. The specimens that were also cleaned inside the ultrasonic bathroom have been waited for 1 day in the 100 mL 5M NaOH + 0.5 mL %35 H_2O_2 solution in drying-oven for being activated. Later on NaOH + H_2O_2 were transferred and the specimens were washed with pure water. After the surface activation, the base specimens are washed with pure water and left for drying at 60 ºC for 24 hours. The specimens are converted ready for thermal treatment by wrapping these with aluminum folio in order to prevent an air contact after they dried. Then the specimens are placed into an oven and kept for 1 hour at a temperature of 600 ºC and left for cooling down at room temperature. After this process is the SBF solution (2 L) stated in Table 4 prepared at a temperature of 37 ºC and a pH value of ~7.4. Later are the coating processes by the biomimetic method performed by subjecting these separately at 37 ºC to a shaking process in resting durations of 24, 48, 72 and 96 hours. The specimens are washed with pure water after the treatment and dried for 24 hours at 60 ºC.

Table 4. Inorganic salts in the synthetic body fluid (total volume = 2 L)

Chemical Material	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
Na – Gluconate	4446.8
$CaCl_2{\cdot}2H_2O$	735.2
$MgCl_2{\cdot}6H_2O$	610.0
Na - Gluconate (76,818 g/L)1M	

3. RESULTS AND DISCUSSIONS

3.1. Mechanical Test Results

Surface roughness values of the coatings have been measured in terms of µm with the Mitutoyo Surftest SJ–301 device that is at Machine Engineering Laboratory in Celal Bayar University. Measurement interval and speed on coating 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. Average surface roughness values obtained by measurement have been shown in Table 5.

Table 5. Variation of the roughness values of the coating surfaces of hydroxyapatite coated specimens depending on the coating durations

Surface Roughness [Ra] (µm)	
24 Hours	1.21 ± 0.785
48 Hours	1.59 ± 0.471
72 Hours	1.62 ± 0.148
96 Hours	2.59 ± 0.114

In the result of this performed study, it is determined that the surface roughness values progressively increased during terms of 24, 48, 72 and 96 hours. Hayakawa et al. have determined as a result of the measurement they performed the mean surface roughness of the HA coating they made on titanium material as 1.3 µm [15]. Yoshinari et al. have measured the mean surface roughness of the HA coating they made on titanium material as 1.1 µm [16]. 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 [12]. Citeau et al. also used titanium and HA in their studies and they have found the average surface roughness of coating as 1.57 µm [17]. Aydın et al. have measured the surface roughness values of the coatings generated by utilizing a new buffer system in the literature in resting durations of 24, 48, 72 and 96 hours in SBF as ~1.20, ~1.90, ~2.60 and ~3.85 µm respectively [18]. Aydın et al. used alanin - alanin sodium salt buffer system in another study and have measured surface roughness values of this study in resting durations of 24, 48, 72 and 96 hours in SBF as ~1.40 µm, ~2.22 µm, ~2.94 µm and ~3.27 µm respectively [19]. Surface roughness

values of coatings achieved through utilization of aminoacetic acid - sodium aminoacetate buffer system by Aydın et al. were found to be ~0.81 µm, ~0.98 µm, ~1.03 µm and ~1.44 µm respectively, at the same waiting period [20].

Thicknesses of HA coatings have been made with using glycolic acid - sodium gluconate buffer system have measured in terms of µm by using the ElectroPhysics Minitest 730/Sensor FN 1.5 HD branded device at Ege University, Ege Vocational School Laboratory. The measurement of each sample realized 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. Averages have been taken and shown in Table 6.

