STUDY OF OSSEOINTEGRATION ON MODIFIED SURFACES OF TITANIUM IMPLANTS

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ABSTRACT

In this work it was obtained a controlled nanotopography by anodization in dental implants, seeking for the optimization of the osseointegration. For this purpose sixty titanium implants were divided into: group 1 (control, machined implant); group 2 (rough, commercial implant); group 3 (experimental, implant anodized with pulsed current). Ten rabbits received an implant of each group into the two tibia bones, and five rabbits were euthanized, 2 and 6 weeks after surgery. Prior to surgery, the surfaces were characterized by Atomic Force Microscopy, Scanning Electron Microscopy and Raman Spectroscopy. The implants of the right tibia underwent Periapical Radiograph (RP) and Computed Microtomography (μCT); while the ones on the left tibia were submitted to reverse torque test and subsequent MTT cytotoxicity assay. Anodizing used in this study positively affected the chemical composition and structure of TiO_2 film, enhancing its biological characteristics in the osseointegration.

Key words: Anodizing, Microtomography, Nanotechnology, Osseointegration.

INTRODUCTION

Several authors have observed that implants surface modifications may be able to improve the speed and quality of osseointegration resulting in increased bone deposition and also a reduction of the repair period [1-10]. These modifications display nano-structural features that can enhance the growth and attachment of mesenchymal cells and osteoblast due to an increased surface area and also provide better conditions for the cell-substrate interaction [10-14].

The interest in obtaining the nanotopography through the anodizing process has been increasing, since this is a low cost and efficient reproducibility technique and exhibits adequate surface modification for cellular activities [15-19]. The anodization process can transform a amorphous oxide film into a crystalline oxide layer and creates nano-roughness on the implant surface, favoring the growth of osteoblastic cells in different orientations, resulting in a more effective osseointegration process [15-18, 20, 21]. According to Yao et al. (2008) [22], the nano-sized features can simulate the cellular environment.

Shokuhfar et al. (2014) [4] analyzed the interaction between osteoblasts and titanium exhibiting amorphous and crystalline titanium dioxide. The authors concluded that the high wettability of the surface due to the crystallinity exhibited greater influence on cell spreading due to the hydrophilicity of the crystalline surface compared to amorphous one. A hydrophilic surface shows a higher protein adsorption to a hydrophobic surface, promoting a positive effect on cell behavior in comparison to a hydrophobic surface.

Kim K et al. (2013) [23] concluded that a relatively thicker and more uniform oxide layer than that formed naturally in the atmosphere enhances the corrosion resistance and abrasion and increases significantly the alkaline phosphatase activity.

The anodization technique is an electrochemical method for surface modification that improves the bioactivity of Ti orthopedic and dental implants, due to the formation of a single nanotopography that promotes positive effects on cellular activities and has adequate capacity to interact with fluid and bone tissue. It is a process of low cost and efficient reproducibility, and its advantages over conventional surface modification methods have been reported in many studies [10, 15-19].

In our study, the goal was to create an appropriate morphology to TiO_2 (roughness at the nanometer scale), and a more biocompatible chemical composition with the advantage of obtaining the anatase phase of TiO_2 through the anodizing process without requiring heat treatment at high temperatures. This goal was achieved successfully, although we used a longer anodization time (4 hours), which is unprecedented in the literature. Thus, it was compared bone formation, osseointegration and in vitro cytotoxicity of machined titanium implant, roughened implant (commercially available), and finally, trial implant subjected to anodizing process.

MATERIAL AND METHODS

Sixty commercially pure titanium implants (grade 4) were provided by Titaniumfix Company - Brazil, measuring 8.5 mm x 3.75 mm in diameter, with rounded conical apex with four cutting chambers, self-drilling screw, external hexagon (HE) were used and divided into three groups: - G1 (smooth, control): machined surface; - G2 (rough, commercial) machined surface subjected to blasting with aluminum oxide followed by subtraction by nitric acid; - G3 (anodized, experimental): machined surface subjected to the anodizing process with application of pulsed current (0.6 A, 30 V and 1000 Hz, for 4 hours).

