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TITANIUM IMPLANT MODIFIED BY PLASMA ELECTROLYTIC FOR NEOINTIMA FORMATION IN BLOOD PUMPS

Rosa Corrêa Leoncio de Sá
Institute Dante Pazzanese of Cardiology and Federal Institute of Technology in Sao Paulo
rosacldesa@gmail.com

Gláucio Furlanetto
Institute Dante Pazzanese of Cardiology
furlanettoglaucio@gmail.com

Nilson Cristino da Cruz
Technological Plasma Laboratory of UNESP- Sorocaba
nilson@sorocaba.unesp.br

Evandro Drigo
Institute Dante Pazzanese of Cardiology
evandro.drigo@gmail.com

Tarcisio Leão
Institute Dante Pazzanese of Cardiology and Federal Institute of Technology in Sao Paulo
leao.tarcisio@gmail.com

Juliana Leme
Institute Dante Pazzanese of Cardiology
pesquisa_bio@fajbio.com.br

Jeison Willian Gomes da Fonseca
Institute Dante Pazzanese of Cardiology
jfonseca@fajbio.com.br

Bruno Utiyama
Institute Dante Pazzanese of Cardiology
brunoutiyama@gmail.com

Edir Leal
Institute Dante Pazzanese of Cardiology
edir.leal@ig.com.br

Aron José Pazin de Andrade
Institute Dante Pazzanese of Cardiology
aandrade**@fajbio.com.br**

Eduardo Guy Perpétuo Bock
Federal Institute of Technology in Sao Paulo
eduardo_bock@yahoo.com.br

***Abstract**. Institute Dante Pazzanese of Cardiology, Federal Institute of Technology in Sao Paulo and Adib Jatene Foundation have developed a Ventricular Assist Device (VAD) for patients with severe heart failure. The Implantable Centrifugal Blood Pump (ICBP) is a VAD basically made of Titanium +4 oxidation state (Ti^{+4}), an important biomaterial with low density and good corrosion resistance. However, when in continuous contact with blood, the released ions can compromise homeostasis and VAD biofunctionality. We are using Plasma Electrolytic Oxidation (PEO) in Ti samples to texturize their blood contacting surfaces. The micro-arcs covered the samples in electrolyte solution (magnesium, sodium phosphate, glycerol and water) for 50 seconds. Current density was maximum of 1.5 A/cm^2, under voltage of 500 V. Scanning electron microscopy revealed the formation of uniform and homogeneous pores. Energy dispersive spectroscopy indicated greater incorporation of magnesium. Contact angle measurements showed a significant hydrophilic character of the surface. Preliminary results showed good blood interaction with treated surface under continuous and laminar flow, as there is in ICBP. Implants made of Ti with plasma treated surface are being developed and will be submitted to In Vivo experiments to prove the formation of coating-endothelial living cells with high resistance to thrombus formation.*

***Keywords**: VAD, titanium, surface modification, plasma electrolyte.*

1. INTRODUCTION

Cardiovascular Diseases (CVD) are the leading cause of death in Brazil, yet the mortality rate decreased in recent decades, mainly in the South and Southeast of the country, for patients aged over 60 years [Mansur et al., 2001]. These diseases accounted for 30% of all deaths recorded in 2012, in our country; one of the factors contributing to this situation are the difficulties related to organ transplantation, which takes about 20-40% of patients on the waiting list for a heart transplant compatible to death [RBT, 2014]. Today, the international biotechnology offers Uni / Biventricular Assistance Devices (VAD) which can be applied in full or partial replacement of the heart functions for patients with chronic Heart Failure (HF). The circulatory support by VAD's can provide hemodynamic stability compatible to the patient's organism until your cardiac system is recovered [Nosé, 2003] and also decreases the dose of intravenous agents applied in the treatment of HF, decreasing mortality in 50% in 6 months, and 80% at 1 year [Mancini, 2005].

