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ELECTROCHEMICAL AND ANTIBACTERIAL CHARACTERIZATION OF THERMALLY TREATED TITANIUM BIOMATERIALS

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The paper aims to investigate the electrochemical and antibacterial behavior of titanium with surface thermally treated at two different temperatures as 400°C and 800°C, respectively. The temperature of 800°C is associated with titanium oxidation, leading to oxide layers with a rutile structure instead of anatase. The electrochemical investigation involves open circuit potential, polarization tests and electrochemical impedance spectroscopy. The characterization of treated surfaces was completed with contact angle determinations. Gram negative and Gram positive bacteria have been used for antibacterial tests. The best thermal treatment is that carried out at 800°C, leading to the titanium with smallest corrosion rate, stronger hydrophilic character and higher index of bacteria inhibition for Escherichia coli and Staphylococus aureus.

Keywords: titanium, thermal treatment, antibacterial effect, contact angle, impedance spectroscopy

1. Introduction

Due to the oxide passive layer formed in reaction with oxygen in the air, titanium and its alloys present high corrosion resistance and biocompatibility [1-3]. Being thermodynamically stable, the TiO_2 oxide (usually with a thickness of 1.5 to 10 nm) has a low tendency to ion release in bioliquids, leading to small corrosion rate [4] of Ti and its alloys. Despite such very good corrosion resistance, in order to improve biomaterials performance in medical applications, many surface modifications have been investigated [5,6], including acid etching [7], thermal treatments [8,9], phosphate masses deposition [10-12] and elaboration of TiO_2 nanoarchitectures on the surface [13,14]. As expected, the thermal

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treatment is a function of time treatment and temperature values [8], taking into account that phase transformations are taking place. However, TiO_2 with a rutile structure was typically formed by thermal procedure at temperatures higher than 400°C. The transition temperatures ranged from 400°C to 1000°C, depending on the kind of titanium [15] were reported in literature. The increase of the surface roughness and *alkaline phosphatase* (ALP) activity as the treatment temperature increased was reported as well [8]. The ALP activity is used as a biomarker, because ALP can mediate bone mineralization by decomposing the phosphate compounds.

Taking into account such data and approach, our aim in this study is focused on electrochemical and antibacterial activity behavior of titanium thermally treated at two temperatures, 400°C and 800°C, respectively. The second temperature was chosen in order to ensure a significant increase of surface energy [8]. The change is seen by XRD patterns of the samples treated at 800°C. Such samples present additional peaks belonging to rutile TiO_2, which indicate the formation of oxide layers with a rutile structure, instead of anatase [9]. It is noticeable that both apatite mineralization and corrosion-resistance are maximized on rutile structure [16]. Thus, the novelty of the paper consists in finding the best thermal treatment of titanium, in order to enhance antibacterial activity and corrosion resistance.

2. Experimental

2.1 Materials and solutions

Test environment was an artificial saliva solution called Canivet solution [17] with the following composition: 0.86 g/L NaCl; 1 g/L Na_2HPO_4; 0.072 g/L KSCN; 0.913 g/L $CaCl_2$. The samples were titanium foils (Advent Research Materials Ltd., UK) of 99.6% purity and 1 cm^2 surface. All experiments were carried out at room temperature.

Titanium samples were polished with successively smoother grade of emery papers (up to 800 grit), then washed with acetone and distilled water, etched for 1.5 hours with a solution of 3:7 H_2O_2 30%:H_2SO_4 98%, washed with deionized water, and dried at room temperature, in order to remove oxides and increase the roughness of the substrates for a better adhesion.

Two different thermal treatments were performed on the titanium samples directly subjected to surface treatment [18] at 400 and 800°C for 10 hours in a LabTechla oven. The surfaces modified by different treatments were subjected to investigations by optical microscopy using Zeiss Scope A1 microscope.

2.2 Electrochemical studies

All electrochemical studies were carried out using an electrochemical cell with platinum electrode as counter electrode, calomel (SCE) electrode as reference, and titanium electrode as working electrode. Electrochemical tests were performed with a Voltalab 40 potentiostat/galvanostrat equipment with a Voltamaster 4 soft.

