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# Effect of the inflammatory conditions and albumin presence on the corrosion behavior of grade 5 Titanium alloy in saliva biological solution

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**Abstract.** The field of biomaterials imposes a multidisciplinary approach that requires the involvement of sciences such as biology, medicine, chemistry and materials engineering so that the material implanted in a living organism does not cause any adverse reaction. This research area of biomaterials is considered as fascinating and challenging. It's fascinating because of its potential applications and the need to improve the quality of life. It is challenging due to the various complexities it faces when biomaterials encounter biological environments for the longevity of life by maintaining or restoring tissue or organ functions. Metallic biomaterials are used as pivots for anchoring dental implants and as parts of orthodontic devices such as crowns and bridges of prostheses. Ti-6Al-4V is well known as a corrosion resistant alloy for dentistry applications due to this film of titanium oxide formed on its surface. However, the inflammatory conditions and protein presence could affect the corrosion resistance of this alloy. Our research work aims to investigate the effect of albumin and inflammatory conditions to corrosion resistance of Ti-6Al-4V in saliva Fusayama Meyer biological solution. In order to simulate the peri-implant inflammatory conditions in vitro studies were conducted with addition of hydrogen peroxide (reactive oxygen species, found during inflammation) and albumin the most typical protein of biological fluids. Electrochemical methods were applied for corrosion investigations. The results provide evidence that titanium's alloy corrosion resistance is affected by inflammatory conditions and albumin presence in saliva biological solution.

## 1. Introduction

Every day, thousands of surgical procedures are performed to replace or repair tissue that has been damaged by illness or trauma. Diseases, injuries and trauma can lead to tissue damage and degeneration in the human body, which requires treatments to facilitate repair, replacement, or regeneration [1].

The physiological environment into which a Ti alloys implant is placed varies with site and with the device application, but is always a complex system containing various inorganic and organic components such as ions, amino acids and proteins and living cells. The specific composition of this environment can also change in either health or disease due to physiological processes such as peri-



implant inflammation [2] or as a direct consequence of the deterioration of the implant itself [3].

Ti-6Al-4V is most commonly used titanium alloy for biomedical applications, such as hard tissue replacement due to their excellent biocompatibility, specific strength and corrosion resistance. In comparison to other metallic materials like stainless steel and Co-Cr alloys, better biocompatibility is the result of instant formation of TiO<sub>2</sub> layer resulting in high corrosion resistance and low toxicity in fluid body environment [4].

It has been widely reported that the activities of Reactive Oxygen Species (ROS) are implicated in many disorders such as diabetes, ageing, neurodegradative disorders, etc. Though the body has efficient antioxidant systems in place to counter the activities of ROS, serious pathological diseases may occur when these highly reactive species overwhelm the activities of the so called antioxidant mechanisms - a condition commonly known as oxidative stress [5].

Albumin is the most abundant protein in the human body (4%). The concentration of albumin in the blood has been used as a major index in health and disease over the years [6]. Structural and functional impairments in albumin are attributed to various pathophysiological conditions like diabetes, osteoarthritis and advanced liver diseases [7].

The presence or absence of different physiological species in simulated physiological environments has been reported to influence corrosion behaviours of Ti alloys, such as pH [8-10], certain inorganic species (e.g. H<sub>2</sub>O<sub>2</sub> or fluoride ions [11-13]), organic species (e.g. lipopolysaccharide (LPS) [14], serum [15, 16], proteins such as albumin [17-20, 21] and albumin and H<sub>2</sub>O<sub>2</sub> [22].

In the literature there are few studies to follow the effect of proteins and reactive oxygen species on Ti-6Al-4V and are still unclear [22].

Our research work aims to investigate the effect of albumin and inflammatory conditions to corrosion resistance of Ti-6Al-4V mirror polished in saliva Fusayama Meyer biological solution.

In order to simulate the peri-implant inflammatory conditions in vitro studies were conducted with addition of hydrogen peroxide (reactive oxygen species, found during inflammation) and albumin the most typical protein of biological fluids. Electrochemical methods such as Open Circuit Potential and Electrochemical Impedance Spectroscopy were applied for corrosion investigations.

