Benedito Rodrigues da Silva Neto
(Organizador)

MEDICINA:

A ciência e a tecnologia em busca da cura

4

Atena
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CIÊNCIAS BIOLÓGICAS E DA SAÚDE

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APRESENTAÇÃO

Ciência é uma palavra que vem do latim, “*scientia*”, que significa conhecimento. Basicamente, definimos ciência como todo conhecimento que é sistemático, que se baseia em um método organizado, que pode ser conquistado por meio de pesquisas. Já a tecnologia vem do grego, numa junção de “*tecnos*” (técnica, ofício, arte) e “*logia*” (estudo). Deste modo, enquanto a ciência se refere ao conhecimento, a tecnologia se refere às habilidades, técnicas e processos usados para produzir resultados.

A produção científica baseada no esforço comum de docentes e pesquisadores da área da saúde tem sido capaz de abrir novas fronteiras do conhecimento, gerando valor e também qualidade de vida. A ciência nos permite analisar o mundo ao redor e ver além, um individuo nascido hoje num país desenvolvido tem perspectiva de vida de mais de 80 anos e, mesmo nos países mais menos desenvolvidos, a expectativa de vida, atualmente, é de mais de 50 anos. Portanto, a ciência e a tecnologia são os fatores chave para explicar a redução da mortalidade por várias doenças, como as infecciosas, o avanço nos processos de diagnóstico, testes rápidos e mais específicos como os moleculares baseados em DNA, possibilidades de tratamentos específicos com medicamentos mais eficazes, desenvolvimento de vacinas e o consequente aumento da longevidade dos seres humanos.

Ciência e tecnologia são dois fatores que, inegavelmente, estão presentes nas nossas rotinas e associados nos direcionam principalmente para a resolução de problemas relacionados à saúde da população. Com a pandemia do Coronavírus, os novos métodos e as possibilidades que até então ainda estavam armazenadas em laboratórios chegaram ao conhecimento da sociedade evidenciando a importância de investimentos na área e consequentemente as pessoas viram na prática a importância da ciência e da tecnologia para o bem estar da comunidade.

Partindo deste princípio, essa nova proposta literária construída inicialmente de quatro volumes, propõe oferecer ao leitor material de qualidade fundamentado na premissa que compõe o título da obra, isto é, a busca de mecanismos científicos e tecnológicos que conduzam o reestabelecimento da saúde nos indivíduos.

Finalmente destacamos que a disponibilização destes dados através de uma literatura, rigorosamente avaliada, fundamenta a importância de uma comunicação sólida e relevante na área da saúde, assim a obra “Medicina: A ciência e a tecnologia em busca da cura - volume 4” proporcionará ao leitor dados e conceitos fundamentados e desenvolvidos em diversas partes do território nacional de maneira concisa e didática.

Desejo uma ótima leitura a todos!

Benedito Rodrigues da Silva Neto

SUMÁRIO

CAPÍTULO 1

ALBUMIN ADSORPTION ON OXIDE THIN FILMS STUDIED BY ATOMIC FORCE MICROSCOPY

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ABSTRACT: Titanium, niobium, and zirconium metals have excellent mechanical properties and corrosion resistance which make them potential candidates for coating implants. In this work, model titanium, niobium, and zirconium coatings were deposited by DC magnetron sputtering on Si(111) substrates at an elevated oxygen flow. The films interacted with bovine serum albumin (BSA) solutions of different concentrations in physiological conditions. The chemical composition, morphology, elastic modulus, and hardness of the three thin films were analyzed by X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), atomic force microscopy (AFM), and nanoindentation. The XPS spectra showed that the surfaces were composed by TiO_2, Nb_2O_5, and ZrO_2. The BSA solutions, with concentrations of 20 and 100 μg/ml, were deposited at 37 °C for 1h. AFM images showed aggregates of adsorbed albumin after interaction with the 100 μg/ml BSA solution. The surface morphology of the adsorbed BSA thin films appeared different on the three surfaces: the AFM images indicated a monolayer adsorption of BSA onto Nb_2O_5 films, the adsorption onto TiO_2 surfaces seemed to result in two layers of adsorbed protein, and the adsorption onto the ZrO_2 surface presented two layers of adsorbed protein with conformational changes.

KEYWORDS: Atomic force microscopy; thin films; biomaterial surfaces; protein adsorption; bovine serum albumin.

