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CORROSION BEHAVIOURS OF HYDROXYAPATITE COATED BY BIOMIMETIC METHOD OF Ti6Al4V, Ti AND AISI 316L SS SUBSTRATES

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Biomimetic method was used to obtain hydroxyapatite (HAP) coatings on Ti6Al4V, Ti and AISI 316L SS substrates. The solutions were prepared as 1.5xSBF and 3.0xSBF similarly to body fluid (SBF). Additionally, three different pretreatment surface operations (HNO_3, anodic polarization, base-acid) were applied to the substrates. HAP coatings were successfully deposited on Ti6Al4V and Ti substrates after immersion periods of 14, 21, 35 days in 3.0xSBF. Corrosion behavior of uncoated and HAP coated substrates was examined in the Ringer and 0.9 % NaCl solutions. The SEM-EDS results pointed out that HAP was formed on the substrates. The best of HAP coated surfaces were obtained in 3.0xSBF and 1.5xSBF kept for 35 days. HAP coatings in 1.5xSBF were less thick than those in 3.0xSBF ones but have higher corrosion resistance.

INTRODUCTION

Ti and its substrates are biomaterials which are selected for many dental and orthopedic applications. These biomaterials have many advantages such as biocompatibility, good corrosion resistance and excellent mechanical properties.[1-7] For heat treatments HAP coated was made with and without glass based in 5xSBF solution. HAP coating was showed better corrosion resistance by using biomimetic method in 5xSBF solution.[5] Adding HCO_3^{-1} into SBF caused forming of apatite plates with β-carbonate.[6] The thickness of the coating increases with increasing the immersion times.[2,4] The presence of Mn^{+2} supported apatite formation.[8] The pH value was changed slightly during immersion. Ca and P ion concentrations were increased and coming to balance with prolongation of the duration of immersion.[9] HAP layers were found a few micrometers thick after immersion for 7 days.[10] Ti substrates were kept in nitric acid PTSO for 10 hours at 60 °C to obtain the best Ca-P coatings.[11] Titania nanotubes formation were decreased NaOH PTSO from 24 hour to 39 minutes.[12] Torres *et al.* obtained homogeneous crystal hydroxyapatite coating on alkali treated titanium substrates.[13] Zhang *et al.* found that biomimetic deposition coatings were obtained thicker and more homogenous than electrophoretic deposition coatings.[14]

EXPERIMENTAL

The preparation of the substrates

Surface properties of the substrates play a major role in the development of biomimetic HAP coatings and their corrosion resistance. Before coating, the substrates were polished and cleaned by using Bandelin ultrasonic bath for 15 minutes in order acetone, alcohol, and bidistilleted water 30 °C. Then, they were dried at 40 °C for one hour in the drying oven. Thus, they were made ready for pretreatment surface operations (PTSO).

Pretreatment surface operations of substrates

Three different pretreatment surface operations were applied to substrates. These are summarized as follows:
a) ***Acid-base (BA) PTSO:*** The substrates were kept in 5 N NaOH solutions for 12 hours at 60 °C and then were kept for 12 hours at 25 °C. Then, they were washed in the ultrasonic bath for 15 minutes by bidistilled water for two times and were dried in the drying oven at 40 °C for one hour. After that

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substrates were kept in 1 N HCl at 60 °C for 12 hours, and then 12 hours at 25 °C. Following the acid treatment, the substrates were rewashed in the ultrasonic bath for 15 minutes with bidistilled water for two times and were dried in the drying oven at 40 °C for one hour.

b) ***Anodic polarization PTSO:*** Anodic polarization was done in 1 N HCI aqueous solution. It was confirmed that optimal treatment time was 300 seconds in 1 N HCl aqueous solution and the potential value was determined as 5 V.

c) ***HNO_3 PTSO:*** Substrates were mechanically polished using emery paper in successive grades and were washed with bidistilled water in an ultrasonic bath. Then substrates were kept for 20 minutes in technical HNO_3 and after the acid treatment, the substrates were washed with running bidistilled water and dried at 40 °C for one hour. The substrates were cleaned in Bandelin ultrasonic bath for 15 minutes in order acetone, alcohol, and bidistilled water 30 °C. Then, substrates were dried at 40 °C for one hour in the drying oven. Surface of substrates was made porous for HAP coating. Substrates were kept in desiccator after put in locked plastic bags.[15-19]

Experimental studies of biomimetic HAP coating

HAP coating with biomimetic method is summarized as follows:

a) SBF solutions were prepared as 3.0xSBF and 1,5xSBF. Special constitutions of SBF, blood plasma, 1.5xSBF and 3.0xSBF[1,3,4,10,15-18,19] were given on Table 1.

BA, HNO_3 and anodic PTSO were applied seperately to substrates. To increase ion concentration and to make easier the composition of the core apatite, solutions were prepared as 1.5xSBF and 3.0xSBF. As pH of solutions would be 7.4 and they were arranged use 0.1 moldm^{-3} tris-(hydroxymethyl) aminomethan (TRIS) and 0.1 moldm^{-3} HCl. TRIS was used to provide stability of solution.[4,20-28] In order to prevent bacterial reproduction NaN_3 was added to the solution as it will be 20 mgdm^{-3}.[12,29]

b) 3 samples of substrates were put in each glass bottle. Surface areas of Ti and Ti6Al4V substrates were 2.0096 cm^2 and totally 75 cm^3 SBF solution was added.[15,26] For that surface area of AISI 316L SS substrates was 0.785 cm^2, totally 40 cm^3 SBF was added.[15,21,30,31]

c) Glass bottles were put into the the shaking water bath and shaking speed was arranged as 80 rpm, bath temperature 37 °C.[32]

d) In tests for 14, 21, 35 days, once every two days SBF solution was changed. SBF were added to bottles after pH was arranged as 7.4 and the solution temperature was set as 37 °C.[6,11,21-25,27,33]

e) After the biomimetic HAP coating in SBF solutions, substrates were washed kindly with bidistilled water, kept in the drying oven at 40 °C for 3 hours and they were sintered in furnace at 850 °C for one hour.[19]

f) Corrosion experiments were studied in 0.9 % NaCl and Ringer solutions. A conventional three-electrode cell was used for all the electrochemical measurements. A saturated calomel electrode (SCE) was used as a reference electrode, platinum foil as a counter electrode and Ti6Al4V, Ti and AISI 316L SS substrates as the working electrode. All potentials referred to the saturated calomel electrode. Solutions were prepared with bidistilled water using Merck grade reagents. Measurements were obtained using a system consisting of a Reference 600 potentiostat/galvanostat/ZRA system. In order to test the reproducibility of the results, the experiments were performed in triplicate.

g) After the corrosion experiments, surface images were taken from by LEO 1430 VP SEM (scanning electron microscope). EDS (energy dispersive spectrum) were also had over same substrates.

RESULTS AND DISCUSSION

Electrochemical experimental results after biomimetic HAP coating

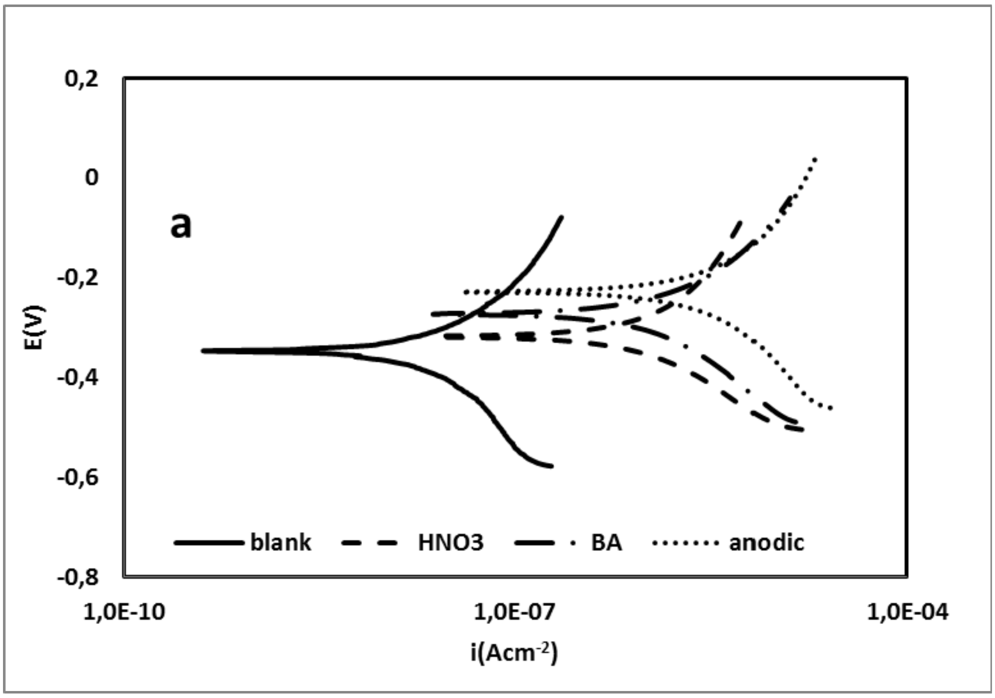
Tafel curves of Ti6Al4V and AISI 316L SS substrates were given in Fig. 1. Corrosion characteristics for Ti6Al4V substrates in Ringer and 0.9% NaCl solutions were given in Table 2.