## 2. Experimental procedures

### 2.1. Materials and methods

The electrochemical measurements were performed on the VoltaLab PGZ 100 with the Volta Master 4 program software. The electrochemical cell with three electrodes working electrode being the surface of the evaluated sample, the counter electrode was a platinum electrode mounted parallel to the working electrode and a reference electrode, the silver electrode (Ag/AgCl/KCl), with a potential of +199 mV vs. NHE (the normal hydrogen electrode) was used. Ti-6Al-4V alloy sheets with the composition presented in the Table 1 were cut into specimens with dimensions of 2.5 x 2.5 cm and 2 mm in thickness.

**Table 1.** Chemical composition of Ti-6Al-4V alloy.

Specification	8-12-05832-1	N	Al	C	V	H	Fe	O	Ti
Ti-6Al-4V Grade 5	Max. [%]	0.003	6.01	0.008	3.83	0.002	0.083	0.088	89.976
	Min. [%]	0.003	5.86	0.008	3.73	0.002	0.068	0.084	90.245

The titanium alloy samples were ground using successively finer SiC abrasive papers (320 - 4000 µm), diamond paste (3 and 1 µm) and a suspension of SiO<sub>2</sub> solution (0.04 µm size of particles), finally achieving a mirror finish.

On each sample, the electrical contact was made by soldering a copper wire, after which all

surfaces, including the contact, were insulated with epoxy resin to obtain a well measurable active surface of 4.32 cm<sup>2</sup>. Before each corrosion test, samples were cleaned with ethyl alcohol and then dried.

In order to simulate the physiological conditions of the human body, Saliva Biological Solution was used for in vitro corrosion studies. Its chemical composition is given in Table 2. The corrosion resistance of the Ti–6Al–4V alloy was determined in saliva Fusayama Meyer doped with 8 mL / L Albumin, saliva Fusayama Meyer doped with 8 mL / L H<sub>2</sub>O<sub>2</sub> and saliva Fusayama Meyer doped with mixed 8 mL / L Albumin and 8 mL / L H<sub>2</sub>O<sub>2</sub>. The albumin used to prepare the testing solutions is a pharmaceutical human albumin 200 mg/mL. The used hydrogen peroxide is of analytical grade having a concentration of 30 %.

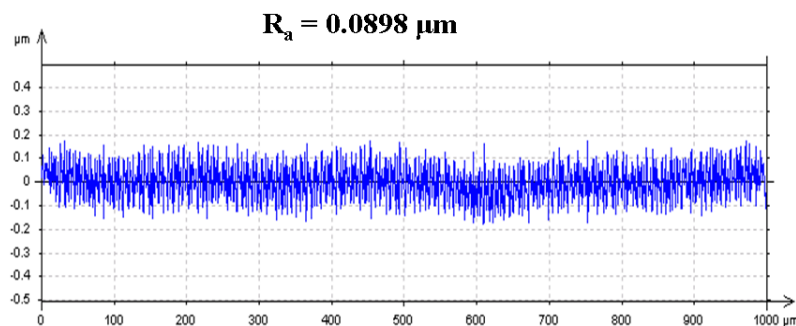
**Table 2.** Chemical composition of saliva Fusayama Meyer.

Nr. Crt.	Compound	Saliva Fusayama Meyer
		g / L
1.	NaCl	0,4
2.	KCl	0,4
3.	CaCl <sub>2</sub>	0,8
4.	NaH <sub>2</sub> PO <sub>4</sub>	0,79
5.	UREA	1
6.	pH	4.7

The volume of solution used for each experiment was 210 mL. All the experiments were conducted at room temperature 25°C and each experiment were repeated at least three times to check the reproducibility. Electrochemical measurements such as Open Circuit Potential (OCP) and Electrochemical Impedance Spectroscopy were applied for corrosion investigations.

### 2.2. Surface roughness measurements

The roughness of titanium alloy samples was measured after polishing using a high resolution microtopography, STIL, equipped with optical fiber for light signal capture and intensity analysis with a side resolution of 1 µm and a vertical resolution of 30 nm. The captured images are transformed into a 2D profile surface and 3D surface with Surface Map software. The surface roughness obtained for titanium alloy samples show a mean value of 0.0898 µm, Figure 1.



