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"DUNĂREA DE JOS" UNIVERSITY OF GALAȚI

DOCTORAL SCHOOL OF FUNDAMENTAL AND ENGINEERING SCIENCES

# PHD THESIS

## PhD Thesis Summary

# THE EFFECT OF SURFACE MODIFICATION OF THE Ti6Al4V ALLOY UPON THE BEHAVIOR IN THE IMPLANT BIOLOGICAL ENVIRONMENT UNDER INFLAMMATORY CONDITIONS

**PhD student,**
**Pharm. Anca RĂVOIU (LUPU)**

**PhD Supervisor,**
**Prof. univ. dr. chem. Lidia BENEA**

**Seria I5: Materials Engineering Nr. 19**
**GALAŢI**
**2023**

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**Domeniul fundamental MATEMATICĂ ȘI ȘTIINȚE ALE NATURII**

Seria C: **Chimie**

**Domeniul fundamental ȘTIINȚE BIOLOGICE ȘI BIOMEDICALE**

Seria M: : **Medicină**

"Everything is theoretically impossible, until it is done."

Robert A. Heinlein.

# CONTENT

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# INTRODUCTION

This thesis is the result of the research studies carried out within the Competences Center: Interfaces-Tribocorrosion and Electrochemical Systems (CC-ITES), Faculty of Engineering of "Dunărea de Jos" University of Galați and whose director is Professor Lidia BENEA, Ph.D.

**The general objective** of the research presented in this thesis is to improve the surface of the Ti6Al4V titanium alloy by forming thin oxide films with the help of the electrochemical method of anodic oxidation, using sulfuric acid of 1M concentration as anodizing electrolyte. The synergistic effect of hydrogen peroxide and albumin on the corrosion resistance of treated and untreated Ti6Al4V alloy was investigated. In order to execute the corrosion tests, Hank's biological solution doped with different concentrations of albumin and hydrogen peroxide was used. In this study, it was specifically aimed that the Ti6Al4V titanium alloy surfaces, on which thin oxide films were formed following the anodic oxidation process, to have biomedical applications by increasing the corrosion resistance in simulated body fluid (SBF).

**The specific objectives** consisted of:

Studying the effect of the electrochemical parameters that were imposed during the anodic oxidation process upon the corrosion resistance of the thin films formed on the surface of the Ti6Al4V titanium alloy.

Studying the corrosion mechanism of thin films formed on the surface of the Ti6Al4V titanium alloy. As an electrolyte, Hank's solution doped with different concentrations of albumin and hydrogen peroxide was used.

The study of morphology (SEM), composition (EDX), roughness, microhardness, hydrophobicity by measuring the wetting degree of the surface of the films formed following anodic oxidation and the layer thicknesses of the thin films formed on the Ti6Al4V titanium alloy.

The structure study by X-ray diffraction (XRD) of the Ti6Al4V titanium alloy and of the thin oxide films formed on the alloy's surface.

The doctoral thesis has 204 pages structured in two parts: a theoretical part that represents approximately 18% of the study and an experimental part that represents personal contributions in the approached field. The thesis contains a bibliographic study with a total of 365 references. Of all 365 bibliographical references that are presented in the thesis, 5 are personal studies, one of them being ISI rated (clarivate Analytics). The doctoral research study has 87 figures and 16 tables.

**The theoretical part** of the study **(Chapter I)**, **SYNTHETIC ANALYSIS OF THE ACHIEVEMENTS IN THE SUBJECT'S FIELD**, presents a synthesis of the research studies presented in the specialised literature in the last time period, both national and international, regarding the field of biomaterials and their applications, materials used in implants, separated per categories, their primary applications as implants, corrosive environments that affect implant materials, biological fluids and inflammatory conditions, different methods and techniques for improving the surface of titanium alloys for biomedical applications, factors that influence obtaining functional layers on biomaterials to improve their corrosion resistance in biological implant environments, specific characterization of functional layers on biomaterials in corrosive biological systems and biomedical applications of functional layers.

In **chapter (II)**, entitled **RESEARCH DIRECTIONS. THE MAIN PROPOSED OBJECTIVES**, the perspectives and the layout of new research directions were presented regarding the approached research theme.

**The 2nd part** of the study presents the sum of own experimental results that are developed in 5 chapters.

In **chapter III**, entitled **MATERIALS, METHODS AND EXPERIMENTAL TECHNIQUES**, the materials, methods and experimental techniques are presented and also structured on two main research directions: the study of the surface modification of titanium-based biomaterials by controlled electrochemical oxidation and the comparative study of nanoporous films of titanium oxide ($TiO_2$) and untreated titanium alloy in biological solutions in the absence and presence of proteins and compounds present in inflammatory conditions.

In order to obtain titanium oxide ($TiO_2$) films on the Ti6Al4V grade 5 implant alloy and to characterise the untreated alloy and the titanium oxide films, it required a rigorous substantiation of the morphological, structural and mechanical in-situ and ex-situ electrochemical characterization methods that are applied in the field of materials science and engineering.

Corrosion resistance evaluation studies in biological solution and/or in the presence of protein and inflammatory compounds were completed with ex-situ methods for evaluating roughness, microhardness, hydrophobicity, morphology, composition and structure of titanium oxide nanoporous films.

In **chapter IV**, entitled **THE INFLUENCE OF THE ELECTROCHEMICAL PARAMETERS FOR THE FORMATION OF THIN OXIDE FILMS UPON THEIR PROPERTIES**, the influence of parameters (potential and duration) used in the anodic oxidation process, comparative analysis of roughness and microhardness of titanium oxide films and untreated alloy is presented. The chapter ends with partial conclusions to the study.

In **chapter V**, entitled **MORPHOLOGICAL, STRUCTURAL, TOPOGRAPHIC AND HYDROPHOBICITY CHARACTERIZATION**, comparative studies are presented regarding the morphological and compositional analysis of titanium alloy surfaces and oxide films by electron microscopy (SEM-EDX) and the composition of treated and untreated Ti6Al4V alloy surfaces through the anodic oxidation process. Also, structural characterisation

by X-ray diffraction (XRD) and surface hydrophobicity of untreated titanium alloy compared to oxide films are presented.

**Chapter VI**, entitled **ELECTROCHEMICAL CHARACTERIZATION OF THE TITANIUM ALLOY AND OXIDE FILMS IN BIOLOGICAL FLUIDS**, contains a comparative evaluation study regarding corrosion resistance of thin oxide films obtained by anodic oxidation with the help of electrochemical methods such as Open circuit potential (OCP), polarization resistance ($R_p$) and Corrosion rate ($V_{cor}$). At the end of the chapter, partial conclusions from this study are presented.

In **chapter VII**, entitled **ELECTROCHEMICAL CHARACTERIZATION OF THE TITANIUM ALLOY AND OXIDE FILMS IN BIOLOGICAL FLUIDS UNDER INFLAMMATORY CONDITIONS**, a comparative analysis was carried out regarding the corrosion resistance of the untreated and anodic oxidized Ti6Al4V titanium alloy. As an electrolyte for testing the corrosion resistance of the analysed samples, Hank's solution was used, doped with different concentrations of albumin and hydrogen peroxide. The Open circuit potential (OCP), polarization resistance ($R_p$) and Corrosion rate ($V_{cor}$) were monitored. At the end of the chapter, partial conclusions of the results of corrosion resistance are presented.

**Chapter VIII**, entitled **GENERAL CONCLUSIONS, PERSPECTIVES, EXPLOIT AND IMPACT OF THE RESEARCH RESULTS**, summarises the conclusions of the experimental results of the doctoral thesis, in the field of characterization of the corrosion resistance of thin films of Ti6Al4V titanium alloy in biological fluids. Also, perspectives and new research directions of the approached theme are presented. The chapter also presents scientific accomplishments that are publications in journals ISI rated (Clarivate Analytics), in conference journals, publications in journals from international databases and participation in international and national scientific events.

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# CHAPTER I

# SYNTHETIC ANALYSIS OF THE ACHIEVEMENTS IN THE SUBJECT'S FIELD

## 1.1. General aspects regarding titanium alloys as biomaterials

The use of metallic materials for medical implants dates since the 19$^{th}$ century [1.2-1.3] and continues to be widely used for manufacturing systems or implants, in order to replace lost or bad biological structures and to restore their form and function [1.4].

The success of biomaterials mainly depends on the human body's reaction to the implant, thus determining the biocompatibility of a material [1.4]. A biomaterial is devised to be used in direct contact with living tissue and is essential that the implanted material is not toxic and does not cause harmful effects such as inflammations or allergies [1.2, 1.4]. Problems generated by biocompatibility are thrombosis, which involves blood coagulation and adhesion of blood clots to the surface of the biomaterial and fibrous tissue encapsulation that confines the implant and prevents it from interacting with the surrounding tissue [1.9]. Rejection of an orthopedic implant due to the toxic release of metal ions will ultimately lead to failure [1.2].

## 1.2. Corrosive environments that affect implant materials

The human body is not a friendly environment for any implanted metallic alloy, as it is a highly oxygenated saline electrolyte that has a pH of approximately 7.4 and a temperature of 37$^{o}$C [1.34]. Although, it is well known that solutions containing chlorine are among the most aggressive and corrosive for metals, the ionic composition and protein concentration of body fluids further complicates the understanding of the phenomenon of biomaterials corrosion [1.35].

To understand the biological degradation of implant materials, the synergic actions that take place have to be taken into account [1.74-1.76]. For example, the cracks associated with stress cracking open a new surface to the electrochemical reaction in metallic biomaterials [1.77]. Inflation and water absorption can simultaneously increase the number of places for the same type of reaction. Degradation products can modify the local pH and stimulate future reactions [1.78, 1.79]. Also, cracks can become places for the initiation of calcification [1.73].

In time, due to the severity of the biological environment, the implant can dissolve,

crumble, or become rubbery or rigid [1.73].

The biological fluids of the human body are solutions with a complex chemical composition that contain various quantities of inorganic salts, amino acids, sugars, proteins and other components and their content depends on many factors [1.80].

## 1.3. Biological fluids and inflammatory conditions

From a clinical point of view, inflammation is characterised by redness, warmth, swelling, sensitivity and loss of function in the affected organ [1.81]. At cellular level, it is characterised by tissue damage associated with a local accumulation of inflammatory leukocytes, including neutrophils, eosinophils, macrophages, lymphocytes and platelets [1.82]. Inflammation is actually an essential defence mechanism against the invasion of the body by microorganisms and elements that it considers foreign [1.83]. Examples of these kinds of inflammations are asthma in the lungs, urticaria in the skin and arthritis in the joints [1.84, 1.85]. Clearly, inflammatory responses can differ in nature to a large extent depending on the stimulus that led to their initiation [1.86].

Inflammation caused by the modification of the tissues' acidity affects the process of passivating the surface of a metal implant during friction [1.87]. One of the most important and at the same time main factors is the change in acidity [1.89]. Inflammation at the level of muscle and bone tissues is caused by the acid that attacks the invading bacteria and foreign bodies [1.90]. Its effect is a significant increase in acidity due to the plasma lysates and lactates.

## 1.4. Different methods and techniques for improving the surface of titanium alloys for biomedical applications

Titanium (Ti) and its alloys continue to be the first choice for biomedical applications, especially for bone anchoring systems like dental, orthopedic implants and osteosynthesis applications [1.112]. The stability of titanium for long implantation times is due to its high resistance to corrosion thanks to the formation of an oxide layer that has a major role in inducing the differentiation of bone cells and the adsorption of proteins [1.113].

The surface of the titanium and its alloys must be modified in order to fulfill all critical aspects necessary for the implant requirements.

The first and major objective for any surface treatment, for changing properties such as specific mechanical structure, topography, surface hardness, various electronic modifications and chemical functionalities, is to improve the biocompatibility of the medical device [1.114].

Also, through surface modifications of titanium and its alloys, indispensable and improved properties can be achieved for an implant, as well as good mechanical attachment between the bone and implant, bone inductivity and conductivity, excellent resistance to corrosion and wear, bioactivity and biocompatibility and shorter healing time [1.115].

## 1.5. Factors that influence the obtaining of functional layers on biomaterials to improve their resistance to corrosion in biological implant environments

The performance of implantable medical devices is dictated by the implant's surface [1.129]. Therefore, the implant's surface must be modified in order to avoid any inflammatory response during implantation. Recent research in surface modification offered various strategies in order to ensure a harmonious relation between the implant and its operating environment.

An implant's capacity to link (connect, tie) with the surrounding host bone is another fundamental requirement for dental and permanent orthopedic implants [1.130]. Osseointegration is defined as a direct structural and functional link between the living bone and the surface of a bearing implant [1.131]. Osseointegration can also be defined as the final stage in the functional reconstruction of an implant. Insufficient osseointegration property can lead to the formation of fibrous tissues that can further bring to the loosening of prostheses. Therefore, a surface that integrates well with the adjacent bone is vital for preventing osteolysis. Factors like the design, chemical composition, surface roughness, as well as stress/loading conditions are very important for good osseointegration of the implants [1.132].

