Characterization and quantification of oxides generated by anodization on titanium for implantation purposes

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Abstract. The use of titanium as implant material is widely known in the surgery field. The formation of natural or artificial compact and protective oxide is a convenient tool for metal protection and a good way to generate phosphate deposits to enhance biocompatibility and bone fixation with the existing tissue. The present work has the aim of superficially modify commercially pure titanium sheets used in orthopedics and odontology, with a potencistatic anodization process with an ammonium phosphate and ammonium fluoride solution as electrolyte. The objective is to generate titanium oxides doped with phosphorous on the surface, to promote bioactivity. The characterization and quantification of the generated deposits is presented as a starting point for the future application of these materials. The applied characterization methods are X ray diffraction, micro-Raman spectroscopy analysis for evaluating the chemical and phase composition on the modified surface and PDI image analysis techniques that allow the segmentation of SEM images and the measurement and quantification of the oxides generated by the anodization process. The samples with polished treated surface at 30V have the deposit of a phosphate rich thick layer covering almost all the surface and spherical-shaped titanium oxide crystals randomly placed (covering more than 20% of the surface area).

1. Introduction
The metallic materials are widely used as orthopedic and odontologic surgical implants due to their excellent mechanical properties and controlled corrosion resistance for these applications [1]. A disadvantage of the prosthetic metallic materials is that most of them cannot make a natural bonding with the existing tissue, so it is necessary a chemical or mechanic fixation. Also the material that is going to be implanted should have good wear resistance and other superficial characteristics [2]. The use of titanium as implant material is widely known in the field. The formation of natural or artificial compact and protective oxide is a convenient tool for metal protection and a good way to generate phosphate deposits to enhance biocompatibility and bone fixation with the existing tissue. The adhesion of these coatings to the metallic substrate and its mechanical properties are of great importance since they are going to be submitted to several mechanical efforts during and after implantation. Also the characterization and quantification of the oxides present in the surface are a key issue for the future calcium phosphate deposition (apatite, first insight of bone formation), to provide bioactivity and mechanical fixation.
Automatic image segmentation is a significant area in Digital Image Processing. One of the most important elements of any automated system of vision is the task of understanding the image, that severely affects the subsequent process of interpretation of the image, providing useful structures such as regions and edges [3].

Mathematical Morphology (MM) has been a successful tool in image segmentation. The MM is a theory based on concepts of geometry, algebra, topology and set theory, created to characterize physical and structural properties of different materials. The main idea of this theory, is to examine if the geometric structures of an image overlap with an small localized pattern (called "structuring element") [4].

In this paper we propose the surface modification of titanium implants of commercial purity, used in orthopedic surgery and dentistry, through potentiostatic anodizing surface treatment, carried out in a solution of ammonium phosphate and ammonium fluoride in order to obtain titanium oxide doped with phosphorus in the surface, to promote bioactivity and generate better interaction with the bone. The characterization and quantification of the oxides generated is presented as an important starting point for further implementation of these materials. For this we use three methods: x-ray diffraction (XRD) and Raman spectroscopy to assess the chemical composition of the surface, and image processing techniques that will enable the segmentation of the image through the use of MM operators for subsequent measurement and quantification of generated oxides on anodized titanium.

2. Materials and Methods

2.1. Sample preparation

Samples of 28 x 13 x 0.3 mm³ grade-2 commercially pure Titanium sheets (CP-Ti, “Unalloyed Commercially Pure Titanium”) were employed. Its chemical composition can be seen in Table 1. Some of its properties (mainly mechanical) are shown in Table 2 comparing with the cortex bone properties.

| Table 1. Chemical composition of CP-Ti (% in weight). |
|-----------------------------------------------|
|       Ti      |   C     | Fe | H      |   N    | O     |
|-------------|--------|----|--------|-------|-------|
| CP-Ti (grade 2) | 99.2   | Max 0.1 | Max 0.3 | Max 0.01 | Max 0.03 | Max 0.25 |

| Table 2. Some properties of CP-Ti comparing with cortical bone. |
|-----------------------------------------------|
| CP-Ti          | Bone                    |
|----------------|-------------------------|
| Tensile Strength (MPa) | 345 | 85 – 150 |
| Yield Limit, σ f (MPa)     | 275 | - |
| Elastic Modulus, E (GPa) | 105 - 140 | 7 – 30 |
| Hardness (GPa)          | Aprox. 2 GPa | - |
| Density (g/cm³)         | 4.51 | 18 – 22 |
| Crystalline Structure   | Hexagonal | Crosslinked macromolecule |

Two surface conditions of the substrates were analyzed: polished to 600 grit paper with water as lubricant (AP samples) and “as-received” (the same condition as the material was received after the fabrication process, AR samples).

