

Anodic oxidation of titanium for implants and prosthesis: processing, characterization and potential improvement of osteointegration

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Abstract—Among all biomaterials used for bone replacement, it is recognized that both commercially pure titanium (Ti c.p.) and Ti₆Al₄V alloy are the materials that show the best *in vivo* performance due to their excellent balance between mechanical, physical-chemical and biofunctional properties. However, one of its main drawbacks, which compromise the service reliability of the implants and its osteointegration capacity, is the thin film of fibrous tissue around the implant due to the bioinert behaviour of titanium. One of the alternatives more studied to improve the titanium osteointegration is the surface modification through the control of the roughness parameters within a specific range which is recognized that improve the osteoblasts adhesion. In this work is investigated the influence of different electrochemical processing conditions for surface modification of c.p. Ti, in their microstructural, morphological, topographical and mechanical properties, as well as in their biological behaviour. The electrochemical anodizing treatment was performed by using different electrolytes based on phosphoric acid (H₃PO₄), sulphuric acid (H₂SO₄) with a fluoride salt; and the Focused Ion Beam (FIB) technique, normally named as *Nanolab*, was used for the microstructural, chemical and morphological characterization, as well as the confocal laser microscopy technique which also served for roughness measurements. The mechanical response of the anodic layers was evaluated through the using of a scratch tester which showed the critical loads for the coating damages. The characterization results showed that both, concentrations and electrolyte species, clearly influenced the morphological and topographical features, as well as the chemical composition of the anodic layer. By using the FIB was possible to detect nanopores within both the surface and the bulk of the coating. Some of the conditions generated a very special coating morphology which promoted a better osteoblasts adhesion. Contrary to what it was *a priori* expected, all anodic coatings showed high critical loads for damages during scratch test, despite their high porosity, which could be related with some defects coalescence mechanism that allows dissipating the high stress concentration applied during the test.

Keywords—*Nanolab*, Biomaterials, Titanium, Surface Modification, Titanium Spark Anodizing.

I. INTRODUCCION

Tissue degradation associated to both traumas and diseases, and its replacement, is currently one of the most important public health problems. In the case of bone tissue, this degradation is evident by the density reduction since the 30 years old, which implies a strength reduction up to 40% that could be increased by both the cyclic load degradation and the surface wear of joints. Of all biomaterials used for bone replacement, it is recognized that commercially pure titanium (CP Ti) medical grade, is one of the biomaterials that show the best *in vivo* behavior due to their excellent balance between mechanical, physical-chemical and biofunctional properties. However, one important disadvantage, which in many cases compromises the implants and prosthesis liability, is that, despite its high osteointegration capability, titanium is surrounded by a fibrous tissue because of its bioinert behavior, which is related with many loosening events.

In order to reduce the loosening risk related with the fibrous tissue presence, there have been several attempts to modify the titanium surface in a favorable manner. In that sense, the physical-chemical nature of titanium surface has been changed from different methods: i. By applying bioactive coatings on the surface [1] or by conversion of the original bioinert surface of titanium to a bioactive one through a biomimetic coating [2]; ii. The topographical characteristics of the surface can be properly adjusted, by using mechanical, chemical and electrochemical methods. With respect to the topographical properties of the surface, there is a general agreement that some specific range of the roughness parameters can improve the bone cells adhesion and, therefore, the titanium osteointegration [3]: a vertical parameter, mean height of the profile, $R_a = 4 - 5 \mu\text{m}$; and an horizontal parameter, mean space of the profile, $P_c = 65 - 80 / \text{cm}$.

