Researches on the Improvement of the Bioactivity of TiO₂ Deposits, Obtained by Magnetron Sputtering - DC

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Abstract. In this paper, layers of TiO₂ were deposited, by magnetron sputtering, on a glass support. The parameters of the deposition process were kept constant except for the O₂/(Ar + O₂) ratio that varied on three levels. The physical and mechanical properties of the layers obtained were investigated by SEM optical microscopy, electronics, AFM and X-ray diffraction. The bioactivity of TiO₂ surfaces was investigated by growing M3C3-E1 osteoblast cells produced by RIKEN Cell Bank (Japan) for a period of 5 days. The modification of the working environment in the enclosure determines both the phasic modification in the TiO₂ film, respectively the amount of the anatase or rutile phase and the decrease of the average roughness of the film from 112.3nm to 56.7nm. The research has demonstrated that the finer layers with a high content of anatase promote the growth of M3C3-E1 cells.

1. Introduction

Due to its physical, chemical and mechanical properties titanium has applications in various fields such as aeronautics, machine building, chemical, and nuclear industries, etc., [1-4]. It is known that in the air, the titanium alloys form on the outer surface a thin layer of TiO₂ with a rutile or anatase structure, which offers a very good corrosion resistance, high thermodynamic stability and reduced sensitivity to ion formation - in aqueous solutions [5-8]. All of these properties contribute to increasing the biocompatibility of Ti and its alloys leading to better bone integration compared to other metallic implants [9-11]. It is known that, any titanium alloy introduced into the human body reacts at interface with the osteoblast cells through an osteointegration process, [12]. The osteointegration capacity of the implant is dependent on surface characteristics, such as: structure, chemical composition, rugosity, topography, and morphology of the implant surface, [13].

In some medical applications, titanium is used as titanium oxide (TiO₂), deposited as a thin layer obtained by various techniques: resistive evaporation, thermal spraying, physical vapor depositions (PVD), magnetron sputtering-reactive DC, reactive magnetron sputtering - RF, vapor electron beam, ion beam, plasma electrolytic deposition, etc., [14-16] obtaining, each time, films which dependent on
the preparation techniques and deposition conditions. Of all these techniques, the method reactive magnetron sputtering - DC reactive is the most used due to its high productivity and due to fact, that allow obtaining of dense and uniform films [17, 18].

In this paper, TiO₂ layers were deposited on a glass substrate - by magnetron sputtering -DC method and the influence of the O₂/(Ar + O₂)mixture on the layer structure and bioactivity of TiO₂ films was studied.

2. Experimental procedure

TiO₂ layers were deposited in vacuum by magnetron sputtering, using an experimental installation -similar to the installation used by Toma et al. [7], whose sketch is represented in Figure 1. Thin films of TiO₂ have been deposited on glass microscope lamellas, having the dimensions of 25x75x1.5mm³.

Before performing the deposition, the glass lamellas were ultrasonically cleaned in an acetone bath and ethanol. At the end, they were rinsed with distilled water and dried by blowing of dry argon (99.999% purity). The argon and oxygen flow rate introduced into the enclosure have varied on three levels and the substrate temperature was 150°C.

The experimental parameters used are presented in Table 1. The target on which deposition was performed was Ti, of 99.9% purity, with a diameter of 50.8mm and a thickness of 4mm [7]. The glass lamellas were fixed on the anode made of the steel plate, cut in the central area, having a thickness of 2 mm.

![Image](Figure 1. Sketch of the installation for deposit of TiO₂ films by DC magnetron sputtering system [7].)

| Parameters                      | Value       |
|---------------------------------|-------------|
| Sputter target                  | Titanium target |
| Target to substrate distance (mm)| 80          |
| Sputter power (W)               | 250         |
| Substrate temperature (°C)      | 150         |
| O₂/Ar + O₂ ratio                | 0.1/0.2/0.3 |
| Sputter pressure (mbar)         | 3.6·10⁻³    |
| Sputter time (sec)              | 26          |
2.1. Characterization of films

The crystalline structure of TiO$_2$ depositions was highlighted by X-ray diffraction using X-ray diffractometer - type XPERT PRO MRD product by PANalytical Holland, using Cu Ka radiation ($\lambda = 0.15406 \text{ nm}$) which works with acceleration voltages of 40kV and an emission current of 40mA. The SEM investigations carried out using the electronic scanning microscope: FEI - Quanta 200 SEM highlighted the deposition morphology. The surfaces of TiO$_2$ films were obtained by atomic sheet microscopy using the microscope: AFM Easyscan2, Nanosurf Switzerland - who operated in tapping mode. The osteoblast cells of MC3T3-E1 type, produced by RIKEN Cell Bank firm (Japan) are placed in the Dulbencco modified Eagle's medium containing 10% fetal Bovine Serum, 500 units/ml penicillin and 500 units/ml streptomycin. All incubations of cells were made in the Co atmosphere at 37°C, with relative humidity of 100%. Medium was changed every 2 days of experiment. The cells investigation was performed by carrying out the proliferation test after a day, 3 and 5 days since of cell cultivation. Proliferated cells were quantified by counting them with a haemocytometer.