ADSORÇÃO DE ALBUMINA EM FILMES FINOS DE ÓXIDOS ESTUDADA POR MICROSCOPIA DE FORÇA ATÔMICA

RESUMO: Os metais titânio, nióbio e zircônio possuem excelentes propriedades mecânicas e resistência a corrosão, o que os torna candidatos a recobrimento de implantes. Neste trabalho, modelos de recobrimento de titânio, nióbio e zircônio foram depositados por sputtering em substratos de Si(111) com um elevado fluxo de oxigênio. Os filmes interagiram com soluções de albumina sérica bovina (BSA) de diferentes concentrações em condições fisiológicas. A composição química, morfologia, módulo de elasticidade e dureza dos três filmes finos foram analisados por espectroscopia de fotoelétrons excitados por raios-X (XPS), espectrometria de massa de tempo de voo de íons secundários (ToF-SIMS), microscopia de força atômica (AFM) e nanoindentação. Os espectros de XPS mostraram que as superfícies são compostas por TiO2, Nb2O5, and ZrO2. As soluções de BSA, nas concentrações de 20 e 100 μg/ml, foram depositadas a 37 ° C por 1h. As imagens de AFM mostraram agregados de albumina adsorvida após com a solução de BSA 100 μg/ml. A morfologia da superfície dos filmes finos com BSA adsorvido foi diferente para cada tipo de oxido na superfície: as imagens de AFM indicaram uma adsorção em monocamada de BSA em filmes de Nb2O5, a adsorção na superfície de TiO2 resultou em duas camadas de proteína adsorvida e a adsorção na superfície do ZrO2 apresentou duas camadas de proteína adsorvida com alterações conformacionais.

PALAVRAS-CHAVE: Microscopia de força atômica, filmes finos, superfície de biomateriais, adsorção de proteínas, albumina.

1 I INTRODUCTION

Most biomaterials with suitable bulk properties do not have surface characteristics that are adequate for some clinical applications such as corrosion resistance, which is an important property of the metallic materials used in orthopedic and dental implants since it determines the device service life. It is also important due to the harmfulness of corrosion products that can be released and then interact with the living organisms. One interesting option to modify the surface functionality is the deposition of a metallic oxide thin film, improving the material corrosion resistance and, therefore, the material biocompatibility.

Sputtering is a straightforward process to obtain a uniform oxide film despite the geometry of the sample. This process provides a strong adherence of the oxide film to the underlying substrate and allows for the formation of nanostructured thin films [Wasa *et al.*]. In the biomedical field, however, this is an innovative technique with great possibilities which is now under extensive research [Olivares-Navarrete *et al.*].

Nanostructured surfaces are interesting for the bone/implant interface since both the surface and the bone have nanoscale particle sizes and similar mechanical properties [Geetha *et al.*]. The combination of these characteristics causes an increase of fracture resistance and biocompatibility for the implants. In addition, the particles generated by wearing the nanostructured implants are not immunoreactive and therefore less harmful to the human body than the microparticles of conventional implants.

Titanium, niobium, and zirconium are potential candidates for coatings due to the combination of good biocompatibility, high mechanical strength, excellent thermal stability, and optimal corrosion behavior [Eisenbarth *et al.*, Aguilar Maya *et al.*]. These materials exhibit a tendency to form a stable surface oxide film. The good in vivo performance of these three metals is mainly due to the presence of protective oxide layers formed in air or in oxygenated electrolytes. Therefore, these layers diminish the corrosion rate, minimizing the metal ion release to the biological media and facilitating osseointegration, leading to an optimal biocompatibility and low cytotoxicity.

The surface chemistry of an implant material and its influence on the interaction with body fluid are crucial to improve implants. When a biomaterial is implanted in the human body a cascade of events occurs on the surface. Proteins adsorb within seconds onto it, followed by cells interacting with the earlier adsorbed proteins rather than with the surface of the implant itself. Interaction of proteins and cells with biomaterials dictates the clinical success of implant devices. During the adsorption process, the proteins may undergo conformational changes. The type, concentration, distribution, and conformation of proteins on material substrates are key parameters of the mechanisms underlying subsequent cell interactions. The number of studies on protein adsorption has grown rapidly over the past few years [Brunette *et al.*, Ratner *et al.*] and research efforts have focused on elucidating the mechanisms that govern protein interactions with various biomaterials including polymers [Werner *et al.*], metals and ceramics. Various strategies have been proposed and used to describe protein adsorption in thermodynamic, molecular, and experimental terms.

In this study, thin films of Ti, Nb, and Zr oxides were deposited by magnetron sputtering on Si (111) substrates and were characterized by XPS, ToF-SIMS, AFM, and nanoindentation. In order to assess the behavior of these materials in physiological medium, the interactions of these surfaces with bovine serum albumin (BSA) were analyzed. BSA is an important protein known to inhibit platelet adhesion and thrombus formation [Carre, Lacarriere].

2 I MATERIAL AND METHODS

The titanium, niobium, and zirconium oxide films were deposited on 1 × 1.5 cm^2 cleaned (111) silicon substrates at room temperature by DC magnetron sputtering using Ti, Nb, and Zr targets (0.060m diameter × 0.003m thick, 99.9% pure). The substrates were mechanically clamped to the DC magnetron cathode of a conventional sputtering system (Balzers BA510). An oxygen (20% vol.) and argon (99.999% pure) mixture was used as the sputtering gas. The target substrate separation was 0.260 m. The films were deposited at the conditions of cathode power and base pressure of: 135 W and 2×10^{-5} Pa to titanium oxide film and 150 W and 4×10^{-5} Pa to niobium and zirconium oxides films. The thickness was 500 nm for all films.

