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Investigation of Fracturing and Adhesion Behavior of Hydroxapatite Coating Formed by Aminoacetic Acid-Sodium Aminoacetate Buffer Systems

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Abstract: Biomaterials utilized in implantation can be categorized into 4 main categories, as ceramics, polymers, metals and composites. Ceramic-based biomaterials are opted for, particularly in the field of orthopedics. These materials, also named as bioceramics, are usually employed by coating them onto the base material, inasmuch as they are far from the mechanical values of bone. In this study, a hydroxyapatite coating that is fully compatible with human blood plasma was applied on Ti6Al4V alloy through a biomimetic technique using aminoacetic acid-sodium aminoacetate buffer system for the first time in the literature, and examinations related thereto were carried out. The surface of the base material Ti6Al4V alloy was activated with various chemicals. Subsequent to activating the surface, a coating process whereby the base material was kept in simulated body fluid for 24, 48, 72, 96 h was carried out. Ultimate microhardness (indentation) tests were performed to determine the average indentation depths in maximum load, vickers hardness and elasticity modulus of the coatings obtained by using the biomimetic method, while scratch tests were performed to measure the surface bonding strengths of the coating layers. Furthermore, the fracture toughness values of the coating were calculated. The results obtained through the study are evaluated and discussed.

Keywords: aminoacetic acid-sodium aminoacetate; biomimetic coating; hydroxyapatite (HA); Ti6Al4V; simulated body fluid (SBF)

1. Introduction

Their strength, moldability, and abrasion resistance, as well as their strong metallic bonds, have led to metallic materials having an important place in biomaterials [1]. The biggest disadvantage of metal prostheses in terms of biocompatibility is their being corroded in body fluids containing protein, oxygen and saline solutions [2]. Titanium and its alloys are frequently utilized in intracorporeal implants because of their low propensity for entering into chemical reactions. Metallic biomaterial Ti6Al4V alloy is widely used in the production of implants, such as hip prostheses, bone plates and bone screws in particular in orthopedic applications. The surfaces of these alloys are coated with ceramic-based biomaterials. Their bioactivity and biocompatibility are increased with this process [3].

Hydroxyapatite (HA) is a calcium phosphate (CaP) ceramic, and has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. It has been used as a coating for metallic implants in medical applications such as the repair of bone defects and bone augmentation, orthopedics, and odontology [4]. Hydroxyapatite coating on metallic biomaterial base materials was performed by the biomimetic method which is accepted as a reflection of natural systems in laboratory environments in this study. The surfaces of the base materials, activated through various chemicals, are covered by precipitation method in the simulated body fluid (SBF), prepared in the laboratory environment. The aim is to produce biomaterials with high biocompatibility by employing this method.

Simulated body fluid has been utilized by many researchers. SBF prepared with inorganic salts must be compatible with the blood plasma. In the literature, the ionic values in human blood plasma have previously been obtained by many researchers. Successful coatings have been achieved through the use of similar compositions in studies carried out by Aydin and Çaglayan [2]. The data for these studies are shown in Table 1.

Table 1. Human blood plasma and ion concentration of simulated body fluid (SBF) [2,3,5].

Ion	Na^+	Cl^-	HCO_3^-	K^+	Mg^{2+}	Ca^{2+}	HPO_4^{2-}	SO_4^{2-}
Kokubu et al. (MM) [6]	142	147.8	4.2	5	1.5	2.5	1	0.5
Taş (mm) [7]	142	125	27	5	1.5	2.5	1	0.5
Sepahvandi et al. (MM) [8]	142	147.8	4.2	5	1.5	2.5	1	0.5
Faure et al. (MM) [9]	154.6	120.5	44	5.37	0.8	1.82	1	0.8
Li et al. (MM) [10]	142	103	27	5	1.5	6	2.4	0.5
Xiaobo et al. (MM) [11]	142	103	10	5	1.5	2.5	1	0.5
Pasinli et al. (MM) [2]	142	103	27	5	1.5	2.5	1	0.5
Aydın (MM) [2]	142	103	27	5	1.5	2.5	1	0.5
Çağlayan (MM) [3]	142	103	27	5	1.5	2.5	1	0.5
Human Blood Plasma (mM)	142	103	27	5	1.5	2.5	1	0.5

In this study, for the first time in the literature, a biomimetic technique has been used to evaluate the hydroxyapatite coating in an aminoacetic acid-sodium aminoacetate buffer environment, which is fully compatible with human blood plasma.