In contribution with the technological scientific development and public health, a project of an Implantable Centrifugal Blood Pump (ICBP) is being developed at the Institute "Dante Pazzanese" of Cardiology (IDPC) to be used as destination therapy (by undetermined time), bridge to transplant (before heart transplantation) or recovery therapy (after transplantation) [Bock, 2008]. We estimate that, only in Brazil, about 2000 patients could be candidates for a VAD [Andrade, 2012]. Implantable medical devices, longer than 5 years, must be made of biomaterials presenting no form of degradation during application time. Thus, the ICBP components that remain in direct contact with the blood, exception to the pivot bearing system, are made of titanium oxide (TiO_2). The titanium is specified as biomaterial by American Society for Testing and Materials (ASTM), the International Organization for Standardization (ISO) and other national standards. Biomaterials must have a combination of mechanical, chemical, physical and biological properties that enable application in the human body without becoming cytotoxic, that is: as fewer as the particles is released by the material they are better accept by local biological tissue [Oréfice, 2005]; material should not cause damage to implant site nor for surrounding tissues, as well as should not cause local or systemic immune responses; they must not be carcinogenic, pyrogenic, toxic, allergenic and still supporting the sterilization process without presenting changes to those properties above mentioned.

Titanium (Ti) is a light transition biometal, atomic number 22 and atomic mass u 47.90; is biocompatible, applied in implants since the late 60's and has excellent mechanic characteristics such as high corrosion resistance, low density (4.54 g/cm^3, 60 % of the iron density) and modulus of elasticity is close to the bone (110 GPa). The titanium oxide is formed spontaneously when Ti comes in contact with oxygen (O_2) and available in three crystallographic forms: rutile and anatase with tetragonal structure; and brookite with orthorhombic structure. The solubility and toxicity of TiO_2 are considered low, but when kept in the midst of bodily fluids for a long time, it releases ionic species triggering electrochemical reactions in the body that can severely compromise the homeostasis of the immune system [Hanawa, 1997].

In this sense, various researches are undergoing in order to increase the biocompatibility of biomaterials to provide better interaction with the human body, for example, through surface modification by coating [Kaneko et al., 2001]. The biocompatibility of an implantable material is considered great when it promotes epithelialization (biological tissue growth) and establishes with the biological environment an interface capable of supporting normal loads at implanted site or there is an appropriate response to the host in a specific application and thus promote the implant biofunctionality [Boshi 1993]. Tissue Engineering investigates the interaction between biomaterials, cells and specific cell growth factors to control organ´s degeneration by diseases, injury or trauma, even tissue coating formation at implants, a kind of biological thin film. For this development one must know the source of appropriate cells, cellular environment, implant period and all characteristics of biomaterials [Godinho, et al, 2011].

Epithelial biocicatricial response starts immediately after the injury through the release of cytokines (inflammatory response) such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-a), FAS ligand system, epidermal growth factor (EGF), platelet growth factor (PGF) among other collagenases and metalloproteinases [Netto, 2005]. Interaction between biological tissue and implant material during biocicatricial process is affected by the topography at the macroscopic level and by roughness at the microscopic level, because the cells need anchoring points at material surface to ensure biofixation and to proliferate [Elias, et al, 2001]. Later, may occur the major cellular phenomena, consisting of apoptosis and necrosis of keratocytes, fibroblasts replication, influx of inflammatory cells and the formation of myofibroblasts [Netto, 2005].

The VAD centrifugal HeartMate II ®, designed by Thoratec Corporation in Pleasanton, CA - USA, uses a coating based on sintered titanium microspheres, which provides a thin layer of living cells at pump surface that becomes endothelia and is highly resistant to thrombus formation (Patent: US 20070299297 A1).

Currently, plasma technology, such as technical Plasma Electrolytic Oxidation (PEO), enables surface treatments into materials to improve the interaction of an implant surface with the living organism [Sá, 2014]. The technique can be applied in cleaning surfaces, heat treatment, change in surface characteristics such as pickling, polishing, texturing and deposits. The formation of a thin coating or film on a substrate can be called Plasma Electrolytic Deposition (PED); Incorporated into the PED there are two processes: Plasma Electrolytic Oxidation by and Saturation by Plasma Electrolytic (SPE) [Kellogg, 2007]. Plasma is defined as an ionized gas containing ion-electron pairs in equal proportions; It can be generated by supplying thermal or electrical energy to a gas system where the atoms and molecules are ionized electrolyte through the ion-electron pairs. When the species of the electrolytic medium is incorporated into the surface of the substrate, it is modified. Such a process can be classified as plasma treatment, deposition or ablation [Sant'Ana, 2014].