2.2.1 Open circuit potential (OCP)

Open circuit potentials were measured for 2000 s in the electrolytes, before carrying out other electrochemical experiments. The shift of corrosion potential in the positive direction corresponds to the formation of a protective oxide film, while moving to negative values may be associated with the reorganization of the surface film in its less resistant configuration.

2.2.2 Tafel studies

Tafel plots were obtained by exposing the Ti samples into electrolytes and polarizing in a narrow potential domain from E_{corr} -250 mV to +250 mV vs. SCE with a scan rate of 0.166 mV/s.

2.2.3 Studies on the extended polarization curve

The specimens were polarized from -0.8 V to 2.5 V vs. OCP. Scan rate during these experiments was 2 mV/s.

2.2.4 Electrochemical Impedance Spectroscopy (EIS)

AC impedance studies were carried out in the frequency range of 100 kHz to 10 mHz. The AC signal imposed during the experiment was of ±25 mV amplitude. The plots were analyzed to obtain electrical equivalent circuits by using Zview software.

2.3 Determination of contact angle

The ability of a liquid to wet a flat solid was evaluated by measuring the contact angle θ that the liquid drop makes with the surface. If the angle is almost zero, the liquid completely wets the solid surface, and it freely disperses on its surface with a speed that depends on its viscosity and solid surface roughness The contact angle determined with a Contact Meter CAM 100 equipment is the average of three measurements.

2.4 Antibacterial activity determinations

Estimating the effect of antibacterial materials was done by using turbidimetric method, because it allows a precise calculation of the antimicrobial effectiveness. Turbidimetric method has several major advantages over the others like being simple, fast, non-destructive, easy and with minimum risks to the user, if the biosafety rules are respected in the laboratory. The principle of the method is based on the fact that the luminous radiation passing through a suspension of microorganisms is dispersed, and the dispersion value is a measure of the biomass in suspension.

We have chosen two reference strains ATCC: *Escherichia coli* -25922, a Gram negative bacillus and *Staphylococcus aureus*-6538, a Gram positive staphylococcus, and a bacterial pathogen isolated from the oropharynx, respectively *S. aureus*, identified from a 6 year old boy who went to the doctor with malaise and fever, and from who a pharyngeal exudate was taken.

Pure cultures were prepared in order to minimize the error sources, as follows: from the primary cultures obtained by direct plating of stock cultures on media, of pharyngeal exudate, respectively, a couple of colonies was taken, and a second passage was made on Columbia Blood agar and CLED. Bacterial suspensions of 0.5 McFarland units were obtained in saline from the grown colonies originated from the second passage ($1.5x10^8$ UFC/mL).

Bacterial suspensions were placed in sterile glass bottles numbered for easy identification. For each type of bacteria, a standard positive control has been preserved. In the obtained suspensions we have introduced the test materials. All the tubes were incubated for 24 hours at 37°C. All determinations were made under vertical laminar flow hood which was sterilized using UV lamps and bactericidal substances before and after each work session.

After incubation, the absorbance was read at 600 nm and bacterial growth inhibition was calculated with the equation (1):

$$I\% = \frac{(C_{24} - C_0) - (P_{24} - P_0)}{(C_{24} - C_0)} \times 100 \tag{1}$$

where C_0 and C_{24} represent the optical density of control at the initial time, and after 24 hours, respectively, and P_0 and P_{24} represent the optical density of the samples at the initial time, and after 24 hours, respectively. Table 1 reveals the correspondence between cellular density and McFarland units.

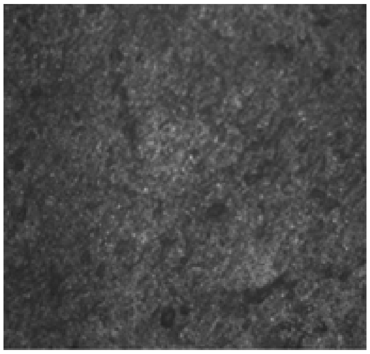
Table1

Cellular density and samples absorbances

Approximate cell density (1×10^8 UFC/mL)	1.5	3.0	6.0	9.0	12.0
McFarland units	0.5	1	2	3	4
Absorbance at the wavelength of 600 nm	0.08-0.1	0.257	0.451	0.582	0.669

3. Results and discussions

The surfaces modified by different treatments were first subjected to an investigation by optical microscopy. Fig 1 shows the Ti surface images obtained after thermal treatments at 400°C and 800°C.