## 1.6. Specific characterization of functional layers on biomaterials in corrosive biological systems

When a metallic device is implanted in the human body, it is permanently exposed to extracellular fluid. Through exposure, the metallic surface of the implant suffers an electrochemical dissolution at a finite rate, due to the interactions with the surrounding environment of the host organism. In the case of the human body, this environment contains water, complex organic compounds, dissolved oxygen, sodium, chloride, bicarbonate, potassium, calcium, magnesium, phosphate, amino acids, proteins, plasma, lymph, saliva, etc. At the time of implantation, the tissue environment is destabilized, disrupting the blood supply to the surrounding tissue and the ionic balance. The initiation of corrosion can be the result of different existing conditions along the implant's surface, whether it is the formation of localized electrochemical cells resulting in pitting corrosion or localized corrosion at the interface between a plate and a locking screw or any of the other forms of corrosion that can occur [1.35]. As body fluids come into contact with the surface of the metal implant, corrosion occurs through an electrochemical redox reaction in which oxidation (loss of electrons to the metal) is coupled with reduction (gain of electrons to electrolyte components) [1.145]. The metallic components of the alloy are oxidized to their ionic forms and then dissolved oxygen is reduced to hydroxyl ions. The types of corrosion that are relevant to alloys currently used in implantology are pitting, crevice, galvanic, intergranular, stress cracking and fretting [1.146].

## 1.7. Biomedical applications for using functional layers

In the last decades, surface coating technology has been transformed into an expanded field of materials research. Today's industry demands additional functions from coatings besides their core values such as protection and decorative appearance. This additional function can be diverse and depends on the actual application of a coating to a substrate. Self-cleaning, anti-dirt, easy cleaning, soft feeling are typical examples of coating with functional properties.

Micro/nano structured coatings have proven to be remarkable as surfaces with anti-bacterial functions, anti-fog, anti-reflection surfaces, low-friction surfaces, and have wide applications in medical and optical devices. There is a great need for highly functionalized surfaces and materials with different functions in medicine and biotechnology, such as functional surfaces/materials to improve cell implant interactions or new coatings for mini-enzymatic bioreactors with small surface areas. This application needs both new materials and new processes, as well as new analytical techniques in order to evaluate the materials' quality and functionality.

## 1.8. Partial conclusions

Metallic biomaterials have been and are used mainly to manufacture medical devices for replacing rough tissues like artificial hip joints, bone plates and dental implants because are very reliable in terms of mechanical performance. Titanium and its alloys are of high interest as implantable materials due to their superior resistance to corrosion, good mechanical properties, remarkably high specific resistance, low modulus of elasticity and excellent biocompatibility compared to other competing biomaterials.

The high corrosion resistance of titanium and its alloys is due to the fact that they form a passive oxide film, stable, protective and adherent on their surfaces. This film forms instantly when the titanium surface is exposed to air or humidity. Also, this film, which consists mostly of $TiO_2$, protects the material and offers chemical inertness in different environments. However, the passive state of titanium is not completely stable and under certain conditions (frictional or sliding wear conditions) it has been found, on a microscopic scale that a localized breakdown of the passive oxide film occurs. Consequently, a strong destructive attack can occur at the surface of the material due to its reaction to the hostile operating environment. Any deficiency in the performance of the $TiO_2$ film on the titanium surface is very detrimental to the corrosion behavior, as well as other characteristics necessary for implantation and biocompatibility.

Thus, engineers and scientists searched for various techniques to increase the biocompatibility and the corrosion behavior related to metallic implants with the purpose to make them adapt better to the implantation environment. Therefore, surface modification became one of the most attractive branches in the field of biomaterials research. Surface modification techniques are the most viable alternatives for increased corrosion resistance and the properties associated with the surfaces of biomaterials. Superior behavior to corrosion, improved resistance to wear, better osseointegration, high biocompatibility and perfect aesthetics are the most important characteristics that can be obtained through surface

modification.

Anodic oxidation of titanium is considered an effective surface coating technology for bioimplants. The creation of biofunctional films of titanium oxide by dielectric breakdown provides improved adhesion of the film to the substrate and helps to obtain high-quality films with porous or non-tubular structures by varying electrolytes, temperature, alloying elements, voltage, density current and time.

Also, the anodic oxidation process can increase the thickness of the native oxide layer on the surfaces of the titanium materials, in order to develop corrosion resistance and decrease the release of metal ions with toxic properties.

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# CHAPTER II

# RESEARCH DIRECTIONS. THE MAIN PROPOSED OBJECTIVES

The main objective of this study is to modify the surface of the titanium based implant alloy, Ti6A4V grade 5, through controlled electrochemical oxidation in order to obtain films (thin layers) of nanoporous titanium oxide ($TiO_2$) and to improve the properties and increase the resistance to corrosion degradation in specific biological environments.

For the motivation of the research study, the behavior of the Ti6A4V grade 5 implant alloy in Hank's biological solution and in various solutions based on Hank's solution in the presence of proteins and inflammatory compounds is studied, in order to observe their effect on the degradation of the implant over time.

This study was carried out within the Competences Center: Interfaces-Tribocorrosion and Electrochemical Systems (CC-ITES) of "Dunarea de Jos" University of Galati and a bibliographic study of the most recent research, national and international, regarding the field of functional surfaces and the modification of the biomaterials surfaces by different methods and especially by electrochemical methods was done.

The $TiO_2$ layers were obtained on the Ti6A4V grade 5 alloy at constant voltage and different times.

The characterization of the titanium oxide layers or films obtained on the Ti6A4V grade 5 alloy, as well as the untreated alloy was carried out by scanning electron microscopy equipped with X-ray scattering spectroscopic analysis (SEM-EDX), X-ray diffraction (XRD), optical microscopy (MO), roughness, microhardness and hydrophobicity (by measuring the contact angle).

The results obtained in this study are interpreted with different experimental data simulation programs followed by their correlation.

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# CHAPTER III

# MATERIALS, EQUIPMENT AND EXPERIMENTAL TECHNIQUES

This chapter describes the experimental configuration and the conditions used for this study. The preparation methods of the used electrolytes, the sample preparation methods and the working conditions are presented. The experimental techniques used to analyze the samples from an electrochemical, structural, morphological and topographical point of view are also described.

## 3.1. Materials

### 3.1.1. Ti6Al4V alloy

Titanium was discovered for the first time in 1790, in England, by William Gregor and in 1795, Klaproth gave the name of *titan* [3.1]. The low density, good biocompatibility, corrosion resistance properties and good mechanical properties determine titanium and its alloys to be applied in industries like aviation, auto, energy, shipbuilding, architecture, medicine, and sports equipment.

Titanium and its alloys do not change when they are implanted in the body. This is the result of their excellent corrosion resistance and that is why are named bio-stable or biologically inert [3.2]. The widespread and successful application of titanium and titanium alloys in biomedical devices (implants) is clearly due to the combination of their high corrosion resistance and adequate mechanical performances which in turn make them biocompatible.

It is reported that over 1000 tons of Ti and titanium alloys are used as biomedical devices each year and in modern dentistry the use of titanium alloy implants has revolutionized patient care, resulting in a global market value for these interventions of over 4.5 billion dollars [3.13].

**Titanium Dioxide ($TiO_2$)**

Titanium dioxide ($TiO_2$) is one of the most important compounds of titanium and oxygen and it is an extremely versatile material, suitable for a variety of technological applications [3.19]. $TiO_2$ is found in three distinct crystallinity structures, namely rutile, anatase and brookite. Rutile is the most naturally common form of titanium [3.20] and the

most studied and well-known of the three polymorphic crystallinity structures.

The anatase crystallographic form, which is rarer, compared to rutile, is also of great interest, especially for its key role in electron injection and transport in photovoltaic devices [3.21]. Brookite is the rarest form of the mineral and is not easily obtained synthetically [3.22].

### 3.1.2. Electrolytes for modifying the titanium alloy surface through anodic oxidation

The specialised literature mentions that the best electrolyte to oxidize titanium and Ti6Al4V is sulfuric acid [3.29-3.31]. When titanium is immersed in an electrolyte solution as an anode and current is absorbed, the oxygen generated on the anode surface combines with the reactive titanium to form titanium oxide. The thickness of the oxide layers is a function dependent on the applied potential [3.32], anodizing time [3.33] electrolyte's temperature [3.33] and the electrolyte's concentration [3.33].

In addition to acids, electrolytes such as sodium phosphate and isopropyl phosphate in ethylene glycol [3.36], ammonium pentaborate [3.37], calcium acetate and calcium glycerophosphate [3.38-3.39], can be used to produce anodic oxides on titanium alloys.

For an efficient high voltage anodic oxidation electrolyte using electrolytes containing calcium and phosphorus (0,1 M calcium acetate, $H_3PO_4$, $Ca(NO_3)_2$) the calcium salt requirements are:

a) good solubility,

b) sufficient passivation of the titanium surface at high positive potentials.

These requirements are met only by a few calcium salts.

Taking into consideration the arguments found in the specialised literature, for anodic oxidation of the studied surfaces, the electrolyte 1M $H_2SO_4$ was used for this study.

### 3.1.3. Solutions and electrolytes used for the comparative characterization of surfaces

#### 3.1.3.1. Biological solutions

The biological environment is surprisingly tough and may lead to rapid or slow degradation of biomaterials. When an implant is introduced into the human body, this will be in contact with biological fluids. If we refer to dental implants, saliva is the most important liquid present in the oral cavity, consisting of biochemical compounds with many individual variations [3.40].

Another biological fluid that plays the most important role in the osseointegration of the implant is blood. The vascular nature of the bone, as with many other tissues, ensures that the first tissue to come into contact with an endosseous implant is blood.

Taking into consideration the abovementioned, Hank's biological solution (the solution that best simulates human blood) was used for the corrosion tests in this study [3.18]. The substances used to prepare the solution were of analytical grade and purchased from Lachner.

The solution was prepared and kept in hermetically sealed vials of 5 L, precisely to keep all the parameters of the solution constant throughout the determination period.

Hank's biological solution parameters were determined using the Consort C533 multiparameter, and the results are summarized in Table 3.1.

**Table 3.1.** Physicochemical characteristics of Hank's biological solution

| Electrolyte | pH | Conductivity [mS/cm] | Salinity [ppt] |
|---|---|---|---|
| **Hank** | **7,41** | **14,6** | **8,4** |

### 3.1.3.2. Protein compounds and compounds with inflammatory effect

**Human serum albumin (HSA)** with the molecular formula $C_{123}H_{193}N_{35}O_{37}$, is the most abundant circulating protein in the plasma of healthy individuals (3.5-5 g/dL) and it represents approximately 60% of the total protein content. It is a small globular protein (molecular weight: 66.5 kDa) formed of a single chain of 585 amino acids organized in three structural homologous domains (sites I, II and III).

The main function of HSA is to control the colloidal osmotic pressure (or the oncotic pressure).

It is also an important transport protein for non-esterified fatty acids and also capable to link an extraordinarily diverse range of metabolites, drugs and organic compounds [3.51].

Albumin ensures more than 50% of the antioxidant processes of normal plasma.

Also, **albumin** interferes with the adhesion of neutrophils to the capillary endothelium [3.55] and thereby, it reduces the effect of endothelial inflammation and maintains its integrity.

Protein is a very important complex organic compound in human fluids and is considered the first component that interacts with biomedical alloys after implantation operations because it adsorbs on the surface of implants and affects the corrosion characteristics of the alloys.

**Inflammatory compound, hydrogen peroxide ($H_2O_2$)**

Ti and Ti alloy implants are exposed to complex physiological environments that contain salts, lipids, proteins, as well as living cells and/or bacteria that can generate a variety of biomolecules as a response to external stimulation [3.75-3.76]. Local inflammation can appear around the implant as a result of infection or even as a result of the presence of debris derived from the implant itself [3.77-3.78].

During inflammation, the levels of $H_2O_2$ can increase as direct products of bacteria (at levels that can surpass 5 mM) or immune cells that have migrated to the inflamed site [3.80-3.81].

It was proven that $H_2O_2$ decreases the corrosion resistance of Ti implants [3.82-3.86],

leading to harsher surfaces [3.86-3.87].

Hydrogen peroxide is a colorless liquid with a boiling point of 150.2°C and a melting/freezing point of -0.432°C. It is mixed with water in any proportion and is soluble in ether and alcohol.

## 3.2. Electrochemical methods and techniques for modifying the titanium alloy surface

### 3.2.1. Electrochemical workstation. Electrochemical cell. Electrodes.

Electrochemical anodizing is one of the simplest techniques used to form rough, porous and uniform films (generally oxide/hydroxide combination) on which oxygen ions can diffuse and oxidize the substrate, increasing the film's thickness.

Anodic oxidation is an electrochemical process that after the application of direct current voltages to the electrodes immersed in the electrolyte leads to the oxidation of the metal anode forming a solid oxide layer on the surface [3.99].