The PEO process is a technique capable of applying coatings on some metals such as Ti, Al, Mg and Nb; it consists of two electrodes and the cathode (-) and anode (+) substrate itself, immersed in electrolyte solution with high electric potential difference; the substrate oxidation occurs by the action of localized electrical discharges, called micro-arcs which determine, in addition the growth rate of the oxide coating, structure, chemical composition and morphology [Antonio, 2011]. The PEO treatment process is controlled and influenced by many factors, both intrinsic and extrinsic; tribological performance of the formed coating are affected by both. The chemical composition of the substrate and of the electrolyte solution are considered to be intrinsic factors responsible for the structure and composition of the coatings formed. The electrical parameters, process temperature, oxidation time and additives are considered and extrinsic factors and are controlled to determine the size and distribution of formed structure and the distribution of chemical species in the coatings [Li, et al., 2004].

This paper describes the production of titanium implants medical grade II by conventional machining; applying of electrochemical process, the electrolytic plasma for formation of uniform pores and well distributed on the surface of titanium implants; and the characterization of the implants by Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and surface wettability by contact angle measurements . The biophysical aspects of biomaterial have been modified by phenomena of conventional electrolysis and the atmospheric plasma for be implanted in pigs as scaffolds, in future experiments In Vivo.

2. MATERIALS AND METHODS

Twenty implants/substrates were made in titanium medical grade II by conventional machining at the Institute "Dante Pazzanese" of Cardiology; the dimensions were adopted according as the morphology of the local implant, internal region of the abdominal aorta of approximately 25 kg pigs, see Fig. 1.

The average normal diameter of the human aorta is 20 millimeters [mm], and the normal thickness of its intima and the media layer is 0.10 mm each [Netter, 2000]. In experiments already made with landrace pigs with an average weight of 20 kilograma [Kg], says if that the mean diameter of the abdominal aorta ranges from 6 to 7 mm [Grüdtner 2001]. It is estimated that for Landrace with maximum weight of 30 kg the inner diameter of the abdominal aorta varies from 10 to 12 mm. Subsequently, the implants were polished by hand by the company INP Biomaterials, using sandpaper 400 and profilometry (Mitutoyo SJ400) to control roughness (Ra) of the substrate, which must be tolerant of 2.249 +/- 0.01 micros [µm] as Preliminary trials performed with POE technique in titanium [Sa, 2014]. The profilometry consists of a nanoindenter what a can be used to obtain transverse profile generated by the wear path of reciprocal motion of tribology test. The tip of monocrystalline diamond which traverses the sample can be used in two different ways: 1. The face of the tip traverses the sample; 2. The vertex is running through the sample [Silva, 2009].