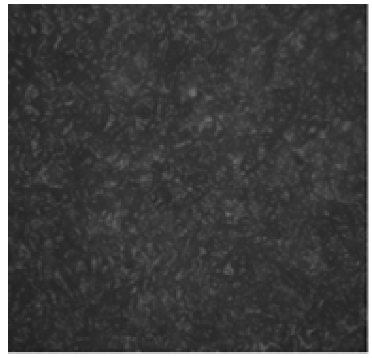


Fig. 1. Optical images for Ti with various treatments: a) thermal treated at 400°C; b) thermal treated at 800°C;

The titanium oxide film formed on the electrode surface at 800°C is denser and more uniform than that obtained at 400°C.

3.1 Open circuit potential

The OCP values (Fig. 2) for the two types of heat treatments of Ti samples immersed in Canivet solution shift in the electropositive positive direction, indicating the formation of protective passive films on their surfaces. Open circuit potential variation in sample annealed at 800°C shows a potential stabilizing after about 600 s of immersion, while in the case of the sample treated at 400°C, stabilization is achieved after 1400 s of immersion.

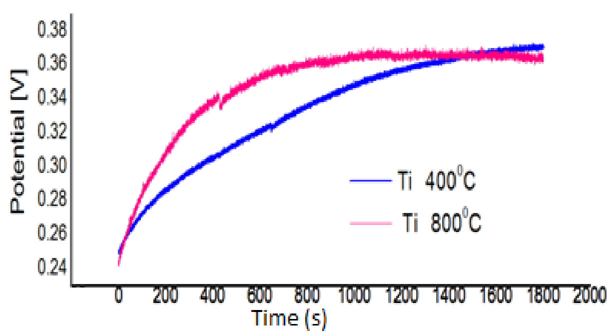


Fig. 2. Variations of OCP in Canivet solution for Ti thermal treated at 400°C and at 800°C

3.2 Polarization studies

Table 2 shows the Tafel electrochemical parameters obtained for treated titanium samples in Canivet solution. The average values of E_{corr}, i_{corr}, β_a, β_c, R_p and v_{corr} determined by Voltamaster 4 soft are presented in Table 1. E_{corr} and i_{corr} represent the corrosion potential and current density, βa - anodic Tafel slope, β_c –

cathodic Tafel slope, R_p - polarization resistance (determined in the potential range ±40 mV in the vicinity of corrosion potential) and v_{corr} is the corrosion rate expressed as a penetration index.

Table 2

Tafel electrochemical parameters

Surface treatments	E_{corr} (mV)	i_{corr} ($\mu A/cm^2$)	β_a (mV/dec)	β_c (mV/dec)	R_p ($K\Omega\ cm^2$)	v_{corr} (μm/year)
Thermal treatment 400^0	-281	1.189	216.7	-120.8	21.13	10.31
Thermal treatment 800^0	-292	0.306	137	-137	73.79	2.655

It is known that a metallic material that has a tendency to passivity has the value of β_a greater than β_c, while the one that corrodes has β_a less than β_c. The higher value of β_a, in comparison with the absolute value of β_c, for titanium treated at 400°C, indicates an anodic control in the corrosion process. For the samples treated at 800°C, the corrosion process takes place under mixed control.
The decrease of i_{corr} with increase of the temperature of thermal treatments suggests an increase in thickness of the oxide layer, and an increase in corrosion protective ability with increase in oxidation temperature. Several researchers have also confirmed the fact that thermally oxidized Ti and Ti6Al4V alloy could offer a better corrosion resistance in a variety of environments [19].

Fig. 3 shows the extended potentiodynamic curves (logarithm of the measured current versus potential) for Ti thermally treated at 400^0C and 800^0C in Canivet solution. These curves represent an indication of the corrosion behavior of the specimen. They show a decrease in corrosion current with increasing the oxidation temperature, and an active-passive transition zone followed by a quasi-passive domain extended to 2500 mV for both thermal treatments. A decrease in passive currents with increasing of oxidation temperature could be observed as well. The decrease of the corrosion current along the large potential domain indicates a thicker film, as well as an increase of the passive protection capacity. Similar results were obtained by Bloyce et al.[19], when comparing the corrosion resistance of untreated Ti and heat-treated Ti in 3.5% NaCl solution.