To form the $TiO_2$ oxide layers for this study, a direct current voltage source TDK LAMBDA GEN 300–8 device available in the CC-ITES research center was used.

All experiments were performed in a specially designed electrochemical cell connected to a direct current source.

### 3.2.2. Experimental protocols for the oxidation of the Ti6Al4V alloy

**Electrochemical cell:**

A copper wire was attached to the anode (working electrode Ti6Al4V) in order to have electrical contact with the samples and after it was insulated with an epoxy resin, having an active surface of ~10 $cm^2$. Before each experiment, the anode was cleaned with alcohol for 5 minutes in the ultrasound bath and rinsed with distilled water.

The used cathode (auxiliary electrode) was also a Ti6Al4V plate that was subjected to the same protocol as the anode, but the active surface of the sample was 54 $cm^2$.

The volume of the $H_2SO_4$ 1M electrolyte was 150 mL with a solution pH of 0,41.

All tests were conducted at room temperature 22°C ± 1°C and repeated 6 times to verify data reproducibility.

## 3.3. Techniques and methods for the characterization of biomaterials and ex-situ oxide films

### 3.3.1. Morphological and compositional characterization of the biomaterials surfaces and oxide films by scanning electron microscopy (SEM-EDX)

Scanning electron microscopies (SEM) have become powerful and versatile instruments

for material characterisation, especially in the last years, as the size of materials used in various applications continues to decrease [3.109-3.110].

Compared to the conventional optical microscope, an electronic microscope offers numerous advantages, including high magnification, large depth of focus, high resolution and ease of sample preparation and observation [3.109-3.110].

Energy dispersive X-ray spectroscopy (EDX) in SEM is a qualitative and quantitative X-ray microanalytical technique that provides information on the chemical composition of a sample for elements with atomic number (Z) >3 [3.109-3.112]. In order to determine the morphological and compositional variations on the samples made for this study, a scanning electron microscope called FEI QUANTA 200 (device of the Faculty of Science and Environment, "Dunarea de Jos" University of Galati) was used. The EDAX GENESIS software was used for the analysis of compositional variations by EDX. A number of five samples from each study were analysed at SEM-EDX for this thesis.

### 3.3.2. Microtopographic analysis of the biomaterials surfaces and oxide films with the profilometer

The roughness of the biomaterial's surface is an important factor and it is known that selectively influences cell adhesion and in some cases supports cell differentiation [3.118-3.120].

The surface topography is a property of an implant that will determine its surface quality. The implant's quality surface will depend on the chemical, physical, mechanical and topographic properties of the surface and it is an important factor for the successful integration of the implant into the human bone. Surface topography refers to the surface's level of roughness and the orientation of irregularities on the surface of a sample.

In order to determine the surface roughness for the samples of this study, the contact method was used. 2D topography was done with portable equipment, Surftest SJ-210 (Mitutoyo).

### 3.3.3. Comparative analysis of microhardness of Ti6Al4V alloy and oxide films

The mechanical properties of biomaterials are of great importance in designing orthopedic and dental implants.

The Vickers procedure is used to test the hardness of metals and other equal materials. Like any other hardness test, the Vickers procedure uses its own hardness unit. This is called Vickers Pyramid Number (HV) or Diamond Pyramid Hardness (DPH).

For the tests of this study, a Leitz microdurimeter (Germany, GMBH Wetzal) equipped with a diamond indenter was used. This is equipped with a standard Vickers type diamond penetrator and the force applied to the surface of the tested samples is 0.1 kgf / $mm^2$ for 15 seconds. A number of 5 samples were analyzed and in order to have an average of the values, 3 measurements were made in different areas on each studied surface. The average values

obtained can be found in chapter IV.

### 3.3.4. Structural analysis with the X-ray diffractometer

X-ray diffraction (XRD) is a powerful tool widely used in research and industry and is a non-destructive technique of significant importance for the characterization of crystalline materials.

XRD is a technique that allows verifying the crystallinity and structure of a sample but does not offer chemical information [3.115].

The XRD analysis for the samples obtained in this study was done with a Dron 3 equipment with an x-ray source of radiation Kα ($\lambda_{Ka}$ = 1,790300 Å). This will analyse and identify crystalline compounds through the Brag Brentano method. The various parameters such as scan step size, collection time, interval, X-ray tube voltage and current must be fixed based on the requirements' analysis of the analyzed sample. For the interpretation of the spectra, the software Match!3 correlated with the standard database Crystallography Open Database (COD) was used, the crystalline phases being identified with a code composed of 9 digits.

Each analysed sample will be exposed to XRD analysis for 3 seconds, on a domain of 15$^o$ – 90$^o$, at a voltage of 35 kV with an intensity of 20mA, the measurement step being 0.02$^o$/s.

### 3.3.5. Comparative analysis of hydrophobicity of Ti6Al4V alloy and oxide films (contact angle measurement)

Biomaterials make contact with living tissue and their success requires adequate properties in order to avoid unwanted answers from the host, like thrombosis, inflammatory reactions and infections [3.125-3.127]. The destiny of metallic biomaterials under biological conditions depends on many factors like wetting - essential property to ensure the desired biological response. The contact angle (θ) of the biomaterials' surface is the angle formed by the intersection of the solid-liquid and liquid-vapor interfaces and it is obtained by drawing a tangent along the liquid-vapor interface.

Water molecules will meet the surface, which is followed by protein absorption. This will allow the attachment and future recognition of the host cells [adapted after 3.118]

In order to determine the contact angle for the samples of this study, the sessile drop method was used with the help of the OCA 15 EC instrument, Dataphysics (shown schematically in figure 3.14), Germany, and the analysis software SCA 20.

To obtain the average values of the contact angle, 5 measurements were performed for each sample. Hank's biological solution was placed in the test syringe, the tests were carried out at human body temperature of 37$^o$C and the volume of solution dispensed for each drop was of 15 μL.

## 3.4. Techniques and methods for the characterization of biomaterials and in-situ oxide films

### 3.4.1. Electrochemical workstation. Electrodes.

The most commonly used electrochemistry system (electrochemical cell) is the system with three electrodes that consist of a WE (the material to be studied), a reference electrode (RE) and an auxiliary electrode (AE).

The working electrode makes contact with the electrolyte because its surface is the place where the reaction takes place. In the electrochemical cell, the tip of the reference electrode must be placed as close as possible to the working electrode.

The reference electrode has a known reduction potential, but no current flows through it. It only acts as a reference when measuring the WE potential.

To study the corrosion behavior of the Ti6Al4V alloy, a PGZ 100 electrochemical station (equipment available in the CC-ITES center) was used for this study.

The experimental setup consisted of an electrochemical cell with three electrodes WE (Ti6Al4V), RE (Ag/AgCl with a saturated solution of KCl, with a potential of + 199 mV vs. SHE) and an auxiliary electrode made of a network of Pt-Rh.

### 3.4.2. Evaluation of corrosion resistance in specific biological solutions through direct current (DC) electrochemical methods

Corrosion resistance evaluation of materials in specific environments of use is the best example of applied electrochemistry. Scientists have developed a series of electrochemical methods to study the phenomenon of material degradation through corrosion.

The great advantages of the electrochemical methods are the relatively short measurement time, high precision and the possibility of continuous monitorization of the corrosion process.

**The open circuit potential (OCP) or the equilibrium potential vs. time** is the starting point for almost all types of electrochemical experiments in order to study the phenomenon of corrosion [3.134].

**Another direct current method is the evaluation of the polarization resistance ($R_p$) and the corrosion rate ($V_{cor}$) from the linear polarization curves (PL)**. Linear polarization can be used to characterise the electrolyte-material pair by scanning the potential-current (i-E) in a narrow domain potential (linear), where Tafel slopes can be drawn.

### 3.4.3. Experimental protocols for the electrochemical characterization

A PGZ 100 electrochemical station connected to a PC equipped with VoltaMaster4 software was used to study the corrosion behavior of the obtained oxide layers.

**Electrochemical protocol:**

When the sample was immersed in the biological solution or the biological solution doped with inflammatory compounds and proteins, sequences of electrochemical measurements of the open potential (OCP) and polarization resistance (Rp) – corrosion rate ($V_{cor}$) were performed.

The working electrode was connected to a copper wire to have electric contact and after, the sample was isolated with epoxy resin in order to limit a measurable and well-defined active surface.

Before each experiment, both the analysed sample and the counter-electrode were washed with distilled water and the electrolyte volume (the biological solution) used for each experiment was kept constant. The tests were repeated three times in order to verify the reproducibility of the experimental data.

## 3.5. Partial conclusions

This chapter describes the main materials used to carry out this research study.

General information about the used solutions and electrolytes are presented in the experimental part and the modification of the surface of the titanium alloy by the electrochemical method of anodic oxidation. From the carried out research, it appears that the best electrolyte used for the controlled growth of oxide layers on titanium alloys is sulfuric acid of 1M concentration.

The biological solutions are described and the choices regarding the use of albumin and of hydrogen peroxide as dopants in Hank's biological solution for the electrochemical characterization of the untreated titanium alloy and titanium oxide films obtained on it, are justified.

The main characterization techniques and methods of biomaterials and ex-situ oxide films (SEM-EDX, XRD, roughness, microhardness, contact angle) are also presented and described.

Also, the characterization techniques and methods of biomaterials and in-situ oxide films are presented. Here direct current electrochemical methods are described for the corrosion behavior evaluation of the alloy and the oxide films obtained in this study.

Finally, the experimental protocols used to obtain oxide layers on the Ti6Al4V alloy are described and also, those for the electrochemical characterization of the obtained oxide layers.

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# CHAPTER IV

# THE INFLUENCE OF THE ELECTROCHEMICAL PARAMETERS FOR THE FORMATION OF THIN OXIDE FILMS UPON THEIR PROPERTIES

In this chapter, the influence of the electrochemical parameters imposed on the anodic oxidation of the Ti6Al4V grade 5 alloy was studied in order to obtain titanium oxide ($TiO_2$) films with improved properties and the comparative characterization of the roughness and microhardness properties.

## 4.1. Variation of the anodizing current density at a constant imposed potential

After the experimental tests, the most efficient potential for obtaining titanium oxide ($TiO_2$) films on the Ti6Al4V grade 5 alloy was 200 V.

A decrease in the current density was observed during the sequencing period for all the times used in the electrochemical oxidation of the Ti6Al4V grade 5 alloy.

## 4.2. Comparative analysis of the surface roughness of the Ti6Al4V grade 5 alloy and the electrochemically formed titanium oxide films

At the moment, titanium implants are the standard treatment in dental implantology. They are approximately divided into three different types according to their surface roughness ($S_a$): machined/minimally rough ((± 0.5 μm), moderately rough (1.0–2.0 μm) and rough (> 2.0 μm). Generally, rougher surfaces of the implant have a bigger contact between the bone and the implant [4.1].

It is well-known the fact that roughness is an important factor that determines the osseointegration of the dental implant. Plus, rough surfaces increase the bone's contact with the implant. In the last years, various studies have been carried out with the purpose to find a surface roughness that maximizes the bone cells' answer during the tissue's healing around the dental implant. The results show a relation between the surface roughness and the cell's behavior [4.2, 4.3].

### 4.2.1. Surface roughness of the untreated Ti6Al4V grade 5 alloy

Figure 4.1 presents the average value of the 2D roughness of the untreated Ti6Al4V grade 5 alloy, values that resulted after five measurements on each sample.

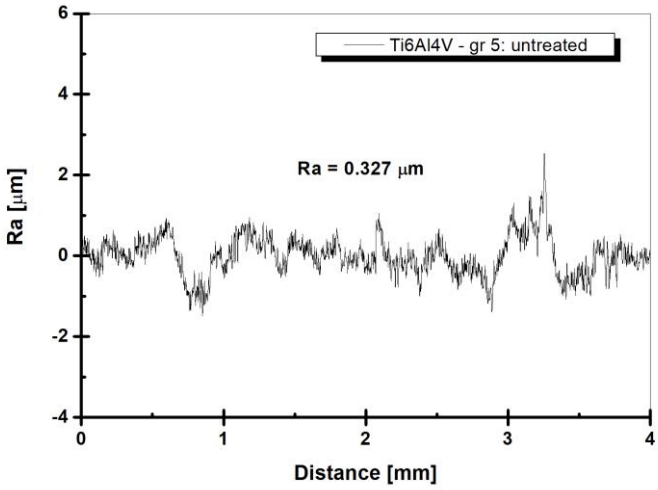


**Figure 4.1.** Surface roughness of untreated Ti6Al4V-grade 5 samples

After the measurements (5 measurements on each sample), the average surface roughness of the untreated Ti6Al4V grade 5 samples was 0.327µm.

### 4.2.2. Surface roughness of electrochemically formed $TiO_2$ films on the Ti6Al4V grade 5 alloy

The 2D roughness of the Ti6Al4V grade 5 alloy electrochemically oxidized at the voltage of 200 V for 1 minute, 2 minutes and 3 minutes was analyzed and it was observed an increase in roughness with the increase in the time imposed on the anodic oxidation process compared to the untreated titanium alloy.