Before the anodizing treatment the samples were cleaned in ultrasonic bath with acetone during 10 minutes and after that with isoprophylic alcohol for another 10 minutes.
The electrolytic solution was prepared with bi-distilled water, 1 mol/L of NH₄H₂PO₄ and 0.5 % weight of NH₄F.

The potenciostatic anodic treatments were carried out at two different voltages: 20 y 30 Volts, for 1 hour at room temperature with a power source Consort EV231. So the samples were: with rough surface (AR20 and AR30) and with polished substrate (AP20 and AP30).

2.2. Surface Characterization

For the morphologic evaluation of the surface deposits several images were taken at different magnifications. A scanning electronic microscope was used to obtain the simple images (SEM, Phillips XL 30).

X-ray diffraction (XRD) and Raman spectroscopy essays were done with the aim of identify the different compounds present in the surface deposits. The XRD experiments were done with a diffractometer PANalytical X’Pert Pro, with a Cu-Kα radiation of 40 kV and 40 mA. The spectra were taken at 0.02 degree per second and with a small angle of 1° from 10 to 70 degrees. The Raman equipment used was an Invia Reflex confocal with a 514 nm wave length laser, at 100 % intensity in a window from 100 to 1500 cm\(^{-1}\), with one accumulation and 50 seconds exposition per spectrum.

2.3. Digital Image Processing

In order to determine the amount of oxide present on the surface of the AP30 samples, MM operators were applied to the images obtained through a scanning electron microscope. The main objective of the MM is to extract information of the geometry and topology of an unknown set present in an image. The key of this methodology is the "structuring element", a small set completely defined and of a known geometry, which is compared, based on a transformation, with the whole image. The shape and size of the structuring element allow quantify if the structuring element is contained in the image or not [4].

A gray level image is a function defined on a subset of \( \mathbb{Z}^2 \) into the natural range \([0,L-1]\), where \(L\) is a number within the interval \([1,256]\). For this work \(f\) denotes a gray level image.

The basic morphological operators of the MM are erosion and dilation. Morphological erosion and dilation of the image \(f\) by the structuring element \(b\) are defined, respectively, as [5, 6]:

\[
\varepsilon (f, b)(x, t) = \min \left\{ f(s + x, t + y) - b(x, y) \middle| (s + x, t + y) \in D_f; (x, y) \in D_b \right\}
\]

\[
\delta (f, b)(x, t) = \max \left\{ f(s - x, t - y) + b(x, y) \middle| (s - x, t - y) \in D_f; (x, y) \in D_b \right\}
\]

where \(D_f\) and \(D_b\) are the domains of the functions \(f\) and \(b\) respectively.

Based on the combination of the previous operators, opening and closing can be defined as follows [5, 6]:

\[
\gamma_b(f) = \delta_b(\varepsilon_b(f))
\]

\[
\Phi_b(f) = \varepsilon_b(\delta_b(f))
\]

The opening is useful to remove small bright details smaller compared to the size and shape of the structuring element, the rest of the image is not modified. When filtering, in the step of erosion, a bright area surrounded by dark one is reduced. Particularly, if the size of the structuring element is greater than the bright area, this bright area totally disappears. Then, in the step of dilation, an bright area surrounded by dark one is expanded and therefore the image recover its current shapes, except of the details removed by erosion in the previous step.

Similarly, the closure is useful to remove small dark details compared to the size and shape of structuring element, the rest of the image is not modified.
The resulting image depends on the gray level, shape and size of the structuring element. Top-hat transform is a well-known and commonly used morphological technique for extracting locally bright (or dark) objects from a grayscale image. Figure 1 shows an opening Top-Hat transform, which emphasizes locally bright objects that have been eliminated from the image by an opening filter. A structuring element larger than the structures to be filtered was used.

This transform is defined as the residual between the identity and a morphological opening [5, 7, 8]:

$$\rho_{\gamma}(f) = f - \gamma_{\gamma}(f) \tag{5}$$

Since this transform emphasizes bright objects on a non-uniform background, it is suitable for segment the oxide deposited on the surface of the samples AP30.

The proposed algorithm was implemented in Matlab, using a graphical interface. It selects and loads images, computes the opening Top-Hat transform, binarizes the image and generates a list that provides: name of the selected image, structuring element dimension, threshold used in the binarization, number of black and white pixels presented in the resulting image and the rate of black and white dots (See Fig. 2-b).

Additional buttons allow: to save the results, to modify the size of the structuring element and the threshold, and to reset the default settings. Figure 2 shows the interface of the program, which can display the original image (top left), the resulting image of Top-Hat transform (top right), the binarization of the image above (bottom left), and overlay of the result on the original image (bottom right).

Figure 1. Top-Hat transform on a one-dimensional signal.

