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Preface

This book covers novel and innovative technologies used for development, modeling, chemical and physical investigation and biomedical (in-vitro and in-vivo) trials of nanomaterials and nanocomposites for medical applications and sensors. Novel method for nanoparticle development and manufacturing highlighted as well as their safety and promising application are under consideration. This book opens a new frontier in metal, metal oxide nanoparticle, hierarchical nanostructures and organic coatings as a sensor for gases, inorganic and organic materials, including biosensors for bacteria and cancer detection. Organic nanoparticle composites for medical application (tissue engineering, tissue replacement, regeneration, etc.) including hydroxyapatite-NPs are under the special focus, including in-vitro and preclinical investigation. Nanoparticle and nanocomposites for antibacterial application are discussed in the present book with a detailed focus on NPs–bacteria interaction and cell toxicity study. Orthopedic and dental implant coatings discussed and detailed described their biological effect and safety.

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Chapter 15
Effect of Surface Modification of Sputtered Ta_2O_5 Magnetron Ceramic Coatings on the Functional Properties of Antigen-Presenting Cells In Vitro Tests

S. Yakovin, S. Dudin, A. Zykova, V. Safonov, A. Goltcev, T. Dubrava, and I. Rassokha

Abstract The effect 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 has been studied. The adhesive potential, indicators of phagocytic and metabolic activity of the studied cells depending on the surface properties of magnetron sputtered tantalum pentoxide coatings were analyzed. Electron irradiation process led to the stimulation of adhesive potential, phagocytic and metabolic activity of cells on the Ta_2O_5 coated surfaces. On the contrary, the surface treatment by argon ions significantly reduced the functional activity of the studied cells.

15.1 Introduction

The biological response to the artificial material is determined by a complex of factors. The important role is played not only by the physico-chemical characteristics of the biomaterial surface, but also by the total cells response on the cell/biomaterial interface [1, 2]. It is known, that first immune response on the implantation was recorded by cells of monocytic-phagocytic system of organism [3–5]. Macrophages play important role in the immune organism reactions on the implanted materials—catheters, stents, femoral and oral implants. Immune response on the artificial implants leads to the postoperative complications, inflammatory processes and the risk of repeated surgery operations. Inflammatory and anti-inflammatory reactions

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can be regulated by biomaterial properties and surface modifications [6, 7]. The aim of present study was to investigate the effect of surface treatment by argon ions and electron beam on the structure and surface properties of tantalum pentoxide (Ta_2O_5) coatings deposited by reactive magnetron sputtering method and further correlation of the surface characteristics with immune cells response.

15.2 Materials and Methods

Magnetron, inductively coupled plasma (ICP) source, and ion source were included in the technological system for synthesis of coatings [8]. The ICP source was designed to create activated flow of reactive gas molecules, as well as flow of low-energy ions and electrons. The ICP source was used to clean the surface of the samples before deposition and, in combination with the magnetron, for reactive deposition of metal oxides and nitrides. The ICP source was located inside the vacuum chamber that allows to choose the optimal ratio between the distances from the magnetron and the plasma source to the samples.

Volt-ampere characteristics of magnetron discharge with tantalum target in argon for different values of reactive gas flow were previously presented [9]. Figure 15.1 shows the dependence of the magnetron discharge voltage and current on the oxygen flow. With the oxygen flow rise, the voltage increases, and the discharge current falls in the case of the tantalum target.

With the oxygen flow increasing, the discharge shifts to the poisoning mode with reduced deposition rate. On the other hand, non-stoichiometric coatings are formed in the case of insufficient oxygen flow. The technological process allows deposition of stoichiometric transparent coatings in the metallic mode of the magnetron target far from the poisoning mode.