This increase in roughness values along with the increase in the time imposed for the anodic oxidation process was also reported in the specialized literature by other authors who studied the influence of the oxidation parameters on the topography of the obtained layers [4.10].

### 4.2.3. Comparisons

The comparative mean value of the 2D roughness of the untreated and anodically oxidized Ti6Al4V grade 5 alloy at the voltage of 200 V for 1 minute, 2 minutes and 3 minutes was analysed and it was observed that the surface

The increase in roughness values correlated with the results from hydrophobicity (see chapter 5) is explained in the specialized literature by the Wenzel equation (equation 4.1.). Actually, this equation shows that increased roughness reduces the value of the contact angle, which means that the surface is more hydrophilic.

$$\cos\theta_W = r\cos\theta_Y \tag{4.1}$$

Where:

$\theta_W$ is the measured contact angle value,

$r$, is the average roughness,

$\theta_Y$, is Young's contact angle value for an ideal smooth surface [4.11-4.14].

The increase in the roughness values with the increase in the time imposed on the anodic oxidation process leads to a decrease in the contact angle values. Similar results are also observed in this study.

## 4.3. Comparative analysis of the surface microhardness of the untreated Ti6Al4V grade 5 alloy and electrochemically formed titanium oxide films

Surface mechanical properties of implants are very important in determining cellular answers and the implant's behavior is dependent on these properties. For example, surface topography and the surface mechanical properties of an implant can be essential for its success in the human body because it was demonstrated that cell adhesion and spreading depend on these factors [4.15].

A simple technique, like microhardness, can offer a possible way to measure the real hardness of the surface layer, which is hard to measure by conventional techniques such as tensile or bending tests.

### 4.3.1. Microhardness of the untreated Ti6Al4V grade 5 alloy surface

After microhardness measurements (3 measurements on each sample, on 5 different samples) that have been carried out with the device and method described in chapter III, the surface microhardness of the untreated Ti6Al4V grade 5 alloy was 347.9 kgf/mm$^2$.

### 4.3.2. Surface microhardness of electrochemically formed $TiO_2$ films on oxidized Ti6Al4V grade 5 alloy

In order to improve the surface of the Ti6Al4V grade 5 alloy, it was subjected to an electrochemical oxidation process at the voltage of 200 V for 1 minute, 2 minutes and 3 minutes.

The obtained results show that the microhardness value increases at once with the increase of oxidation time.

### 4.3.3. Comparisons

A comparative analysis was performed in order to better highlight the microhardness results of the untreated Ti6Al4V grade 5 alloy to that of the Ti6Al4V grade 5 alloy electrochemically oxidized at 200 V for 1 minute, 2 minutes and 3 minutes. After this, an increase in microhardness is observed with the increase in the time imposed on the anodic oxidation process compared to the untreated titanium alloy.

This increase of the microhardness value with the increase in the time imposed on the anodic oxidation process was reported in the specialized literature by other authors who studied the influence of the oxidation parameters on the topography of the obtained layers [4.16].

## 4.4. Partial conclusions

From the analysis of the obtained layers' roughness compared to the untreated alloy, an increase in the roughness of the layers is observed with the increase in the time imposed on the anodic oxidation process.

From the analysis of the layers' microhardness, it is also observed an increase in the microhardness values with the time imposed on the anodic oxidation process compared to the untreated alloy.

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# CHAPTER V

# MORPHOLOGICAL, STRUCTURAL AND HYDROPHOBICITY CHARACTERIZATION

This chapter presents the analysis of the layers obtained by anodic oxidation of the Ti6Al4V grade 5 alloy at different parameters from a structural, morphological and hydrophobicity point of view. Also, the time effect on the wetting properties of the obtained porous $TiO_2$ layers was studied.

## 5.1. Morphological analysis of the untreated titanium alloy surfaces and electrochemically obtained oxide films by optical microscopy

The morphological characterization of the Ti6Al4V grade 5 samples surfaces was done with the help of the optical microscope (OPTIKA XDS 3 MET within the Competences Center: Interfaces-Tribocorrosion and Electrochemical Systems and using the Vision Pro Plus 5.0 pilot program) both before and after the anodic oxidation process. The Ti6Al4V samples were anodically oxidised in static mode at 200 V, varying the time from 1 minute to 3 minutes. More potentials from 100 – 300 V were tried, but the layers' quality was the best for the presented parameters.

With the help of optical microscopy, the influence of time on the quality of the obtained layers was studied.

From the results, it was observed that with the increase in the time imposed on the anodic oxidation process, the titanium oxide layer is much more compact and evenly distributed on the surface of the samples.

## 5.2. Morphological and compositional analysis of the untreated titanium alloy surfaces and electrochemically formed oxide films by electron microscopy (SEM-EDX)

### 5.2.1 Untreated Ti6Al4V grade 5 alloy

The morphological and compositional analysis (SEM-EDX) was done with the help of the equipment described in chapter III of this thesis.

The EDX analysis of the untreated Ti6Al4V implant alloy indicates the presence of the

main elements from the studied alloy, i.e. Ti, Al and V. Oxygen is also present but in a small percentage because on the surface of the untreated alloy, a thin layer of titanium oxide natively forms.

From the surface morphology of the untreated Ti6Al4V grade 5 alloy, it can be seen that surface is relatively smooth and does not show surface defects.

### 5.2.2. Titanium oxide films formed electromechanically on the surface of the Ti6Al4V alloy

From the analysis of the surface morphologies of the Ti6Al4V grade 5 alloy electrochemically oxidized at 200 V for 1 minute, 2 minutes and 3 minutes, obtained at a magnitude of 2000x, it can be observed that the surface of the grade 5 titanium alloy electrochemically oxidized at different time intervals presents a compact and dense $TiO_2$ layer with agglomeration patches of formed oxide but without interruptions and surface defects, confirming that the voltage and times imposed to the anodic oxidation process were optimal.

To highlight the growth of nanopores on the surface of the titanium alloy, the obtained surface morphologies of the Ti6Al4V grade 5 alloy electrochemically oxidized at 200 V for 1 minute, 2 minutes and 3 minutes, were analysed at a magnitude of **20.000 x**. It was noticed that with the increase in the duration of the anodic oxidation process, the surface becomes denser and the size of the nanopores tends to decrease.

In order to back up this affirmation, surface morphologies of the Ti6Al4V grade 5 samples electrochemically oxidized at 200 V for 1 minute, 2 minutes and 3 minutes were analysed at the magnitude of **50.000x** with direct SEM pore diameter measurement.

To highlight the increase of the $TiO_2$ layer, the morphological analysis of the electrochemically oxidized Ti6Al4V grade 5 alloy was completed by the compositional analysis.

It can be mentioned the fact that for better electrical conductivity, the samples were gilded with a thin gold layer of 5 nm and on the EDX specter at 2.10 keV the golden pick is present. Even though it also was identified on the specter, it can be seen from the compositional analysis that it has been removed in order not to influence the mass percentage of the identified elements. The EDX analyses were made on morphologies obtained at a magnitude of 50.000x.

After the EDX analysis, it can be seen the presence of the main elements of the studied biomaterial, i.e. Ti, Al and V. At the same time, it can be seen that for the 1 minute time there is an increase in the percentage of oxygen compared to the untreated Ti6Al4V grade 5 alloy, which confirms the **increase of the $TiO_2$ layer** on the surface of the sample. Increasing the duration of the anodic oxidation process to 3 minutes determines the highest percentage of oxygen present on the surface of the sample and the thickest layer of **$TiO_2$**. Therefore, it can be confirmed that with the increase of the time of the anodic oxidation process, a continuous increase in the mass percentage content of the oxygen element takes place, thus confirming the effectiveness of the anodic oxidation process.

Another step after the elementary spectral analysis was to make the element distribution

(the map of the elements Ti, Al, V, O) identified on the SEM micrographs obtained at a magnitude of 50.000x.

From the analysis of the figures, the most dense distribution of oxygen is observed on the Ti6Al4V grade 5 alloy surface electrochemically oxidized at 200 V for 3 minutes compared to the Ti6Al4V grade 5 alloy surface electrochemically oxidized at 200 V for 1 minute and 2 minutes.

### 5.2.3. Comparisons

From the analysis of the obtained results, it is observed that the surface morphology of the Ti6Al4V grade 5 alloy electrochemically oxidized at 200 V between 1 minute and 3 minutes changes, compared to the surface of the untreated Ti6Al4V grade 5 alloy. If before the anodic oxidation process the surface of the untreated Ti6Al4V grade 5 alloy presents a thin native oxide layer that instantaneously forms on the surface of the untreated alloy in air, the samples electrochemically oxidized at 200 V show growth of nanopores on the surface of the Ti6Al4V grade 5 alloy. At the same time, a decrease in the dimensions of the nanopores can be observed with the increase in the imposed time for the electrochemical oxidation process. From the comparative analysis of the EDX spectra, the efficiency of the electrochemical oxidation process can be noticed by increasing the mass percentages of the oxygen element.

## 5.3. Structural characterization by X-ray diffraction (XRD)

X-ray diffraction (XRD) is a technique used on large scale to evaluate the crystallinity and structure of solid samples. Briefly, the phenomenon of crystal X-ray diffraction results from a scattering process in which X-rays are scattered by the electrons of the atoms present in the sample without changing the wavelength. Because X-rays have wavelengths (between 0.2 and 10 nm) comparable to the interatomic spacing of a solid crystalline, the incident X-ray beam diffracts in specific directions predicted by Bragg's law. The resulting diffraction pattern, given by the positions and intensities of the diffraction effects, is a fundamental physical property of the material, providing not only the identification but also the complete elucidation of its structure [5.2].

### 5.3.1. Structural characterization of the untreated Ti6Al4V grade 5 alloy surface

From the analysis of the XRD spectrum before the electrochemical process, the following crystalline phases were identified using the software Match! 3, (1) titanium (Ti) with crystallographic planes ((100), (002), (101), (012), (110), (013) and (200)), corresponding to 2θ angles (42,33°, 44,68°, 47,17°, 62,61°, 74,90°, 84,49° and 89,20°). This detected phase is registered in the database of the used program, namely Crystallography

Open Database (COD) 96-901-6191, and belongs to the hexagonal crystallization system, space group P63/mmc. Also, (2) aluminum (Al) with crystallographic planes ((200) and (202)) was identified, corresponding to 2θ angles (49,00° and 76,86°), identified with the program database COD 96-431-3211, as belonging to the cubic crystallization system, space group F m -3 m.

Another identified phase is (3) vanadium (V) with the crystallographic plane (200), corresponding to 2θ angles (74,12°), registered in the database of the Crystallography Open Database (COD) 96-410-5684, belonging to the cubic crystallization system, space group I m -3 m. The last identified phase is (4) titanium dioxide ($TiO_2$) brookite with crystallographic planes ((111), (002), (211), (131), (231), (023) and (200)), corresponding to 2θ angles (40,24°, 41,03) °, 55,93°, 67,44°, 81,30°, 78,82° and 45,87°) that belongs to the orthorhombic crystallization system, space group (P b c n), registered in the database with COD 96-153-0027.

### 5.3.2. Structural characterization of the electrochemically formed $TiO_2$ films on the surface of Ti6Al4V grade 5 alloy

From the analysis of the XRD spectrum, the diffraction angles and identified phases for Ti, Al, V and $TiO_2$ are kept as in the case of the untreated titanium alloy. In addition to these phases identified in the structural analysis of the untreated titanium alloy, the crystalline phase of anatase $TiO_2$ appeared, identified with the database COD 96-900-8215, which belongs to the tetragonal crystallization system, space group I 41/a m d (141) with the crystallographic planes ((011), (110), (101), (020), (220), (002), (130), (112), )132)), corresponding to the 2θ angles (24,73°, 29,60°, 34,86°, 49,41°, 58,73°, 64,24°, 65,55°, 69,48°, 83,76°). Also, it can be noticed that for the analysed samples of Ti6Al4V grade 5, the titanium oxide (anatase $TiO_2$) appeared with significant diffraction peaks on the XRD pattern. This is proof that the surface of the titanium alloy oxidizes and predominantly forms a passive $TiO_2$ film.

### 5.3.3. Comparisons

From the XRD diagrams, it is observed that anatase $TiO_2$ electrocrystallizes preferentially according to the planes ((011), (110) (020) and (002)), which have the highest intensity. The peak corresponding to the (110) crystallization plane of anatase $TiO_2$ slightly increases in intensity with the increase of the time imposed on the anodic oxidation process. It is also observed that at the time of 3 minutes, the peak of anatase $TiO_2$ with the (110) crystallization plane appears. A peak that does not appear at lower times or with the untreated alloy. This fact demonstrates that the increase in the time imposed on the anodic oxidation process it forms more titanium oxide ($TiO_2$) on the surface of the samples.