The surface chemical composition obtained by XPS analyses were performed under ultrahigh vacuum (low 10^{-7} Pa range) employing a SPECS Phoibos Hs 3500 spectrometer with an Al Kα (hν = 1486.6 eV) monochromatized, focused X-ray source. The spectrometer was calibrated against the reference binding energies (BEs) of clean Cu (Cu $2p_{3/2}$ at 932.6 eV), Ag (Ag $3d_{5/2}$ at 368.2 eV), and Au (Au $4f_{7/2}$ at 84.0 eV) samples. The analyzed area had a diameter of about 500 *μ*m. In addition to the survey spectrum (pass energy of 100 eV, step energy of 1 eV), the following core level spectra were systematically recorded at higher energy resolution (pass energy of 20 eV): C 1s, O 1s, Ti 2p, Zr 3d, Nb 3d, and Si 2p (step energy of 0.1 eV), with a take-off angle of 90°. To take into account surface charging effects, the core level spectra were referenced by setting the lowest BE component of the resolved C 1s peak (corresponding to adventitious carbon in a hydrocarbon environment) to 285.0 eV. Core level peak decompositions were performed with the CasaXPS© program. All peaks were fitted using the Shirley background and a 70% Gaussian/ 30% Lorentzian peak shape.

The surface chemical composition and the distribution in depth of the different elements were determined by ToF-SIMS using a TOF.SIMS V spectrometer (ION-TOF GmbH) with the following configuration: the analysis chamber was maintained at less than 5×10^{-7} Pa in operation conditions, the total primary ion flux was below 10^{12} ions × cm^{-2} to ensure static conditions, and a pulsed 25 keV Bi^+ primary ion source (Liquid Metal Ion Gun, LMIG) at a current of about 1 pA (high current bunched mode for spectrometry), rastered over a scan area of 100 µm × 100 µm, was used as the analysis beam. The exact mass values of at least five known species, from H^-,C^-, O^-, C_2^-, and C_3^- were used for calibration of the data acquired in the negative ion mode. The sputtering was performed using a 2 keV Cs^+ ion beam at a current of 90 nA and rastered over an area of 300 µm × 300 µm. Data acquisition and processing analyses were performed using the commercial IonSpec© program.

The surface morphology and roughness were determined using a commercial atomic force microscope (MultiMode 8 Bruker AXS). The instrument was operated in tapping mode in air, and the image sizes were 500 × 500 nm^2. Grain size and the average roughness (Ra) were obtained.

Nanoindentation tests were carried out with a TriboIndenter nanoindenter (Hysitron Inc.) using a Berkovich diamond tip. Each specimen was tested at room temperature and the measurements were done at extremely small penetration depths. The statistical analysis of the measured results allowed for the elastic modulus (E) and hardness (H) values. To eliminate any influence of the substrate material in the measurement of elastic modulus, the penetration range of the indenter was limited to a depth <0.2d, where d is the film thickness.

The BSA (Sigma, 98% purity) solution was used in physiological-like conditions (pH 7.4 and 37 °C). This protein was dissolved in ultrapure water (2 g/L) and the stock solution was diluted in a phosphate buffer-saline (PBS). The solution concentrations of BSA utilized were 20 μg/ml and 100 μg/ml. For each concentration, the surface of the thin film was immersed in the protein solution at 37ºC for 1 h. After 1 hour, each thin film was washed three times with ultra-pure water and stored at controlled room temperature.

3 I RESULTS AND DISCUSSION

3.1 XPS analysis

The XPS survey spectra for the three oxides films are shown in Figure 1, and they present only carbon (contaminant), oxygen, and metal peaks: Ti (Fig. 1 (a)), Nb (Fig. 1 (b)), and Zr (Fig. 1 (c)). For all three films, no traces of the substrate elements were detected, indicating the formation of continuous oxide coatings.

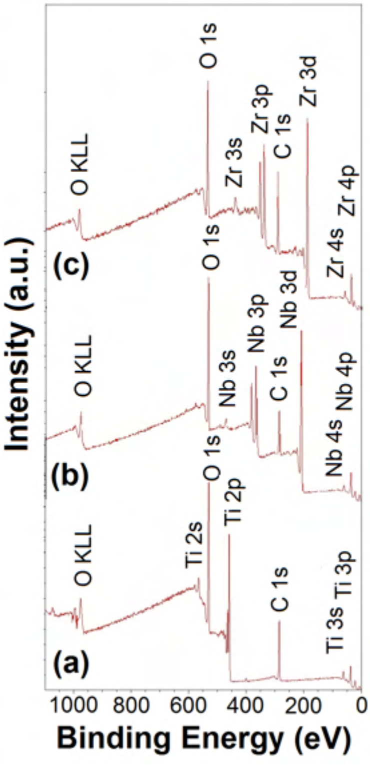

Figure 1. XPS survey spectra of (a) Ti, (b) Nb, and (c) Zr thin films deposited on Si(111).