The ion source "Radical M" of medium energies (0.5–3 keV) was used to clean and activate the surface of the samples before the coatings deposition, as well as

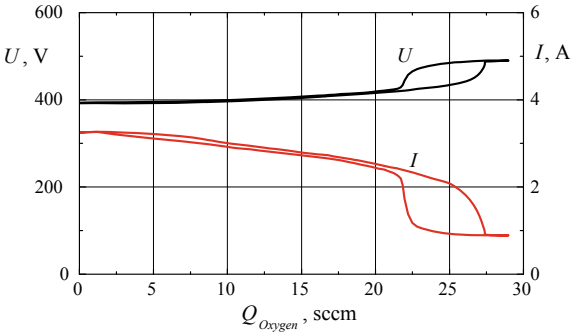

Fig. 15.1 Dependencies of the magnetron discharge voltage and current on the oxygen flow Q_{Oxygen} in the case of the tantalum target

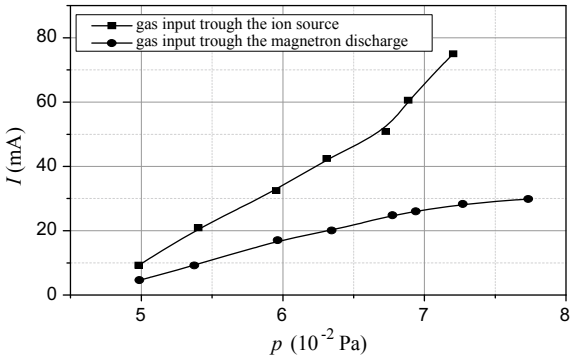

Fig. 15.2 Dependence of the discharge current of the ion source on the gas pressure in the process chamber upon gas input into the magnetron discharge or to the ion source

ion assistance in the process of film synthesis. There is a possibility to change the density, morphology and stoichiometrical composition of the coatings during deposition process by controlling the energy, current density and composition of the ion beam. The influence of gas pressure and flow rate on the current-voltage characteristics was analyzed.

The plots shown in Fig. 15.2 correspond to the technological regime of coatings deposition starting from the minimum gas pressure of 5×10^{-2} Pa for the gas input to the magnetron discharge. As can be seen from the figure, it is possible to control the current density of ions in wide range regulating the amount of gas passing through the ion source or through the magnetron discharge. The allowable range of the current density regulation reaches from 0.1 to 10 mA/cm^2 at a constant average energy of ions.

Ta_2O_5 coatings were deposited on Petri dishes glass substrates in a high vacuum pumping system with a base pressure of about 10^{-4} Pa by ion-assisted magnetron sputtering. Oxygen for the reactive deposition was delivered through the ICP plasma source $Q = 60$ sccm, magnetron voltage was $U = 700$ V, magnetron current was about $I = 5.7$ A.

For other substrates, the deposition process was carried out with simultaneous bombardment of the growing film by argon ions using the ion source. The ion source parameters were as follows: magnetic coil current 1.5 A, ion acceleration voltage 2.5 keV, ion source current 30 mA.

In electron bombardment research the electron beam was created by electron gun of type UL-119. The electrons energy was 20 keV, current density on the sample surface 14 μA/cm^2, the irradiation time 1500 s.

The coating thickness was measured by Calotest. The roughness parameters of the oxide ceramic coatings were evaluated by profilometer Hommel. The surface morphology and topography were observed by scanning electron microscope JSM 5500 LV. The chemical composition of the coatings was analyzed by energy dispersive X-ray (EDX) spectroscopy (Oxford Link ISIS 300). Advancing contact

angles and wettability of the coated surfaces were evaluated by tensiometric measurements (Kruss 12). The total surface free energy, polar and dispersion parts were estimated by Owens-Wendt-Rabel-Kaeble methods.

Adhesive and proliferative activity of immune cells was evaluated by standard protocols. Phagocytes of peritoneal cavity of mice CBA/H line were isolated and cultivated on the coated substrates. The phagocytes cells were cultured with density 1×10^7 cells per dish on the control and oxide coated glass Petri dishes in an CO_2 incubator (5% CO_2) at 37 °C and 95% humidity. Cell adhesive potential was evaluated after 30 min of cultivation on coated/uncoated substrates. After the indicated time, non adhesive cells were removed by washing the tested substrates twice with Hanks solution. Adherent cells were fixed with methanol for 5 min and counted. Visual control of the cell cultures was carried out using a light phase contrast microscope «Primo Star» («Zeiss», Germany) and inverted microscope «Axiovert 40C» («Zeiss», Germany). Statistical analysis of test results was performed using the nonparametric Mann-Whitney U test. The difference was considered statistically significant at $P < 0.05$.

