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Surface modification of tantalum pentoxide coatings deposited by magnetron sputtering and correlation with cell adhesion and proliferation in *in vitro* tests

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Abstract. The effect was analyzed of surface treatment by argon ions on the surface properties of tantalum pentoxide coatings deposited by reactive magnetron sputtering. The structural parameters of the as-deposited coatings were investigated by means of transmission electron microscopy, atomic force microscopy and scanning electron microscopy. X-ray diffraction profiles and X-ray photoelectron spectra were also acquired. The total surface free energy (SFE), the polar, dispersion parts and fractional polarities, were estimated by the Owens-Wendt-Rabel-Kaeble method. The adhesive and proliferative potentials of bone marrow cells were evaluated for both Ta_2O_5 coatings and Ta_2O_5 coatings deposited by simultaneous bombardment by argon ions in *in vitro* tests.

1. Introduction

In recent years, a new branch of medicine has undergone rapid development based on the use of nanocomposite coatings that promote positive biological processes in a living organism. Medical products with ceramic coatings form normal biopotentials in damaged areas of tissue and organs, prevent the necrosis, tissue atrophy, significantly accelerate the healing process and the post-operative rehabilitation [1-5]. The novel implant and stent types coated by oxide and oxynitride coatings have demonstrated excellent biocompatibility. The positive effect results in lower rates of postoperative infections, inflammatory reactions and other complications [6-8].

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Tantalum and tantalum-based compounds are promising for biomedical applications due to their good dielectric properties. The results of tests on implanting Ta in both soft and hard tissue of rats showed the good biocompatibility and osteogenesis of this metal [9]. TaC and TaN materials possess relatively high hardness due to the covalent nature of their bond [10] and demonstrate high corrosion resistance [11]. The blood compatibility of TaN films was shown to be better than that of Ta [12]. The aim of the present work was to study the effect of surface treatment by argon ions on the structure and surface properties of tantalum pentoxide (Ta_2O_5) coatings deposited by reactive magnetron sputtering and, further, the correlation of the surface characteristics with the cell's adhesion and proliferation.

2. Materials and methods

Ta_2O_5 coatings were deposited on Petri dishes glass substrates in a high vacuum pumping system with a base pressure of about 10^{-2} Pa by ion-source-assisted magnetron sputtering. For the reactive deposition, oxygen was delivered through the ICP plasma source, q = 60 sccm, the magnetron voltage was U_m = 700 V, and the magnetron current was about I_m = 5,7 A. The sputtering process was conducted in modes far from the target poisoning one for further oxide coatings deposition with highly stoichiometric composition. Also, such deposition conditions allowed us to avoid micro-arcs and micro-drops formation. The optimum conditions were implemented in the upper part of the volt-ampere characteristic curves of a magnetron discharge in argon with oxygen (figure 1). At sufficiently high mass-flow rates of oxygen, a hysteresis effect was observed.

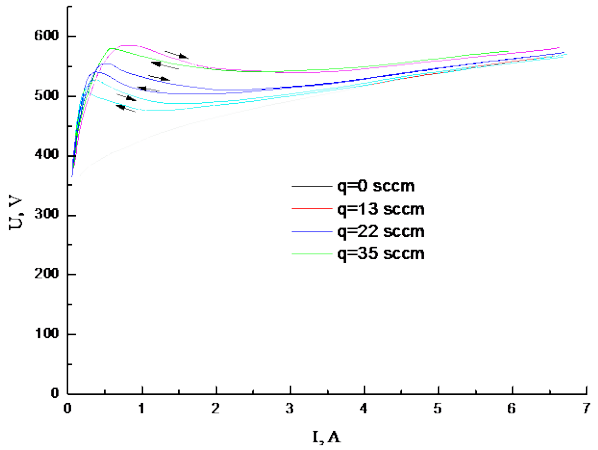


Figure 1. Volt-ampere characteristics of the magnetron discharge with a tantalum target in argon for different values of the oxygen flow: q = 0 sccm, q = 13 sccm, q = 22 sccm, q = 35 sccm.

For other substrates, the deposition process was carried out by a simultaneous bombardment of the growing film by argon ions using an ion source. The ion source parameters were: ion acceleration voltage of 2.5 kV, and ion current source of 30 mA.