Figure 2 displays the (a) Ti 2p, (b) Nb 3d, and (c) Zr 3d spectra for the oxide films. The Ti 2p doublet was fitted with only one component for each spin-orbit peak, having Ti $2p_{3/2}$ at 458.5±0.1 eV, which corresponds to TiO_2. The Nb 3d spectrum was deconvoluted with only one component for each peak, with Nb $3d_{5/2}$ at 207.1±0.1 eV, which is associated to Nb_2O_5. The Zr 3d doublet was fitted by using only one component for each spin-orbit peak, with Zr $3d_{5/2}$ at 182.0±0.1 eV, corresponding to ZrO_2.

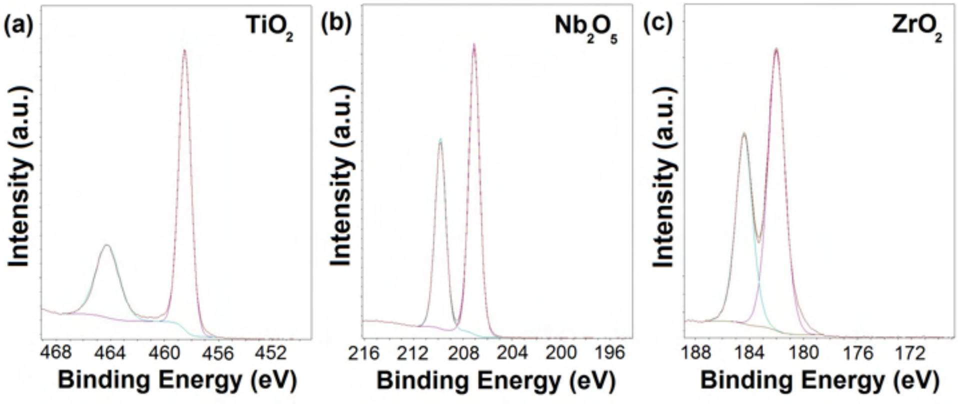

Figure 2. High-resolution XPS spectra of (a) Ti 2p, (b) Nb 3d, and (c) Zr 3d regions.

3.2 Tof-SIMS analysis

Figure 3 presents a typical ToF-SIMS negative ion depth profile for: (a) TiO_2, (b) Nb_2O_5, and (c) ZrO_2 thin films. All the films were oxidized and two main regions can be identified. The first one corresponds to the formed oxide film and the other, to the Si(111) substrate. It is possible to detect two main oxides, TiO^- and TiO_2^-, in the Ti film depth profile (Fig. 3 (a)). The outer layer, which corresponds to approximately the first 15 seconds in the horizontal axis of the graphic, is predominantly constituted by TiO_2^-. This outer layer was the one that was probed by XPS. The inner layer, which corresponded to the range of 15 to approximately 160 seconds in the horizontal axis, comprises of both TiO^- and TiO_2^- phases; the signal of TiO^- was approximately 67% higher than that for TiO_2^-.

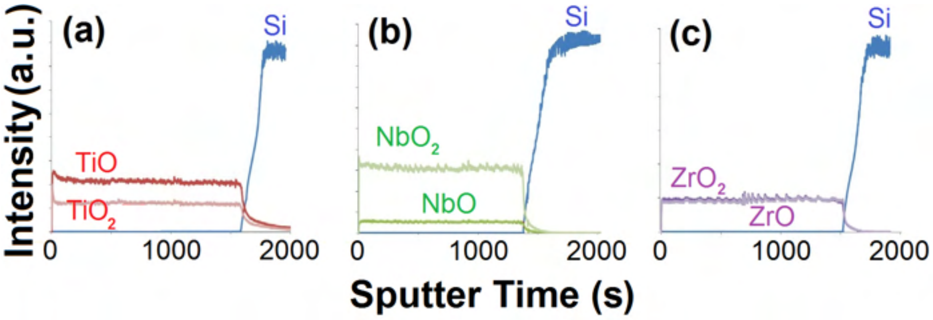

Figure 3. Depth profile for TiO_2, Nb_2O_5, and ZrO_2 thin films deposited on Si(111).

Figure 3 (b) shows the Nb^- oxide film depth profile, and it is possible to identify two main oxides: NbO_2^- and NbO^-. The NbO^- signal represents only 11% of the total signal. It was not possible to identify the Nb_2O_5 oxide.

Figure 3 (c) depicts the Zr oxide film depth profile, which is very uniform, with the same signal intensities for ZrO^- and ZrO_2^- oxides. It should be mentioned that there is a predominance of ZrO_2^- in the outer layer, which is associated to approximately the first 15 seconds in the horizontal axis of the graphic and was the one that was probed by XPS.

3.3 AFM analysis

The surface roughness and morphology for the TiO_2, Nb_2O_5, and ZrO_2 films were characterized by AFM. It is possible to observe the low roughness and the nanometric grain sizes of the films, which can be analytically described by the parameters (Ra roughness and grain size) extracted from the image analyses, presented in Table 1.