From the comparative analysis of the peaks of interest, it is noted that the peak corresponding to the titanium dioxide crystallized according to plane (131) increases in intensity with the increase of the time imposed on the anodic oxidation process, and the

smallest peak corresponds to the untreated titanium alloy.

For each studied system, depending on the time imposed on the oxidation process, the average size of the $TiO_2$ crystallites was calculated using the Debye–Sherrer formula. After the calculation, it can be said that for the untreated Ti6Al4V grade 5 alloy the crystallite size corresponding to the crystallization plane (131) has an average size of 182.25 nm. In the case of the oxidized samples, it can be seen that with the increase in the time required for the electrochemical oxidation process, the size of the $TiO_2$ crystallites increases.

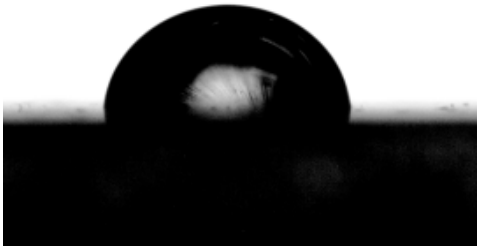
## 5.4. Surface hydrophobicity of the untreated titanium alloy compared to oxide films

The performance of an in-situ biomaterial is generally evaluated by its "biocompatibility", which refers to the quantified success of the biomaterial interaction with biological cells. The biomaterial surface is the first component of the implant that comes into contact with cells or biological fluids. Thus, biocompatibility will be firstly influenced by the surface, biomaterial's characteristics, especially wettability, surface chemistry of exposed atoms, surface energy and surface topography. In order to determine the wetting of the Ti6Al4V samples before and after the anodic oxidation process, a device OCA 15 EC, Dataphysics, Germany, was used, connected to a PC and piloted with the help of a software named SCA20. In its testing syringe, distilled water was introduced and the dosed solution volume for each measurement was 10 µL.

### 5.4.1. Surface hydrophobicity analysis of the untreated Ti6Al4V grade 5 alloy

The average value and the image of the droplet obtained from the hydrophobicity measurements with Hank's biological solution on the untreated Ti6Al4V grade 5 alloy are shown in table 5.1.

**Table 5.1.** Average value of contact angle measurement on untreated titanium alloy

| Studied sample | Contact angle [degrees] | Images of the droplet on the sample surface |
|---|---|---|
| Ti6Al4V – grade 5 untreated | 85.13 |  |

### 5.4.2. Surface hydrophobicity analysis of the electrochemically formed $TiO_2$ films on Ti6Al4V grade 5 alloy

From the analysis of the average values and the images of the drop obtained as a result of the hydrophobization measurements on the tested sample after the electrochemical oxidation process, a decrease in the contact angle values is observed with the increase in the duration of the electrochemical oxidation process.

### 5.4.3. Comparisons

From the analysis of the contact angle values before and after the electrochemical oxidation process, a decrease in the contact angle values is observed with the increase in the time imposed on the electrochemical oxidation process, a behavior that indicates that after the anodic oxidation process, the surfaces become more hydrophilic.

## 5.5. Roughness - contact angle comparison

From the analysis of the average values of the roughness and contact angle of the untreated Ti6Al4V grade 5 alloy and electrochemically oxidized at the voltage of 200 V for 1 minute, 2 minutes and 3 minutes, it is observed that with the increase of the surface roughness of the material, the average value of the contact angle decreases.

This surface improvement of titanium based biomaterials is done through experimental research within this doctoral thesis.

Wetting can be evaluated by the contact angle. Wetting is recognised as good when the contact angle is lower than 90º. On the other hand, wetting becomes inconsistent when the contact angle is higher than 90º.

## 5.6. Partial conclusions

From the present chapter, entitled Morphological, structural, topographic and hydrophobicity characterization of the untreated titanium alloy and oxidised at different parameters, we can draw the following conclusions:

From the morphological analysis of the samples with the help of optical microscopy, it is observed that with the increase in the time imposed on the anodic oxidation process, the oxide layer becomes much more compact and dense compared to the lower times imposed on the process of controlled growth of the oxide layer and with the untreated alloy.

From the SEM analysis, a change in the surface morphologies is observed with the increase in the duration of the electrochemical oxidation process. At the same time, from the morphologies presented at 50.000x magnification, a decrease in the size of the nanopores formed on the surface of the Ti6Al4V grade 5 alloy can be observed.

From the EDX analysis of the untreated and electrochemically oxidized Ti6Al4V grade

5 alloy, an increase in the mass percentage of oxygen is observed with the increase in the duration of the electrochemical oxidation process.

From the analysis of the contact angle, it can be observed that with the increase in the time required for the anodic oxidation process, the surfaces become more hydrophilic, indicating better osseointegration of the treated alloy, which is intended to be used in possible biomedical applications.

From the structural analysis (XRD), it was observed that with the increase in the time imposed on the anodic oxidation process, the peak corresponding to the Ti element decreases.

An increase in the intensity of the $TiO_2$ peaks is observed with the increase in the time imposed on the anodic oxidation process, a behavior that confirms the growth of the $TiO_2$ layer through the anodic oxidation process on the surface of the studied titanium alloy. Also, the size of the $TiO_2$ crystallites calculated for the most intensive peak shows us that with the increase in the time required for the electrochemical oxidation process, the size of the $TiO_2$ crystallites increases.

From the comparative analysis of the roughness and angle contact, it is observed that with the increase of the surface roughness of the Ti6Al4V alloy, the value of the contact angle decreases, which indicates the fact that the material's surface is hydrophilic.

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# CHAPTER VI

# ELECTROCHEMICAL CHARACTERIZATION OF THE TITANIUM ALLOY AND OXIDE FILMS IN BIOLOGICAL FLUIDS

## 6.1. Evolution of the open circuit potential (OCP) vs. time in Hank's biological solution of the untreated alloy and the electrochemically formed oxide film

The open circuit potential (OCP) for the untreated Ti6Al4V alloy and the electrochemically titanium oxide film formed on it, immersed in Hank's solution for 75,5 hours was measured at different time intervals. This was done especially to monitor the necessary time to reach the stationary state of the tested surfaces since they are intended to be used as implants.

To simulate the conditions of the human body, the corrosion tests were done in Hank's physiological solution.

Following the analysis of the obtained results, it can be noticed that in the case of both studied surfaces the open circuit potential values move towards nobler (more positive) values with the increase of the immersion period with the specification that since immersion, the open circuit potential of the oxidized surfaces registers more positive (nobler) values. A shift of the open circuit potential to more positive values indicates the formation of a passive film, while a decrease indicates film breaks, film dissolution or no film formation.

## 6.2. Evolution of the polarization resistance ($R_p$) vs. time in Hank's biological solution of the untreated alloy and the electrochemically formed oxide film

After immersing the untreated and electrochemically oxidized Ti6Al4V grade 5 alloy samples to form the protective film of titanium oxide ($TiO_2$), the open circuit potential (OCP) was recorded in several sequences for 75,5 hours. Between the open circuit potential measurement sequences, 7 recordings of 100 points of polarization resistance were made, calculated from as many linear polarization curves by using the Stern Geary equation and Tafel slopes.

For a better highlighting of the results obtained, the average values of all polarization resistance values recorded at different immersion periods were calculated for the untreated Ti6Al4V grade 5 alloy compared to the electrochemically oxidized Ti6Al4V grade 5 alloy. Based on the results, it can be stated that in the case of the electrochemically oxidized

Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the polarization resistance values are superior, compared to the untreated Ti6Al4V grade 5 alloy at all studied times. Also, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, an increase in the values of the polarization resistance is observed with the increase in the immersion time. This behavior demonstrates the efficiency of the electrochemical oxidation process of the titanium alloy for improving the resistance of the implant to the aggresive action of biological fluids in the human body.

## 6.3. Evolution of the corrosion rate ($V_{cor}$) vs. time in Hank's biological solution of the untreated alloy and the electrochemically formed oxide film

Polarization resistance is the only monitoring method of corrosion that makes possible the direct measurement of the corrosion rate (expressed as thickness loss over time) in real time. The corrosion current determined through this method represents the current that appears at the corrosive metal/medium interface when the metal is immersed in the solution.

After analyzing the results, it can be stated that the corrosion rate values ($V_{cor}$) for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution are higher than the corrosion rate values recorded for the controlled oxidized Ti6Al4V alloy and having the titanium oxide film ($TiO_2$) on its surface for all measurement periods during immersion.

From the analysis of the average values evolution of the corrosion speed of the two surfaces immersed in Hank's biological solution, during the entire period, at different times after immersion, it is observed that in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the corrosion speed values are lower compared to the untreated Ti6Al4V grade 5 alloy at all studied times (high polarization resistance means low corrosion rate, desired behavior for all types of biomaterials to be inserted in the human body). Also, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, a decrease in the corrosion rate values is observed with the increase in the immersion time.

## 6.4. Partial conclusions

To investigate the electrochemical behavior of the untreated Ti6Al4V grade 5 alloy and the titanium oxide film electrochemically formed on it in Hank's biological solution, methods such as the evolution of the open circuit potential vs. time for a period of 75,5 hours from immersion, the evolution of the polarization resistance ($R_p$) and the corrosion rate ($V_{cor}$) vs. time were used.

From the evolution of the open circuit potential, it can be observed that in the case of the untreated Ti6Al4V grade 5 alloy, it has a more negative immersion potential value compared to the electrochemically oxidized alloy. At the same time, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, a continuous shift of the open circuit potential towards more positive values is observed, but it does not reach the equilibrium state

even at the end of the immersion state. This behavior indicates the efficiency of the electrochemical oxidation process of the titanium alloy for improving the resistance of the implant to the aggressive action of biological fluids in the human body.

From the analysis of the evolution of the polarization resistance, it can be seen that in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the values of the polarization resistance are superior, compared to the untreated Ti6Al4V grade 5 alloy at all studied times. Also, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, an increase in the values of the polarization resistance is observed with the increase in the immersion time.

From the evolution of the corrosion rate in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the corrosion rate values are low compared to the untreated Ti6Al4V grade 5 alloy at all studied times. While for the untreated Ti6Al4V grade 5 alloy, it is observed that the corrosion rate values show a slight increase after 8 hours of immersion compared to the value obtained after 360 minutes and a slight decrease at the end of the immersion period.

Following these results, we can state that the electrochemical modification of the titanium alloy (Ti6Al4V grade 5) by the anodic oxidation method confirms the possibility of improving its anticorrosive properties for testing the implant in Hank's biological solution.

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# CHAPTER VII

# ELECTROCHEMICAL CHARACTERIZATION OF THE TITANIUM ALLOY AND OXIDE FILMS IN BIOLOGICAL FLUIDS UNDER INFLAMMATORY CONDITIONS

This chapter includes the study of the untreated Ti6Al4V grade 5 alloys' behavior in Hank's biological solution doped with different concentrations of albumin as protein, different concentrations of hydrogen peroxide as inflammatory compound and combinations of albumin and inflammatory compound. Thus, the influence of these compounds on the behavior of the untreated Ti6Al4V grade 5 alloy in Hank's biological solution can be observed.

## 7.1. The influence of doping the biological solution with albumin on the untreated Ti6Al4V grade 5 alloy's behavior

### 7.1.1. Evolution of the open circuit potential (OCP) vs. time in Hank's biological solution with the addition of different concentrations of albumin on the untreated Ti6Al4V grade 5 alloy

When interacting with fluids in the human body, most metals and alloys are subjected to corrosion, as these fluids are highly chlorinated due to the presence of chlorine ions and proteins. Electrochemical methods such as OCP show the thermodynamic tendency of electrochemical oxidation of a material in a corrosive environment. Generally, after a certain period from immersion, this value stabilizes around a stationary value, reaching the steady value. As a result of the changes that may occur on the surface of the investigated material, the potential's value may vary due to the following causes: formation of the passive layer, dissolution, oxidation, etc. OCP is a method used to compare the oxidation tendency behavior of different materials in a corrosive environment [7.1-7.7].

Proteins are known to interact with metal oxides, thereby altering the corrosion resistance of implants. Albumin is the most abundant protein (4.2 – 5.3%) in blood plasma and extracellular tissue fluid. In general, albumin inhibits cathodic reactions on Ti by absorbing on the metal surface and covering the reaction sites and/or blocking the mass transport of dissolved $O_2$.

Measurements of the potential time function of the untreated Ti6Al4V grade 5 alloy

immersed in Hank's biological solution and in Hank's biological solution with addition of different concentrations of albumin were performed. Thus, in the case of the untreated titanium immersed in Hank's solution, the potential value has a shift towards more positive values, a fact that indicates the formation of a $TiO_2$ oxide layer on the surface of the sample. In the case of the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution doped with the highest concentration of albumin, a tendency of the potential towards higher values is observed. At the same time, it is observed that with the decrease in albumin concentration, the potential value has a tendency to move towards more negative values compared to the alloy immersed in Hank's colution. A behavior that indicates the inability of the material to form a protective oxide layer on its surface.