The structural parameters of the as-deposited coatings were investigated by means of transmission electron microscopy (TEM) using a JEOL JEM 2100 instrument. The surface topography of the tantalum pentoxide ceramic coatings were evaluated by scanning electron microscopy (QUANTA 600 FEG) (SEM) and by atomic force microscopy (NTEGRA-AURA) (AFM). The coating thickness and cross-section structure were obtained using SEM (Nova NanoSEM, FEI) cross-section measurements. The X-ray diffraction profiles were acquired by means of a DRON-3 diffraction device. X-ray photoelectron spectroscopy (XPS) was carried out by an ESCALAB MkII (VG Scientific) electron spectrometer using an AlKalpha X-ray source (excitation energy hν = 1486.6 eV).

The advancing contact angles were measured by Wilhelm's method (Kruss K12) at a temperature of 20 ^{o}C. Standard liquids with well-known values of the surface tension, dispersion component and polar interaction were used. The estimation of the surface free-energy SFE and its polar and dispersion parts were performed by means of the Owens-Wendt-Rabel-Kaeble' method for thee liquid system α-bromonaphthalene-formamide-ethylene glycol-diidomethane-glycerol-water.

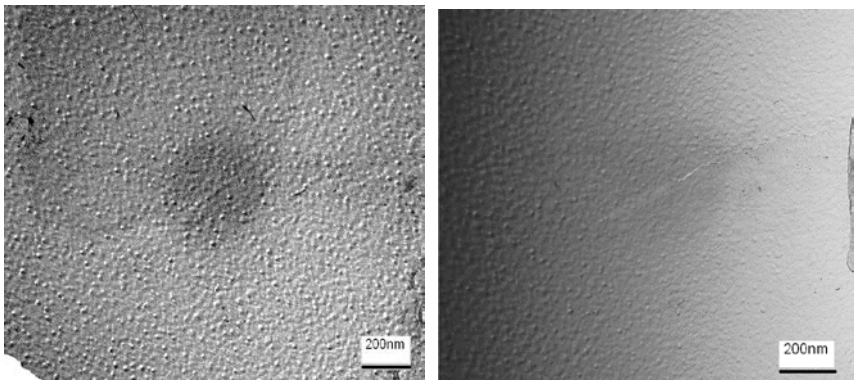
Culturing of bone marrow (BM) cells (CBA/H) was performed with densities of 0.5–1×10^4 cells/cm^2. After passage, the cells were removed by a trypsin-EDTA solution («Sigma-Aldrich», USA). The structural and functional characteristics of the BM cells were then evaluated. Visual control of the cell cultures was carried out using a Primo Star light phase-contrast microscope (Zeiss,

Germany) and an Axiovert 40C inverted microscope (Zeiss, Germany). The adhesive potential of the BM cells was also evaluated.

A statistical analysis of test results was performed using the non-parametric Mann-Whitney method. The difference was considered statistically significant at $P < 0.05$.

3. Results and discussion

The difference of the structural parameters of the deposited coatings was investigated by means of TEM (figure 2). The coatings had a nano-globular structure with characteristic sizes of the structural elements about 15 nm in the case of as-deposited tantalum pentoxide film and 20 nm after bombardment of the growing film. The deposition process carried out by simultaneous bombardment by argon ions resulted in surface structure changes of the growing films.



a) b)

Figure 2. Brightfield TEM images of as-deposited oxide ceramic coationgs: a) Ta_2O_5 coatings, b) Ta_2O_5 coatings deposited with simultaneous bombardment by argon ions.

X-ray diffraction profiles and XPS spectra of ceramic Ta_2O_5 coatings have been analyzed previously [13]. The as-deposited magnetron sputtered Ta_2O_5 coatings demonstrated an amorphous nature, i.e, no peaks were observed. All XPS spectra consisted of well-defined XPS lines of Ta 4f, 4d, 4p and 4s; O 1s; C 1s. Ta 4f doublets are typical for Ta_2O_5 coatings and have two peaks: Ta 4f7/2 at ~ 26.5 eV and Ta 4f5/2 whose binding energy is higher by 1.9 eV. The Ta 4f lines of the deposited films were in a good agreement with the Ta 4f doublet that is representative for the Ta-0 bond in Ta_2O_5. The Ta/O ratio estimated from the spectra was about 0.4 for all coatings investigated. The O 1s peaks were centered at binding energies of 530.6 eV for the deposited coatings.