The Ra values for the samples were established from the AFM images before and after deposition, over a surface region of 500 × 500 nm^2. Each sample was analyzed at five randomly chosen locations. Statistical analyses were performed using the standard deviation. The Nb_2O_5 film has the smoothest surface (Ra = 0.17±0.04 nm) and the smallest grain size (13±2 nm).

	Ra roughness (nm)	Grain size (nm)
TiO_2	0.21±0.01	17±1
Nb_2O_5	0.17±0.04	13±2
ZrO_2	1.10±0.02	35 ±3

Table 1. Values of roughness and grain sizes for TiO_2, Nb_2O_5, and ZrO_2 thin films.

Nanoscale topography is an important characteristic of biomaterials surfaces, because it is believed that it may be related to cell proliferation [Hansen *et al.*]. In this work, all surfaces are nanostructured and, according to Stanford, propitious to a better bioactivity, because the topographical surface features at the nanoscale level on the surface have the advantage that the conventional properties of materials are very different for a nanomaterial (e.g., increased number of atoms at the surface, surface grain boundaries, electron delocalization, etc.). At the nanoscale level, molecular interactions with the surface can be targeted to create specific cell level responses. Studies suggest this effect may be related to protein orientation to the nanophase structures and specifically the mode of orientation of adhesion proteins such as vitronectin to the grain boundaries which in turn alters osteoblast adhesion and shape; both critical to formation of bone.

In a work with MG63 cells and TiO_2 thin films, Vandrovcova et al. reported that the material surface roughness was inversely correlated with the size of the cell spreading area. With the smoother TiO_2 films (Ra = 0 and Ra = 40 nm) being the most appropriate of all the materials studied for forming new bone tissue.

3.4 Nanoindentation

The elastic modulus (E) and the hardness (H) of TiO_2, Nb_2O_5, and ZrO_2 thin films obtained by using the nanoindentation technique are depicted in Figure 4 and Table 2 as a function of indentation depth. Each value is an average of 15 measurements. For metallic biomaterials, it is desirable low elastic modulus, close to the values of the elastic modulus of nature bone (10 to 40 GPa), to enable a better bone-implant mechanical cohesion. Figure 4 shows a non-uniform behavior near the surface (approximately first 40 nm). Since these differences were observed for the three thin films used in this study, they were associated with the experimental conditions. As suggested by Cáceres *et al.* in a similar study with titanium alloys, this could be a combination of errors in depth determination at the very small contact displacement and the greater effect of the surface roughness, which can set a lower limit to the useful nanoindentation size. After the first nanometers, the measurements showed a constant value (plateau region). The plateau region for the three thin films described in this study was considered from 40 to 140 nm contact displacement.

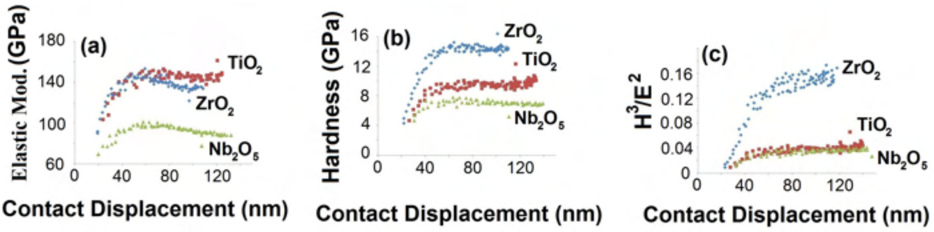

Figure 4. Elastic modulus (a), Hardness (b), and H^3/E^2 (c) for TiO_2, Nb_2O_5, and ZrO_2 thin films.

Figures 4 (a) and (b) show that the Nb_2O_5 film presented the lowest elastic modulus (90 GPa) and hardness (7 GPa). Figure 7 (c) shows the H^3/E^2 ratios as a function of indentation depths for the TiO_2, Nb_2O_5, and ZrO_2 films. This ratio is a parameter which controls the resistance to plastic deformation during contact events [Musil]. The optimum H^3/E^2 ratio is obtained for the ZrO_2 thin film, which also showed to be the hardest sample.

Thin film	Elastic modulus(GPa)	Hardness (GPa)
TiO_2	150	10
Nb_2O_5	90	7
ZrO_2	140	14

Table 2. Elastic modulus and hardness for TiO_2, Nb_2O_5, and ZrO_2 thin films.

The TiO_2, Nb_2O_5, and ZrO_2 thin films presented adequate values of elastic modulus, hardness, and resistance to plastic deformation for biomedical applications. All films had values of elastic modulus lower than those obtained for the 316L stainless steel (200 GPa) and cobalt-chromium alloys (220-230 GPa). The hardness value obtained for the three thin films was greater than the hardness of commercially pure titanium (1.6 GPa), traditional titanium alloys such as Ti-6Al-4V (4.9 GPa) and 316L stainless steel (2 to 6.7 GPa).