3. Results and discussions

In figure 2 are presented diffractograms of TiO$_2$ layers deposited by magnetron sputtering at different working reports. The diffractograms present two diffraction peaks, well defined at 2$\theta$ values, corresponding to the planes (101) and (200), of the crystalline phase of the anatase. The films have a crystal structure - in accord to other works using magnetron sputtering deposition techniques, [19, 20]. It can be seen in Figure 1 that the intensity of the anatase peaks increases with the increase in O$_2$ content. It can also be observed that the excess presence of O$_2$ quantity in the enclosed workplace impedes the crystallization of the anatase phase.

![Figure 2. XRD patterns of TiO2 thin films deposited at p=3.6·10$^{-3}$ mbar and T=150°C.](image)

In Figure 3 are presented AFM images of the three samples prepared at different O$_2$/(Ar+O$_2$) ratios, in the Table 2 there are presented the average grain size (D), average roughness ($R_{ave}$), and root mean square roughness ($R_{rms}$) obtained by five determinations - by AFM analysis. It is observed an an obvious increase of the physical dimension of the grains with the addition of O$_2$. In addition, by analyzing the roughness variation, it can be observed that when increasing the O$_2$/(Ar + O$_2$) ratio from 0.1 to 0.3, the average roughness decreases from 112.3nm to 56.7nm.

To investigate the interaction of cells with the surface of TiO$_2$ film, the culture layers were scanned using the electron microscope. Figure 4 are presented the morphologies of the culture deposited on the surface of the TiO$_2$ film on after 3 day.
Table 2. Experimental result.

| No | O₂/(Ar+O₂) ratio | D[nm] | R_{ave}[nm] | R_{rms}[nm] |
|----|------------------|-------|-------------|-------------|
| 1  | 0.1              | 46.5  | 112.3       | 128.2       |
| 2  | 0.2              | 122.4 | 86.4        | 104.5       |
| 3  | 0.3              | 182.2 | 56.7        | 69.4        |

Figure 3. AFM images of TiO₂ thin films deposited at different ratio O₂/(Ar+O₂): (a) 0.1; (b) 0.2; (c) 0.3.

Figure 4. SEM images of osteoblasts cultured directly on TiO₂ layers obtained at different ratio O₂/(Ar+O₂): (a) 0.1; (b) 0.2; (c) 0.3.

Figure 5. Proliferation cells MC3T3 after a day, 3 and 5 days since the cell cultivation.
As it can have observed, the film obtained is porous and the cells have the morphology rounded. Increasing of larger coatings are recorded on the TiO$_2$ surface, obtained at a ratio of O$_2$/ (Ar + O$_2$) = 0.2. It can be suggested that the anatase phase present in the TiO$_2$ film favours cell growth.

The result of MC3T3 cell proliferation on the surface of TiO$_2$ film after a day, 3 and 5 days since the cell cultivation it is presented in Figure 5. As it is observed, the surface of TiO$_2$, obtained at 0.1 value of the O$_2$/ (Ar + O$_2$) ratio has the lowest cell proliferation.

A higher cell proliferation has been recorded on TiO$_2$ surfaces, obtained at an O$_2$/ (Ar + O$_2$) ratio of 0.2. It can be suggested that the anatase phase - present in the TiO$_2$ film and the existence of a smaller roughness - Table 2 and Figure 5, favours cell growth.

4. Conclusions
Following experimental research on the bioactivity of thin films of TiO$_2$ obtained in vacuum by magnetron spraying we can state:
- the increase in oxygen content in the working chamber used to obtain the TiO$_2$ layers - favours the increase of the phase of the anatase – see figure 1. However, the increase in excess of the O$_2$/ (Ar + O$_2$) ratio, prevent the crystallization of anatase phase;
- the grain size and the roughness of the TiO$_2$ deposition decrease with the increase of the O$_2$ content in the enclosure – see figure 2;
- the presence of the anatase phase in the TiO$_2$ layer and of a smaller roughness - see Table 2, favour the growth of osteoblast cells of MC3T3-E1 type.

5. References
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