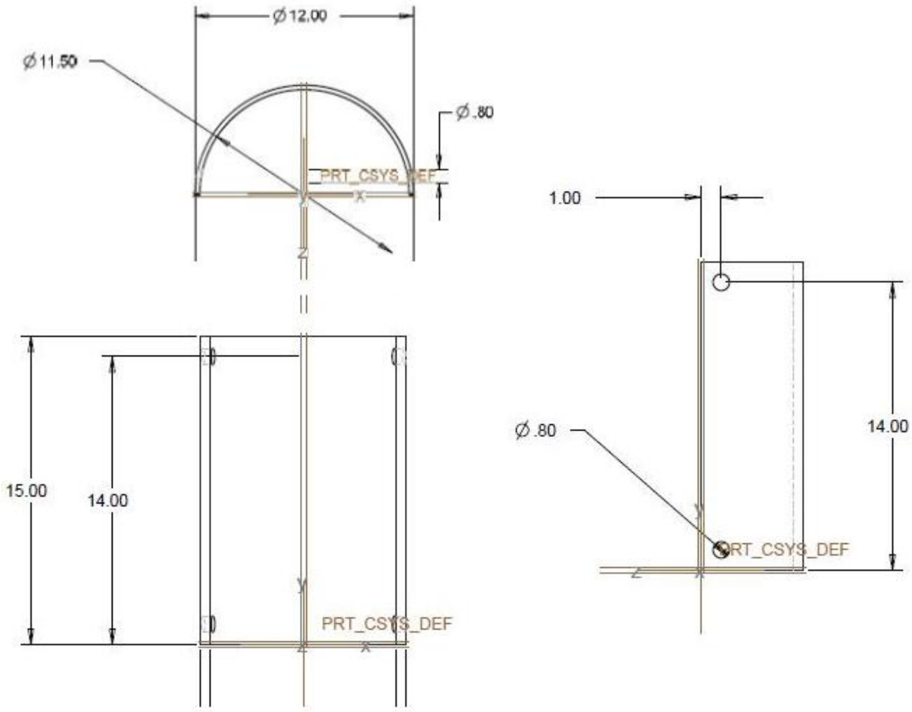

Figure 1. Bidimensional drawings of titanium implant.

The surface modification by plasma electrolytic and the chemical physical characterization of titanium implants were performed in the Experimental Campus of the Technological Plasma Laboratory of the Universidade Estadual Paulista - UNESP Sorocaba. First, we performed a process of cleaning the substrates with enzymatic detergent in distilled water (20 grams ‘g’ / l for 900 seconds ‘s’) and isopropyl alcohol for disposal the residues ; the implants were stored in petri dishes and air dried . Each implant was kept immersed in electrolyte solution for 50 seconds; The solution was comprised of glycerol phosphate disodium salt ($C_3H_7Na_2O_6P$ x H_2O) and magnesium acetate tetrahydrate ($C_4H_6MgO_4$ x $_4$ H_2O) in specific concentrations, diluted in deionized water; the voltage applied during the treatment time was fixed at 500 Volts and 400 Hz frequency [Sa, 2014].

During treatment, the characteristics of the reactions take place as shown in Fig. 2 where, curve "a" represents the reactions that occur at the metal - electrolyte system and the curve "b", as in the formation of the oxide film on the anode, which is the implant itself. At low voltages, the kinetics of the processes occurring in the electrodes can be equated the law of Faraday and the voltage and current characteristics, under the laws of Ohm [Yerokhin 1999].

The physicochemical characterization of titanium implants was performed by analysis of Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) in a JEOL / EO microscope, version 1.11. The SEM produces high magnification and resolution images (up to 300,000 x); its working principle consists in the issuance of electron beams by a tungsten capillary (negative electrode), by applying a potential difference which can range from 0.5 to 30 kV [Hans-Jürgen, et al, 1997]; EDS in the identification of molecular bonds is performed based on the comparison of frequencies absorbed by the sample with tabulated frequencies [Scheinmann, 1970].

The hydrophilic or hydrophobic character of the surface of TiO_2 implants has been defined by wetting tests determined by contact angle measurements; one Ramé Hart Goniometer, Model 100-00 was used to deposit a drop of deionized water on the surface of each sample and then observed whether the drop scattering angle and counted drop on the substrate. The RHI 2001 Imaging software has been adopted for the contact angle measurements.