The low elastic modulus and high hardness values obtained for the three films can be attributed to the nanostructure film, which gives them the unique properties. Studies have shown that nanostructured surfaces exhibit superior mechanical properties of hardness and wear resistance compared to conventional materials [Hansen *et al.*].

3.5 AFM analysis to BSA adsorption

The surface roughness and morphology for the TiO_2, Nb_2O_5, and ZrO_2 films were characterized by AFM before(a) and after(b) adsorption of 100 µg/ml BSA, and the images are displayed in Figures 5, 6, and 7, respectively.

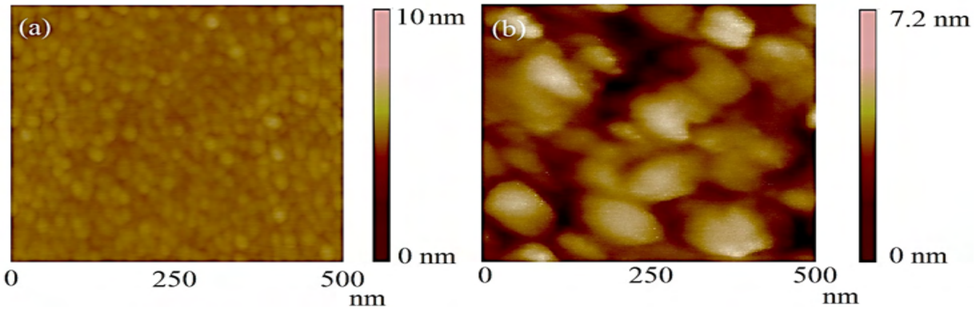

Figure 5. AFM images (500 nm x 500 nm) for TiO_2 film (a) before and (b) after 100 μg/ml BSA adsorption.

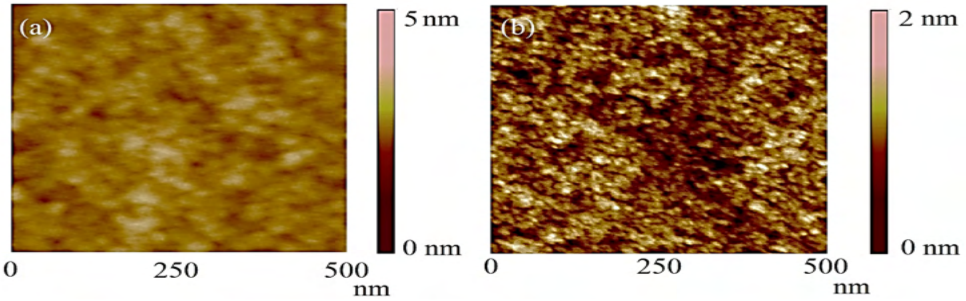

Figure 6. AFM images (500 nm x 500 nm) for Nb_2O_5 film (a) before and (b) after 100 μg/ml BSA adsorption.

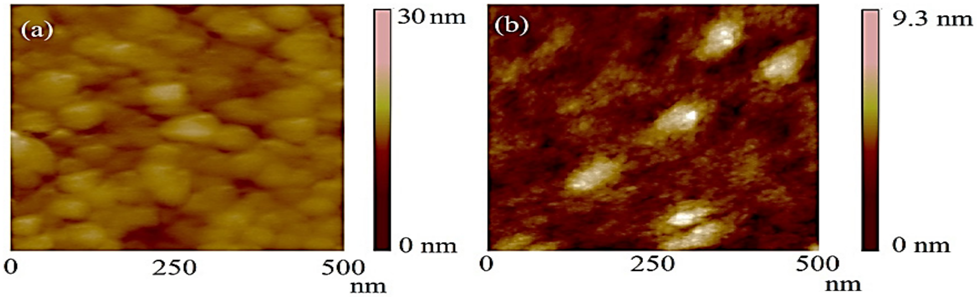

Figure 7. AFM images (500 nm x 500 nm) for ZrO_2 film (a) before and (b) after 100 μg/ml BSA adsorption.

The surface of all films showed a significant increase in its roughness and grain size after the adsorption of BSA, thus these parameters presented in Table 3 confirmed the presence of BSA in all surfaces analyzed. All surfaces showed protein clusters, indicating unequal coating of the surfaces. This is due to the fact that the surfaces are not uniform and there are therefore preferential sites where adsorption occurs. This associated with the poor mobility of the adsorbed molecule hinders the formation of a uniform layer.

	Roughness Ra (nm)	**Grain size (nm)**
TiO_2 film	0.21 ± 0.01	17 ± 1
TiO_2 after BSA adsorption	2.8 ± 0.1	107 ± 8
Nb_2O_5 film	0.17 ± 0.04	13 ± 2
Nb2O$_5$ after BSA adsorption	0.48 ± 0.02	30 ± 5
ZrO_2 film	1.10 ± 0.02	35 ± 3
ZrO_2 after BSA adsorption	1.80 ± 0.02	90 ± 4

Table 3. Values of roughness and grain sizes for Ti, Nb, and Zr films.