### 7.1.2. Evolution of the polarization resistance ($R_p$) at different time intervals in Hank's biological solution with the addition of albumin on the untreated Ti6Al4V grade 5 alloy

After the immersion of the untreated Ti6Al4V grade 5 alloy, the open circuit potential (OCP) was recorded in several sequences. Between the open potential measurement sequences, 7 recordings of 100 points of polarization resistance were made, calculated from as many linear polarization curves by using the Stern Geary equation and Tafel slopes.

From the values analysis of the polarization resistance evolution of the untreated alloy immersed in Hank's biological solution with the addition of different concentrations of albumin, it can be seen that the values of the polarization resistance of the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution are higher than the recorded polarization resistance values for the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution with the addition of different concentrations of albumin.

For a better highlighting of the obtained results, the average comparative values of all polarization resistance values recorded at different immersion periods were calculated for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution and Hanks biological solution doped with the 3 studied concentrations of albumin. Based on the results, it can be stated that in the case of the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the polarization resistance values are higher compared to the untreated Ti6Al4V grade 5 alloy immersed in the solutions with added albumin, at all studied times. It is also observed that with the increase in the albumin concentration in Hank's biological solution, the values of the polarization resistance increase. This behavior demonstrates that the addition of albumin inhibits cathodic reactions by absorbing on the metal surface an albumin layer that acts as a protective film.

### 7.1.3. Evolution of the corrosion rate ($V_{cor}$) at different time intervals in Hank's biological solution with the addition of albumin on the untreated Ti6Al4V grade 5 alloy

The variation of the corrosion rate expressed as a penetration index was evaluated for the untreated alloy immersed in Hank's biological solution and in Hank's solution doped with different concentrations of albumin.

From the analysis of the obtained results, it can be observed that the corrosion rate values ($V_{cor}$) for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution are lower than the corrosion rate values recorded for the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution doped with different concentrations of albumin for all measurement periods during immersion.

From the analysis of the average values of all corrosion rate values ($V_{cor}$), it can be observed that in the case of the untreated Ti6Al4V grade 5 alloy immersed in Hank's biologocal solution, the corrosion rate values are lower, compared to the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution doped with different concentrations of albumin, at all studied times.

It is also observed that with the increase in albumin concentration in Hank's biological solution, the corrosion rate values decrease. This behavior demonstrates that the addition of albumin inhibits cathodic reactions by adsorbing on the metal surface an albumin layer that acts as a protective film, increasing the polarization resistance values.

## 7.2. The influence of doping the biological solution with hydrogen peroxide on the behavior of the untreated Ti6Al4V grade 5 alloy

### 7.2.1. Evolution of the open circuit potential (OCP) vs. time in Hank's biological solution with the addition of different concentrations of hydrogen peroxide on the untreated Ti6Al4V grade 5 alloy

Measurements were made of the potential time function of the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution and Hank's biological solution with the addition of different concentrations of hydrogren peroxide. It is observed that the highest value of the potential corresponds to the titanium alloy immersed in Hank's solution doped with the highest concentration of hydrogen peroxide.

The tendency to move the potential towards more positive (nobler) values was observed in the specialised literature and by other authors, as well. This behavior could be explained by the formation of a thin film of $TiO_2$ since hydrogen peroxide is known to be a strong oxidizing compound [7.3]. The addition of hydrogen peroxide promotes the growth of $TiO_2$ on the surface of the titanium alloy, but it can also have the effect of dissolving the oxide formed on the surface of the material following exposure to oxygen, as expressed by the possible reactions [7.3, 7.25-7.26]:

$$Ti + H_2O_2 \rightarrow TiO_2 + H_2 \quad (7.1)$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (7.2)$$

$$TiO_2 + 2H_2O \rightarrow Ti(OH)_4 \quad (7.3)$$

$$TiO_2 + nH_2O \rightarrow TiO_2nH_2O \quad (7.4)$$

$$(Ti-OH) + H_2O \rightarrow [Ti-O]^- + H_3O^+ \quad (7.5)$$

$$TiO_2nH_2O + OH^- \rightarrow HTiO_3^-nH_2O \quad (7.6)$$

Titanium from an alloy can react with hydrogen peroxide to form a titanium oxide film (Equation (1)). Moreover, some of the $TiO_2$ could be hydrolyzed (Equations (2) and (3)). A hydration reaction (Equation (4)) simultaneously occurs with the formation of Ti-OH (Equation (5)). Hydrated and hydrolyzed titanium oxide ($TiO_2$) can cause the formation of negatively charged surfaces (Equations (5) and (6)), which could be involved in the decrease of the open potential to a more negative value during the immersion time, followed by the transition to a more positive value, as explained by equation (1) [7.3].

### 7.2.2. Evolution of the polarization resistance ($R_p$) at different time intervals in Hank's biological solution with the addition of hydrogen peroxide on the untreated Ti6Al4V grade 5 alloy

From the analysis of the evolution of the polarization resistance, it can be noticed that the values of the polarization resistance for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution are higher compared to the values of the polarization resistance recorded for the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution with the addition of different concentrations of $H_2O_2$.

For a better highlighting of the obtained results, the average comparative values of all the polarization resistance values recorded at different immersion periods were calculated for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution and Hank's biological solution with the addition of different concentrations of $H_2O_2$. It was noticed that in the case of the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the values of the polarization resistance are higher, compared to the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution with the addition of different concentrations of $H_2O_2$, at all studied times. It is also observed that with the increase in the concentration of hydrogen peroxide in Hank's biological solution, the values of polarization resistance decrease. This behavior demonstrates that the addition of hydrogen peroxide increases the cathodic and anodic reactions by dissolving the $TiO_2$ layer on the surface of the material, thus decreasing the corrosion resistance of the untreated Ti6Al4V grade 5 alloy.

### 7.2.3. Evolution of the corrosion rate of the untreated Ti6Al4V grade 5 alloy in the presence of the inflammatory compound

The variation of the corrosion rate expressed as a penetration index was evaluated for the untreated alloy immersed in Hank's biological solution and Hank's solution with the addition of different concentrations of $H_2O_2$.

From the analysis of the results, it can be seen that the corrosion rate ($V_{cor}$) values for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution are lower than the corrosion rate values recorded for the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution doped with different concentrations of $H_2O_2$, for all measurement periods during immersion.

Also, it can be observed that with the increase of the $H_2O_2$ concentration in Hank's biological solution, the corrosion rate values increase.

## 7.3. The synergistic effect of the protein and the inflammatory compound

### 7.3.1. Evolution of the open circuit potential (OCP) of the untreated Ti6Al4V grade 5 alloy in the presence of the protein and the inflammatory compound

Open circuit potential measurements were performed for the titanium alloy immersed in Hank's solution and Hank's solution doped with 4 different concentrations of albumin and hydrogen peroxide in order to highlight the synergistic effect of the two compounds.

After the analysis of the obtained results, it can be said that the addition of hydrogen peroxide to Hank's solution in the first phase favors the growth of the $TiO_2$ layer, after which the formed layer dissolves, thus reducing the corrosion resistance of the untreated Ti6Al4V grade 5 alloy. Instead, albumin inhibits the cathodic reactions of the untreated Ti6Al4V grade 5 alloy by absorbing on the metal surface a layer of adsorbed albumin acting as a protective film, a behavior that leads to an increase in the corrosion resistance of the untreated Ti6Al4V grade 5 alloy. The results are consistent with the electrochemical results from the determination of $R_p$ and $V_{cor}$, as well as with the SEM-EDX morphology presented in this chapter.

### 7.3.2. Evolution of the polarization resistance of the untreated Ti6Al4V grade 5 alloy in the presence of the protein and inflammatory compound

Measurements were made of the polarization resistance evolution for the untreated alloy immersed in Hank's biological solution and for the untreated titanium alloy immersed in Hank's solution in which 4 different concentrations of albumin and hydrogen peroxide were added to observe the synergistic effect of the two compounds. It was observed that the polarization resistance values for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution are much higher compared to the polarization resistance values recorded for the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution with the addition of different concentrations of $H_2O_2$ and albumin.

For a better display of the obtained results, the average comparative values of all the polarization resistance values recorded at different immersion periods were calculated for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution and Hank's biological solution doped with the 4 studied albumin concentrations and hydrogen peroxide. It was noticed that in the case of the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the polarization resistance values are higher compared to the untreated Ti6Al4V grade 5 alloy immersed in the solutions with the addition of albumin and $H_2O_2$, at all studied times.

It is also observed that with the increase in the concentration of hydrogen peroxide in the mixture, the values of the polarization resistance decrease, while the increase in the concentration of albumin in the mixtures increases the values of the polarization resistance, but no more than those obtained for the untreated alloy in Hank's biological solution. Making a comparative analysis of the results presented in this chapter, it is observed that the highest values of the polarization resistance are obtained by the untreated grade 5 titanium alloy immersed in Hank's biological solution, followed by the untreated grade 5 titanium alloy immersed in the solutions with the addition of different concentrations of albumin. The next system in terms of polarization resistance values obtained is represented by the alloy immersed in different concentrations of albumin + $H_2O_2$ mixture and the lowest values of polarization resistance are recorded for the system with the addition of different concentrations of $H_2O_2$.

### 7.3.3. Evolution of the corrosion rate of the untreated Ti6Al4V grade 5 alloy in the presence of the protein and inflammatory compound

Measurements were made of the corrosion rate variation expressed as penetration index for the untreated alloy immersed in Hank's biological solution and in Hank's solution with 4 different concentrations of albumin and $H_2O_2$. It was observed that the values of the corrosion rate ($V_{cor}$) for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution are much lower compared to the ($V_{cor}$) values recorded for the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution with the addition of different concentrations of albumin and $H_2O_2$.

For a better highlighting of the obtained results, the average comparative values of all the ($V_{cor}$) values recorded at different immersion periods were calculated for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution and Hank's biological solution doped with the 4 studied concentrations of albumin and $H_2O_2$. Based on the results, it can be stated that in the case of the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution, the corrosion rate values are lower compared to the untreated Ti6Al4V grade 5 alloy immersed in the solutions with added albumin and $H_2O_2$, at all studied times. It is also observed that with the increase in the concentration of hydrogen peroxide in the solutions, the $V_{cor}$ values increase, while the increase in the albumin concentration in the solutions causes a decrease in the $V_{cor}$ values. However, these low $V_{cor}$ values do not decrease more than those obtained for the untreated alloy in Hank's biological solution. Making a comparative analysis

of the results presented in this chapter, it is noticed that the lowest values of $V_{cor}$ are obtained by the untreated grade 5 titanium alloy immersed in Hank's biological solution, followed by the untreated grade 5 titanium alloy immersed in the solutions with the addition of different concentrations of albumin. The next system in terms of $V_{cor}$ values obtained is represented by the alloy immersed in different concentrations of albumin and $H_2O_2$ mixture, and the highest $V_{cor}$ values are recorded for the system with the addition of different concentrations of $H_2O_2$.

## 7.4. Increasing the resistance of the Ti6Al4V grade 5 implant to the inflammatory process in the presence of protein through the electrochemically controlled formation of titanium oxide films

### 7.4.1. Evolution of the open circuit potential (OCP), polarization circuit ($R_p$) and corrosion rate ($V_{cor}$) vs. time in Hank's biological solution doped with albumin of the untreated alloy and electrochemically formed oxide film

Measurements of the evolution of the open circuit potential were performed for the untreated alloy and the electrochemical titanium oxide film formed on it immersed in Hank's solution doped with albumin. It was observed that in the case of the electrochemically oxidized surface, the values of the open circuit potential move towards higher values with the increase of the immersion period, while for the untreated alloy immersed in Hank's solution doped with albumin, a continuous shift towards more negative values is observed until the end of the measurements. A shift of the open potential to more positive values indicates the formation of a passive film, while a decrease indicates film breaks, film dissolution or no film formation. Comparatively, the values of the electrochemically oxidized titanium alloy immersed in Hank's solution doped with albumin are more positive than those of the untreated alloy.

Also, measurements were made of the comparative evolution of the polarization resistance for the untreated alloy and for the oxidized alloy with titanium oxide film ($TiO_2$) immersed in Hank's biological solution doped with albumin. Based on the results obtained, it can be stated that the polarization resistance values for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with albumin are lower than the polarization resistance values recorded for the controlled oxidized Ti6Al4V alloy with the titanium oxide ($TiO_2$) film formed on its surface, for all measurement periods during immersion.

For a better highlighting of the results, the average values of all the polarization resistance values recorded at different immersion periods were calculated for the untreated Ti6Al4V grade 5 alloy compared to the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with albumin. It was observed that in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with albumin, the polarization resistance values are higher compared to the untreated Ti6Al4V grade 5 alloy, at all studied times.