Before surface treatment, the machined implants (screws) were properly fixed on a titanium plate. After cleaning the surface, the implants were anodized. For this procedure, the titanium plate and a copper plate were used as anode and cathode, respectively. Both plates were immersed in 1.0 mol/L H_2SO_4 solution as electrolyte. The parameters used for the anodization were: 0.6 A of resulting current, 30 V of applied potential, and 1000 Hz of frequency pulses, for 4 hours. For the monitoring of electrical parameters under this condition, there were used: a digital oscilloscope, model MO2061, Minipa brand; a pulsating square wave rectifier, GI21P-10/30 model, of the company General Inverter and also a multimeter, ET-2615A model, of the brand Minipa.

The implants were analyzed by Scanning Electron Microscope (SEM) and Atomic Force Microscopy (AFM) to characterize the surface morphology. The scanning electron microscopy analysis was performed on Structural Characterization Laboratory DEMa/UFSCar through Philips XL-30 FEG equipment. The AFM analysis was performed in the Associated Laboratory of Sensors and Materials - LAS, National Institute for Space Research - INPE, using an atomic force microscope Veeco V Nanoscope.

In order to determine the chemical composition of the anodic film, Raman spectroscopy and Energy-dispersive X-ray spectroscopy (EDS) analysis were performed. A Raman Spectrometer Horiba Scientific T64000 was used, aiming to find the positioned bands in the anatase region. The implants were analyzed prior to surgical installation, and after their removal by reverse torque, in order to observe any surface damages and alteration of their chemical compositions.

Prior to the implantation surgery, the animals were weighed and intramuscularly anesthetized with a mixture of 13 mg/kg of aqueous solution of 2.3 g of xylazine hydrochloride (Anasedan - Vetbrands), analgesic and muscle relaxing sedative substances, with 33 mg/kg of ketamine (Dopalen® - Agibrands of Brazil Ltda.) as a general anesthetic; and local anesthetic composed of prilocaine hydrochloride 3% associated with felypressin 0.03 IU / mL (Citanest® 3% - Dentsply). The surgical sites of the right and left tibia were submitted to scaling and antisepsis with iodine alcohol. The incision was performed with number 15 scalpel blade in the region corresponding to the medial surface of the tibia in its proximal third. The cortical tibia was exposed and the surgical procedure was performed. Throughout this procedure, a copious irrigation with sodium chloride 0.9% was kept, in order to avoid heating because of the drill friction against the bone Thus, in accordance with standard drilling sequence for implants of 3.75 m in diameter and 8.5 mm in height, drilling for the installation of the implants were performed. The implants were installed manually in order to obtain a primary stability and then they were adapted to the cover screws. The rabbits were subjected to six implants installation surgery, one from each group in each tibia.

After the surgical procedure of implant placement, the muscle tissue was sutured with absorbable Number 4 (Monoglyde® Poliglecarpone 25), the skin sutured with Number 4 silk suture (Ethicon® / Johnson & Johnson) and then it was made the antisepsis with the use of iodine alcohol. The animals received antibiotic therapy of benzathine benzyl penicillin, procaine benzyl penicillin, potassium benzyl penicillin and dihydrostreptomycin sulphate base, in a glass vials of 6,000,000 IU (Pentabiotic - Fort Dodge), intramuscularly at a dose of 1,35 mL / kg in the immediate postoperative (48 h). After surgery, the rabbits were placed into individual cages with food and water *ad libitum*, and monitored until the time of their euthanasia, at 2 and 6 weeks. Each period of sacrifice was of a group of five rabbits per time.

For euthanasia procedure, the animals underwent deep general anesthesia (propofol 10 mg/kg) intravenously. Then, they were submitted to the administration of a vial of intravenous potassium chloride in order to sacrifice them. The right tibia was stored in buffered formaldehyde solution, so they were evaluated by RP and µCT. The left tibia with the implants were stored in Ringer solution, placed inside a freezer at -20 °C ± 1 °C, for a further torque removal test.