The AFM imaging in Figures 8, 9, 10, and 11 showed the conformation of BSA on thin films. Single molecules and aggregates of albumin were observed. Figure 8, 9, 10, and 11(a) shows a topographical view of adsorbed BSA molecules on TiO_2, Nb_2O_5, ZrO_2 and ZrO_2 surfaces respectively, obtained by AFM. Single molecules and aggregates of BSA were distinguished. Figure 8, 9, 10, and 11 (b) shows a higher magnification to better visualize the individual BSA molecules, as well as, a cross-sectional image of a single BSA molecule.

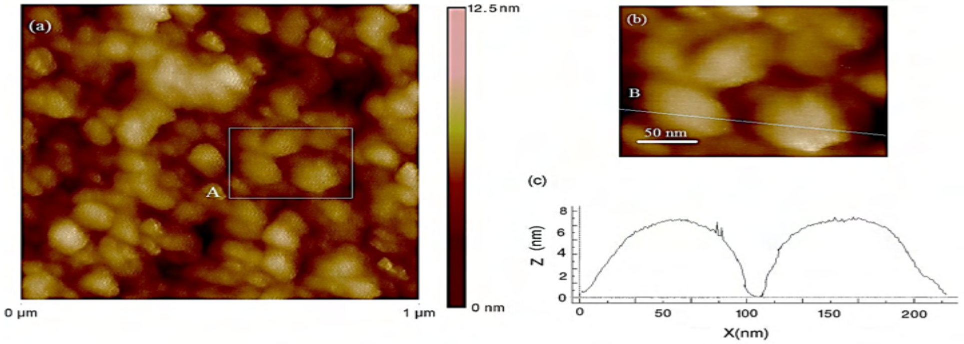

Figure 8. (a) AFM image (1 μm x 1 μm) for BSA adsorbed on the TiO_2, (b) Selected area, and (c) Side view of BSA molecules (**B** line).

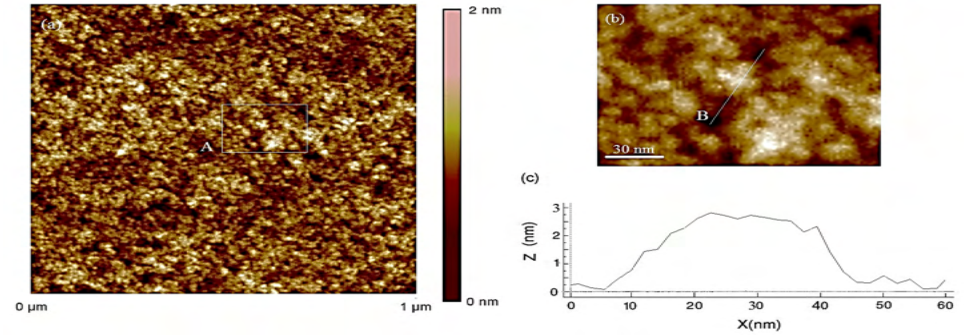

Figure 9. (a) AFM image (1 μm x 1 μm) for BSA adsorbed on the Nb_2O_5 film, (b) Selected area, and (c) Side view of BSA molecules (**B** line).

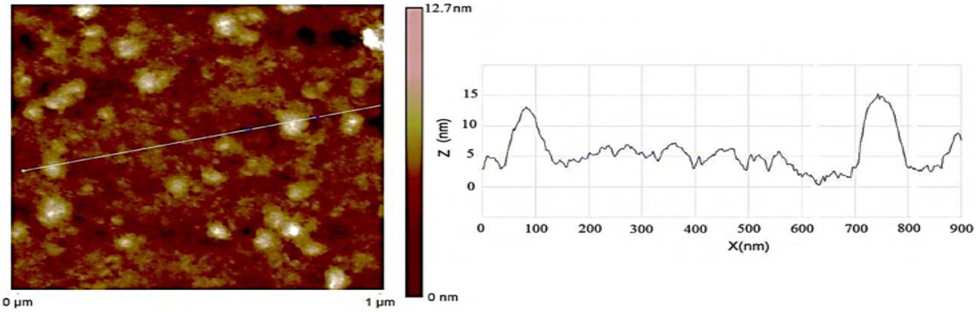

Figure 10. AFM images (1 μm x 1 μm) for ZrO_2 film after BSA adsorption, the select line showed the non-uniform adsorption.