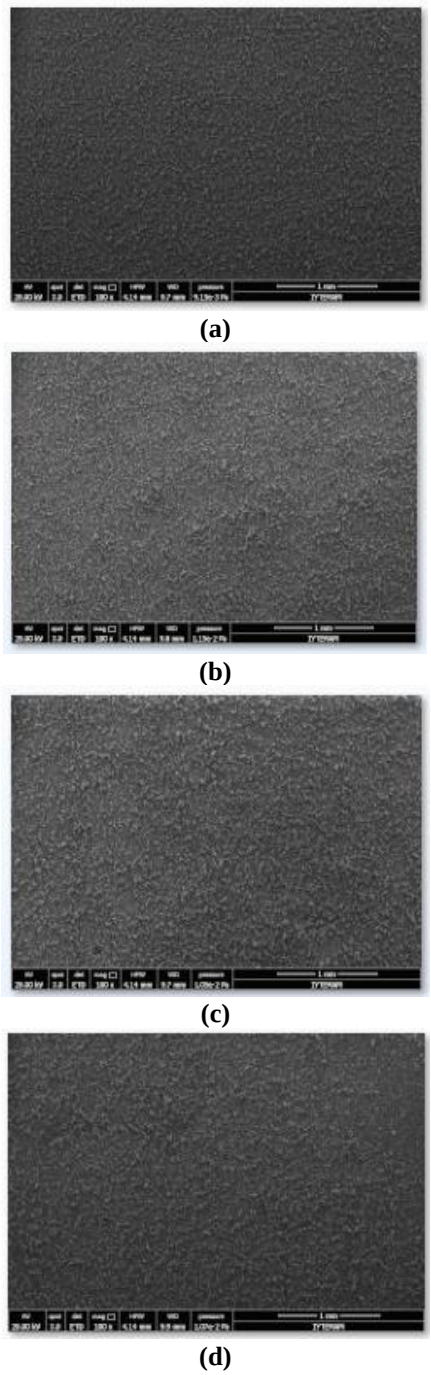
Table 6. Variation of the thicknesses values of the coating surfaces of hydroxyapatite coated specimens depending on the coating durations

Coating Thicknesses (µm)	
24 Hours	3.99 ± 0.783
48 Hours	4.47 ± 0.655
72 Hours	4.95 ± 0.743
96 Hours	5.49 ± 0.332

Examining the coating results, it is, as evident from the table, to be seen that the thicknesses of the HA coating generated on the sample surface are increasing proportional with the resting duration in SBF. Şimşek has stated in their study that he has established an HA layer in SBF with a thickness varying between 10 to 100 µm [21]. Li et al. have used NaH_2PO_4 with the biomimetic method and converted the solution saturated by adding $NaHCO_3$ to the solution with high calcium and phosphate ion concentration and reported in their study that there was coating with a thickness of nearly 40 µm on the sample surface by the end of 24 hours [10]. Nagano et al. have noted in their study that they have generated a hydroxyapatite layer of 20 µm with the biomimetic method [22]. Pasinli et al. have obtained HA coatings with thicknesses of approximately 6.78 µm, 8.93 µm and 19.13 µm for 1 SBF, 1.5 SBF and 3 SBF respectively by using biomimetic technical [12]. Aydın et al. have stated that they have measured the coating thicknesses within the intervals 7-8 µm, 9-11 µm, 13-14 µm and 18-20 µm for the waiting periods of 24, 48, 72, and 96 hours in SBF [18]. Thicknesses of the coatings in the alanin – alanine sodium salt buffer solution were found to be 8.25 µm, 8.85 µm, 9.10 µm and 10.35 µm respectively, in work of Aydın et al. within the same periods of time [19]. Thicknesses of the coatings of Aydın et al., employing the aminoacetic acid - sodium aminoacetate buffer system also in their works, were reported to be 4.13 µm, 4.73 µm, 5.47 µm and 5.55 µm respectively [20].