Corrosion rates of Ti6Al4V substrates after biomimetic (Table 2 and Fig. 1 a,d) HAP coating were observed as being higher than for uncoated Ti6Al4V substrates. This showed that the biomimetic HAP coating could not prevent the corrosion. Polarization resistance of biomimetic HAP coated Ti6Al4V substrates were decreased as can be seen in Table 2. Corrosion behaviour of biomimetic HAP coated HNO_3 PTSO Ti6Al4V substrates in waited 1.5xSBF solution were studied in Ringer solution; corrosion rate was increased by the time. E_{cor} values acted variable. The less current density in Ringer solution was 1.49 μAcm^{-2} anodic PTSO Ti6Al4V substrate which was kept for 35 days in 1.5xSBF solution. The corrosion rate of the anodic PTSO Ti6Al4V substrate which was kept for 14 days in 1.5xSBF solution was 1.72 μAcm^{-2} in 0.9 % NaCl solution. The corrosion rate increased after the substrate was kept for 21 days and then again it was decreased in waiting 35 days in 1.5xSBF solution.

Table 1

Special constitutions of SBF, blood plasma, 1.5xSBF and 3.0xSBF

Ions	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF concentration (mmoldm^{-3})	142	5.0	2.5	1.50	147.8	4.2	1.0	0.50
Blood plasma concentration (mmoldm^{-3})	142	5.0	2.5	1.50	103.8	27.0	1.0	0.5 0
1.5xSBF concentration (mmoldm^{-3})	213	7.5	3.75	2.25	221.7	6.3	1.5	0.75
3.0xSBF concentration (mmoldm^{-3})	426	15.0	7.5	4.50	443.4	12.6	3.0	1.50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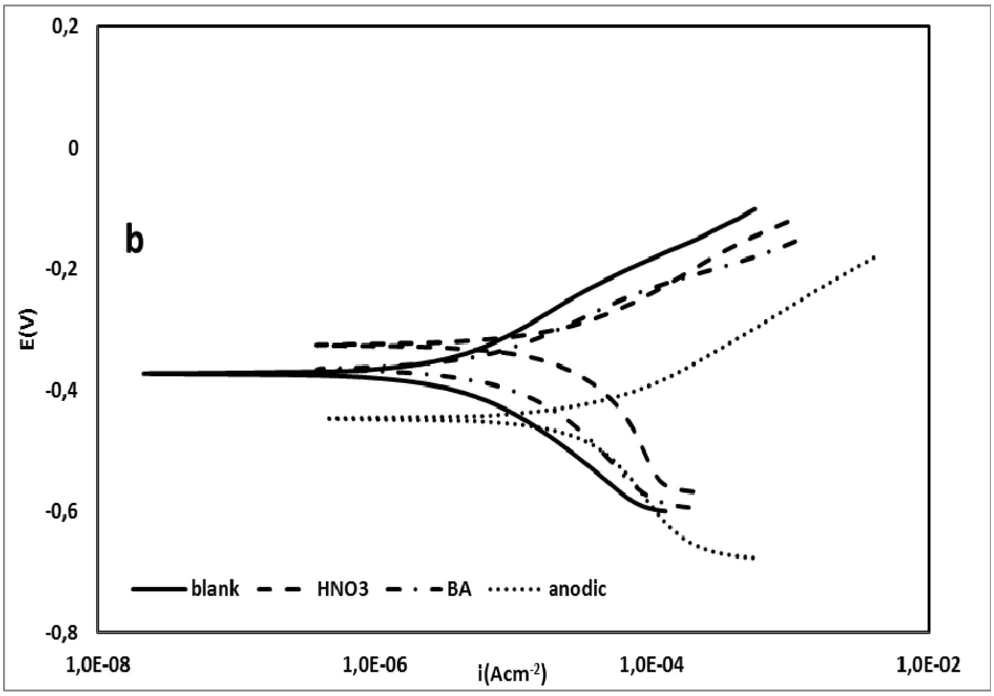
HNO3
BA
anodic