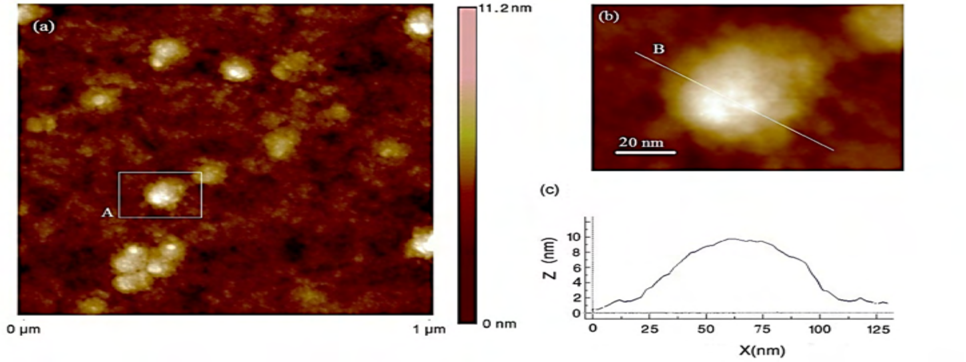

Figure 11. (a) AFM image (1 *μ*m x 1 *μ*m) for BSA adsorbed on the ZrO_2, (b) Selected area, and (c) Side view of BSA molecules (**B** line).

For TiO_2 surface (Figure 11), the BSA adsorbed molecules were approximately 100 nm in diameter and 8 nm in height. To ZrO_2 film (Figures 13 and 14) the BSA adsorbed molecules were approximately 100 nm in diameter and variable height: while most of the surface was covered with molecules at the height of approximately 5 nm (Fig. 13), some dots show clusters of 10 nm in height (Fig. 14). As the AFM-height measured of the agglomerates of adsorbed BSA molecules on these two surfaces is greater than the size of the protein molecule (4 nm x 4 nm x 14 nm), BSA multilayers formed on these two surfaces. In TiO_2 the BSA preserved its native shape and on the surface of the ZrO_2 film experiment conformational change.

The BSA adsorbed on the Nb_2O_5 surface (Figure 12) also suffer conformational change since the height of the AFM-measured layer of the BSA molecules adsorbed on this film is less than the size of the protein molecule (4 nm x 4 nm x 14 nm). The measured thickness of the adsorbed BSA layer (less than 4 nm) supports the possibility that spreading of the protein molecules on the material surface occurred with low protein adsorption, making the Nb_2O_5 the thin film with the lowest affinity with BSA.

While the TiO_2 preserved its native shape, ZrO_2 and Nb_2O_5 films experimented conformational change. Conformational changes of adsorbed proteins are important because they may subsequently lead to either accessibility or inaccessibility of bioactive sites which are ligands for cell interaction and function relevant to physiology and pathology. This explanation agrees with other reports regarding the adsorption of soft proteins such as albumin (both bovine and human) to titanium dioxide [Andrade et al., Xiu-Mei Li] and other material surfaces [Dabkowska *et al*., Indesta *et al*.].

Wertz and Santore reported that the more hydrophobic the surface becomes, the stronger the interaction is between the surface and the proteins. This would cause a larger spread of the protein on the surface and thus result in a larger perturbation of the native structure of the protein [Roach *et al*.].

As ZrO_2 film presents BSA multilayers and conformational change, it follows that ZrO_2 demonstrate higher affinity for BSA than TiO_2 and Nb_2O_5 surfaces,

4 I CONCLUSIONS

In this work, titanium, niobium, and zirconium oxide thin films were deposited by DC magnetron sputtering on Si(111) substrates. The chemical, morphological, and mechanical properties of these coatings were evaluated. XPS analysis showed that the surfaces of the three films were constituted by TiO_2, Nb_2O_5, and ZrO_2. ToF-SIMS results indicated the formation of an outer layer of TiO_2^- and inner layer of both TiO^- and TiO_2^-, for the oxidized Ti film, combined NbO^- and NbO_2^- oxides, for the oxidized Nb film, and ZrO^- and ZrO_2^-, with a predominance of ZrO_2^- in the outer layer, for the oxidized Zr film. AFM images showed that the oxidized Ti, Nb, and Zr films had nanostructured grains and low roughness. The elastic modulus and hardness were measured and the lowest elastic modulus was 90 GPa (Nb_2O_5 film) and the largest recorded hardness was 14 GPa (ZrO_2 film). All thin films produced in this study were completely oxidized, had nanostructured grains, bulk uniformity and good mechanical properties, thus sputtered TiO_2, Nb_2O_5, and ZrO_2 thin films can be considered as materials with great potential for coating of implants.

After covering the thin films with the protein, the AFM images showed the conformation of BSA, and single molecules and aggregates of albumin were observed. The conformation of the adsorbed BSA molecules appears to be different on the three surfaces. The results obtained by AFM indicated TiO_2 and ZrO_2 surfaces presented the formation of BSA multilayers while Nb_2O_5 surface presented low protein adsorption. In TiO_2 film the BSA preserved its native shape and on ZrO_2 and Nb_2O_5 surfaces underwent conformational change with more remarkable spreading of the protein molecules on Nb_2O_5 films. The ZrO_2 surface presented the highest adsorption with partial conformational change and the prevalence of the native protein structure.

The present study supports the use of AFM as a valid technique to study the dynamic adsorption of proteins to nanostructured material surfaces as well as enrich current knowledge of protein interactions with nanomaterials.

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