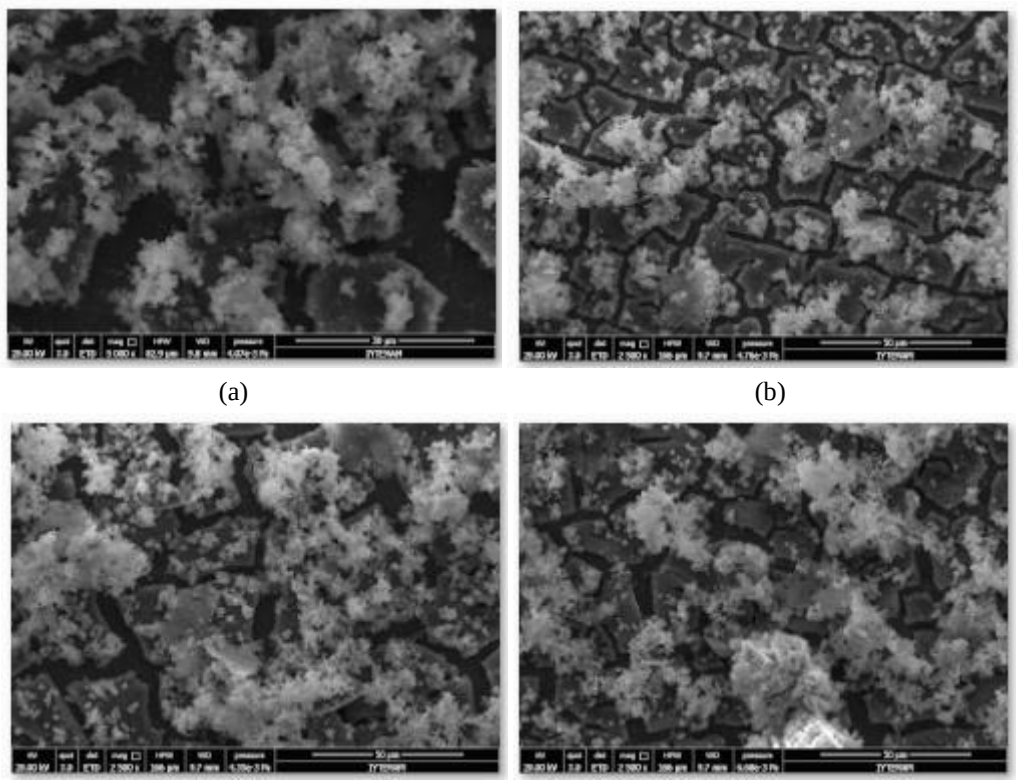
3.2. Results of Metallographic Analysis

Microscopic examinations of the surfaces of the hydroxyapatite coatings are performed by utilizing a Philips XL 30S FEG (SEM) electron microscope with model scanning available at the Izmir High Technology Institute Material Research Centre. The 100X and 2500X images for each of the sample surfaces with performed 24, 48, 72 and 96 hours HA coating are given in Figure 1 and Figure 2.



(a) **(b)** **(c)** **(d)**

Figure 1. The SEM images of coatings generated in synthetic body fluid in a) 24 hours b) 48 hours c) 72 hours d) 96 hours resting durations (100X)



(a) (b)

(c) (d)

Figure 2. The SEM images of coatings generated in synthetic body fluid in a) 24 hours b) 48 hours c) 72 hours d) 96 hours resting durations (2500X)

For conducting elemental analysis 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 calcium and phosphate structures on HA coated surfaces.