The scanning electron microscopy images of the magnetro-sputtered Ta_2O_5 ceramic coatings revealed a relatively flat surface without cracks. The coating thickness and cross-section structure were obtained from the SEM cross-section measurements (figure 3 a, b). The surface topography and roughness parameters were studied by AFM (figure 3 c, d). The ions bombardment resulted in a change of the surface roughness from $S_a = 0.9$ nm, $S_q = 1.2$ nm to $S_a = 0.6$ nm, $S_q = 0.7$ nm. The difference in the structural parameters of the Ta_2O_5 coatings and the Ta_2O_5 coatings deposited by simultaneous bombardment by argon ions was in a good agreement with the TEM results.

The changes in the surface structure parameters may lead to changes in the surface characteristics and functional properties of deposited coatings. An analysis has been previously performed of the capacity, dielectric constant, and dielectric loss tangent depending on the frequency of the electromagnetic field for tantalum pentoxide ceramic coatings deposited in different sputtering modes [13,14]. The magnetron-sputtered Ta_2O_5 ceramic coatings demonstrated high dielectric parameters. The effect of the deposition process conditions on the electrical behavior of the magnetron-sputtered Ta_2O_5 coatings was analyzed.

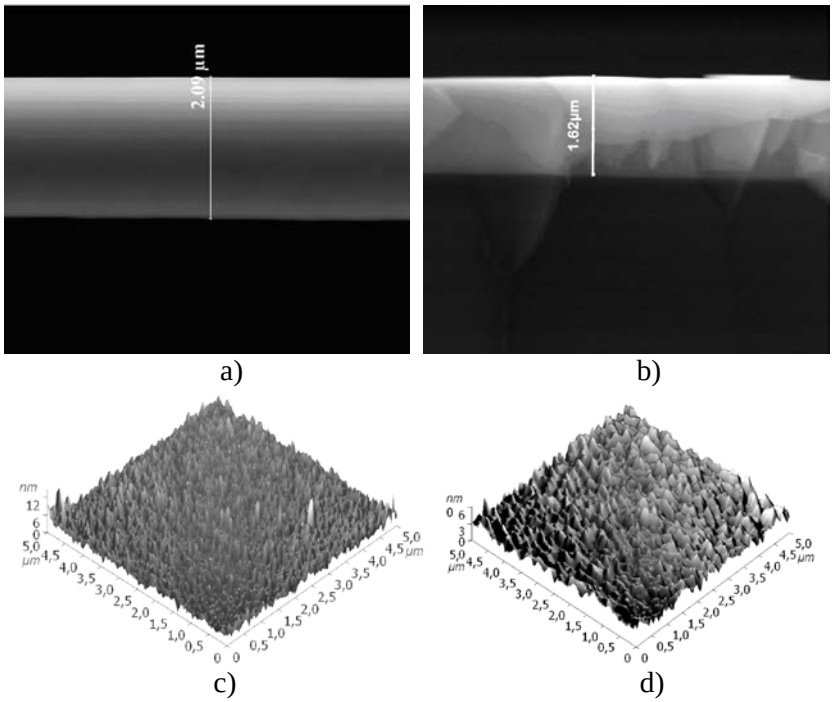


Figure 3. SEM cross-section images (a, b) and AFM surface images (c, d) of as-deposited oxide ceramic coatings: (a, c) Ta_2O_5 coatings, (b, d) Ta_2O_5 coatings deposited by simultaneous bombardment by argon ions.

The surface free energy (SFE) plays an important role in the mechanism of cell/biomaterial response [15]. The total SFE and its polar and dispersion parts and fractional polarities were estimated by the Owens-Wendt-Rabel-Kaeble method for the liquid system α-bromonaphthalene-formamide-ethylene glycol-diidomethane-glycerol-water (table 1) at a temperature of 20 °C.

The data demonstrated that the surface free energy of Ta_2O_5 coatings thus calculated was in the range 41 – 44 mN/m, the SFE polar parts were in the range 11 – 13 mN/m, and the fractional polarity values were in the range 0.26 – 0.29.

Table 1. Surface free energy components by the Owens-Wendt-Rabel-Kaeble method.

