Surface treatment of magnetron-sputtered tantalum pentoxide coatings and its effect on the phenotype and function of immune cells *in vitro*

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Abstract. The effect was analyzed of surface treatment by argon ions and electron beam on the properties of tantalum pentoxide coatings deposited by reactive magnetron sputtering and the treatment's correlation with immune cells response. The characteristics of the coatings were determined by means of energy dispersive X-ray (EDX) scanning electron microscopy (SEM), X-ray photoemission spectroscopy (XPS), and atomic force microscopy (AFM). The results demonstrate that the electron-irradiation post-treatment process stimulates the adhesive potential and phagocytic activity of antigen-presenting cells.

1. Introduction

Antigen presenting cells, such as macrophages may affect the immune response after implantation. Implanted biomaterials are in a close contact with macrophages so that characteristics such as surface chemistry and topography may play a critical role in initiating an anti-inflammatory response [1-3]. The immune response to artificial implants leads to postoperative complications, inflammatory processes and a risk of repeated surgical interventions. Inflammatory and anti-inflammatory reactions can be regulated via modifying the biomaterial's properties and surface [4, 5]. Surface modifications are a direct way of changing the cells' phenotype and function with the aim of reducing immune reactions and promoting a beneficial healing response.

This study was devoted to the effect of surface treatment by argon ions and electron beam on the structure and surface properties of Ta_2O_5 coatings deposited by reactive magnetron sputtering and its correlation with immune cells' response in *in vitro* tests.

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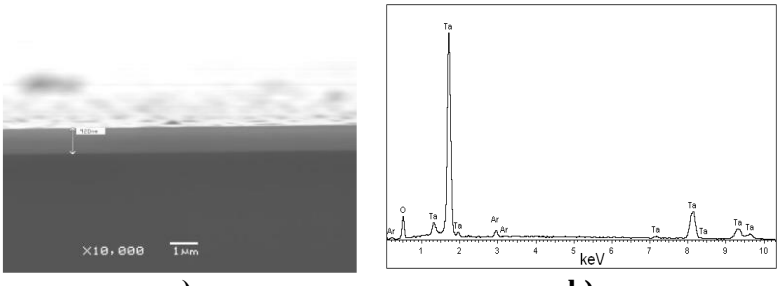
2. Materials and Methods

Ta_2O_5 coatings were deposited by ion-source-assisted magnetron sputtering on Petri-dish glass substrates in a high-vacuum pumping system with a base pressure of about 10^{-2} Pa. Oxygen for the reactive deposition was delivered through an ICP plasma source with q = 60 sqcm; the magnetron voltage was U_m = 700 V and the magnetron current was about I_m = 5.7 A. For part of the substrates, the deposition process was carried out through simultaneous bombardment of the growing film by argon ions using an ion source. The ion-source parameters were as follows: magnetic field current 1.5 A, ion acceleration voltage 2.5 keV, ion source current 30 mA. A primary electron beam was created by an UL-119 electron gun type. The reflected electrons energy was 20 keV, the current density on the sample surface, 14 mA/cm^2, the irradiation time, 1500 s. The coatings thickness was measured by a Calotest device. The surface morphology and topography were observed by a JSM 5500 LV electron scanning microscope and an atomic force microscope. The chemical composition of the coatings was analyzed by energy dispersive X-ray (EDX) spectroscopy (Oxford Link ISIS 300). The coated surfaces' advancing contact angles and wettability were evaluated by tensiometric measurements (Kruss 12). The total surface free energy and its polar and dispersion parts were estimated by using Wu and Owens-Wendt-Rabel-Kaeble methods.

The immune cells' adhesive and proliferative activity was evaluated by standard protocols. The study was conducted on the phagocytes of the peritoneal cavity of 15-week old CBA/H mice. The phagocytes cells were cultured with a density of 1×10^7 cells/cm^2 on control and oxide-coated glass Petri dishes in a CO_2 incubator (5 % CO_2) at 37 °C and 95 % relative humidity. The cells' adhesive potential was evaluated after 30 minutes of cultivation on coated/uncoated substrates. After the indicated time, unattached cells were removed by washing the tested substrates twice with Hanks solution (PanEco, Russia). The adherent cells were fixed with methanol for 5 min and counted. The statistical analysis of the test results was performed using the nonparametric Mann-Whitney method. To study the phagocytic activity, a suspension of cells (1×10^7) in 2 ml of medium 199 with 3% ETS was placed in a Petri dish with coated and control samples; 0.9 ml of inactivated staphylococcus (*Staphylococcus aureus*) with 1×10^9 cell/ml dilution were added and incubated in a thermostat at 37 °C for 45 minutes. The dishes were then washed twice with Hanks solution, fixed with methanol, colored, and the percentage of phagocyte cells and the number of staphylococcal particles absorbed by each cell were calculated. 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).