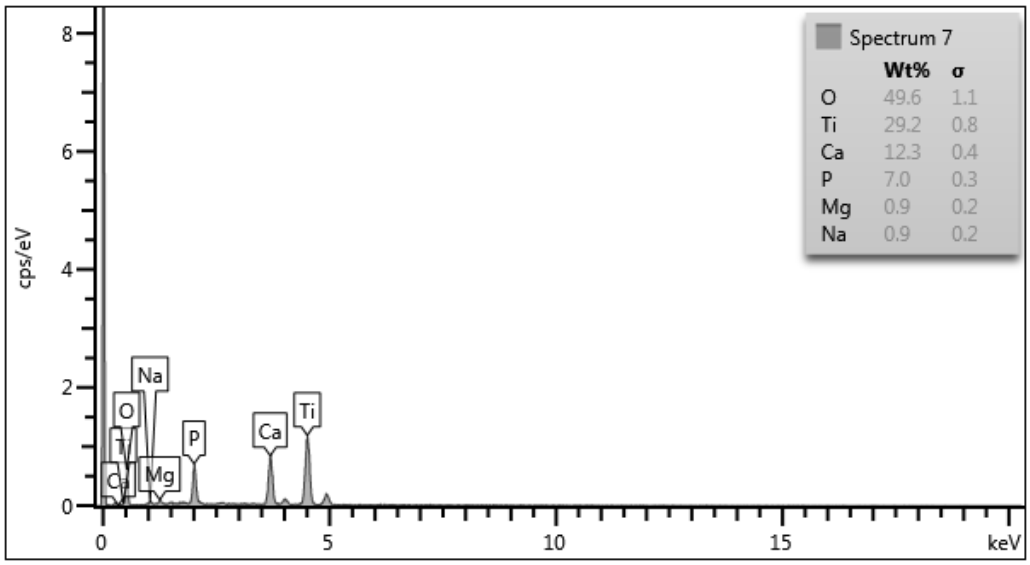


Figure 3. EDS analysis results of coating surfaces obtained by waiting for 24 hours in synthetic body fluid

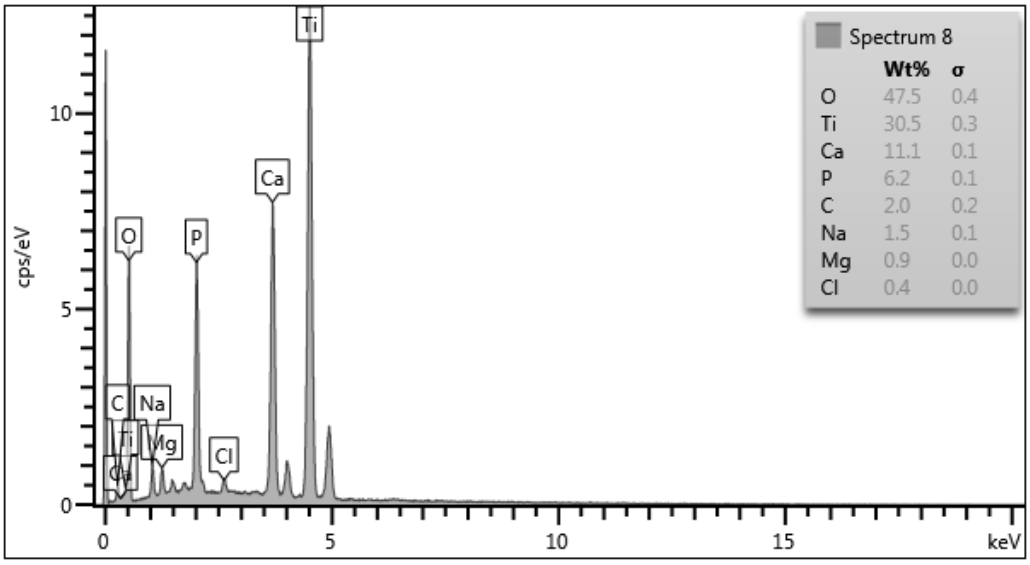


Figure 4. EDS analysis results of coating surfaces obtained by waiting for 48 hours in synthetic body fluid