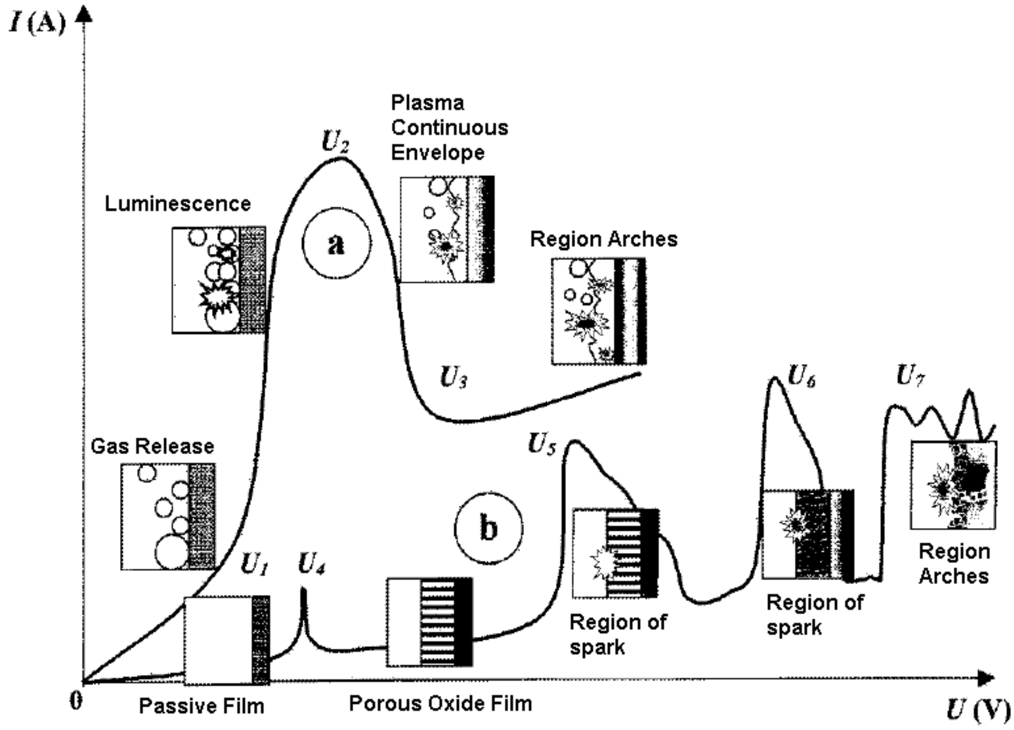

Figure 2. Features of the reactions of treatment Plasma Electrolytic Oxidation. Curve "a" - reactions that occur at the metal-electrolyte system. Curve "b" - oxide film formation at the anode.

3. RESULTS

Of the twenty implants made in TiO_2, he separated two to be configured as Reference Material in future experiments In Vivo. The Reference Material is characterized by the common use of the material in the making of implants, where the bioactive principle and other properties are already known and consecrated. The superficial aspect presented by machined and polished titanium is smooth, shiny and of color silver.

Figure 3. Pictures from titanium implant with medical grade II; A. Reference Material and B. Implant treated with electrolytic plasma.

The electrical parameters and of electrolyte concentration adopted for the PEO technique, again proved ideal for achieving the necessary conditions of the effects of conventional electrolysis and atmospheric plasmas on titanium substrates; the current density was controlled within the band from 0.01 to 1.5 A/cm^2 under high voltage; and then it there was excessive gas formation, accompanied by luminescence, followed by micro-arcs what appear around the implants submerged in the electrolyte. Thus, 50 seconds of treatment were sufficient to transform glossy and smooth surface of the TiO_2, machined and polished into a matt and rough surface, see Fig. 3, with the presence of uniform pores and frameworks well distributed; Fig. 4 shows two SEM micrographs of one of titanium implants modified by electrolytic plasma; due the implant be concave, it was decided to study the interactions of treatment in two areas of the implant, the center and the edge.

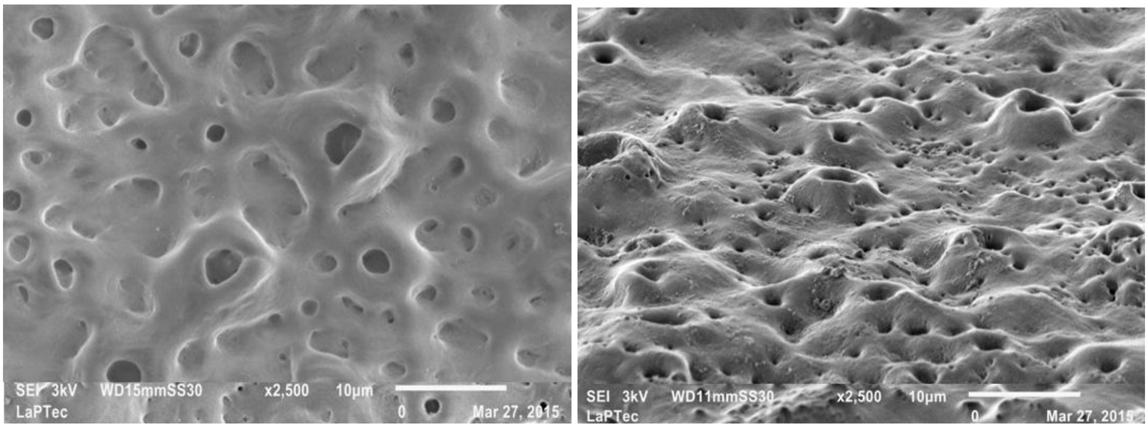

Figure 4. Micrographs generated by SEM the modified titanium surface by electrolytic plasma; the left, the center of the implant and the right the edge of the implant.