**Figure 1.** 2D surface profile of surface roughness for Ti-6Al-4V.

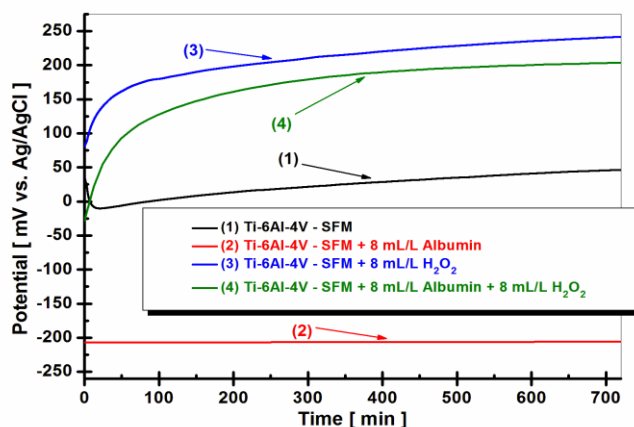
## 3. Results and discussions

### 3.1. Open circuit potential (OCP)

The open circuit potential analysis is an electrochemical method which indicates the tendency of a material to electrochemical oxidation immersed in a corrosive environment. After a period of

immersion, it stabilizes around a stationary value. This potential may vary with time, because changes occur of the nature of the electrode surface such as (oxidation, passive layer formation or immunity).

The open circuit potentials were monitored during the exposure time of 12 h until it has been reached a stable state value vs. Ag/AgCl reference electrode used. The potential time measurements of Ti-6Al-4V alloy determined in saliva Fusayama Meyer doped with 8 mL / L Albumin, saliva Fusayama Meyer doped with 8 mL / L  $\text{H}_2\text{O}_2$  and saliva Fusayama Meyer doped with mixed 8 mL / L Albumin and 8 mL / L  $\text{H}_2\text{O}_2$  are shown in Figure 2.



**Figure 2.** Open circuit potential evolution during immersion time of 12 h for: (1) Saliva Fusayama Meyer; (2) Saliva Fusayama Meyer with 8 mL / L albumin; (3) Saliva Fusayama Meyer with 8 mL / L  $\text{H}_2\text{O}_2$  and (4) Saliva Fusayama Meyer with mixed 8 mL / L albumin and 8 mL / L  $\text{H}_2\text{O}_2$ .

From Figure 2 it can be seen that the addition of 8 mL / L  $\text{H}_2\text{O}_2$  to Fusayama Meyer saliva shifted the open circuit potential value to more positive value as compared with saliva Fusayama Meyer without addition of  $\text{H}_2\text{O}_2$ . This trend can be explained by the fact that in the presence of  $\text{H}_2\text{O}_2$  the cathodic reaction is likely to be dominated by the reduction reaction of  $\text{H}_2\text{O}_2$  since  $\text{H}_2\text{O}_2$  is a strong oxidizing compound and has a high standard reduction potential leading to a higher cathodic current density and also resulting in a more positive OCP value [22, 23].

For Ti-6Al-4V immersed in Fusayama Meyer doped with 8 mL / L Albumin the OCP value is almost constant throughout the immersion period, and the stationary potential is already reached starting from the immersion time, being shifted to more negative value as compared with the potential measured in saliva solution without any addition. This almost constant potential is associated with improved corrosion resistance. The lower OCP value attained by titanium alloy in Fusayama Meyer doped with 8 mL / L Albumin in comparison with saliva Fusayama Meyer doped with 8 mL / L  $\text{H}_2\text{O}_2$  can be attributed to the presence of albumin into saliva which significantly inhibits the cathodic reaction of Ti-6Al-4V resulting in a lower value of OCP.