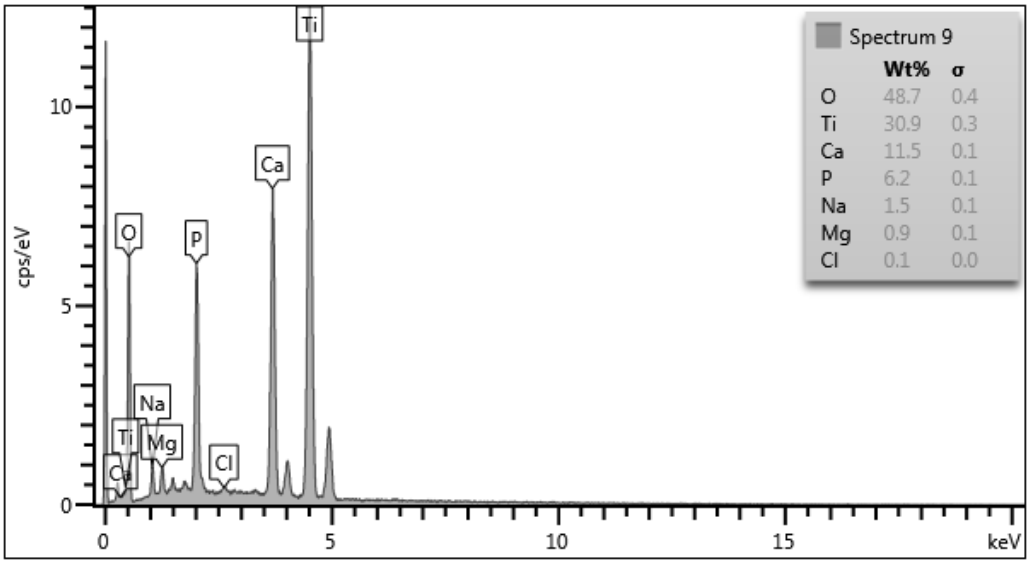


Figure 5. EDS analysis results of coating surfaces obtained by waiting for 72 hours in synthetic body fluid

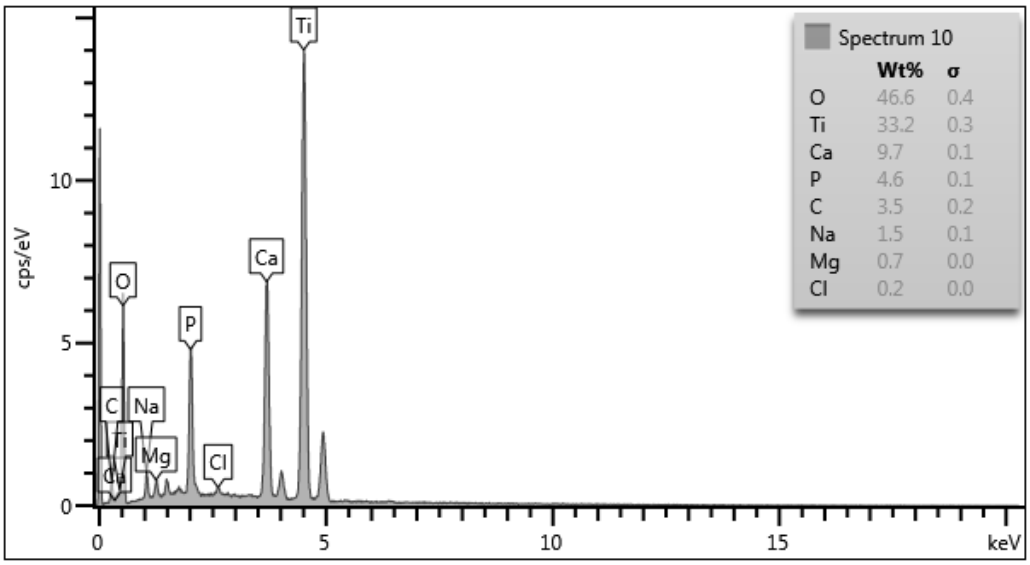


Figure 6. EDS analysis results of coating surfaces obtained by waiting for 96 hours in synthetic body fluid

According to the study of Urist et al. is bone in nature consisting of bone cells with extracellular matrix of which the carrier system for morphogenetic proteins is embedded in an apathetic calcium phosphate skeleton at a ratio of 1.66 Ca/P [23]. With EDS analysis, weight percentages of atomic structures on the coating surfaces have been obtained. The ratios of % Ca values to % P values were calculated and shown in Table 7.