Fig. 1 a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BA
anodic

Fig. 1 b

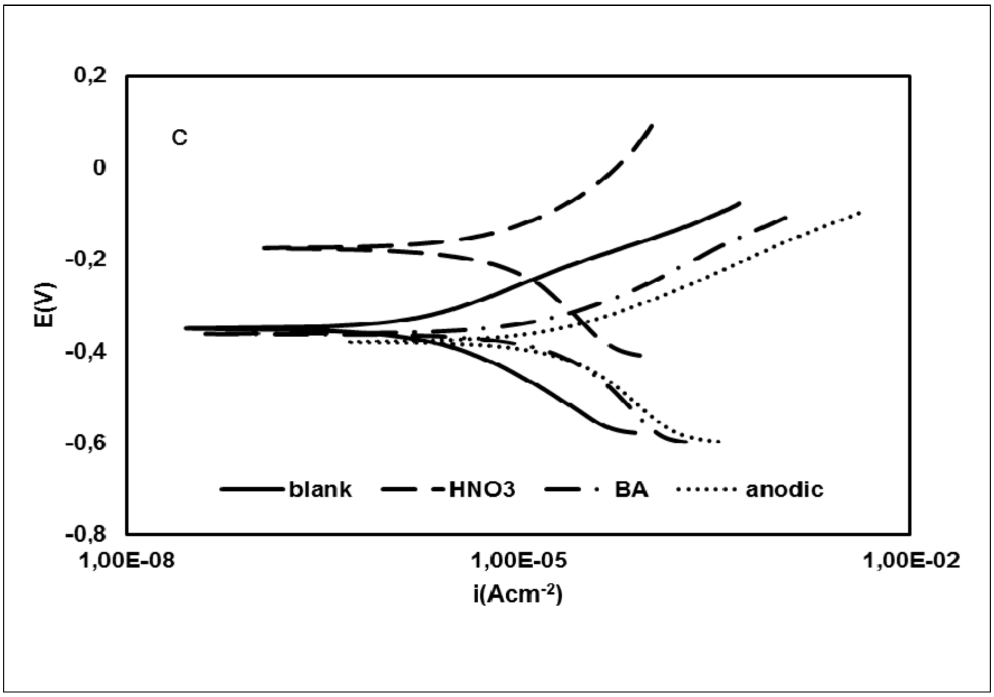

Fig. 1 c

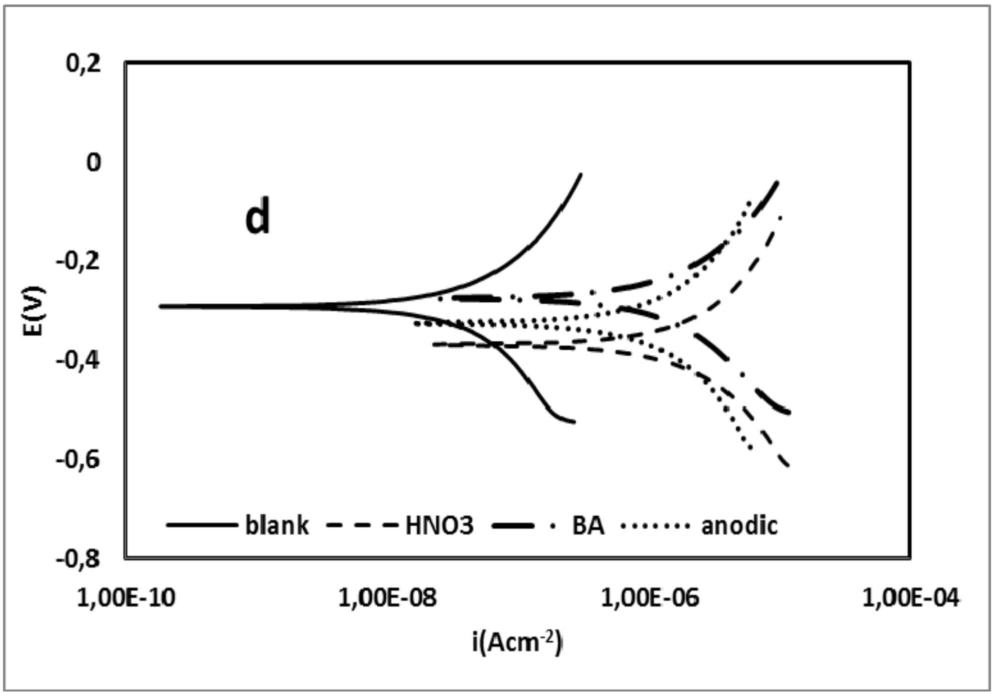

Fig. 1 d

Fig. 1 – Tafel plots were obtained for Ti6Al4V and AISI 316L SS substrates in Ringer and %0.9 NaCl solutions a) for Ti6Al4V substrates in %0.9 NaCl solution kept 35 days in 3.0xSBF b) for AISI 316L SS substrates in %0.9 NaCl solution kept 35 days in 3.0xSBF c) for AISI 316L SS substrates in Ringer solution waited 35 days in 1.5xSBF d) for Ti6Al4V substrates in Ringer solution kept 35 days in 1.5xSBF.

Table 2

Corrosion characteristics in Ringer and 0.9 % NaCl solutions for Ti6Al4V substrates

			1.5 X SBF			**3.0 x SBF**		
		Pretreatment surface operations	$-E_{cor}$ (mV)	i_{cor} (μAcm^{-2})	R_p ($k\Omega.cm^2$)	$-E_{cor}$ (mV)	i_{cor} (μAcm^{-2})	R_p ($k\Omega.cm^2$)
	Ringer solution	blank	276	0.097	600.87	276	0.097	600.87
	0.9 % NaCl solution	blank	351	0.172	721.45	351	0.172	721.45
35 days	Ringer solution	HNO_3	303	6.41	17.26	307	1.73	27.01
		anodic	305	1.49	26.06	259	2.42	24.98
		BA	253	2.62	38.02	309	3.48	23.59
	0.9 % NaCl solution	HNO_3	243	4.44	36.27	316	2.22	28.54
		anodic	256	2.45	27.97	251	5.87	11.23
		BA	297	4.27	15.76	293	1.65	33.26
21 days	Ringer solution	HNO_3	184	3.45	32.70	307	7.21	14.25
		anodic	250	2.63	16.50	222	11.82	16.56
		BA	220	6.19	23.41	192	14.59	10.65
	0.9 % NaCl solution	HNO_3	333	8.61	11.99	211	14.18	8.86
		anodic	299	7.37	27.05	242	6.84	12.42
		BA	212	1.28	28.19	243	11.23	11.43
14 days	Ringer solution	HNO_3	353	1.41	35.89	259	4.06	20.90
		anodic	199	1.63	23.57	244	6.34	11.88
		BA	220	1.08	79.84	279	9.14	8.10
	0.9 % NaCl solution	HNO_3	269	0.93	40.84	254	6.71	19.73
		anodic	295	1.72	17.99	339	6.08	23.79
		BA	263	4.57	30.83	220	3.33	67.90

5 V voltage was applied for 300 seconds to electrode surface for anodic PTSO. Titanium oxides formed on the surface when positive potential was applied to the surface. These formed oxides led to the corrosion rate decrease of the by getting stable in time and closing the surface. TiO_2 contributed to form apatite on the surface by interacting with SBF. After the corrosion experiments in Ringer solution, the corrosion rate of HNO_3 PTSO Ti6Al4V substrate was 1.41 μAcm^{-2} for waiting 14 days in 1.5xSBF solution, the corrosion rate was increased by the time. When the values of corrosion of HNO_3 and anodic PTSO Ti6Al4V substrates in 0.9 % NaCl were examined, the corrosion rate was increased until waiting for 21 days in 1.5 xSBF. This is because the oxides formed on the surface, after waiting 21 days in 1.5xSBF, Ti6Al4V surface can be closure and corrosion of Ti6Al4V decreased. 35 and 14 days kept in 1.5xSBF solution stand by values very close to each other in BA PTSO Ti6Al4V substrates. The lowest value of i_{cor} in 0.9 % NaCl solution was obtained 1.28 μAcm^{-2} BA PTSO Ti6Al4V substrate which was kept for 21days in 1.5xSBF solution.

Sodium titanate in SBF relased Na^+ ions to replace with H_3O^+ ions in the solution to form Ti-OH groups on the titanium surface. These Ti-OH groups conjugate with Ca^{2+} ions in SBF solution to form amorphous calcium titanate. Then these calcium titanates conjugated with phosphate ions presence in the solution to form amorphous calcium phosphate with low Ca/P ratio. Calcium phosphate turns to apatite.[20] When corrosion characteristics of Ti6Al4V substrates in Ringer and 0.9 % NaCl solutions after being kept in 3.0xSBF solution were examined, corrosion rates increased until kept for 21 days in all PTSO substrates and then they decreased. This is in accordance with information related with oxides mentioned above. Rp values were in accordance with i_{cor} values. When 1.5xSBF and 3,0xSBF compared to each other, all corrosion rates of Ti6Al4V substrates increased except HNO_3 and anodic PTSO Ti6Al4Vsubstrates which were kept for 35 days in 3.0xSBF solution. It had acted variable in 0.9 % NaCl solution. Corrosion rates decreased in BA PTSO Ti6Al4V substrates surfaces after kept for 35 and 14 days in 3.0xSBF solution.

When SEM images were examined, the best apatite coatings were seen in BA PTSO Ti6Al4V substrates after corrosion experiment in Ringer solution (Figs. 3-4). Also, corrosion current density of BA PTSO Ti6Al4V substrates after corrosion experiments in Ringer solution kept 1.5xSBF solution was smaller than in Ringer solution kept 3.0xSBF. This state may be explained as follows. The amount of Cl^- ions in 3.0xSBF solution was 443.4 $mmoldm^{-3}$. This was actually a high value. Because of the Cl^- ions were corrosive ions, it may be important for corrosion increasing. E_{cor} values in Ringer solution after being kept in 3.0xSBF were shifted to negative potentials in HNO_3 PTSO Ti6Al4V substrates (except 14 days). E_{cor} values were shifted to positive potentials after corrosion experiments in 0.9 %NaCl solution in kept 3.0xSBF and 1.5xSBF solutions.

Corrosion characteristics of AISI 316L SS substrates in Ringer and 0.9 % NaCl solution were given in Table 3, after biomimetic HAP coating kept 3.0xSBF and 1.5xSBF solutions.

When Table 3 and Fig. 1 b, c were examined, the current density of AISI 316L SS substrates increased actually higher than Ti6Al4V substrates. After corrosion experiments, HAP coating did not stay on the surface of AISI 316L SS substrates. Because of this reason, SEM-EDS analysis could not provide for the AISI 316L SS substrates. HAP coating on the AISI 316L SS substrates was formed kept for 35 days in 3.0xSBF solution.

When corrosion characteristics of AISI 316L SS substrates in Ringer solution were examined, they were seen that, the corrosion rate of HNO_3 and BA PTSO AISI 316L SS substrates, which were kept for 21 days in 1.5xSBF, were the lowest. Also, the corrosion rate of HNO_3 PTSO AISI 316L SS substrates which were kept for 14 and 21 days in 1.5xSBF, were same and increased kept for 35 days in 1.5xSBF, after corrosion experiments in 0.9 % NaCl. Since the passive film formed on the surface after kept 21 days broke, AISI 316L SS substrates surfaces passed to transpasive region and corrosion rate was getting higher again. When corrosion characteristics in 0.9 % NaCl solution were examined, it was seen that the corrosion rate of anodic and BA PTSO AISI 316L SS substrates, which were kept for 21 days in 1.5xSBF, were the lower.