In this work, has been evaluated the influence of different electrochemical processing conditions for surface

modification of CP Ti, in their microstructural, morphological, topographical and mechanical properties, as well as in their *in vitro* biological behaviour. The electrochemical anodizing treatment was performed by using different electrolytes based on phosphoric acid (H_3PO_4) and sulphuric acid (H_2SO_4), with small additions of a fluoride salt, according to the plasma-chemical anodization technique, which allows incorporating phosphate ions into a titanium oxide matrix in a one single anodic oxidation step [4]. This anodic plasma-chemical treatment (APC) is based on the dielectric breakdown of an insulating oxide film at the surface of a metal anode in contact with a suitable electrolyte and is accompanied by visible plasma-like sparking at the anode surface. The analysis of the different anodic coating was focused on the changes of surface morphology for different electrochemical conditions, and its relationship with both mechanical and *in vitro* biological response. In that sense, it was used the Focused Ion Beam (FIB) technique, normally named as *Nanolab*, as well as the confocal laser microscopy which also served for roughness measurements. The mechanical response of the anodic layers was evaluated through the using of a scratch tester which showed the critical loads for the coating damages.

II. EXPERIMENTAL PROCEDURE

A. Samples processing

The specimens of CP Ti grade 2 (ASTM F67-00) were cut in discs of 2 cm of diameter and 2 mm of thickness, and then were properly prepared before the anodizing process: grinding and polishing by using SiC abrasive up to sandpaper No. 600, cleaning by ultrasound with acetone during 15 min and etching in a mix of 3% en HF y 30% en HNO_3 . The electrolytes used were based on orto-phosphoric (H_3PO_4), sulphuric acid (H_2SO_4) and a fluoride salt. The electrochemical conditions consisted of a process initially galvanostatic with a constant current density, up to the system reached the desired voltage, moment from which the process continue potentiostatically at the same voltage, during certain time. All the electrochemical conditions are. The anodizing process were performed by using a DC power supply Hewlett Packard 6035A, controlled via a GP-IB interface with LabView software running on a personal computer. The coating process was carried out in a double-walled glass beaker in order to control the temperature with an external thermostat (Lauda Ecoline E119, IG Instrumenten Gesellschaft, Zurich, Switzerland). A cylindrical platinum mesh was used as counter cathode.

After being anodized, specimens were thoroughly rinsed with distilled water several times and dried by air blowing.

B. Microstructural characterization and mechanical testing

The SEM analysis of the CP Ti anodizing coatings was performed in a JEOL JSM-6490LV, by using 25 kV and a field-emission gun. The focused ion beam (FIB) analysis was carried out to characterize both, the top and cross sectional microstructure, as well as the chemical composition of the anodic layers, through a Carl-Zeiss Neon40 equipment by using decreasing ion currents until 50 pA at 30 kV. Observations were done in FE-SEM (Gemini column). The confocal laser was used to analyze, qualitative and quantitatively, the surface characteristics of the anodic layers by using an equipment LEXT-OLS Series Standard type (resonant galvano mirror scanner mode and High speed, high precision auto focus unit)and it was also used to analyze the results after the scratch testing for which was used an equipment CSM (Revetest scratch tester) coupled with a digital camera, a suitable system for capture the load and displacement data and a sensor of acoustic emission. For scratch testing it was used a Rockwell C indenter with a spherical tip (radius of 200 μm) and were applying different values of maximum load between 5N and 30 N, with a displacement velocity of 1 mm/min and for scratch lengths between 2 mm and 10 mm. The damages produced after the scratch test, were observed optically through the camera and also by confocal laser microscopy in order to determine both, the features of the damage and the critical loads for different damages of the anodic layers.

C. *In vitro* biological testing

The primary cell culture of osteoblasts was performed by extracting bone biopsies from femoral epiphysis of rabbits Balb/c of aprox. 8 mm³. This bone was subjected to enzymatic activity of a mix of collagenasa type I (Sigma C9772), concentration of 1 mg/ml, and trypsin EDTA (Sigma), concentration of 0.05 % in a culture plate of 24 POZOS, during 1 h at 37 °C with permanent agitation. Afterwards, the enzymes were removed and it was added a complete media of MEM modified by Dulbeco (DMEM), 10 % of SFB, 2 mM of L-glutamine, 100 $\mu g/ml$ of estreptomycine, 100 UI/ml of peniciline and 0.05 mg/ml of ascorbic acid (Sigma). Afterwards, it was necessary to wait up to formation of a cell monolayer in order to change it to other recipient. The cells fixation was performed with 2 % of glutaraldehyde overnight and then, several cleaning steps were carried out with PBS and the cells were dehydrated. For this last step, it was added different concentrations of ethanol (30%, 50 %, 60 %, 70 %, 80%, 90 %, 100 %),

during 15 min at room temperature and, then, they were left in ethanol of 100 % at 4°C, to finally dry them with the critical point dryer before SEM analysis.