Table 7. Ca/P values that change depending on waiting periods in synthetic body fluid

Ca/P ratio	
24 Hours	1.75
48 Hours	1.78
72 Hours	1.85
96 Hours	2.11

It is determined that the ideal Ca/P value in resting durations of 24, 48, 72 and 96 hours in our solution we prepared for the first time in the literature in an glycolic acid - sodium gluconate environment was achieved in 24 hours and that it was departed from this ratio in the other time periods. Despina et al. have reported the Ca/P ratio of the hydroxyapatite coating they prepared as 1.65 [24]. Xiaobo et al. have applied a calcium phosphate coating in SBF on Ti120, Ti240, Ti600 and Ti1200 materials and obtained Ca/P ratio results of 1.73 for Ti120 material, 1.72 for Ti240 material, 1.69 for Ti600 material and 1.70 for Ti1200 material. The Ca/P ratios for calcium phosphate solutions in 10xSBF solutions are calculated to be 1.67, 1.62, 1.65 and 1.63 respectively [11]. Han et al. have determined calcium phosphate components ($Ca(OH)_2$, $CaHPO_4$ and HA) inside synthe-sised powder with a ratio of 1.57 Ca/P using the hyro-thermal method (for 30 minutes with 600 bar and 300 ºC) [25]. Pasinli et al. have reported the Ca/P ratio of the calcium phosphate coating they realized on Ti6Al4V as 1.26 [12]. Aydın et al. have obtained with a resting duration of 24, 48, 72 and 96 hours in SBF solution Ca/P ratios of 1.58, 1.66, 1.69, 2.19 respectively [18]. 2.02, 2.25, 2.85 and 2.51 were determined, respectively, within the same periods of time in SBF in the coatings of Aydın et al, made by employing the aminoacetic acid - sodium aminoacetate buffer system [20]. Aydın et al. have reported that the Ca/P ratios of the coatings they made in the alanin – alanine sodium buffer environment were 1.81, 1.93, 2.01 and 2.10, respectively [19].

The device of the model PANalyticalEmpyrean available at the Celal Bayar University, Experimental Natural Sciences Application and Research Centre (DEFAM) is utilized for the XRD test. The test results obtained with the performed application are given in Figure 7.

According to the test results developed the HA crystals at peak (002) 26.030º, peak (120) 29.65°, peak (121) 31.956°, peak (030) 33.08°, peak (310) 39.641°, peak (312) 47.897°, peak (123) 49.63° and (004) 53.519°. The 2 Theta crystals have been formed at peak (101) 15.758º, peak (201) 24.664º, peak (002) 26.096º, peak (030) 32.485º, peak (310) 39.641º, peak (312) 47.897º, peak (014) 54.611º and peak (224) 67.090º. Takadama et al. have construed the peaks at 2 Theta = 23.31° and 48° at their XRD analysis in addition to the Ti peaks due to the sodium titanate ($Na_2Ti_5O_{11}$) and rutile (TiO_2) crystals [26]. Barrere et al. have noted in their study that after a resting of 24 hours in their solution with high concentration (5 SBF) at the 2 Theta = 32.03° peak (211), (112), (300) and (202) HA crystals developed [27].