Also, the corrosion rate of HNO_3 PTSO AISI 316L SS substrates, which were kept for 14 and 21 days in 1.5xSBF were close to each other, and increased within 35 days. Since the passive film formed on the surface after 21 days broke, steel surface passed to transpasive region and corrosion rate was getting higher again.

When corrosion characteristics of AISI 316L SS substrates were examined in Ringer and 0.9 % NaCl solution which were kept for 14 and 21 days in 3.0xSBF solution, have high corrosion rates in all pretreatment surface process compared to kept in 1.5xSBF solution. The lowest corrosion rates of BA PTSO AISI 316L SS substrates were seen % 0.9 NaCl and Ringer solutions kept 35 days in 3.0xSBF. E_{cor} values were shifted to more positive potentials.

Tafel curves obtained from Ringer and 0.9 % NaCl solutions for Ti substrates after being kept in 1.5xSBF and 3.0xSBF solutions were given in Fig. 2. Corrosion characteristics of Ti substrates were given in Table 4.

Table 3

Corrosion characteristics in Ringer and 0.9 % NaCl solutions for AISI 316L SS substrates

			1.5 x SBF			**3.0x SBF**		
		Pretreatment surface operations	$-E_{cor}$ (mV)	i_{cor} (μAcm^{-2})	R_p ($k\Omega.cm^2$)	$-E_{cor}$ (mV)	i_{cor} (μAcm^{-2})	R_p ($k\Omega.cm^2$)
	Ringer solution	blank	375	4.44	7.05	375	4.44	7.05
	0.9 % NaCl solution	blank	322	1.27	23.35	322	1.27	23.35
35 days	Ringer solution	HNO_3	276.33	20.44	2.67	380	35.07	2.83
		anodic	325	21.57	2.17	418	37.32	1.21
		BA	404	32.31	1.30	376	17.41	2.89
	0.9 % NaCl solution	HNO_3	290.67	17.18	3.96	372	21.19	2.35
		anodic	365.5	37.45	3.37	435	27.89	1.82
		BA	377.67	18.512	2.21	376	17.41	3.32
21 days	Ringer solution	HNO_3	288.67	4.47	4.46	362	53.29	2.73
		anodic	323.33	13.37	7.68	320	60.34	1.99
		BA	343.67	9.399	5.46	279	53.89	2.88
	0.9 % NaCl solution	HNO_3	286	13.12	5.19	393	105.73	1.38
		anodic	384.67	27.45	2.05	343	57.77	1.06
		BA	324.3	13.06	4.41	364	66.20	1.62
14 days	Ringer solution	HNO_3	350.67	30.96	1.40	386	29.59	1.84
		anodic	314.67	13.29	4.38	337	33.50	1.52
		BA	383	40.26	2.16	416	52.44	1.64
	0.9 % NaCl solution	HNO_3	249.33	13.12	1.85	388	58.13	1.78
		anodic	316.67	34.21	2.08	423	26.84	2.21
		BA	368.33	47.77	2.16	443	23.18	2.65

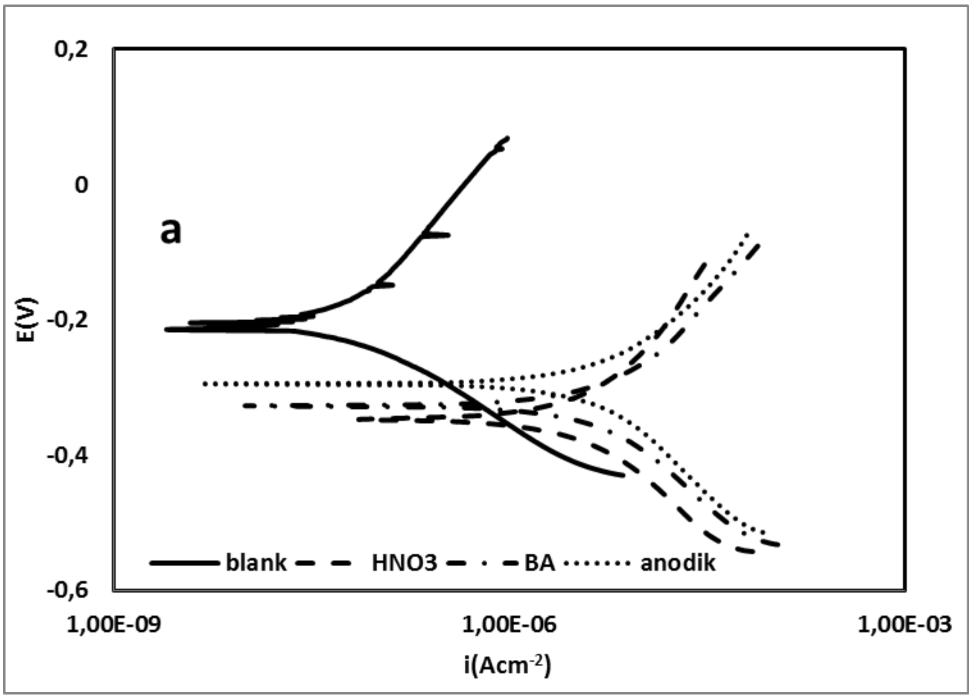
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Fig. 2 a

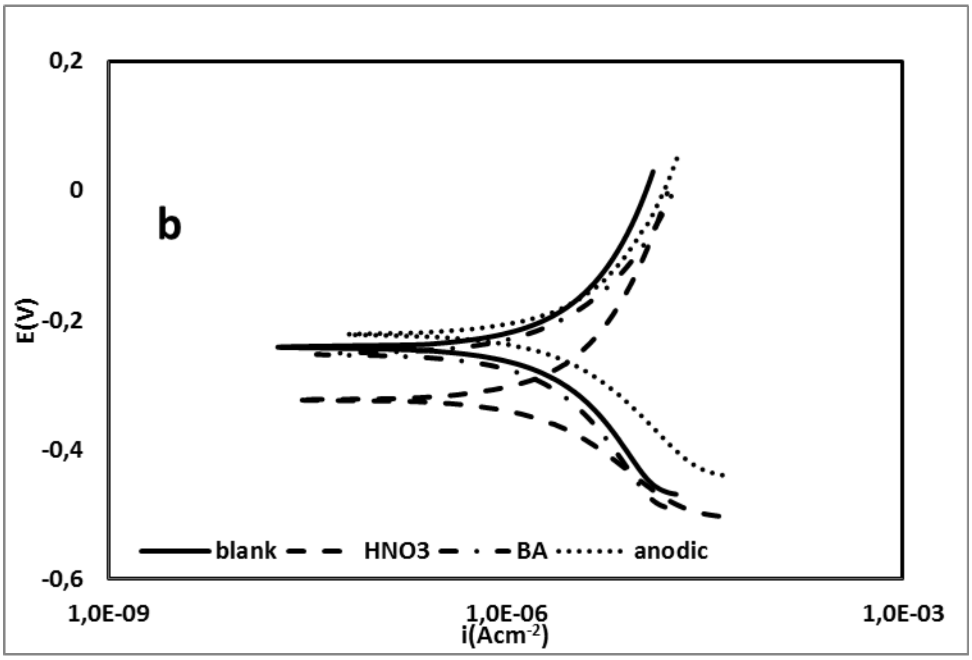
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Fig. 2 b