Substrate /coating	Total γ	Components of SFE mN/m		
		Dispersion part γ^d	Polar part γ^p	Fractional polarity $\gamma^p / (\gamma^d + \gamma^p)$
Glass	56.28	30.31	25.97	0.46
Glass/ Ta_2O_5	43,96	31.06	12.90	0.29
Glass/ Ta_2O_5 (argon ions bombardment)	41,11	30,03	11,08	0.26

The tantalum pentoxide ceramic coatings deposited by a simultaneous argon ion bombardment showed the minimal SFE and fractional polarity values. The properties of the oxide ceramic coatings shifted to the more hydrophobic region after the surface treatment by argon ions.

The BM cells were cultured during 18 – 20 days. The medium was changed every three days to achieve a sub-confluent monolayer, and then the culture was prepared for one passage. At the end of each passage, the cells were removed by a solution of trysin-EDTA (Sigma-Aldrich) using a standard method, inactivated by a medium containing 10% FCS, washed out and counted. The effect of the

conditions of depositing oxide ceramic coatings on the adhesive potential of the BM cells was registered (table 2).

During the culturing on the Ta_2O_5 coated substrates, a reduction of the BM cells adhesion potential was observed (table 2).

Table 2. Adhesive potential of the BM cells on glass and Ta_2O_5 coated substrates.

Substrate/coatings	Adhesive potential of BM cells, %	Percent of attached cells,%
Glass (control)	7,12±0,64	100
Glass/ Ta_2O_5	5,73±0,53*	80,46[a]
Glass/ Ta_2O_5 (argon ions bombardment)	3,00±0,24*	42,22*

[a]Statistically significant difference to control, $P < 0,05$.

The minimal values of cell adhesive potential were found in the case of Ta_2O_5 coatings deposited by a simultaneous bombardment by argon ions (figure 4). Therefore, the low adhesive and proliferative activity suggest the possibility of applying the Ta_2O_5 coatings investigated to angioplasty and vascular surgery in view of reducing the likelihood of thrombosis.

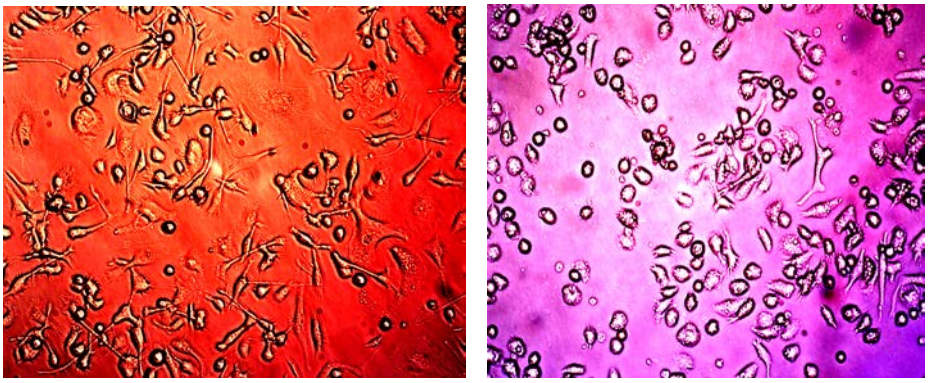


Figure 4. Morphology of adhesive BM cells by means of inverted microscopy: a) Ta_2O_5 coatings, b) Ta_2O_5 coatings deposited by simultaneous bombardment by argon ions (phase contrast, magnification 1000).

4. Conclusions

The deposition process carried out by a simultaneous bombardment by argon ions resulted in a surface modification of the growing films. The surface roughness parameters decreased from S_a = 0.9 nm to S_a = 0.6 nm following the ion bombardment. The changes in the surface structure parameters led to changes in the surface characteristics. The minimal values of SFE and the fractional polarity were exhibited by the tantalum pentoxide ceramic coatings deposited by a simultaneous bombardment. The properties of the oxide ceramic coatings shifted to the more hydrophobic region after a surface treatment by argon ions.

It was shown that the argon ions bombardment during tantalum pentoxide coating deposition process reduces the adhesive and proliferative potential of BM cells. Therefore, the Ta_2O_5 coatings are promising for application to angioplasty and vascular surgery due to the low adhesive and proliferative activity.

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