III. RESULTS AND DISCUSSION

A. Samples processing

In Fig. 1 is presented the Evolution of the CP Ti anodizing process with time: a) and b) Colored aspect of anodized surfaces corresponding to conventional anodizing; c) Initial dielectric breakdown of the insulating oxide film at the surface of the anode; d) visible plasma-like sparking at the anode surface. Whilst the conventional sample presents the typical colored aspect due to interference phenomenon inside the nanometric pores, the spark anodized samples show a very dull finishing as a consequence of the roughness created by the new porous layer produced due to dielectric breakdown of the insulating oxide film at the surface of the CP Ti samples. According to well known roughness parameters for improving osteointegration, these rough anodized surfaces are much more suitable than the conventional ones and, therefore, they were selected to develop the work of evaluate the osteointegration potential of CP Ti anodized surfaces.

B. Microstructural characterization and mechanical testing

In Fig. 2 is presented the top view of samples a, b and c (different contents of H_3PO_4 , H_2SO_4 and fluoride salt).

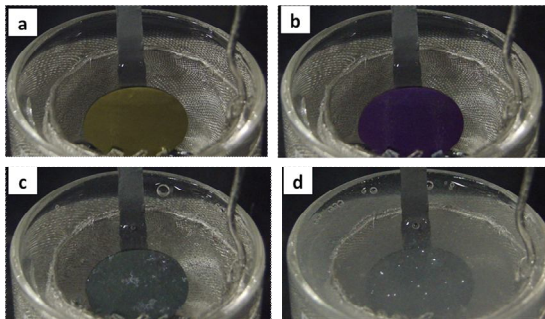


Fig. 1. In situ aspect of CP Ti anodized discs: a) and b) galvanostatic conditions; c) and d) potentiostatic conditions.

The general topographical features in this figure, clearly explain that dull finishing mentioned before, with respect to that one of colored conventional anodizing: micro-porosity uniformly distributed and a clear rough surface with micro-irregularities. The pores that can be observed within this surface can be divided into three types: i. the biggest ones

(~2-3 μm) which show a spherical shape as well as elongated shape, this most probably associated to coalescence of the formers; ii. the intermediates ones (~0.5-1 μm) which are mostly spherical; iii. the smallest ones, which could be considered nano-pores (less than 0.5 μm), mostly spherical and more or less well distributed in the solid matrix of the porous layer. Note that some of these smallest pores not only appear on the very top side, but also on the more inner non-flat zones of the solid matrix. The EDX pattern associated to this top view of the surface, also depicted in Fig. 2, shows the normal peaks associated to CP Ti grade 2; however, note the presence of a P peak which is clear evidence that one substance from the electrolyte is being part of the final anodic coating (3% - 5% wt). Therefore, it is necessary to clarify if this P is related with the strong tendency of phosphates to chemisorb on oxide surfaces [4].

In Fig. 2 is also shown the microstructural aspects of the anodized samples processed with H_3PO_4 + fluoride salt electrolyte. This surface shows a structure clearly different compared with the previous one in which the electrolyte was only H_3PO_4 ; in this case it can be observed two main morphology characteristics: i. the coating has, as its basic structure, a mesh of grooves of different lengths between 5 μm and 20 μm , which seems to be a consequence of a prolonged coalescence of a previous line of neighbor pores; ii. on the other hand, there are small pores (~2-3 μm) which are located mostly inside and between the mentioned grooves.

From the mechanical point of view, a first approach obtained by scratch test of the samples corresponding to all conditions, showed that these anodic layers present strong resistance to fail by this kind of load (Fig. 3). Note that, interestingly, the critical load for delamination damage was increased for the samples with the more grooved layer.