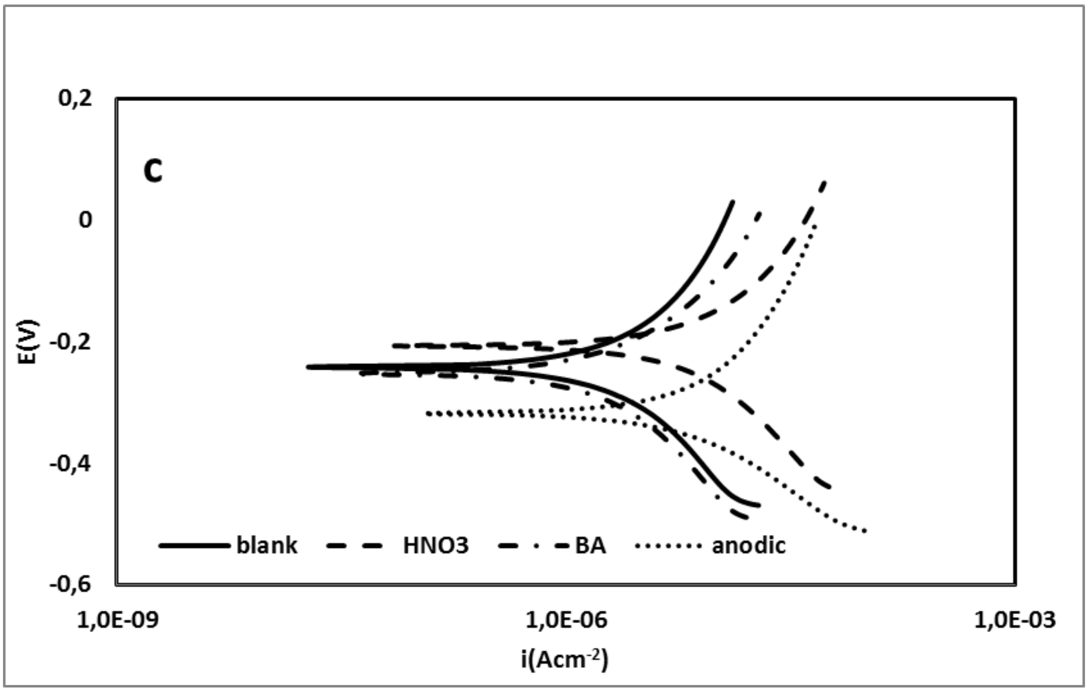

Fig. 2 c

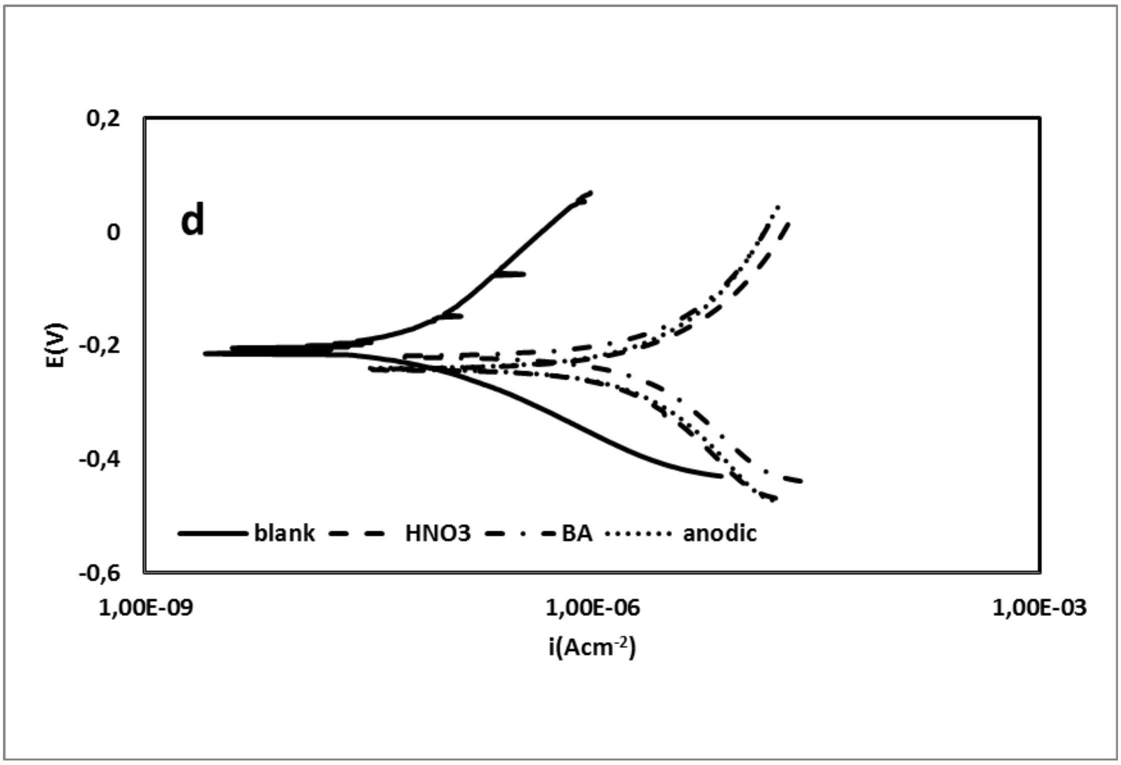

Fig. 2 d

Fig. 2 – Tafel plots were obtained for Ti substrates in Ringer and %0.9 NaCl solutions a) for Ti substrates in Ringer solution kept 35 days in 3.0xSBF b) for Ti substrates %0.9 NaCl solution kept 35 days in 3.0xSBF c) for Ti substrates in 0.9 % NaCl solution kept 35 days in 1.5xSBF, d for Ti substrates in Ringer solution kept 35 days in 1.5xSBF.

Table 4

Corrosion characteristics in Ringer and 0.9 % NaCl solutions for Ti substrates

			1.5 x SBF			**3.0 x SBF**		
		pretreatment surface processed	$-E_{cor}$ (mV)	i_{cor} (μAcm^{-2})	R_p ($k\Omega.cm^2$)	$-E_{kor}$ (mV)	i_{cor} (μAcm^{-2})	R_p ($k\Omega.cm^2$)
	Ringer solution	blank	231	0.015	516.47	231	0.015	516.47
	0.9% NaCl solution	blank	191	0.211	592.83	191	0.211	592.83
35 days	Ringer solution	HNO_3	259.3	5.173	14.91	330	8.04	10.69
		anodic	241.67	1.79	18.31	313	7.89	9.20
		BA	209	7.08	13.62	335	5.95	8.92
	0.9% NaCl solution	HNO_3	235.3	4.08	19.94	290	7.38	15.59
		anodic	247	6.89	11.93	217	7.08	12.64
		BA	238.3	5.26	16.84	300	4.51	13.06
21 days	Ringer solution	HNO_3	193.67	2.57	13.10	247	6.89	10.23
		anodic	206.67	4.54	13.30	234	6.49	9.83
		BA	198	5.07	21.74	236	2.79	16.84
	0.9% NaCl solution	HNO_3	230.67	7.11	15.01	207	3.93	13.52
		anodic	227.33	8.15	20.80	213	18.13	4.40
		BA	246.33	7.71	11.56	229	9.34	3.92
14 days	Ringer solution	HNO_3	314	9.61	13.87	286	8.39	12.03
		anodic	261.7	1.35	16.84	205	7.81	25.78
		BA	208.33	6.99	9.81	256	2.17	24.44
	0.9% NaCl solution	HNO_3	372.7	0.98	11.39	244	1.45	27.25
		anodic	213.7	2.47	8.54	282	11.18	5.02
		BA	214.5	12.5	7.43	262	5.16	24.56

When Table 4 was examined, corrosion rates of HNO_3, anodic and BA PTSO Ti substrates were increased in Ringer and 0.9% NaCl solutions after 21 days on kept in 1.5xSBF and then decreased (in Ringer solution except BA PTSO Ti substrate kept 14 days in 1.5xSBF). This showed that oxidation continues up to 21 days on these substrates, after 21 days surfaces were covered by completing oxidation and corrosion was decreased. When corrosion rates of all pretreatment surface processes of Ti substrates in Ringer solution compared to each other, the lowest corrosion rate was anodic PTSO Ti substrate for 14 and 35 days hold in 1.5xSBF. When corrosion rates of all days in 0.9 % NaCl solution were compared to each other, the lowest corrosion rate of Ti substrate was seen HNO_3 PTSO for waiting 14 days in 1.5xSBF. The lowest corrosion rate of Ti substrate in Ringer solution was seen for waiting 14 days in BA pretreatment surface processed in 3.0 x SBF. Corrosion rates of HNO_3 and anodic PTSO of Ti substrates were decreased until 21 days and after 21 days corrosion rates were again increased in Ringer solution for kept in 3.0xSBF. It can be said that the permeability of HA structure formed on this surface increased and passive layer formed by oxides on the surface were broken, by this way the corrosion increased. Corrosion rate was increased for HNO_3 PTSO Ti, BA PTSO Ti (in 0.9% NaCl solution) with immersion time in 3.0xSBF. Corrosion rate was increased until 21 days and then decreased in anodic PTSO Ti in waited 3.0xSBF. E_{cor} values of HAP coated Ti behaved variable in corrosion studies after waited 1.5xSBF.