2. Materials and Methods

2.1. Selection of Implant Material

Ti6Al4V alloy, which is frequently preferred in medical applications due to its high biocompatibility, has been utilized in this study, in which we performed the HA coating process on a metallic coating. The chemical composition of the Ti6Al4V alloy used is shown in Table 2, while the mechanical properties are shown in Table 3.

Table 2. Chemical composition of Ti6Al4V alloy [2].

Ti	N	C	H	Fe	O	Al	V	Other
Remainder	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 properties of Ti6Al4V alloy [2].

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

2.2. Preparation of the Coating

The HA coating process using a biomimetic method employed in this study consists of 3 stages: cleaning and chemical treatment of base materials, heat treatment in aluminum foil, and keeping in prepared simulated body fluid. The materials were firstly sanded, then washed with distilled water and kept in acetone to dissolve the oil particles for the surface cleaning of the Ti6Al4V alloys selected as base materials. The materials, which were cleaned again by distilled water, were kept in an ultrasonic bath, and their surface cleaning was completed. The materials were kept in 100 mL of 5 M NaOH + 0.5 mL % 35 H_2O_2 at 40 °C for 24 h to increase their potential to form chemical bonds by activating the surface of the base materials. After this process, the materials were again washed with distilled water and allowed to dry at 60 °C for 24 h. Material surfaces, which were dehumidified, were wrapped

with aluminum foil for protect them against air contact, and were kept at 600 °C for 1 h and cooled to room temperature. This process activated apatite nucleation on the base surface. Materials, the heat treatment stages of which had been completed, were subjected to agitation for 24, 48, 72 and 96 h at 37 °C in the simulated body fluid, the inorganic salt values of which are shown in Table 4. Following the coating process using the biomimetic method, the materials were washed with distilled water and allowed to dry at 60 °C for 24 h.

Table 4. Inorganic salts in simulated body fluid (SBF) [5,12].

Chemical Matter	Amount (mg/2 L)
KCl	746.0
NaCl	10519.2
$Na_2HPO_4 \cdot 2H_2O$	356.0
Na_2SO_4	142.0
$NaHCO_3$	4536.6
NA-Glycinate	4313.4
$CaCl_2 \cdot 2H_2O$	735.2
$MgCl_2 \cdot 6H_2O$	610.0
Glycine (75.818 g/L) 1 M	-

2.3. Preparation of Simulated Body Liquid

Approximately 1.5 L of purified water was put in a large beaker and the salts given in the first 6 lines of Table 4 were added in the indicated amounts in order to prepare 2 L of simulated body fluid with the same ionic values as blood plasma. A pH electrode was immersed in the solution to determine the pH value of the solution mixed by magnetic stirrer with heater. After this process, the pH value was decreased to 8 at 37 °C by slowly adding 1 M glycine to the solution. 2 L of distilled water, the mixture, and $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ salt in the amounts indicated in Table 4 were added to the solution. Finally, 2 L of the simulated body fluid, which was obtained by adding glycine again until the pH value of the mixture reached 7.4, was taken.

2.4. Test Methods

The tests required for determining the average indentation depths at maximum load, hardness and elasticity modulus of the obtained HA coatings were carried out with the IBIS Nanoindentation System DME-DS 95 Series AFM device at Electronic Materials Manufacturing and Application Center (EMUM) of the Dokuz Eylül University. The indentation process was performed using a Berkovich brand type tip at a depth of 2 µm under 2 mN load. Nine measurements were taken for each sample at waiting times of 24, 48, 72 and 96 h, and the average of the results was taken.