RESULTS AND DISCUSSION

SEM analysis was performed with increases from 1000 times to G1; from 2000 times to G2; and from 100000 and 200000 times to G3. In these increases, it was established that the Group 1 demonstrated micro grooves, while Group 2 implants showed a textured surface on a micrometric scale, and Group 3 implants showed the presence of nanotexturized surface of the anodized implant (Figure 1). According to the AFM images, it was possible to observe the risks that are inherent to the dental implants machining stage (Figure 2A), the textured surface in the micrometer range (Figure 2B), and good uniformity of a nanotexturized surface from the anodization process (Figure 2C).

The analysis of implant surfaces was conducted by Raman spectroscopy, which provides information about the nanoparticles structures, because it is sensitive to changes in local structural order in a given material. According to the spectra in Figure 3, bands were observed positioned in regions of anatase in Group 3 implants in the amount of 146.4 cm^{-1} as recorded by Alhomoudi and Newaz [(24)], wherein the frequency bands were identified as: 147 (± 2.8) cm^{-1}, 392.8 (± 4.3) cm^{-1}, 515.2 (± 5.3) cm^{-1}, 513.14 (±7.4) cm^{-1}, 628.8 (± 10.2) cm^{-1}.

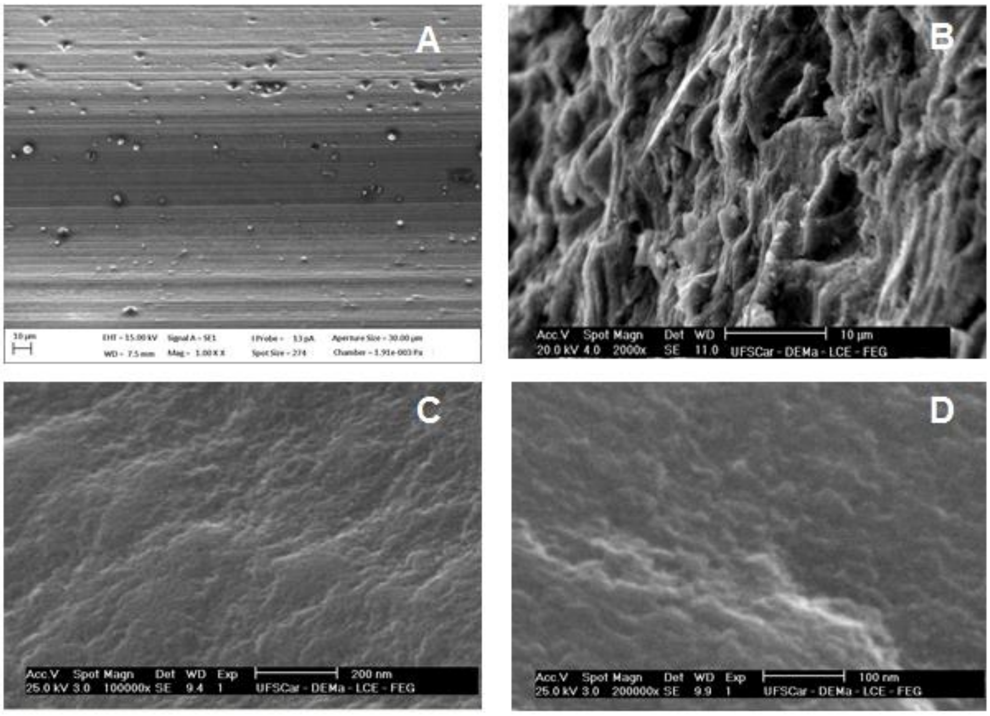

Figure 1 - A) G1 implant surface at a 1000x magnification (10μ). Note the presence of grooves on the surface inherent to the machining process. B) G2 textured implant surface by inkjet technique of aluminum oxide (Al_2O_3) followed by acid etching. It is possible to see some metal grooves and also some cuts caused by the shock of the oxide particles against the surface of the implant, resulting in increased roughness in 2000x (10μ). C) nanotexturized implant surface G3 increased in 100,000 times (200nm) and D) nanotexturized implant surface G3 increased in 200,000 times (100nm).