SEM analysis of biomimetic HAP coatings after corrosion experiments

HAP coatings were formed on the surface of Ti6Al4V and Ti substrates by biomimetic method. There has been very little coating on the surface of 316 steel or not. So, SEM images just for Ti6Al4V were given in Figs. 3 and 4.

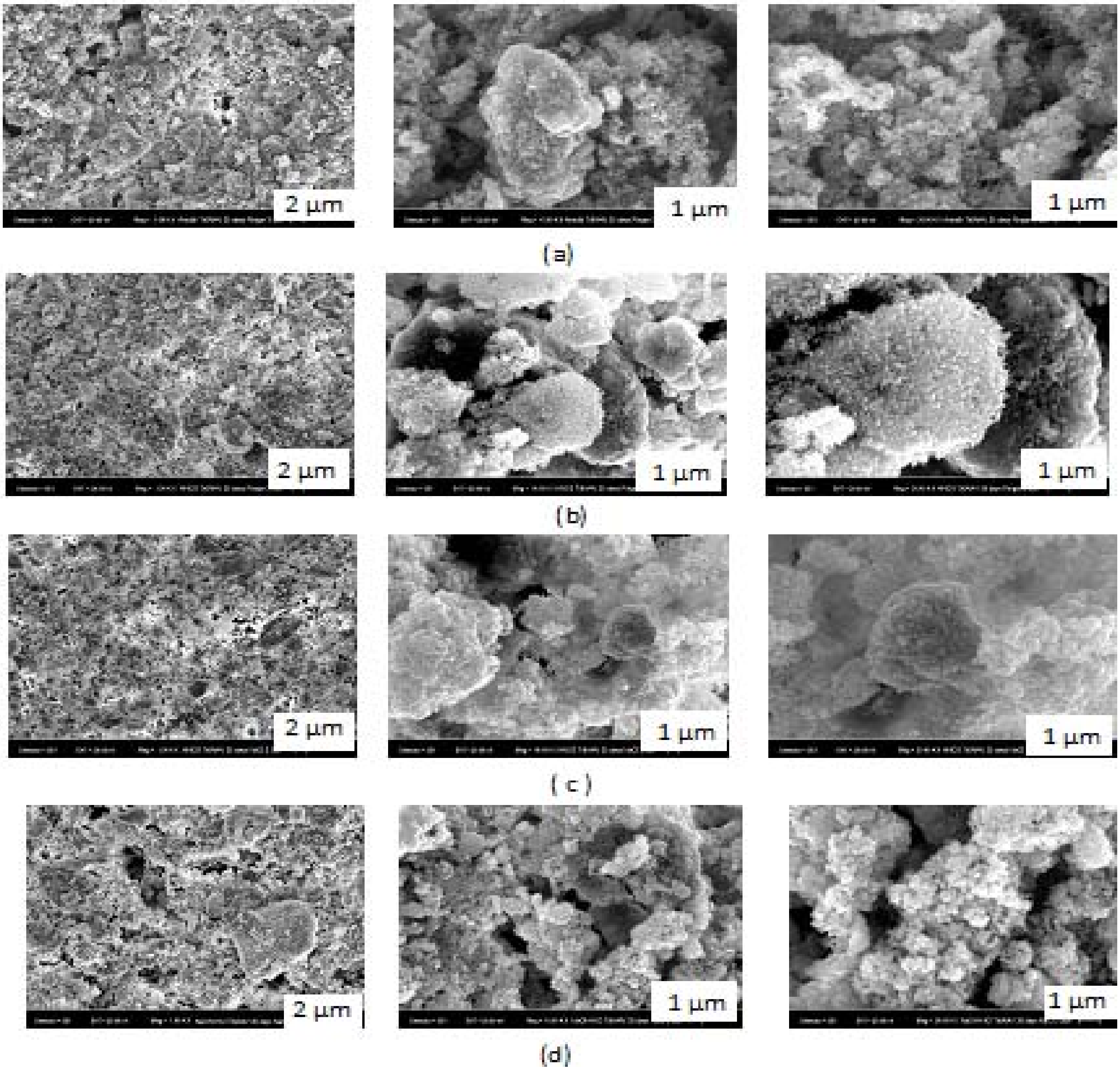

Fig. 3 – Surface images of biomimetic HAP coated Ti6Al4V substrates after corrosion experiments kept 35 days in 3.0xSBF solution a) Ringer (anodic PTSO) b) Ringer (HNO_3 PTSO) c) 0.9 % NaCl (HNO_3 PTSO) d) 0.9 % NaCl (BA PTSO).

When the surface images of biomimetic HAP coated substrates after corrosion experiment were examined in Fig. 3, it was observed that biomimetic HAP coating was formed on the surface of Ti6Al4V substrates. HAP coating appear to be similar to the grape collected cauliflowers. Cauliflower storey images combined with salts formed by sediments presence in 3.0xSBF were obtained (Fig. 3-4).[21] Increased surface roughness of the coating surface treatment with nitric acid pre-adhesion and provides good adhesion.[34] This bone-like apatite layer biomimetic synthesis of bioactive surfaces of biomaterials may be an effective way to produce.[29] When HAP coatings in 35 days and 21 days in 1.5xBF (Fig. 3 b) were compared with each other, it was observed that HAP coating was better after 35 days. SEM observation showed that Ca-P plate deposition was formed onto Ti6Al4V substrates.

Surface images of Ti substrates in Ringer and 0.9 % NaCl solutions after corrosion experiments were given in Fig. 5.

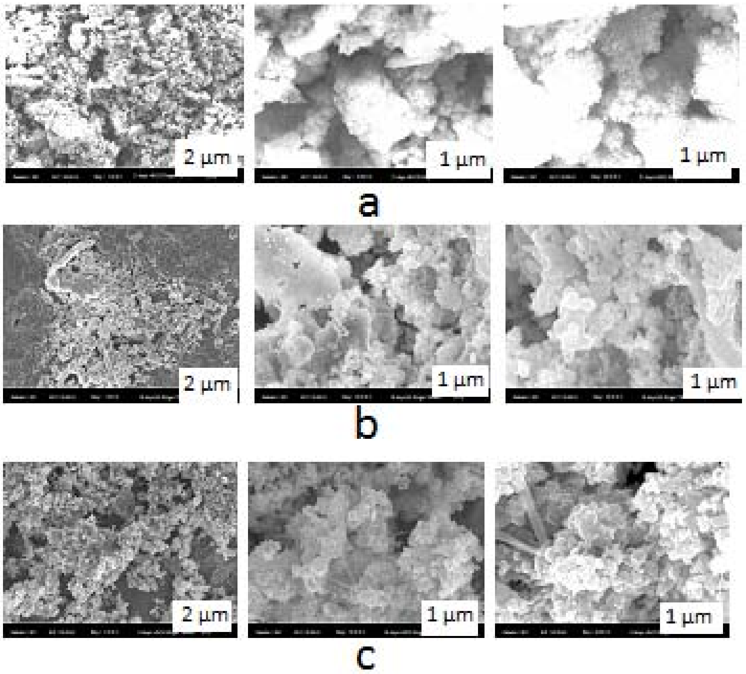

Fig. 4 – Surface images of biomimetic HAP coated Ti6Al4V after corrosion experiments (Ringer solution) kept in 3.0xSBF solution a) waited 21 days (HNO_3 PTSO) b) waited 14 days (BA PTSO) c) waited 14 days (HNO_3 PTSO).