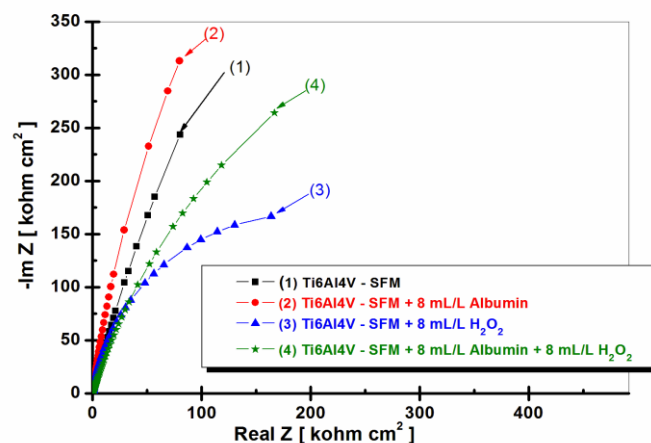
The OCP value of Ti-6Al-4V alloy immersed in Fusayama Meyer doped with mixed 8 mL / L Albumin and 8 mL / L  $\text{H}_2\text{O}_2$  was close to that in Fusayama Meyer saliva doped only with 8 mL / L  $\text{H}_2\text{O}_2$ , but shifted slowly to more negative values, suggesting that the presence of albumin inhibits the cathodic reaction of Ti-6Al-4V in  $\text{H}_2\text{O}_2$  containing solutions.

### 3.2. Electrochemical impedance spectroscopy (EIS)

EIS measurements were performed in the frequency range between 100 kHz and 10 mHz, with an AC sine wave amplitude of 10 mV, 10 Hz frequency per decade and 0.1 s delay before integration. The

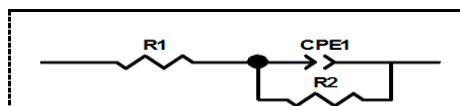
electrochemical impedance spectroscopy diagrams were recorded after 720 min of immersion at free potential. All the recorded impedance spectra were analyzed as Nyquist and Bode diagrams. The obtained data were recorded using ZView 3.4e software and the quality of the fitted results was evaluated with the chi-square value that was lower than  $10^{-3}$ .

The impedance data recorded for Ti-6Al-4V immersed in saliva Fusayama Meyer, saliva Fusayama Meyer doped with 8 mL / L Albumin, saliva Fusayama Meyer doped with 8 mL / L  $H_2O_2$  and saliva Fusayama Meyer doped with mixed 8 mL / L Albumin and 8 mL / L  $H_2O_2$  are displayed in the form of a Nyquist plot in Figure 3.



**Figure 3.** The Nyquist representation of EIS results for titanium alloy immersed in: (1) Saliva Fusayama Meyer; (2) Saliva Fusayama Meyer with 8 mL / L albumin; (3) Saliva Fusayama Meyer with 8 mL / L  $H_2O_2$  and (4) Saliva Fusayama Meyer with mixed 8 mL / L albumin and 8 mL / L  $H_2O_2$ . The plain symbols represent the experimental data and the continuous lines represent the fitted results.

In order to fit the EIS specters recorded for Ti-6Al-4V immersed in saliva Fusayama Meyer, saliva Fusayama Meyer doped with 8 mL / L Albumin, saliva Fusayama Meyer doped with 8 mL / L  $H_2O_2$  and saliva Fusayama Meyer doped with mixed 8 mL / L Albumin and 8 mL / L  $H_2O_2$  it was used a simple Randles equivalent circuit showed in Figure 4 composed by  $R_1$  as the electrolyte resistance,  $CPE_1$  and  $R_2$  as the constant phase element and the specific resistance instead of polarization resistance (the semicircle did not intersect the real axis of Nyquist EIS diagram of Ti-6Al-4V alloy).



**Figure 4.** Schematic representation of Randles equivalent circuit of Ti-6Al-4V alloy for fitting of electrochemical impedance spectroscopy experimental data.

The constant phase element (CPE) was used instead of a pure capacitor to explain the deviation of impedance data from a semicircle to a depressed semicircle due to inhomogeneous surfaces, which

could have different adsorbed compounds connected with tested solutions. The impedance expression with a constant phase element instead of capacitance, can be written with the equation [24]:

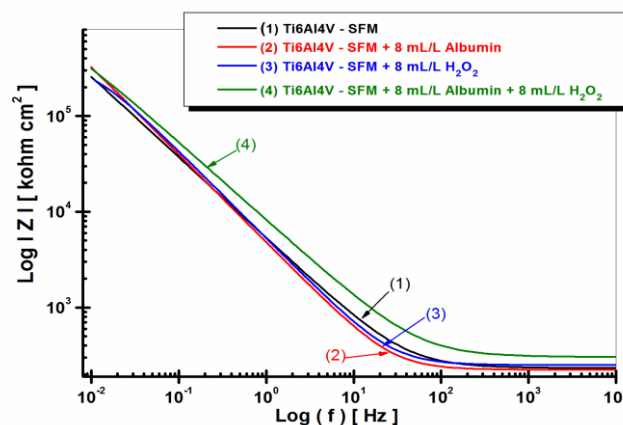
$$Z_{CPE} = \frac{1}{Q(j\omega)^n} \quad (1)$$

where:  $Q$  is a constant measured in  $F \cdot cm^{-2} \cdot s^{(n-1)}$ ,  $n$  is a value representing the deviation from purely capacitive behavior,  $j = \sqrt{-1}$  is an imaginary number,  $\omega$  represent the angular frequency. When the  $n$  value is 1 pure capacitive behavior is observed and  $Q$  is the layer capacitance.

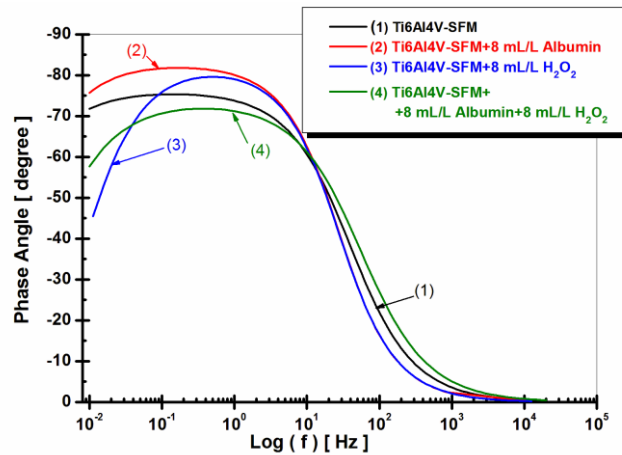
The specific polarization resistance resulting from fitted results using the proposed equivalent circuits reveals that for Ti-6Al-4V alloy immersed in saliva Fusayama Meyer, the value is around  $10972 \text{ kohm} \cdot cm^2$ . The addition of 8 mL / L  $H_2O_2$  into saliva Fusayama Meyer increases the rate of anodic and cathodic reactions and the specific polarization resistance decreases up to  $385.40 \text{ kohm} \cdot cm^2$  being the smallest one. On the other hand, the addition of 8 mL/L albumin in saliva Fusayama Meyer causes the inhibition of anodic and cathodic reactions and the specific polarization resistance increases until  $17000 \text{ kohm} \cdot cm^2$ . In the presence of both,  $H_2O_2$  and albumin in saliva Fusayama Meyer, the specific polarization resistance of Ti-6Al-4V alloy decreases up to  $798.56 \text{ kohm} \cdot cm^2$ . The value is ranged between the polarization resistance of titanium alloy resulted in purely Fusayama Meyer solution and those resulted from immersion in saliva Fusayama Meyer with 8 mL / L  $H_2O_2$  added. The mixed  $H_2O_2$  and albumin added into saliva Fusayama Meyer increases the rate of anodic reaction simultaneously with the inhibition of cathodic reaction rate and as a result an increase of the corrosion rate of Ti-6Al-4V alloy (decrease of specific resistance).

Similar results were obtained by other authors [22] when evaluated the synergetic effect of bovine serum albumin and  $H_2O_2$  added in physiological solution on corrosion behavior of Ti-6Al-4V.

The EIS diagrams were fitted very well also for Bode representations of EIS results as logarithm of module  $Z$  versus logarithm of frequency and phase angle versus logarithm of frequency as they are shown in Figure 5 and Figure 6.