3. Results and Discussion

The volt-ampere characteristics of the magnetron discharge with tantalum target in argon for different values of the reactive gas flow were presented previously [6]. The deposition process carried out with simultaneous bombardment by argon ions resulted in structural changes of the growing films. The coating thickness and cross-sectional structure were obtained by SEM cross-section measurements. The chemical composition of the magnetron-sputtered coatings grown with simultaneous bombardment by argon ions were analyzed by energy dispersive X-ray (EDX) spectroscopy (Oxford Link ISIS 300 (figure 1)

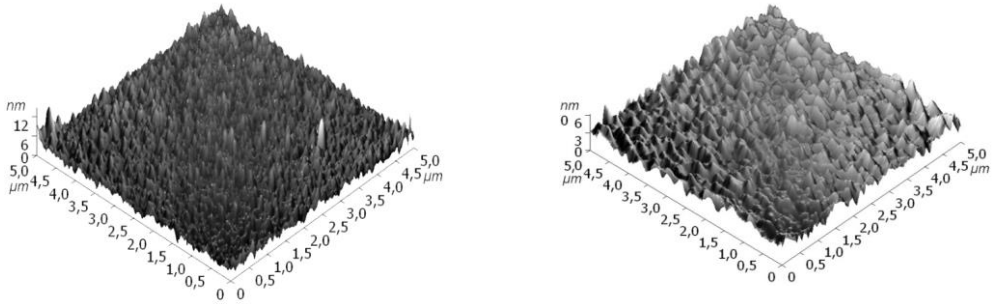


a) **b)**

Figure 1. SEM cross-section image **a)** and EDX spectrum of Ta_2O_5 coatings deposited by simultaneous bombardment with argon ions **b)**.

Bombarding the surface of the growing coatings with high-energy argon ions during the deposition process led to considerable changes in the relief due to sputtering which increases the surface roughness. For comparison, the other coated samples were treated by an electron beam after deposition. The process parameters (electron energy and dose) did not affect significantly the morphology of the deposited coatings, i.e., they were identical with the original ones. A possible effect of this process would be a change in the surface free energy and the surface charge state. These factors have a significant impact on the biological interaction and the cell/material response.

The changes in the ceramic oxide coatings' surface topography and roughness parameters were evaluated by atomic force microscopy (figure 2 a), b).



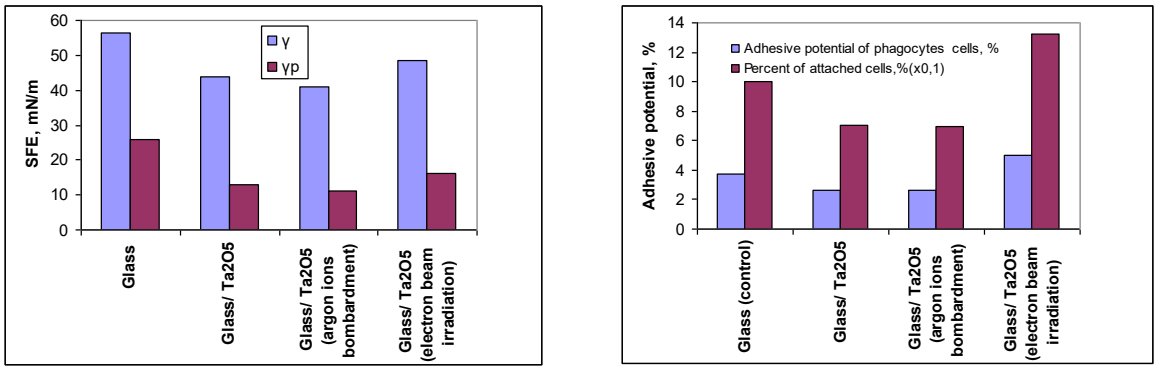
a) **b)**

Figure 2. AFM images **a)** surface topography of Ta_2O_5 coatings, **b)** surface topography of Ta_2O_5 coatings after surface treatment by argon ions.