The EDS technique used for chemical characterization of the surface of the implants revealed the incorporation of magnesium and phosphorus; incorporation of magnesium (Mg) on the surface of the scaffolds is very promising, because it is a mineral essential to life, which participates in the regulation of fluxes through the cell membrane, various enzymatic processes helps and participates in the formation of new proteins [Feltre, 1990]; and may play an important role as adhesion and cell growth factor in Vivo experiments.

The wettability of surfaces can be quantified by the theory of the contact angle between the droplet and the surface of the substrates/implants; when the touched drop on the surface under study, it was observed that the scattering shown in Fig. 5. Software RHI 2001 imaging revealed the average contact angle measurements implant machined and polished titanium and titanium 77.367 ° modified plasma 34.367 °.

Figure 5. Comparative analysis of the contact angle in titanium surface; the right, titanium machined and polished; the left, modified titanium plasma.

4. CONCLUSION

Techniques such Electrospinning Fiber bonding, Phase Reversal, Solvent Evaporation and Rapid Prototyping are studied and applied for making Scaffolds, but does not apply in biometais as titanium. The oxidation process by Plasma Electrolytic met the expectations for fabrication of Scaffolds titanium, even with the slight disproportion texture, pointed out by SEM, between regions of the implant edge and center. The structure of the substrate, in this case concave, is considered an extrinsic factor, which should be studied and suitable to obtain uniformity of pores and of frameworks formed. In texture, the technique provided incorporation of chemical species of the electrolyte solution to the TiO_2 surface such as magnesium and phosphorus; such species derived biofixation and stimulate cell proliferation in vivo growth factors.

Increased surface roughness leads to obtaining a hydrophilic surface; thus, the higher the wettability, the greater the interaction of the material with the biological environment. The biophysical aspect of the implants in study is characteristic of metallic scaffold by having pores with a diameter of 5-10 μm and frameworks with a diameter greater than 10 μm; these dimensions may be suitable for providing adhesion and cell proliferation with vascularisation and removal of metabolites generated through a process epithelialization in vivo; in a specific application, the in ICBP biofilm formation by implant time and continuous blood flow [Sa, 2014].

The superficial aspect of titanium implants can provide the anchoring of blood cells such as erythrocytes with Ø 7.5 μm and hamper access of monocytes with diameter 20 to 30 μm; monocytes are large cells, formed by monoblasts able to enter the loose connective tissue and develop in phagocytic cells, macrophages, responsible for expelling bacteria and other foreign substances from the body. beyond the red blood cells and monocytes, 55% of the blood is composed of plasma [water (92%), proteins (fibrinogen, albumin and globulins), sodium (7%), gases, nutrients, excretions, hormones and enzymes]; normally, such components are at the nanoscale; blood plasma plays an important role in the balance of intravascular osmotic pressure, electrolyte balance, and as drainage and distribution of proteins. In addition, the pore diameter and scaffolds formed on the surface of TiO_2 implants are related to the diameter of capillaries blood, approximately 5-10 µm enough to pass a single blood cell; capillaries comprise the largest surface area of all types of blood vessel responsible for vascularization, there wave exchange oxygen, nutrients and waste products between the blood and local tissue.

The implants will undergo acute In Vivo experiments, where they will remain fixated to the aorta inner layer of pigs, during a minimum period and sufficient to prove its ability to provide a well adhered thin biofilm in function of implantation time under continuous blood flow. This technique will be applied for coating titanium surface of ICBP, to improve its biofunctionality.

5. ACKNOWLEDGEMENTS

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