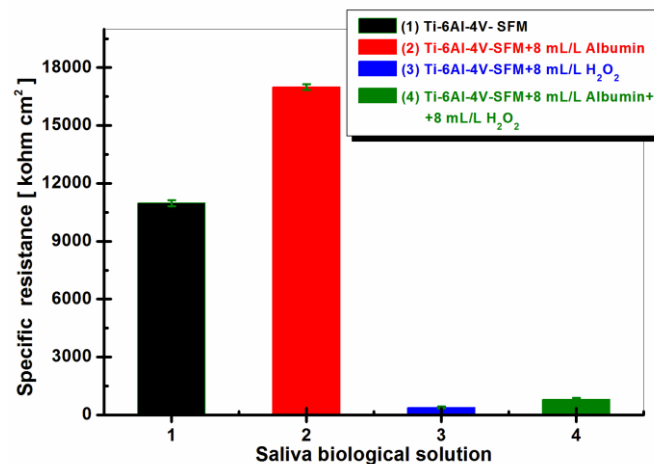


**Figure 5.** Bode representation of EIS data as Module  $|Z|$  versus frequency in logarithmic scale for Ti-6Al-4V immersed in: (1) Saliva Fusayama Meyer; (2) Saliva Fusayama Meyer with 8 mL / L albumin; (3) Saliva Fusayama Meyer with 8 mL / L  $H_2O_2$  and (4) Saliva Fusayama Meyer with mixed 8 mL / L albumin and 8 mL / L  $H_2O_2$ .



**Figure 6.** Bode representation of EIS data as Phase Angle versus logarithm of frequency in for Ti-6Al-4V immersed in: (1) Saliva Fusayama Meyer; (2) Saliva Fusayama Meyer with 8 mL / L albumin; (3) Saliva Fusayama Meyer with 8 mL / L H<sub>2</sub>O<sub>2</sub> and (4) Saliva Fusayama Meyer with mixed 8 mL / L albumin and 8 mL / L H<sub>2</sub>O<sub>2</sub>.

For a better understanding of the results explained above, in Figure 7 are highlighted the effect of the addition of 8 mL / L albumin, 8 mL / L H<sub>2</sub>O<sub>2</sub> and the synergistic effect of these two mixed components on the specific polarization resistance resulted from electrochemical impedance spectroscopy measurements of Ti-6Al-4V alloy immersed in saliva Fusayama Meyer.



**Figure 7.** The effect of albumin and reactive oxygen species as H<sub>2</sub>O<sub>2</sub> resulted from inflammatory conditions on specific polarization resistance obtained from EIS data and fitted results.

From Figure 6 it can be seen that with the addition of 8 mL / L albumin into saliva Fusayama Meyer the specific polarization resistance of Ti-6Al-4V increase in comparison with Ti-6Al-4V immersed in saliva Fusayama Meyer. It is generally accepted that the addition of albumin decreases the cathodic reaction on Ti alloys, indicating that albumin serves as a cathodic inhibitor [22].



#### 4. Conclusion

The aim of this research paper was to investigate the effect of albumin and inflammatory conditions to corrosion resistance of Ti-6Al-4V in saliva Fusayama Meyer biological solution. In order to simulate the peri-implant inflammatory conditions in vitro studies were conducted with addition of hydrogen peroxide (reactive oxygen species, found during inflammation) and albumin the most typical protein of biological fluids.

The experimental results reveal that the addition of 8 mL / L  $H_2O_2$  into saliva Fusayama Meyer increases the rate of anodic and cathodic reactions and the specific polarization resistance decreases up to 385.40 kohm  $cm^2$ . On the other hand, the addition of 8 mL / L albumin in saliva Fusayama Meyer cause the inhibition of anodic and cathodic reactions and the specific polarization resistance increases until 17000 kohm  $cm^2$ .

In the presence of both,  $H_2O_2$  and albumin in saliva Fusayama Meyer, the specific polarization resistance of Ti-6Al-4V alloy decreases up to 798.56 kohm  $cm^2$  proving the dominant effect of reactive oxygen species. The  $H_2O_2$  and albumin added into saliva Fusayama Meyer increases the rate of anodic reaction simultaneously with the inhibition of cathodic reaction rate and increases the corrosion rate of Ti-6Al-4V alloy.

The research work conclude that the addition  $H_2O_2$  into saliva Fusayama Meyer decreases the corrosion resistance of Ti-6Al-4V alloy while the addition of albumin in saliva Fusayama Meyer increases the corrosion resistance of Ti-6Al-4V alloy, the presence of albumin strongly inhibits the cathodic reaction and slightly inhibits the anodic reaction of Ti-6Al-4V alloy in saliva Fusayama Meyer resulting in a lower OCP value.

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