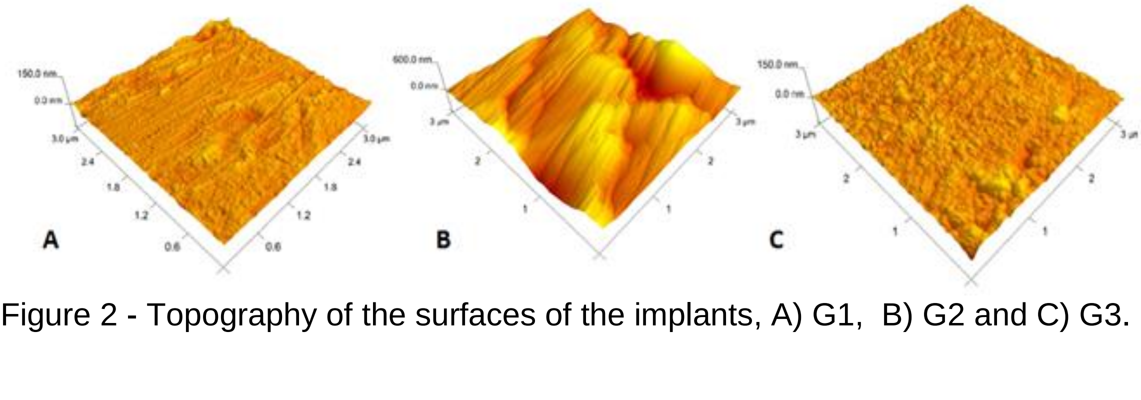

Figure 2 - Topography of the surfaces of the implants, A) G1, B) G2 and C) G3.

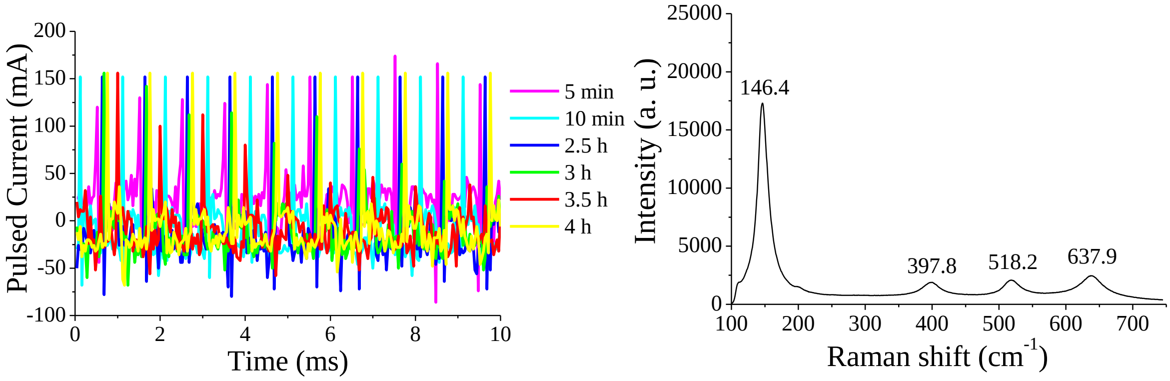

Figure 3 - Oscilloscope data during anodization of the Group 3 implants (A); Raman spectrum after anodization of the Group 3 demonstrating the presence of anatase phase (B).

Analysis by Periapical Radiograph (PR)

All images were evaluated and the absence of radiolucent halo was found, indicating that all implants osseointegrated, even within a period of 2 weeks. Then, the images of the implants were analyzed, in the computer program Image J, and a histogram of newly formed bone was conducted around the implant threads. These values were submitted to descriptive analysis and ANOVA analysis of variance. In Figure 4, it can be observed the descriptive data of the histograms of the implants at 2 and 6 weeks period, where the mean values of histograms of G3 implants (anodized-experimental) were higher. The ANOVA variance analysis showed no statistically significant difference between groups in periods of two weeks ($p = 0.99$) and 6 weeks ($p = 0.38$).