15.3 Results and Discussion

The deposition process carried out with simultaneous bombardment by argon ions was resulted in the structural changes of the growing films.

Also, the changing of surface topography and roughness parameters of the oxide ceramic coatings was evaluated by scanning electron microscopy. The coating thickness and cross-section structure were obtained from the SEM cross-section measurements (Fig. 15.3).

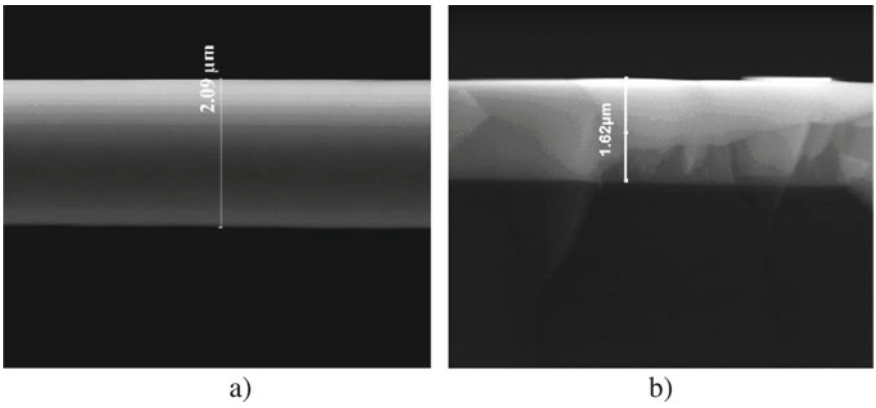

Fig. 15.3 SEM cross-section images of as-deposited oxide ceramic coatings: **a** Ta_2O_5 coatings, **b** Ta_2O_5 coatings deposited with simultaneous bombardment by argon ions

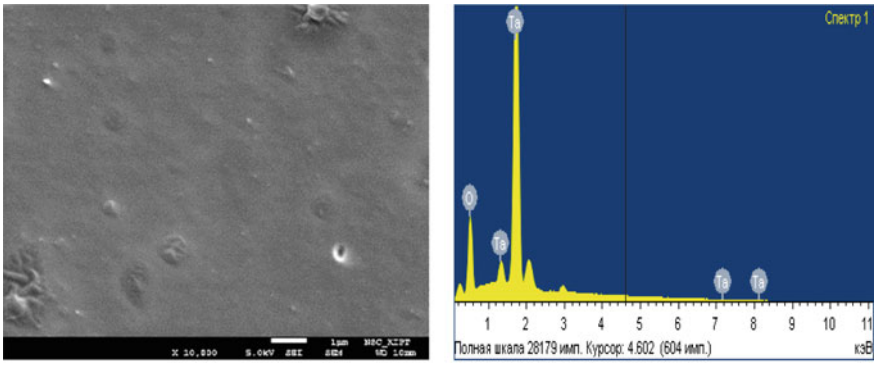

Fig. 15.4 SEM surface images and EDX spectra of Ta_2O_5 coatings: **a** Ta_2O_5 coatings surface, **b** EDX spectra

Scanning electron microscopy images of the magnetron sputtered Ta_2O_5 ceramic coatings have revealed a relatively flat surface with no cracks. EDX spectra demonstrate the stoichiometric composition of magnetron sputtered tantalum pentoxide coatings (Fig. 15.4).

The difference of structure parameters of Ta_2O_5 coatings and Ta_2O_5 coatings deposited with simultaneous bombardment by argon ions was described in [10]. The coatings had a nano-globular structure with characteristic sizes of structural elements of about 15 nm in the case of tantalum pentoxide films without additional treatment and 20 nm with argon ion bombardment of the growing films.

The thickness and roughness parameters of as-deposited coatings, after simultaneous bombardment by argon ions and electron beam post treatment are presented (Table 15.1).