Figure 2. (a) Graphical user interface. (b) Parameter list.
3. Results
In the polished substrate samples (AP's) and the AR20 samples can be observed a high amount of phosphates over all the surface. This statement is demonstrated with the XRD spectra showed in Figure 4, where is very clear the presence of phosphorous compounds coming form the electrolytic solution.
From comparing the morphology of the different deposits on the titanium substrates, can be denoted that the final surface roughness highly influences the shape, quantification and Quality of the deposits. The Figure 3 (c) shows the AR20 sample surface by SEM. It shows mostly a non homogeneous deposit, leaving some sites with a clear view of the rough titanium substrate below it. The AR30 sample does not show the presence of cloudy-like deposits by SEM, just a rough surface (Figure 3 (d)).
The polished samples, in other side, have different and maybe better superficial characteristics than the AR treated sheets. Both the AP20 and the AP30 (Figure 3 (e) and (f)) samples have an homogeneous deposited layer in all the surface extension. The AP20 and AR20 treatment show a similar final surface.

![Figure 3. SEM images obtained for the different substrate roughness and anodization conditions. (a) AR20, (b) AP20, (c) AR30, (d) AP30](image-url)
The AP30 sample (Figure 3 (d)) presents a distinctive characteristic from the other samples: some spherical-shape white deposits. The identification of the compound was done by X-ray diffraction (Figure 4) showing the presence of two of the characteristics peaks of anatase, a crystallographic phase of titanium oxide. The spectra of the AP20 and AR20 samples also showed the presence high amount of ammonium phosphate at the surface but from the AR30 sample couldn’t be obtained the same results. This could be due to a very thin layer of the deposit present in the surface.

The qualitative analysis of the components of the anodic deposit could also be done by backscattered electron images (Figure 5). Back-scattered electrons (BSE) are beam electrons that are reflected from the sample by elastic scattering. BSE are often used in analytical SEM along with the spectra made from the characteristic X-rays. Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images can provide information about the distribution of different elements in the sample. Since heavy elements (high atomic number) backscatter electrons more strongly than light elements (low atomic number), and thus appear brighter in the image, BSE are used to detect contrast between areas with different chemical compositions [9]. In this case the phosphorous-rich compounds are lighter so appear as dark clouds on the surfaces and the titanium substrate and the titanium oxide deposits are lighter points in the image, as it can be seen in AP20 and AP30 samples (Figure 5)

The presence of titanium oxide with anatase structural phase deposits is also denoted by microRaman analysis. The AP30 sample spectrum shows the presence of some characteristic peaks of the anatase phase in 144-148, 400, 516 y 640 cm\(^{-1}\) [10, 11]. In Figure 6 there are also shown some Raman spectra of other zones of the sample, where also can be denoted the presence of a characteristic phosphate peak at 925 cm\(^{-1}\) [12].

Figure 4. XRD spectra of all the analyzed samples.
Figure 5. Backscattering images on the AP30 and AP20 samples.
(a) AP20, (b) AP20 BSE, (c) AP30, (d) AP30 BSE

Figure 6. Raman spectra of the AP30 sample showing different areas (a) and the associated optical image (b).
With the aim of quantify the amount of spherical deposits on the samples, the SEM images were processed by a costume-made Mathlab program. Two sample magnifications were used for the mathematical image analysis. Figures 7 and 8 show the analyzed images of the **AP30** sample at 100 and 500X of magnification of SEM microscopy (a), the binary images (b) and both images superposition. The results give that the percentage of oxides at low magnification was 24% and at high, 42%. The calculated proportion shows a high variation since the Figure 8 was taken in a denser particle zone of the **AP30** sample. The Figure 7 can be taken as the more representative one since it takes a larger zone of the sample. Even though the mathematical image analysis is a powerful tool that can be applied to different images with similar contrast and color, it has to be taken into account that in this case the real oxides proportion could be more since they grow under and between the phosphate layer.

![Figure 7](image1.png)

**Figure 7.** (a) SEM image of the **AP30** sample (100X). (b) Binary image. (c) Superposed image.

![Figure 8](image2.png)

**Figure 8.** (a) SEM image of the **AP30** sample (500X). (b) Binary image. (c) Superposed image.

### 4. Conclusions

This work presented the surface modification of commercially pure titanium sheets used in orthopedics and odontology, with a potencistatic anodization process with an ammonium phosphate and ammonium fluoride as the electrolytic solution. The process allowed to generate titanium oxides doped with phosphorous on the surface, thought as a way promote bioactivity and implant fixation as the deposits can bond directly with the existing bone. The characterization and quantification of the generated deposits is presented as a staring point for the future application of these materials. The applied characterization methods (X ray diffraction, micro-Raman spectroscopy) allowed to identify phosphate and titanium oxides phases present after the anodic treatment. The PDI analysis with segmentation of SEM images technique permitted the measurement and quantification of the oxides generated by the anodization process. The samples with polished initial surface and with an anodic treatment of 30V presented a phosphate/titanium oxide layer that could enhance bioactivity and bone fixation.
5. References

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