Scratch tests were performed by the IBIS Nanoindentation System DME-DS 95 Series AFM device at the Electronic Materials Manufacturing and Application Center (EMUM) of Dokuz Eylül University to determine the surface bonding strengths of the HA coatings on the Ti6Al4V alloy selected as the base material. Both indentation tests and scratch tests can be performed by the same type of device. Tests carried out by 100.00 µm/h speed freight and 1–30 mN load were repeated three times for each sample, and the average of the values obtained was taken.

0.245 N load was applied on the coatings by the HVS-1000 Digital Display Microhardness Tester device in Ege Vocational Training School of Ege University. The length of the fracture that occurred as a result of the load application (c) (see Figure 1) was measured and was is placed into Equation (1).

$$K_{1c} = \propto \left(\frac{E}{H}\right)^{0.5} \left(\frac{P}{c^{1.5}}\right) \quad (1)$$

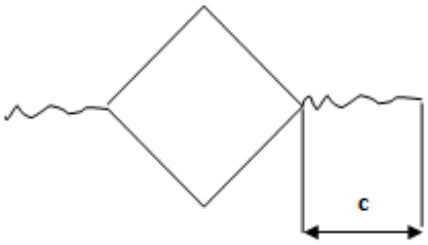

Figure 1. The crack length that occurred in the coating (c).

In the above formula, K_{1c} is the fracture toughness values, P is the load applied during testing in N, H is the hardness value in GPa, E is the elasticity modulus in GPa and c is the crack distance in m. The value of "α" in the equation was taken as 0.016 based on the literature studies [12].

3. Results

3.1. Ultra Microhardness (Indentation) Tests

The average maximum indentation depths obtained at the end of the measurement are shown in Table 5, while the Vickers hardness and elasticity modulus values are given in Table 6.

Table 5. Average Indentation Depths of HA coating surfaces under 2 mN load applied in nano-indentation device [12].

Average Indentation Depths at Maximum Load (μm)	
24 h	3.10
48 h	3.55
72 h	4.10
96 h	4.16

Table 6. Vickers hardness of HA coating surfaces and the changes in elasticity modulus by different holding periods in SBF [12].

HA Coating Period (Hours)	Vickers Hardness (H) (GPa)	Elasticity Modulus (E) (GPa)
24 h	0.0163	1.238
48 h	0.0111	0.351
72 h	0.0089	0.339
96 h	0.002	0.173

When the data in Table 5 are examined, it can be observed that the average indentation depths (ht) at maximum load increase continuously with the increase in holding period (24, 48, 72 and 96 h).

When the data in Table 6 are examined, it can be seen that the Vickers hardness and modulus of elasticity of HA-coated surfaces decrease continuously depending on the holding times in SBF. Mechanical properties vary in each region of the human bone. For example, the elasticity modulus value of crustal bone is between 7 and 30 GPa, while the values in spongiform bone are 0.05–0.5 Gpa; it is 0.001–0.01 GPa in joint cartilage, and 1 GPa in the tendon bone [13–16]. It is observed that mechanical properties close to those of bone are achieved by the HA coating process on the implant material in this study when the obtained results and the mechanical values of the bone are examined.

3.2. Scratch Tests

The results obtained from the tests are shown in Figure 2. The mean values of the results are given in Table 7.