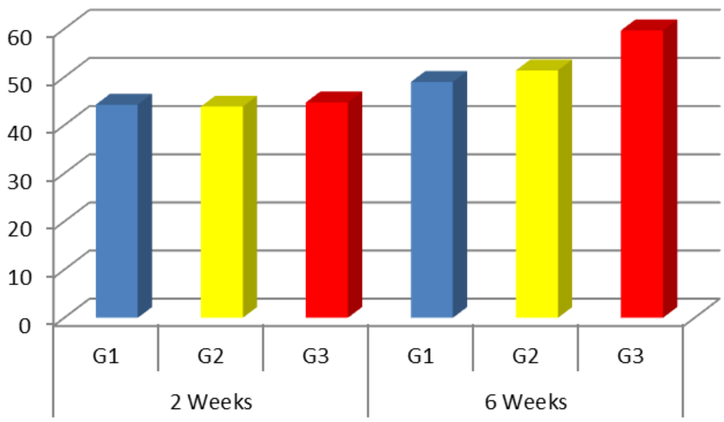

Figure 4 - Graph 1of the averages of the histogram data of the implants in the 2 and 6 week period.

Two implants from Group 1 (machined) were not osseointegrated, one within a period of 2 weeks, and another within 6 weeks of bone healing, which were excluded from the study.

From the removal torque testing values, a descriptive analysis was performed for each group (Figure 5). According to the results, it was possible to observe that the anodized implants demonstrate greater removal torque values than the implants of other groups, for both 2 and 6 weeks of osseointegration period.

There was a statistical difference between Group 1 (machined) and Group 3 (anodized), within a period of 2 weeks ($P<0.05$), indicating that the anodized implant osseointegration showed higher removal torque values than those of machined implants. It means that at the initial period of bone formation, the experimental implants (Group 3) have greater cell types which are responsible by formation of such tissue. Within 6 weeks of osseointegration, there was no statistical difference between groups ($p = 0.14$).

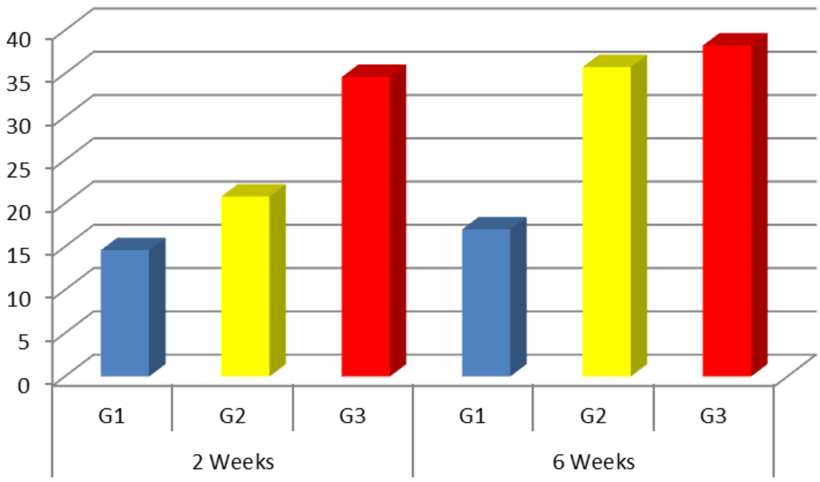

Figure 5 - Graph of the averages of the removal torque values for the implants machined, anodized and treated after 2 and 6 weeks from the surgery implant placement.

CONCLUSIONS

It is possible to conclude that the anodizing procedures used in this study positively affect the chemical composition and structure of the titanium oxide film, enhancing its biological behavior in osteogenesis, which enables its use in the practice of dental clinic.

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