The ion bombardment resulted in a change of the surface roughness parameters from S_a = 0.9 μm, S_q = 1.2 μm in the case of Ta_2O_5 coatings to S_a = 0.6 μm, S_q = 0.7 μm in the case of Ta_2O_5 coatings deposited with simultaneous bombardment by argon ions. In contrast, the electron-beam post-treatment process did not result in any significant changes in the oxide ceramic coatings' surface and structural parameters. The structure of the electron-beam irradiated coatings was similar to that of the as-deposited coatings – roughness parameters S_a = 0.8 μm, S_q = 1.1 μm

The changes in the structural parameters affected the surface characteristics and functional properties of the deposited coatings. As previously demonstrated and analyzed in [6], the X-ray diffraction profiles of as-deposited Ta_2O_5 coatings prove the amorphous nature for as-deposited magnetron sputtered Ta_2O_5 coatings – no peaks are observed. The Ta 4f lines of the deposited films are in a good agreement with the Ta 4f doublet representative of the Ta-O bond in Ta_2O_5. The Ta/O ratio estimated from the spectra is about 0.4 for all coatings investigated.

The surface free energy (SFE) plays an important role in the mechanism of antigen presenting cell/biomaterial response [7, 8]. The SFE and its polar part were estimated by Owens-Wendt-Rabel-Kaeble method for the following liquid system: α-bromonaphthalene-formamide-ethylene glycol-diidomethane-glycerol-water (figure 3 a) at a temperature of 20 °C. The data demonstrated that the Ta_2O_5 coatings' SFE calculated by means of the quoted method was in the range of 40 – 50 mN/m, with the SFE polar part in the range 11 – 16 mN/m. The SFE minimal values were exhibited by the tantalum pentoxide ceramic coatings deposited with simultaneous bombardment by argon ions. The oxide ceramic coatings' properties shifted to the more hydrophilic region; also, following the electron beam-irradiation post-treatment of the oxide-coated substrates, their SFE increased. The topographic and physico-chemical characteristics of the surface act as factors that modulate the cells' adhesive potential [9, 10]. Figure 3 b) shows the results of studying the adhesive potential of the phagocytes of the peritoneal cavity of CBA/H mice on glass (control) and Ta_2O_5 coatings before and after surface treatment by electrons and argon ions.

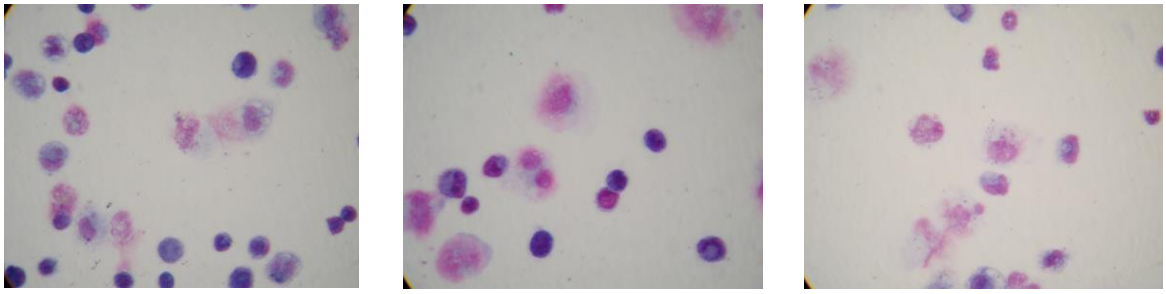


a) **b)**

Figure 3. a) Surface free energy (SFE) (γ and its polar part γ_p; **b)** adhesive potential of the phagocytes cells on glass and on Ta_2O_5-coated substrates.

The Ta_2O_5 coating reduced the adhesive potential of phagocytic cells by 29 % in comparison with the control (glass). The deposition process with simultaneous bombardment by argon ions did not increase the cells' adhesive ability in comparison with the Ta_2O_5 coatings. In contrast, the electron-beam treatment of the Ta_2O_5 coating contributed to a 32.7-% increase in the adherent cells content, in a good agreement with previous results [11].

Further, the phagocytic activity of phagocyte cells was studied. Figure 4 shows phagocytes stained by Romanovsky after a 45-minute exposure to inactivated staphylococcus. In figure 4, the macrophages cells with sizes in the range 15 – 20 μm show a foamy cytoplasm with jagged edges; the lymphocytes with sizes in the range 5 – 10 μm have a compact core; and the staphylococcus particles with sizes in the range 0.6 – 1.0 μm are absorbed by the macrophages cells. The main quantitative results, namely, percentage of phagocytic cells (PI) and number of staphylococcal particles absorbed by each cell (PN) are presented in table 1.



a) **b)** **c)**

Figure 4. a) Phagocyte activity after a 45-minute exposure with inactivated staphylococcus glass (control), **b)** Ta_2O_5 after ion treatment, **c)** Ta_2O_5 after electron irradiation, magnification ×100.

Table 1. Phagocytic activity on glass and Ta_2O_5 coated substrates.