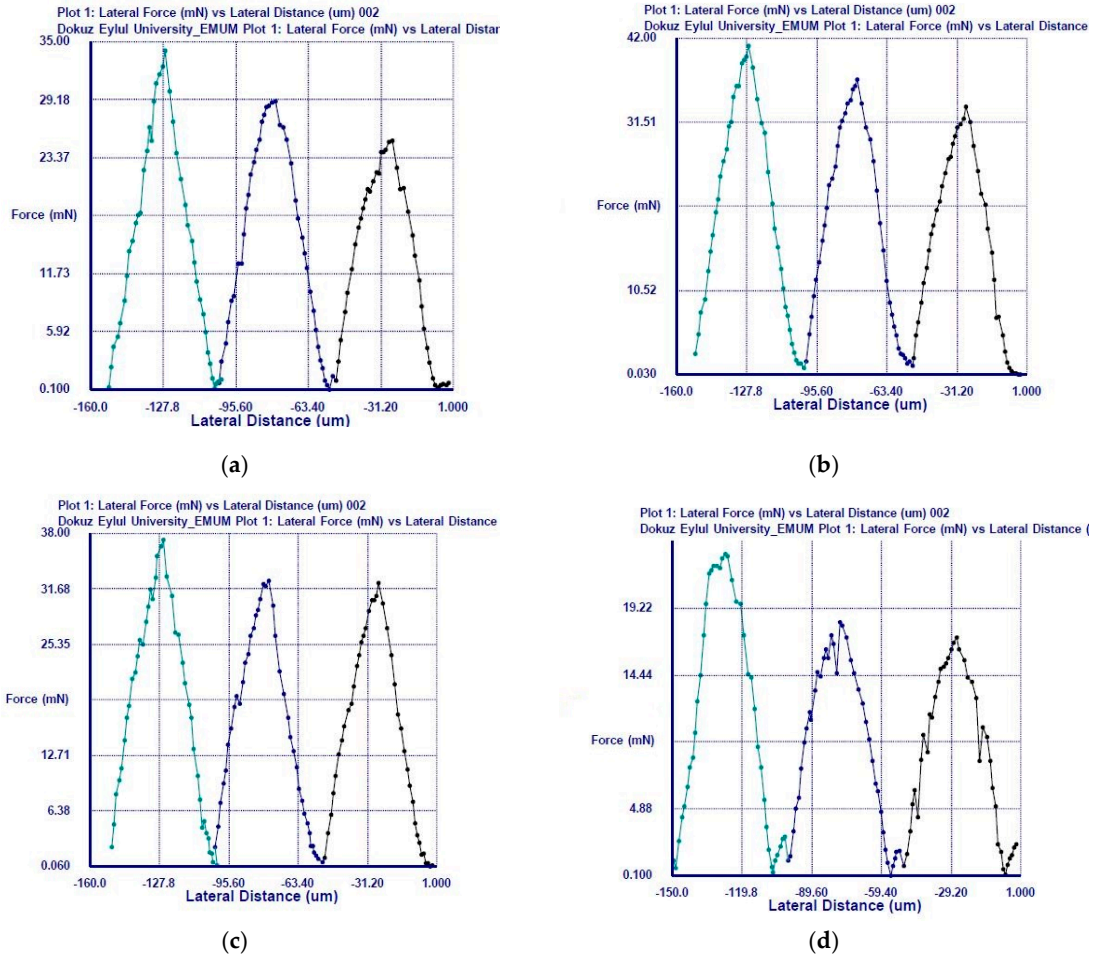

Figure 2. The critical load average values of the coatings held in SBF for (**a**) 24 h (**b**) 48 h (**c**) 72 h (**d**) 96 h critical load values held [12].

Table 7. The critical load average values of the HA coating depending on the holding time in SBF [12].

	Critical Load (Lc) (mN)
24 h	29.42
48 h	37.12
72 h	34.05
96 h	19.04

The critical load values of the coatings prepared using citric acid-sodium citrate buffer system for the first time by Aydın in the literature were found to be 54.79 N, 8.85 N, 8.69 N and 39.2 N for 24, 48, 72 and 96 h, respectively [17]. Kui et al. reported that the critical load values for surface adhesion of the HA coatings they created in their work varied between 390 and 478 mN [18]. Xiang et al. reported the critical load values of HA coatings they prepared as 27.85 mN and 68.74 mN in their study [19]. Dunstan et al. reported that the critical load value of the HA coatings they prepared was 2.4 N, while Pasinli reported the critical load value of the HA coating prepared as 8 mN [20,21]. Caglayan found the critical load values of the HA coatings prepared in the Alanine-Alanine sodium salt environment to be 22.23 mN, 24.93 mN, 20.76 mN and 7.74 mN, respectively, at 24, 48, 72 and 96 h [22].