Also, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, an increase in the values of the polarization resistance is observed with the increase in the immersion

time. This behavior demonstrates the efficiency of the electrochemical process of the titanium alloy for improving the resistance of the implant to the aggressive action of biological fluids in the human body. The increase in the polarization resistance values in the case of the electrochemically oxidized titanium alloy is due to the presence of the $TiO_2$ layer that has superior insulating properties.

Electrochemical measurements were also performed to analyze the variation of the corrosion rate expressed as a penetration index for the untreated alloy and for the oxidized alloy with titanium oxide film ($TiO_2$) immersed in Hank's biological solution doped with albumin. Based on the results obtained, it can be stated that the corrosion rate values ($V_{cor}$) for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with albumin are higher than the corrosion rate values recorded for the controlled oxidized Ti6Al4V alloy having an oxide film of titanium ($TiO_2$) formed on its surface for all measurement periods during immersion.

### 7.4.2. Evolution of the open circuit potential (OCP), polarization resistance ($R_p$) and corrosion rate ($V_{cor}$) vs. time in Hank's biological solution doped with hydrogen peroxide of the untreated alloy and the electrochemically formed oxide film

Measurements of the open circuit potential evolution were performed for the untreated alloy and the electrochemically titanium oxide film formed on it, immersed in Hank's solution doped with $H_2O_2$. Comparatively, the values corresponding to the electrochemically oxidized titanium alloy immersed in Hank's solution doped with $H_2O_2$ are more positive than those of the untreated alloy.

Subsequently, measurements were made for the evolution of the polarization resistance for the untreated alloy and for the oxidized alloy, immersed in Hank's biological solution doped with $H_2O_2$. Based on the results obtained, it can be stated that the polarization resistance values for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with $H_2O_2$ are lower than the polarization resistance values recorded for the controlled oxidized Ti6Al4V alloy, the titanium oxide film ($TiO_2$) formed on its surface for all measurements periods during immersion.

For a better highlighting of the obtained results, the average values of all the polarization values recorded at different immersion periods were calculated for the untreated Ti6Al4V grade 5 alloy compared to the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with $H_2O_2$. It was observed that in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's solution doped with $H_2O_2$, the polarization resistance values are superior, compared to the untreated Ti6Al4V grade 5 alloy, at all times studied.

Also, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, an increase in the values of the polarization resistance is observed with the increase in the immersion time. This behavior demonstrates the efficiency of the electrochemical oxidation process of the titanium alloy for improving the resistance of the implant to the aggressive action of biological fluids in the human body. The increase in the polarization resistance values in the

case of the electrochemically oxidized titanium alloy is due to the presence of the $TiO_2$ layer which has superior insulating properties.

In addition, measurements were made of the corrosion rate variation expressed as a penetration index for the untreated alloy and for the alloy oxidized with titanium oxide ($TiO_2$) film. After analysing the obtained results, it was observed that the corrosion rate values ($V_{cor}$) for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with $H_2O_2$ are higher than the corrosion rate values recorded for the controlled oxidized Ti6Al4V alloy, having the oxide film of titanium ($TiO_2$) formed on its surface for all measurement periods during immersion.

From the comparative analysis of the corrosion rate, it can be observed that in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's solution doped with $H_2O_2$, the corrosion rate values are low compared to the untreated Ti6Al4V grade 5 alloy at all studied times. Also, in the case of the electrochemically Ti6Al4V grade 5 alloy, a decrease in the corrosion rate values is observed with the increase in the immersion time. This behavior demonstrates the efficiency of the electrochemical oxidation process on the titanium alloy in order to improve the corrosion resistance of the implant to the aggressive action of the biological fluids in the human body.

### 7.4.3. Evolution of the open circuit potential (OCP), polarization resistance ($R_p$) and corrosion rate ($V_{cor}$) vs. time in Hank's biological solution doped with albumin and hydrogen peroxide of the untreated alloy and the electrochemically formed oxide film

Measurements of the open circuit potential evolution were performed for the untreated titanium alloy and the electrochemical titanium oxide film formed on it immersed in Hank's solution doped with albumin and $H_2O_2$. Following the analysis of the obtained results, it is observed that in the case of both studied surfaces, the values of the open potential move towards higher values with the increase of the immersion period. Comparatively, the values of the electrochemically oxidized titanium alloy immersed in Hank's solution doped with albumin and $H_2O_2$ are more positive than those of the untreated alloy. This behavior can be explained by the formation of a more stable and protective thin layer of oxide on the surface of the electrochemically oxidized alloy, which acts as a protective film to prevent the release of metal ions.

Subsequently, measurements were made of the polarization resistance evolution for the untreated alloy and for the alloy oxidized with a titanium oxide ($TiO_2$) film immersed in Hank's biological solution doped with albumin and $H_2O_2$. Based on the results obtained, it can be stated that the polarization resistance values for the controlled oxidized Ti6Al4V grade 5 alloy with the titanium oxide ($TiO_2$) film formed on its surface, immersed in Hank's biological solution doped with albumin and $H_2O_2$, are higher than the polarization resistance values recorded for the untreated Ti6Al4V alloy for all measurement periods during immersion.

For a better highlighting of the obtained results, the average values of all the values of the polarization resistances recorded at different immersion periods were calculated for the

untreated Ti6Al4V alloy compared to the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with albumin and $H_2O_2$. It was observed that in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's solution doped with albumin and $H_2O_2$, the polarization resistance values are higher compared to the untreated Ti6Al4V grade 5 alloy, at all studied times.

Also, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, an increase in the values of the polarization resistance is observed with the increase in the immersion time. This behavior is due to the non-uniformity of the natively formed titanium oxide film on the surface of the untreated alloy that leads to a much faster degradation process of the material compared to the electrochemically oxidized titanium alloy.

In addition, measurements were made of the corrosion rate variation expressed as a penetration index for the untreated alloy and for the oxidized alloy with a film of titanium oxide ($TiO_2$) immersed in Hank's biological solution doped with albumin and $H_2O_2$. From the analysis of the obtained results, it can be observed that the corrosion rate values ($V_{cor}$) for the untreated Ti6Al4V grade 5 alloy immersed in Hank's biological solution doped with albumin and $H_2O_2$ are higher than the corrosion rate values recorded for the controlled oxidized Ti6Al4V alloy, having the oxide film of titanium ($TiO_2$) formed on its surface for all measurement periods during immersion.

From the comparative analysis of the corrosion rate, it is observed that in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy immersed in Hank's solution doped with albumin and $H_2O_2$, the corrosion rate values are low, compared to the untreated Ti6Al4V grade 5 alloy at all studied times. Also, in the case of the electrochemically oxidized Ti6Al4V grade 5 alloy, a decrease in the corrosion rate values is observed with the increase in the immersion time. This behavior demonstrates the efficiency of the electrochemical oxidation process on the titanium alloy in order to improve the implant's corrosion resistance to the aggresive action of the biological fluids of the human body.

These studies show us that electrochemical oxidation is an efficient method to improve the corrosion behavior of the Ti6Al4V grade 5 alloy in solutions that simulate the human body's fluids and also in the presence of metabolic compounds and inflammatory reactions.

## 7.5. Morphological and compositional (SEM-EDX) characterisation before and after corrosion

Subsequently, for the samples immersed in the 6 solutions, tested to determine the corrosion behavior of the untreated Ti6Al4V grade 5 alloy, SEM-EDX analyses were made (on the most conclusive samples).

The EDX analysis of the untreated Ti6Al4V grade 5 implant alloy before corrosion indicates the presence of the main elements in the studied alloy, namely: Ti, Al and V, O, with their mass percentage values.

From the EDX analysis of the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution, it can be seen that compared to the EDX analysis of the untreated Ti6Al4V grade 5 alloy before corrosion, the relative atomic mass of Ti decreases. In addition, an increase is

observed in the oxygen element, which indicates an oxidation effect on the titanium alloy when the alloy surface is immersed in Hank's biological solution.

From the SEM-EDX analysis of the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution doped with albumin, the concentration of $O_2$ is small compared to the concentration of hydrogen peroxide added in the same amount.

For the addition of hydrogen peroxide in the highest concentration, it is observed that the percentage of mass $O_2$ is the highest. This is due to the strong oxidizing effect of hydrogen peroxide, which increases the $TiO_2$ film on the sample surface, combined with a corrosion attack.

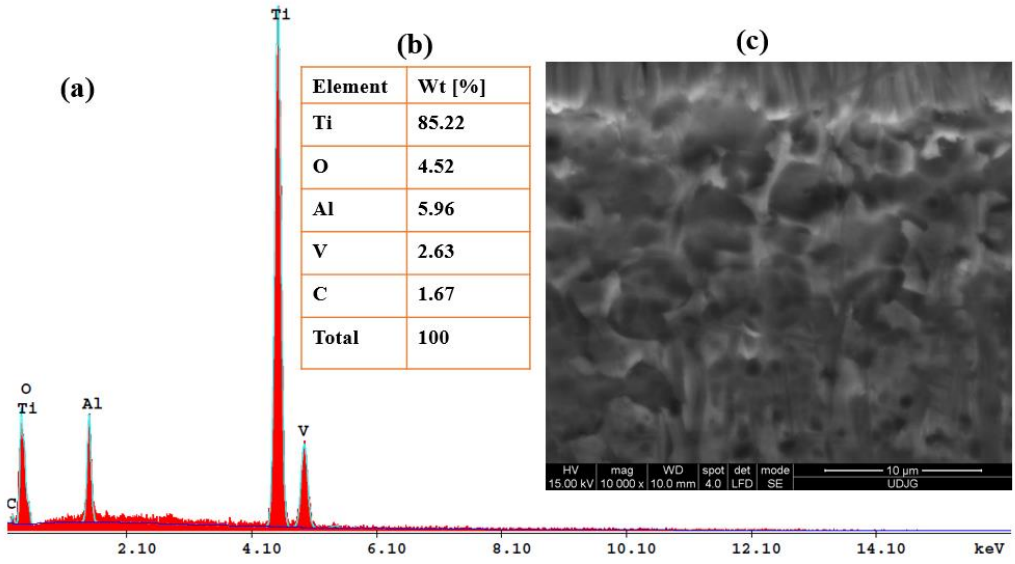


**Figure 7.1.** SEM surface micrographs and EDX analysis of untreated Ti6Al4V grade 5 alloy in Hank's solution + 4 mL/L albumin + 4 mL/L $H_2O_2$: (a) EDX spectrum; (b) EDX elemental analysis; (c) SEM surface morphology.

From the SEM-EDX analysis of the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution doped with the lowest concentrations of albumin and hydrogen peroxide, it is observed that the concentration of $O_2$ and carbon is also lower, compared to the untreated Ti6Al4V grade 5 alloy immersed in the solutions with the highest addition of albumin and $H_2O_2$. These results are consistent with the obtained electrochemical results.

## 7.6. Partial conclusions

This chapter highlights the effects of time, albumin (metabolic compound), hydrogen peroxide (as an inflammatory product) and the effect of the two compounds (synergistic effect) added to Hank's biological solution, on the corrosion behavior and stability of the untreated and electrochemically oxidized titanium alloy implant (Ti6Al4V grade 5).

The electrochemical methods used to assess the corrosion resistance of the alloy used as

an implant (untreated and electrochemically oxidized Ti6Al4V grade 5) were: the evolution of the open circuit potential (OCP), determination of the polarization resistance ($R_p$) and the corrosion rate ($V_{cor}$). The electrochemical results are completed by the morphological characterization using (SEM-EDX) analysis.

From the analysis of the open circuit potential evolution, it is noticed that for the untreated titanium alloy immersed in Hank's solution, the potential value has a shift towards more positive values, which indicates the formation of a $TiO_2$ oxide layer on the sample surface. In the case of the surface of the untreated Ti6Al4V grade 5 alloy immersed in Hank's solution doped with albumin, a tendency of the potential to move towards nobler values is also observed.

With the decrease in albumin concentration, the potential value has a tendency to move towards more negative values compared to the alloy immersed in Hank's solution, a behavior that indicates the inability of the material to form a protective oxide layer on its surface.

For the Hank solution in which different concentrations of $H_2O_2$ were added, it is observed that the value of the immersion potential has a slight increase in the first 300 minutes after which it stabilizes, remaining almost constant until the end of the measurement. The highest value of the potential corresponds to the titanium alloy immersed in Hank's solution doped with the highest concentration of hydrogen peroxide. This behavior is due to the fact that hydrogen peroxide is a strong oxidizing medium.

In the case of Hank's solution with the addition of albumin and $H_2O_2$, it is observed that the displacement of the potential towards more positive values is lower compared to the untreated titanium alloy immersed in the solutions with the addition of hydrogen peroxide, but an increase in the potential is observed, compared to the untreated titanium alloy immersed in solutions with added albumin.

In the case of the electrochemically oxidized samples for all the studied solutions, it is observed that the values of the open circuit potential move towards more positive values compared to the values of the untreated alloy immersed in the same solutions.