Substrate/coatings	Phagocytic index, %	Phagocytic number, %
Glass (control)	92.0±1.43	8.8±0.52
Glass/ Ta_2O_5	89.4±1.60	8.4±2,41
Glass/ Ta_2O_5 (argon ion bombardment)	77.0±1.35*	4.7±0.38*
Glass/ Ta_2O_5 (electron-beam irradiation)	97.0±2.48*	10.8±0.56*

*Difference statistically significant to control, P <0.05.

The analysis of the data in table 1 shows that the PI and PN of cells cultured on the substrate with a Ta_2O_5 coating did not have statistically significant differences from the control indicators (glass). The Ta_2O_5-coated surface deposited with simultaneous argon ions bombardment reduced both the percentage of phagocytic cells and the number of staphylococcal particles absorbed by each cell in comparison with the control. In contrast, the Ta_2O_5-coated substrate demonstrated a statistically significant increase of the main indicators of phagocytosis after electron-beam irradiation. The effect of material surface modification on several inflammatory events *in vivo* was previously reported in [8, 12]. In the present study, the surface roughness changes after argon ions bombardment did not lead to a significant increase of the adhesive potential and on the activity of phagocytes in *in vitro* tests. Conversely, the changes in the surface free energy and the shift to the more hydrophilic region induced by electron-beam processing had the effect of increasing the cells' adhesive potential and phagocytic activity.

4. Conclusions

The effect was analyzed of surface treatment of Ta_2O_5 nanostructured coatings by argon ions and electron beam on the functional potential of antigen-presenting cells of the monocyte-phagocytic system. It was shown that the electron irradiation post-treatment process leads to a stimulation of the adhesive potential and the phagocytic activity of antigen-presenting cells on the Ta_2O_5 coated surfaces. On the contrary, the surface treatment by argon ions significantly reduces the functional activity of the cells, which can be important in vascular surgery. The changes in the parameters of the surface free energy and its polar part and the shift to the more hydrophilic region after electron-beam irradiation results in an increase of the functional activity of cells of the monocytic-phagocytic system. The modification of biomaterial surfaces with respect to their immune response can pave the way to a new generation of materials with immunomodulatory properties for a wide range of clinical applications.

Acknowledgments

The research was supported by the international scientific cooperation project # 08-03-20 of the National Academy of Sciences of Ukraine and by the program or international scientific cooperation between the Bulgarian Academy of Sciences and the National Academy of Sciences of Ukraine.

References

[1] Rostam H M, Fisher L E, Hook A L, Winkler D A, Alexander M R and Ghaemmaghami A M 2020 *Matter* **2** 1564
[2] Higgins D M, Basaraba R J, Hohnbaum A C, Lee E J, Grainger D W and Gonzalez-Juarrero M 2009 *Am. J. Pathol*. **175** 161
[3] Vishwakarma A, Bhise N S, Evangelista M, Rouwkema J, Dokmeci M R, Ghaemmaghami A M, Vrana N E and Khademhosseini A 2016 *Trends Biotechnol*. **34** 470
[4] MacLauchlan S, Skokos E A, Meznarich N, Zhu D H, Raoof S, Shipley J M, Senior R M, Bornstein P and Kyriakides T R 2009 *J. Leukoc. Biol*. **85** 617
[5] Jenney C R and Anderson J M 2000 *J Biomed Mater.Res*. **49** 435
[6] Zykova A, Safonov V, Goltsev A, Dubrava T, Rossokha I, Donkov N, Yakovin S, Kolesnikov D and Goncharov I 2016 *J. Phys. Conf. Series* **700** 012027
[7] Hallab N J, Bundy K J, O'Connor K, Moses R L and Jacobs J J 2001 *Tissue Eng.* **7** 55
[8] Rostam H M, Singh S, Vrana N E, Alexander M R and Ghaemmaghami A M 2015 *Biomater. Sci.* **3** 424
[9] Anselme K, Linez P, Bigerelle M, Le Maguer D, Le Maguer A, Hardouin P, Hildebrand H F, Alain I and Leroy J M 2000 *Biomaterials* **21** 1567
[10] Diener A, Nebe B, Luthen F, Becker P, Beck U, Neumann H G and Rychly J 2005 *Biomaterials* **26** 383
[11] Yakovin S, Dudin S, Zykova A, Safonov V, Goltcev A, Dubrava T and Rassokha I 2020 *Springer Proceedings in Physic* **244** 151
[12] Werthen M, Sellborn A, Kalltorp M, Elwing H and Thomsen P 2001 *Biomaterials* **8** 827