The surface adhesion strengths of HA coatings in the aminoacetic acid-sodium aminoacetate buffer environment were examined by scratch test for the first time in the literature in this study. When we look at the values, we observe that the highest critical load value, and therefore the best

adhesion strength value, was 37.12 mN, obtained at 48 h. It is observed that very successful results were achieved for each time period when compared with other studies in the literature.

3.3. Fracture Toughness

The fracture toughness values obtained are shown in Table 8. In the literature, when the calculated fracture toughness values of the HA coating surfaces were examined, the fracture toughness values of hydroxyapatite coating surfaces made by Zhang et al., Marcelo et al., Tsui et al., Li et al. Mohammadi et al. and Bharat et al. were reported as between ~0.12 and 0.31 MPa $m^{1/2}$ [23], 1.18 MPa $m^{1/2}$ [24], between 0.23 and 1.20 MPa $m^{1/2}$ [25], between 0.49 and 0.67 MPa $m^{1/2}$ [26], between 0.99 and 1.27 MPa $m^{1/2}$ value [27] and 0.74 MPa $m^{1/2}$, respectively [28]. Çağlayan found the fracture toughness values of coatings obtained in the Alanine-Alanine sodium salt buffer medium as 1.57 MPa $m^{1/2}$, 1.73 MPa $m^{1/2}$, 2.29 MPa $m^{1/2}$ and 2.31 MPa m^{1} for 24, 48, 72 and 96 h, respectively [22]. Aydin found the fracture toughness values of HA coatings prepared by using a citric acid-sodium citrate buffer system for first time in the literature to be within the range of 1.98–2.075 MPa $m^{1/2}$ [17]. Bonfield has reported that fracture toughness values calculated in different regions on the bone shell is between 2 and 12 MPa $m^{1/2}$ [13]. A good fracture toughness value was found when compared with those in the literature, and the fracture toughness values on bone reported by Bonfield et al. were observed.

Table 8. Fracture toughness values of HA coating held in SBF for different periods of time [12].

Fracture Toughness (K_c) (MPa $m^{1/2}$)	
24 h	1.02
48 h	1.25
72 h	1.35
96 h	2.51

4. Conclusions

As a result of this study, a simulated body fluid (SBF) solution biocompatible with human body was prepared by using a biomimetic method in an aminoacetic acid-sodium aminoacetate buffer environment for the first time in literature, and HA coating was performed. HA coating was realized at 37 °C and pH = 7.4 by using the lactic acid/Na-lactate buffer system which was first proposed by Pasinli et al. in an environment that is fully compatible with human blood plasma. Successful results were obtained by working at 37 °C and pH = 7.4 in an environment that is fully compatible with human blood plasma, and which is non-toxic for the human body in a citric acid/Na-citrate buffer environment within the context of Aydın's PhD thesis, and a contribution was made to the literature. These two pioneering studies were accepted as a 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 + H_2O_2 mixture was used instead of NaOH to activate the chemical base. A better and faster activation was performed in this way. When the data obtained by the mechanical tests performed were examined, it was found that the produced coatings showed successful results when compared with data in the literature. It was also seen that the coatings are compatible with the mechanical properties of bone. Based on the results of this study, biomaterials of a quality whereby they could be applied in the industry were obtained, and the biomimetic method was used, and a further step was taken towards moving to production of hydroxyapatite-coated implant in a biocompatible environment.

Author Contributions: İ.A. conceived and designed the experiments; İ.A. and M.K. performed the experiments; İ.A. and M.K. analyzed the data; İ.A. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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