C. In vitro biological testing

From Fig. 4 it can be observed the Osteoblasts aspect for different anodic layers on CP Ti. It is clear that the cells exhibit a better adhesion on the more rough and grooved surface, implying a great potential for improving the osteointegration. These results are especially remarkable considering that the roughness parameters of these surfaces ($R_a = 2 - 3 \mu\text{m}$) are lower than the searched ones ($R_a = 4 - 5 \mu\text{m}$) which indicates the important role of the special morphologies that have been found in this work.

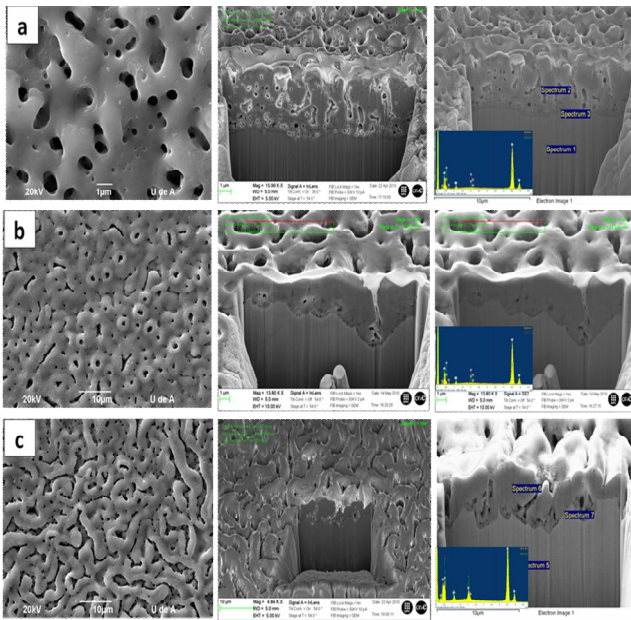


Fig. 2. Influence of electrolyte composition in the anodic coating structure: the increment in H_3PO_4 , a) to b), has not considerable role, whilst small additions fluoride salt (c) change clearly the structure.

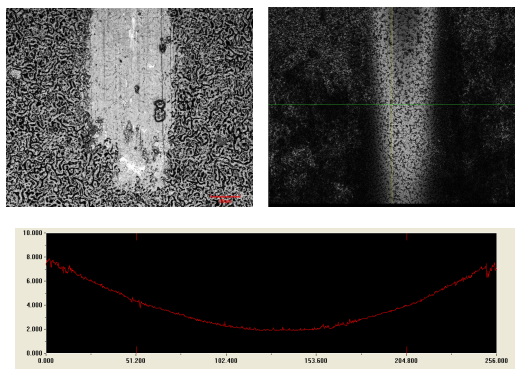


Fig. 3. Confocal laser analysis of the scratch pattern produce don the anodic layer from different processing conditions..

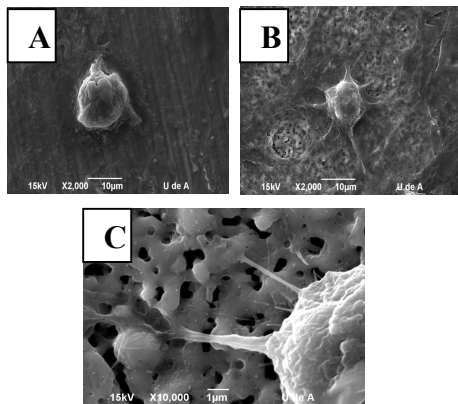


Fig. 4. Cells culture results on different CP Ti surfaces: a) Machined surface; b) Isolated pores surface; c) Grooved Surface.

IV. CONCLUSIONS

1. The spark anodizing process applied on CP Ti samples allowed to obtain porous and rough surfaces which can be modified by controlling both electrochemical conditions and electrolyte composition of a galvanostatic/potentiostatic process.
2. In vitro biological testing of osteoblasts cells culture has shown the osteointegration potential of these porous and rough surfaces, specially for the modified morphology by changing the electrolyte composition with additions of fluoride salts.

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