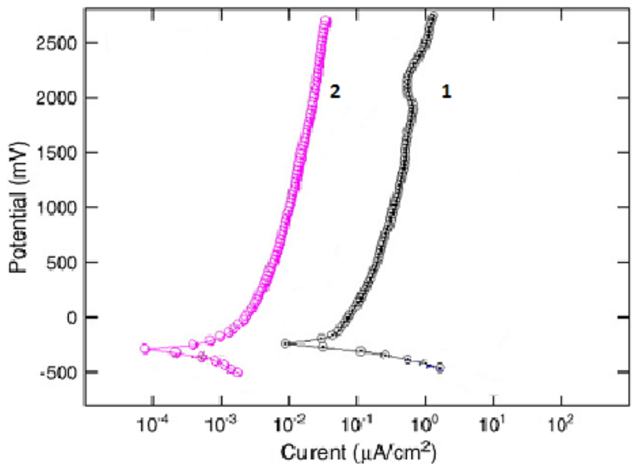


Fig. 3. Potentiodynamic polarization curves for Ti in Canivet solution. Samples were thermally treated at 400°C (curve 1) and 800°C (curve 2)

3.3 EIS studies

As a non-destructive and sensitive technique, EIS was widely applied to investigate the passive layer properties, and solid/liquid interfaces of the corrosion systems. EIS measurements have been performed on the passive film in order to study the corrosion mechanism, as well as the passive layer properties of Ti thermal treated immersed in Canivet solution. The Nyquist plot for samples oxidized at 400^0C (curve 1 in Fig. 4) exhibits a single semicircle continued with a straight line. The samples subjected to oxidation at 800^0C exhibit a semicircle in the high frequency region, followed by a loop in the low frequency region (curve 2 in Fig. 4). The diameter of the semicircle increases with increase of the oxidation temperature. The presence of one single semicircle for thermally oxidized samples at 400^0C indicates the implication of a single time constant.
The circuit used to fit the EIS experimental data is shown in Fig. 5.

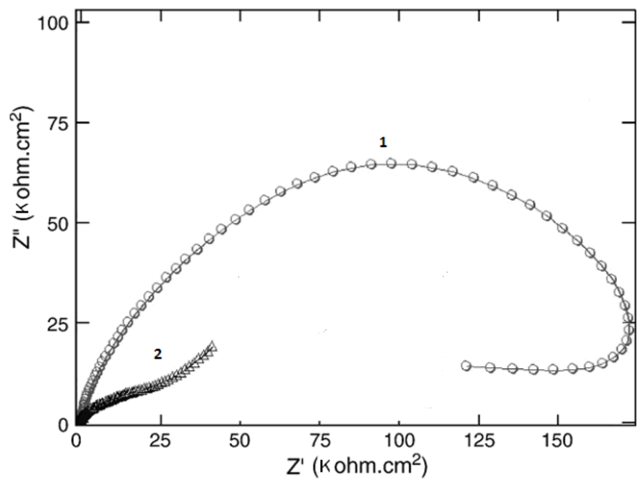


Fig. 4 Nyquist plots for titanium thermally treated at: (1) 400°C; (2) 800°C

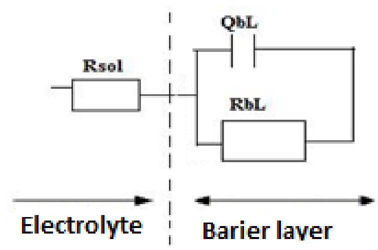


Fig. 5. Circuit used for simulating the interface Ti/Canivet solution

The simulation of Nyquist diagrams was used to determine the solution resistance (R_{sol}), polarization resistance at the interface electrode/solution (R_{bL} which is similar with R_p determined from Tafel curves), and of the double layer capacitance at the interface (Q_{bL}). Q_{bL} is expressed as a constant phase element with a capacitance Q and an exponent n of jω, where j^2= -1 and ω –angular frequency of a.c. voltage. R_{bL} is the parameter that illustrates the corrosion resistance of alloys. Values of the electrical parameters determined by fitting the data with equivalent circuit are shown in Table 3.