Making a comparative analysis of the results presented in this chapter, it is noted that the highest values of the polarization resistance ($R_p$) are obtained by the untreated grade 5 titanium alloy immersed in Hank's biological solution, followed by the untreated grade 5 titanium alloy immersed in the solutions with the addition of different concentrations of albumin. The next system in terms of polarization resistance values obtained is represented by the alloy immersed in different concentrations of albumin + $H_2O_2$ mixture. The lowest values of the polarization resistance are recorded for the system with the addition of different concentrations of $H_2O_2$.

From the comparative analysis of the ($V_{cor}$) values, it can be seen that the lowest values of the corrosion rate are recorded by the untreated grade 5 titanium alloy immersed in Hank's biological solution, followed by the untreated grade 5 titanium alloy immersed in the solutions with the addition of different concentrations of albumin. The next system of obtained ($V_{cor}$) values is represented by the alloy immersed in different concentrations of albumin + $H_2O_2$ mixture and the lowest ($V_{cor}$) values are recorded for the system with the addition of different concentrations of $H_2O_2$. This behavior demonstrates that the addition of hydrogen peroxide increases the cathodic and anodic reactions by dissolving the $TiO_2$ layer on

the surface of the material, thus decreasing the corrosion resistance of the untreated Ti6Al4V grade 5 alloy. The addition of albumin increases the corrosion resistance of the untreated Ti6Al4V grade 5 alloy because it inhibits the cathodic reactions by adsorbing on the metal surface of the used alloy a layer of albumin that acts as a protective film.

In the analysis of the polarization resistance values for the controlled oxidized Ti6Al4V grade 5 alloy with the titanium oxide ($TiO_2$) film formed on its surface, it is seen that they are higher, compared to the values of the untreated Ti6Al4V alloy for all measurement periods during immersion. This behavior confirms the efficiency of the electrochemical oxidation process of the titanium alloy for improving the resistance of the implant to the aggressive action of human body fluids.

Making a comparative analysis of the results presented in this chapter, it is observed that the highest values of the polarization resistance ($R_p$) are obtained by the electrochemically oxidized grade 5 titanium alloy immersed in Hank's biological solution, followed by the electrochemically oxidized grade 5 titanium alloy immersed in the solution with added albumin. The next system in terms of obtained resistance values is represented by the electrochemically treated alloy immersed in Hank's solution doped with albumin and $H_2O_2$, the lowest values of the polarization resistance for the electrochemically oxidized grade 5 titanium alloy are recorded for the $H_2O_2$ added system.

From the comparative analysis of the ($V_{cor}$) values, it is observed that the lowest corrosion rate values are recorded by the electrochemically oxidized grade 5 titanium alloy immersed in Hank's biological solution, followed by the oxidized grade 5 titanium alloy immersed in Hank's solution doped with albumin. The next system in terms of obtained ($V_{cor}$) values is represented by the oxidized alloy immersed in Hank's solution with the addition of albumin and $H_2O_2$ and the highest ($V_{cor}$) values are recorded for the oxidized Ti6Al4V grade 5 alloy immersed in Hank's solution with the addition of $H_2O_2$. It can be observed that the same trend is maintained in the case of the untreated titanium alloy, only that the corrosion rate values are lower for the oxidized titanium alloy compared to the untreated titanium alloy.

The presented results are highlighted by the SEM-EDX comparative analysis before and after the corrosion process and confirm the electrochemical test results.

## Selective bibliography chapter VII

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# CHAPTER VIII

# GENERAL CONCLUSIONS, PERSPECTIVES, EXPLOIT AND IMPACT OF THE RESEARCH RESULTS

## 8.1. General conclusions

The field of biomaterials is very diverse and constantly developing. Over time, scientists have sought various techniques to achieve medical safety standards in the use of biomaterials, to improve the quality of human life and to increase lifespan. Titanium and its alloys are of great interest in terms of implantable materials.

The main purpose of the research carried out in this study was to improve the surface properties of the Ti6Al4V grade 5 alloy used in biomedical applications, through the formation of biofunctional films of high quality titanium oxides with the help of electrochemical methods.

The importance of surface characterization is constantly increasing as the interest in miniaturization and nanotechnology progresses. Surface modification is a very attractive field of biomaterials research.

In **chapter I** of this study, the general aspects regarding titanium alloys as biomaterials are presented. Also, corrosive environments that affect implant materials (as the human body is known to be unfriendly to any implanted metal alloy), biological fluids, inflammatory conditions, methods and techniques for improving the surface of titanium alloys for biomedical applications are described. Furthermore, the factors that influence the obtaining of functional layers on biomaterials to improve their corrosion resistance in biological implant environments and biomedical applications that use functional layers are described.

In **chapter II** of this study, the research directions and the main proposed objectives are presented. The study sought to obtain a deep understanding of the corrosion resistance of the oxide layers (films) obtained in the presence of albumin, as a metabolic product and hydrogen peroxide, as a compound of inflammatory processes in the body, but also the synergistic effect of the presence of the two compounds in Hank's biological solution that simulates human blood.

**Chapter III** describes the materials, equipment and experimental techniques used to carry out this research study. Therefore, general information is presented about the Ti6Al4V alloy and the solutions and electrolytes used to modify the titanium alloy surface through anodic oxidation.

**Chapter IV** describes the influence of the electrochemical parameters to form thin oxide films on their properties. The variation of the anodizing current density at a constant applied potential and the comparative analysis of the surface roughness and microhardness of the Ti6Al4V grade 5 alloy and the electrochemically formed titanium oxide films are presented.

In **chapter V**, the morphological analysis of the layers obtained by anodic oxidation was carried out using optical microscopy and electron microscopy (SEM-EDX); structural characterization by X-ray diffraction (XRD) and surface hydrophobicity analysis of the untreated Ti6Al4V grade 5 alloy.

In **chapter VI**, the electrochemical characterization of the titanium alloy and oxide films in biological fluids was carried out using electrochemical methods such as the open circuit potential (OCP), the evolution of the polarization resistance ($R_p$) and the corrosion rate ($V_{cor}$) vs. time. All the obtained results reinforce the fact that the surface modification of the Ti6Al4V grade 5 alloy by the anodic oxidation method improves its anticorrosive properties.

**Chapter VII** presents the electrochemical characterization of the titanium alloy and oxide films in biological fluids under inflammatory conditions. It is important to know the corrosion behavior of the Ti6Al4V alloy in biological fluids under inflammatory conditions because most metals react when they come into contact with fluids in the human body. Thus, in this chapter, the effect of time, albumin (protein), hydrogen peroxide (added to simulate the inflammatory environment in the body), but also of the two compounds (the synergistic effect) added to Hank's biological solution was analsyed on the corrosion behavior and stability of the Ti6Al4V alloy through electrochemical methods such as the open circuit potential (OCP), the determination of the polarization resistance ($R_p$) and corrosion rate ($V_{cor}$). The electrochemical results are completed by the morphological characterization using (SED-EDX) analysis. The results obtained are consistent with those obtained from the SEM-EDX comparative analysis before and after the corrosion process and confirm that the surface modification of the Ti6Al4V grade 5 alloy by the anodic oxidation method improves its anticorrosive properties.

## 8.2. Future perspectives

Although, this study addresses 2 important issues of implant biomaterials:
(1) The study of the albumin protein effect, the effect of the hydrogen peroxide inflammatory compound, as well as the synergistic effect of their presence in the biological fluid and
(2) The modification effect of the surface of the Ti6Al4Vgrade 5 implant alloy on the improvement of its properties by increasing the resistance to corrosion degradation both in biological fluids and in the presence of proteins and inflammatory compounds in the human body.

The research in this field could continue by addressing other aspects as well:

Thus, it is proposed to increase the times imposed on the electrochemical oxidation

process, in order to have more parameters and to be able to make a more complex characterization of the untreated and oxidized Ti6Al4V alloy.

Several values of the potential imposed on the electrochemical oxidation process correlated with the oxidation times can also be researched.

All implant biomaterials starting from pure titanium to other titanium-based alloys can be improved by electrochemically controlled oxidation.

Another proposed research direction is to approach other test solutions such as doping Hank's biological solution or other solutions with other compounds, in order to more correctly evaluate the corrosion resistance of implant biomaterials and thus predict their lifespan in a specific given environment.

## 8.3. Personal contributions

From a scientific point of view, the personal achievements following the research during the preparation of this doctoral thesis are:

- ✓ Preparation of the biological solutions used for the electrochemical characterization of the untreated titanium alloy and the titanium oxide films obtained on them.
- ✓ Preparation of the experimental protocols used for the electrochemical characterization of the Ti6Al4V grade 5 alloy and for obtaining the titanium oxide layers.
- ✓ Analysis of the influence of the electrochemical parameters imposed on the anodic oxidation of the Ti6Al4V grade 5 alloy to obtain titanium oxide ($TiO_2$) films with improved properties and the comparative characterization of roughness and microhardness.
- ✓ Analysis of the surfaces of the untreated titanium alloy but also of the oxide films electrochemically formed on its surface, by optical microscopy, electron microscopy (SEM-EDX).
- ✓ X-ray diffraction structural characterization and hydrophobicity analysis for both untreated and electrochemically oxidized alloy.
- ✓ Electrochemical characterization of the titanium alloy and oxide films in biological fluids (such as Hank's solution) by methods such as the open circuit potential, evolution of the polarization resistance ($R_p$) and the corrosion rate ($V_{cor}$) vs. time.
- ✓ Electrochemical characterization of the titanium alloy and oxide films in biological fluids under inflammatory conditions, through methods such as the open circuit potential, evolution of the polarization resistance ($R_p$) and the corrosion rate ($V_{cor}$)vs. time. I evaluated the influence of doping the biological solution with albumin (the most abundant protein in the human body) with hydrogen peroxide (to simulate the inflammatory environment in the body) and the combination of the two compounds to highlight their synergistic effect.

- ✓ Interpretation of the experimental results and forming the final conclusions.

## 8.4. Scientific achievements in the field of the research topic

### 8.4.1. Articles published in ISI (Clarivate Analytics) ranked journals with Impact Factor

**8.4.1.1.** Lidia Benea, **Anca Răvoiu**, Jean-Pierre Celis, **Anticorrosion Performance of the Electrochemically Grown Mixed Porous Oxide Films on Titanium Alloy in Biological Solution**, *ACS Biomaterials Science & Engineering (ACS Biomater. Sci. Eng.*) 2019, 5, 11, 5925–5934. Impact Factor = 5.395, **Q2.**
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WOS: 000496344800039

**8.4.1.2**. Simionescu Nicoleta, **Răvoiu Anca**, Lidia Benea, **Electrochemical in vitro Properties of 316L Stainless Steel for Orthodontic Applications,** *Revista de Chimie*, 2019, Vol. 70, Issue 4, p. 1144-1148. Impact Factor = 1.605, Q3
https://doi.org/10.37358/RC.19.4.7081
WOS:000469387200008

### 8.4.2. Publications in journals indexed in international databases (BDI)

**8.4.2.1**. **Anca Răvoiu**, Nicoleta Simionescu, Lidia Benea, **Influence of different concentration of hydrogen peroxide on the corrosion behavior of Ti-6Al-4V alloy immersed in physiological solution,** *IOP Conf. Ser.: Mater. Sci. Eng.* 572 (2019) 012006.
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**8.4.2.2.** L Dragus, L Benea, N Simionescu, **A Răvoiu**, V Neaga**, Effect of the Inflammatory Conditions and Albumin Presence on the Corrosion Behavior of Grade 5 Titanium Alloy in Saliva Biological Solution,** *IOP Conf. Ser.: Mater. Sci. Eng.* 572 (2019) 012005.
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DOI 10.1088/1757-899X/374/1/012077

**8.4.2.4**. Nicoleta Simionescu, Lidia Benea, **Anca Răvoiu**, **Effect of hydrogen peroxide addition to phosphate buffered saline solutions on corrosion resistance of 316l stainless steel,** *Proceeding Volume of 18th International Multidisciplinary Scientific GeoConference SGEM* 2018, Volume:18, Book number: 6.1, Pages:169-176, ISSN: 1314-2704, 24. Section Micro and Nano Technologies, 30 June - 9 July, 2018, Albena, Bulgaria.
Doi: 10.5593/sgem2018/6.1.

**8.4.2.5. Anca Răvoiu**, Lidia Benea, **The pH value effect of a simulated physiological solution on the corrosion resistance of Ti-6Al-4V alloy,** *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM*, 2017, 17(61), pp. 419–426,
Doi: 10.5593/sgem2017/61/S24.055

### 8.4.3. Conferences and scientific papers presented at international congresses

**8.4.3.1. Anca Răvoiu**, Lidia Benea, Nicoleta Lucica Simionescu, Alexandru Chiriac, **Influence of inflammatory conditions on the corrosion behavior of titanium alloy in buffered biological solution,** *Oral presentation. The Vth international conference "New trends in environmental and materials engineering" (TEME 2019)*, 23-25 October 2019, Galați, Romania.
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