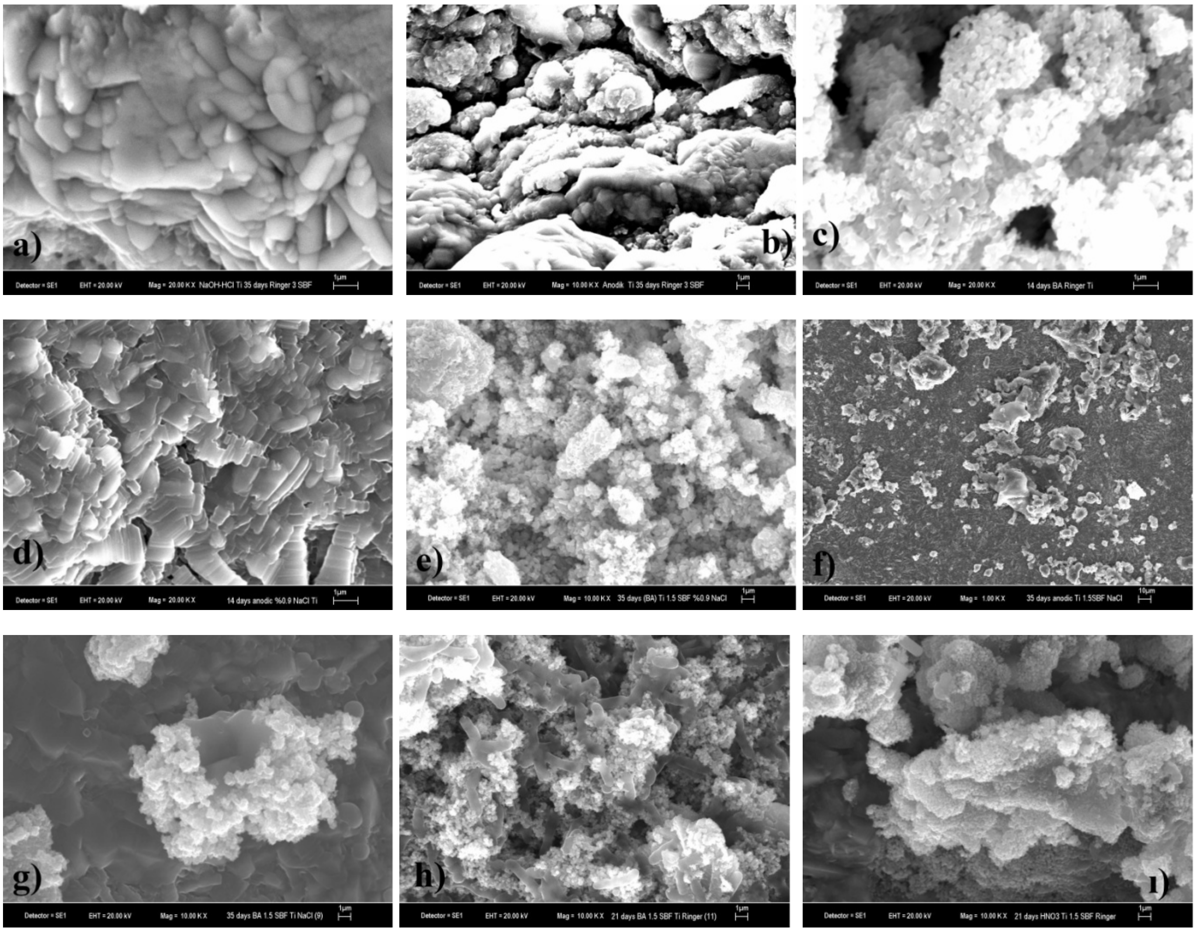

Fig. 5 – Surface images of HAP coated Ti after corrosion experiments in Ringer and 0.9% NaCl solutions (a: 35 days 3xSBF Ringer BA PTSO, b: 35 days 3xSBF Ringer Anodic PTS, c: 14 days 3xSBF Ringer BA ÖY, d: 14 days 1.5xSBF 0.9% NaCl Anodic PTSO, e: 35 days 1.5xSBF 0.9% NaCl BA PTSO, f: 35 days 1.5xSBF 0.9% NaCl Anodic PTSO, g: 35 days 1.5xSBF 0.9% NaCl, h: 21 days 1.5xSBF Ringer Anodic PTSO, ı: 21 days 1.5xSBF Ringer HNO_3 PTSO).

When Fig. 5 was examined, it was observed that biomimetic HAP coating was formed on the Ti substrates surface. HAP coated substrates appear to be similar to grapes as collected cauliflowers. Storied cauliflower images were formed combined with sediment which was consisted by salts found in 3.0xSBF.[35] Instead in Fig. 5 a, b, d, it was seen only TiO_2 on the surface. After corrosion experiments there was no HAP on the surface. In vitro bioactive titanium surface was adsorbed OH^- group chemically involved in SBF and generated negative charged anatase TiO_2.[2] Surface of anatase TiO_2 carries faster negative charged ions in physical environment.[1,4,10,11,29,36] Ca-P coatings in nature were very porous and make substrates increased surface roughness significantly.[20] Therefore, they contribute to a complete change in surface topography.[1] The surface of the substrates with NaOH-HCl pretreatment surface became partially hidratized and Na^+ containing groups were formed at the surfaces. Na^+ ions located in these groups replaced with Ca^{2+} ions located in SBF.[37] This result showed as a first step, Ca^{2+} and Mg^{2+} ions combined with titanium surface. Studies indicated that Ti metal and its substrates were usually coated with a thin passive layer of TiO_2.[1,10,15] If Na_2O surface merged with TiO_2 layer, Ti metal treated with NaOH pretreatment surface was kept in SBF solution, this metal creates Ti-OH groups on their surfaces in their living bodies. Na^+ ions were released by replacing with H_3O^+ and Na^+ ions in body fluids. The negative charged surface created calcium titanates with positive Ca^{2+} ions in SBF. As a result of accumulation of Ca^{2+}, the surface charges with positive and creates amorphous calcium phosphate with negative charged phosphate ions. This calcium phosphate was semi stabile and as a result it changed to stabile crystal structure similar to bone. Finally, it was expected that they were creating apatite similar to bone by bonding living bone. When Ti substrate metal was placed into 5 N NaOH solution at 60 ^oC for 24 hours, a part of 1 μm passes through the surface of sodium and oxygen titanium. Sodium and oxygen amounts were decreased according to increasing depth.[37] It was observed that they had apatite spheres based Ca-P and was especially accumulated form and showed weak crystal features, the thickness of layer increased by the incremental time of immersion.[34] It was showed that having crystal hydroxyapatite coating on titanium bases alkali-process has been applied successfully.[36]

EDS analysis of biomimetic HAP coatings after corrosion experiments

After corrosion studies also, EDS spectrums were taken from the surfaces of Ti6Al4V which SEM images taken. The obtained quantitative % atom values of the spectra were given in Table 5.

The corrosion experiments of biomimetic HAP coated substrates were done in Ringer and 0.9 % NaCl solutions waited 35, 21 and 14 days in 1.5xSBF and 3.0xSBF solutions. Ca/P ratio of HAP coating formed on Ti6Al4V changed depends on SBF solution concentration and immersion time. When Table 5 was examined, especially Ca, O, P, Cl^- ions were observed on the surface of Ti6Al4V substrates. Forming of Ca, O, and P on the surface indicates forming of HAP. Also presence of Cl^- was a result of the combination of 3.0xSBF and 1.5xSBF solutions, 0.9 % NaCl and Ringer solutions.

Cl^- reduces the corrosion resistance of coatings composed of HAP. It increases the corrosion of the surface by creating a porous structure. Calcium phosphate can be classified into groups as a specific Ca / P ratio. Ca/P ratio of hydroxyapatite was 1.67.[2, 15, 30-33] Various calcium phosphates were classified according to differences ranging from 0.5 to 2.0. Tetracalciumphosphate ($CaO.Ca_3(PO_4)_2$) was hilgenstockite with the highest Ca/ P ratio.[4, 15, 30- 32, 35-42] The lowest Ca / P ratio of calcium phosphate was calcium meta-phosphate (CMP). Calcium phosphate bioactivity depends on the resolution of Ca^{2+} ions left by one of these compounds. If Ca/P ratio was 1.5, it was tricalcium phosphate (TCP).[35-42] Ca/P proportions were actually very high in 3.0xSBF. It was related with density and waiting for time of 3.0xSBF. Hilgenstockite structure was formed on the surface. The closest value of HAP structure was in 3.0xSBF. In the waiting for time within 14 days, the ratio of Ca/P was seen as 1.66 HNO_3 PTSO Ti6Al4V Ringer solutions waited in 3,0xSBF. It was also seen 1.61 in 1.5xSBF within 35 days BA PTSO Ti6Al4V. After corrosion studies, on the surfaces with SEM imaging, also EDS spectrums were taken on HAP coated Ti substrates. Quantitative % atom values of obtained spectrum for titanium substrates were given in Table 6.