Table 3

Values of EIS electrical parameters

Sample	R_{sol} (Ω cm^2)	R_{bL} (Ωcm^2)	Q ($Ω^{-1}$ $cm^{-2}s^n$)	n
Ti at 400^0C	77.7	$28×10^3$	$3.1×10^{-6}$	0.744
Ti at 800^0C	79.5	$78×10^3$	$2.2×10^{-9}$	0.827

Both the increase of R_{bL} and decrease of Q with the oxidation temperature increase suggest the enhancement of the protection capacity of the surface oxide formed on Ti after the heating treatment. EIS data support the results obtained by Tafel diagrams. In the case when n (a parameter independent on frequency) is less than 0.7, interactions between the passive oxide and the electrolyte ions occur. In our case, n is only a bit higher for the sample treated at 400^0C, but much higher than 0.7 for the sample treated at 800^0C, as it can be seen from Table 3.

We propose a scale of stability in Canivet solution as:

$$Ti\ (800°C) > Ti\ (400°C)$$

3.4 Contact angle

It has been shown that osteoblasts adhere and proliferate preferentially to hydrophilic substrates [10]. The surface modifications of titanium by thermal treatment led to changes in contact angle values (Fig. 6). The calculated surface energies were 56.5 mJ/m^2 for Ti thermal treated at 400^0C, and 64.27 mJ/m^2 for Ti thermal treated at 800^0C, while the contact angle is 39° and 28°, respectively. As it is already known that a material which shows contact angles of less than 30^0 can be successfully osteointegrated [10], it can be concluded that the thermal treatment ensure a better integration of the samples.

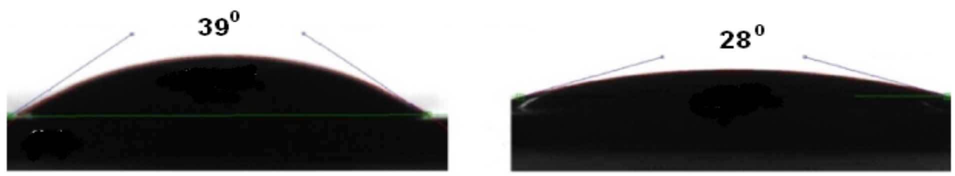


Fig. 6. Contact angle values for Ti thermally treated at 400°C (left) and 800°C (right)

3.5 Antibacterial activity

No antibacterial activity for untreated titanium has been observed (Table 4).

Table 4

Antibacterial activity inhibition

Sample / I (%)	Ti	TiO_2(400^0C)	TiO_2(800^0C)
E.coli	-	41.6	49.4
S.aureus–reference strain	-	46.2	51.02
S.aureus–pharyngeal exsudate	-	44.3	50.11

For thermally treated titanium, a higher value has been observed for Staphylococcus aureus. At higher temperature treatment the antibacterial activity is also more significant, as literature confirms for other systems [21].

With respect to the smaller antibacterian effect on patogen staphylococcus comparing to the one from reference culture, this one could be explained by a defence mechanism developed at the pacient, in comparison with the reference strain. The difference between inhibition growth values for *S.aureus* and *E.coli* is probably due to the different structure of cellular wall, which is more complex in the case of gram negative bacteria. Gram-positive bacterium has a relatively thick cellular wall with more peptidoglican layers, and only one plasmatic membrane. Gram negative bacterium has a thinner peptoglican layer, but a more complex cellular wall with two cellular membranes [22], an exterior one and a plasmatic one, which can influence the permeability for various molecules. It is to mention that Gram-negative bacteria present more resistence to chemical agents, comparing to Gram-positive bacteria. The increase of antibacterial activity of TiO_2 (800) comparing to TiO_2(400) is probably due to the increase of cristallinity structure with formation of the predominant rutile phase.

4. Conclusions

After investigating the electrochemical stability and antibacterial activity of titanium biomaterial thermally treated at 400°C and 800°C, the treatment leading to better performance was selected. The treatment at 800°C was the best one, leading to the smallest corrosion rate, higher hydrophilic character and antibacterial activity. The electrochemical tests by open circuit potential, Tafel plots method and electrochemical impedance spectroscopy sustain the best stability behavior of samples heated at 800°C. The antibacterial activity was around 50% for both *Escherichia coli* and *Streptococcus aureus* bacteria, respectively.

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