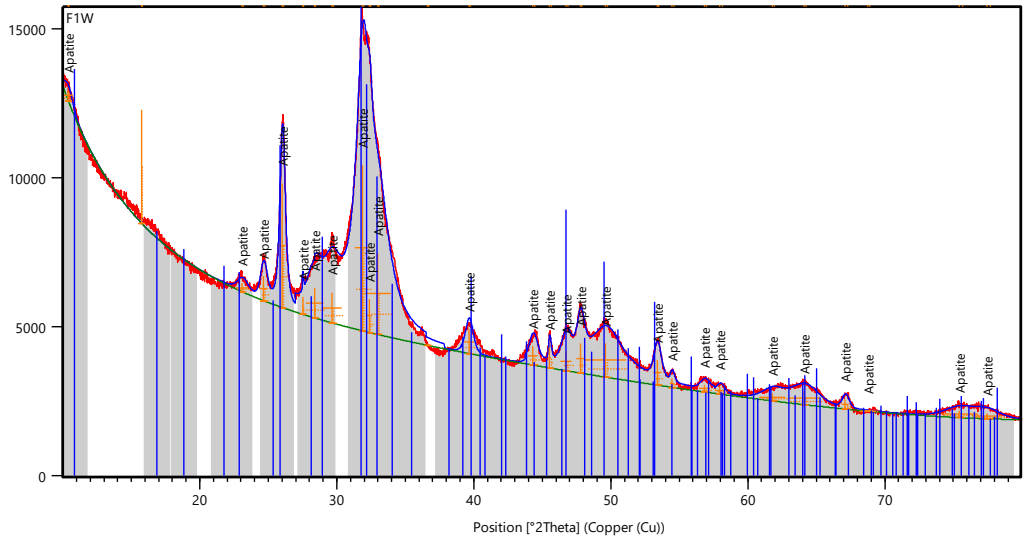


Figure 7: XRD analysis results

Pasinli et al. have stated in their study that in the XRD results of the Ti6Al4V alloys with hydroxyapatite surface coating performed by resting these in SBF solutions at different concentrations the hydroxyapatite crystals of the titanium crystals developed at peak points of (002) 25.70º, (210) 29.32º, (211) 32.14º, (310) 40.34º and (113) 43.30º respectively [12]. HA crystals of the coatings

created during 24 hour waiting period in the SBF solution were (102) 53.90° peak, (110) 62.88° peak, (103) 70.53° peak, (112) 76.12° peak respectively, 48 hour waiting period in the SBF solution were (102) 52.79° peak, (110) 62.80° peak, (103) 70.43° peak and (201) 76.01° peak respectively, 72 hour waiting period in the SBF solution (102) 52.89° peak, (110) 62.84° peak and (103) 70.49° peak respectively, 96 hour waiting period in the SBF solution (211) 52.80º peak, (119) 62.83º peak, (0210) 70.46º peak and (128) 76.67º peak respectively in the coatings made by Aydın [3]. HA crystals created (201) 25.336° peak, (201) 25.40° peak, (121) 29.55° peak, (121) 29.625° peak, (301) 35.9765° peak, (302) 42.636° peak, (113) 42.747° peak in the coatings made by Aydın et al. through utilization of aminoacetic acid - sodium aminoacetate buffer system [20]. Aydın et al. have reported that HA crystals created (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 in the coatings made in the alanin – alanine sodium salt buffer environment [19].

4. RESULTS

As a result is at our study is a SBF solution in glycolic acid - sodium gluconate environment not to have a toxic effect in human body for the first time in the literature is prepared and HA coating processes by the biomimetic method are performed. HA coating was realized at 37 °C and pH = 7.4 by using lactic acid / Na - lactate buffer system by Pasinli et al. for the first time in literature at an environment which is fully compatible with human blood plasma. Successful results were obtained by working at 37 ºC and pH = 7.4 at an environment which is fully compatible with human blood plasma which is nontoxic for the human body in citric acid / Na - citrate buffer environment within the context of Aydın's PhD thesis and contribution was made to the literature. These two pioneering studies were accepted as guide and a similar recipe was applied in this study and all values in human blood plasma were realized in this new buffer system. Furthermore NaOH was used with H_2O_2 to activate the chemical base. When the test results are examined, it is understood that the produced coatings give successful results compared to the data in the literature. Based on the results of this study, biomaterials were obtained that could be applied to the industry and one more step was taken hydroxyapatite coated implant production in a biocompatible environment by using the biomimetic method.

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