Table 5

EDX quantitative element analysis (% atom) of Ti6Al4V substrates after corrosion experiments in Ringer and 0.9 % NaCl solutions

			O	**Na**	**Mg**	**P**	**Cl**	**Ca**	**Ti**	**Ca/P**
						3.0xSBF				
35 days	Ringer solutions	anodic	62.12	0.38	0.54	11.05	1.44	24.47	---	2.21
		HNO$_3$	63.29	0.70	0.83	10.91	1.88	22.28	0.12	2.13
	0.9 %NaCl solutions	anodic	56.76	0.56	---	7.43	1.29	14.52	8.76	1.95
		HNO$_3$	63.48	---	0.58	10.91	1.03	24.01	---	2.20
		BA	61.99	---	1.11	11.74	1.58	23.57	----	2.00
21 days	Ringer solutions	HNO$_3$	65.15	0.59	---	10.05	2.59	21.60	----	2.15
14 days	Ringer solutions	HNO$_3$	46.17	0.23	---	10.31	1.35	17.17	0.19	1.66
		BA	58.30	---	---	3.33	---	4.89	33.47	1.47
						1.5xSBF				
35 days	Ringer solutions	BA	53.80	5.02	---	6.66	4.76	10.12	19.64	1.52
	0.9 % NaCl solutions	BA	62.79	0.89	---	11.27	1.48	18.20	5.37	1.61
21 days	Ringer solutions	BA	61.66	3.15	---	3.92	2.10	5.53	23.65	1.41

Table 6

EDX quantitative element analysis (% atom) Ti substrates after corrosion experiments in Ringer and 0.9 % NaCl solutions

			O	**Na**	**Mg**	**P**	**Cl**	**Ca**	**Ti**	**Ca/P**
						3.0 x SBF				
35 days	Ringer solutions	HNO$_3$	63.99	1.30	0.66	11.10	1.63	20.67	0.65	1.86
		BA	--	2.25	---	21.92	4.74	34.20	36.89	1.56
	0.9% NaCl solutions	HNO$_3$	55.92	2.42	0.45	13.17	3.38	23.74	0.93	1.80
14 days	Ringer solutions	BA	46.80	0.93	---	9.89	2.25	16.76	3.21	1.69
						1.5 x SBF				
35 days	0.9% NaCl solutions	BA	60.76	7.61	0.51	7.24	10.40	11.11	2.23	1.53
21 days	Ringer solutions	HNO$_3$	50.20	5.21	0.21	10.97	11.75	18.80	1.98	1.71
		BA	64.59	0.63	---	0.95	0.16	1.38	32.29	1.45

Corrosion studies of HAP coated substrates by biomimetic method were examined in Ringer and 0.9% NaCl solutions. Ca/P molar ratio of human blood plasma was 2.5. SBF was not stoichiometric against to carbonate precipitation, has a weak crystallization feature or super saturated with calcium phosphate similar to nanocrystal apatite.[28] When Table 6 was examined, it was observed that Ca, O and P were in excess on the electrode surface. This indicates that coating created form Ca-P phase. Presence of Na$^+$ indicates that they combined with apatite. Presence of Ti indicates that the coating was not thick enough and EDS ray goes to the surface.[4] It also shows why corrosion increases in biomimetic coating finally. Presence of Cl$^-$ comes from the combination of 3.0xSBF solution with 0.9% NaCl and Ringer solution. Cl$^-$ makes decreasing corrosion resistance of formed

HAP coatings. It increases corrosion by forming a porous structure on the surface. Bohner was grouped CaP into two according to synthesizing temperature.[36] Sintered form at high temperature (>900 °C) and at low temperature (~37 °C). Compounds synthesized at low-temperature showed more resolution, more surface area, more active features in biological systems. Ca/P ratio changes between 1.45 to 1.86 on titanium. The high ratio of Ca/P with a holding in 3.0xSBF was due to the concentration of solution. The closest value to Ca/P ratio of HAP was seen as 1.69 after being held for 14 days in 3.0xSBF BA PTSO Ti substrates.

FTIR analysis after corrosion experiments

After corrosion studies in Ringer solution, FTIR spectrum was taken anodic PTSO Ti substrates waited 35 days in 3,0xSBF. Also FTIR spectrum was taken commercial HAP powder. FTIR spectrum was given in Fig. 6.

As a result of especially Ringer solution, it was seen that the HAP coating on the surface was not damaged. Fig. 6 was investigated, hydroxl group bands 3750, 630, 335 cm^{-1} were seen on HAP structure. The characteristic bands of phosphate components were performed on the region of 900-1200, 563-601 cm^{-1}. Ca-PO_4 275, 295 cm^{-1} and Ca-OH 335 cm^{-1} bands were characterized. The availability of some carbonat groups 1420-1455 cm^{-1} can be determined. The peaks which were seen on 3571-632 were marked as OH^- ion. It was understood that the peaks seen on 1090-962-601-473-569 were against PO_4^{3-} ion.

CONCLUSIONS

HAP coatings were performed on Ti, Ti6Al4V and AISI 316 L SS substrates by the method of biomimetic. Ca/P ratio of HAP coatings which were performed on Ti, Ti6Al4V and AISI 316 L SS substrates change according to the concentration of SBF solution and period of immersion. HAP coatings which were performed by the method of biomimetic did not prevent corrosion. It was observed during the analysis of FTIR that there was HAP on the surface of Ti substrates. During the analysis of EDS of Ti substrates, it was seen that the closest value in the ratio of Ca/P hydroxyapatit was 1.69 waited 14 days in 3.0xSBF. In EDS analysis of Ti6Al4V, Ca/P rates was 1.66 waited 14 days in 3.0xSBF and it was 1.61 waited 35 days in 1.5xBF. HAP coating was formed on 316 L steel after waiting for 35 days in 1.5xSBF and 3.0xSBF.

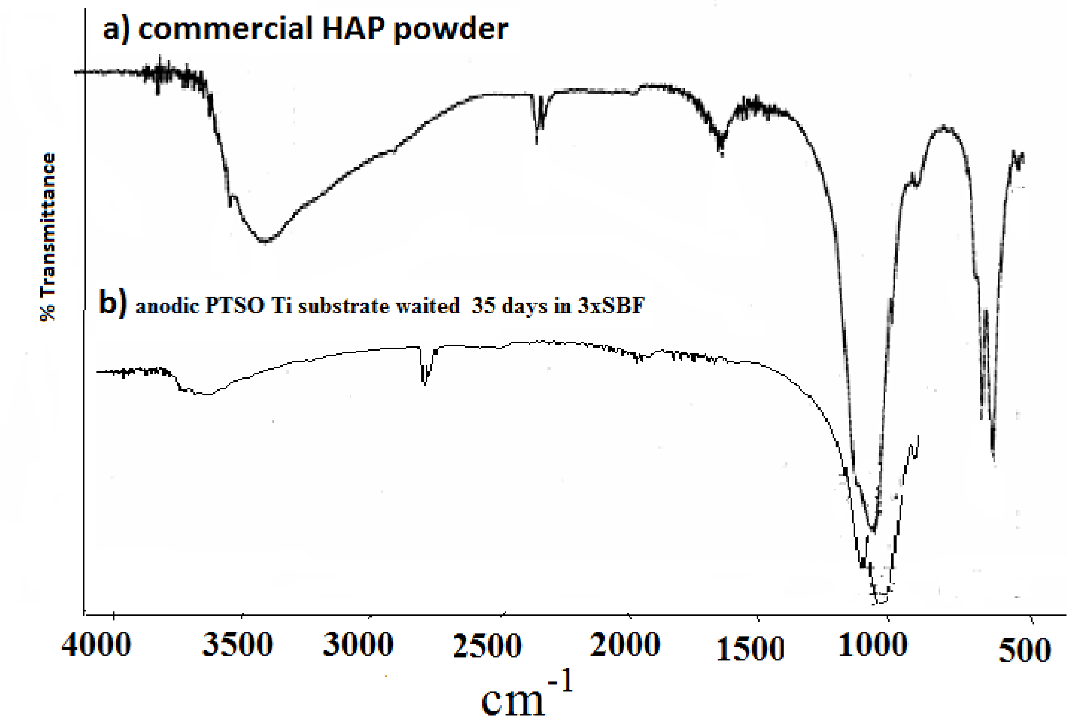

Fig. 6 – After corrosion experiments in Ringer solution, FTIR spectrum of anodic PTSO Ti substrate kept 35 days in 3.0xSBF and commercial HAP powder.

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