The roughness parameters were principally changed during argon ions bombardment: roughness $Ra = 0.017$ μm, $Rz = 0.240$ μm in the case of Ta_2O_5 coatings, $Ra = 0.035$ μm, $Rz = 0.490$ μm in the case of Ta_2O_5 coatings deposited with simultaneous bombardment by argon ions. In contrast, the post-treatment processing by electron beam created by electron gun was not resulted in the principal changing of surface and structural parameters of oxide ceramic coatings. The structure of electron beam irradiated coatings was similar to as-deposited coatings, roughness parameters

Table 15.1 Thickness and roughness parameters

Substrate/coating	Thickness (μm)	Roughness Ra (μm)	Roughness Rz (μm)
Glass/Ta_2O_5	2.09	0.017	0.240
Glass/Ta_2O_5 (electron beam irradiation)	2.09	0.014	0.210
Glass/Ta_2O_5 (argon ions bombardment)	1.62	0.035	0.490

Table 15.2 The surface free energy SFE and its polar and dispersion parts by Owens-Wendt-Rabel-Kaeble method

Substrate/coating	γ (mN/m)	γ^d (mN/m)	γ^p (mN/m)
Glass	56.28	30.31	25.97
Glass/Ta_2O_5	43.96	31.06	12.90
Glass/Ta_2O_5 (electron beam irradiation)	48.37	32.16	16.21
Glass/Ta_2O_5 (argon ions bombardment)	41.11	30.03	11.08

were $Ra = 0.014$ μm, $Rz = 0.210$ μm close to the case of Ta_2O_5 coatings in good agreement with previous results [9].

Variation of structural parameters may lead to a change of surface characteristics and functional properties of deposited coatings. X-ray diffraction profiles of as-deposited Ta_2O_5coatings demonstrated an amorphous nature for as-deposited magnetron sputtered Ta_2O_5 coatings, no peaks were observed.

XPS survey spectra of the ceramic Ta_2O_5 coatings deposited by magnetron sputtering method were previously analyzed [11]. All spectra consist 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 with two main peaks. 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 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 was about 0.4 for all investigated coatings. The O 1s peaks are centred at binding energies 530.6 eV for the deposited Ta_2O_5 coatings.

The surface free energy (SFE) plays an important role in the mechanism of cell/biomaterial response [12]. The SFE and its polar and dispersion parts were estimated by Owens-Wendt-Rabel-Kaeble method for liquid system: α-bromonaphthalene–formamide–ethylene glycol–diidomethane–glycerol–water (Table 15.2) at temperature 20 °C.

The data demonstrate that the surface free energy of Ta_2O_5 coatings was in the range 40-50 mN/m and SFE polar parts were in the range 11–16 mN/m. Tantalum pentoxide ceramic coatings deposited with simultaneous bombardment by argon ions possess the minimal values of SFE.

The data demonstrate (Table 15.2) that the properties of oxide ceramic coatings shift in the more hydrophilic region and values of the surface free energy increase for the oxide coated substrates after electron beam irradiation post-treatment.

The topographic and physico-chemical characteristics of the surface act as factors that modulate the adhesive potential of cells [13]. The adhesive potential of phagocytes cells of the peritoneal cavity of CBA/H mice depending on the surface properties of tantalum pentoxide coatings was analysed. Table 15.3 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.

Table 15.3 Adhesive potential of the phagocytes cells on glass and Ta_2O_5 coated substrates

Substrate/coatings	Adhesive potential of phagocytes cells, %	Percent of adhesive cells, %
Glass (control)	3.76 ± 0.34	100
Glass/Ta_2O_5	$2.66 \pm 0.20^*$	70.74^*
Glass/Ta_2O_5 (electron beam irradiation)	$4.99 \pm 0.43^*$	132.70^*
Glass/Ta_2O_5 (argon ions bombardment)	$2.61 \pm 0.14^*$	69.41^*

*The difference statistically significant to control, $P < 0.05$

The minimal values of cell adhesive potential in the case of Ta_2O_5 coatings deposited with simultaneous bombardment by argon ions were observed (Table 15.3). Adhesive potential of phagocyte cells on the Ta_2O_5 coated surface after electron irradiation was 30% higher than on the uncoated surface (glass substrates). Thus, the electron irradiation process led to the stimulation of adhesive potential, phagocytic and metabolic activity of cells on the Ta_2O_5 coated surfaces. On the contrary, the surface treatment by argon ions significantly reduced the functional activity of the antigen-presenting cells. The effect of material surface modification on the several inflammatory events in vivo was previously observed [14, 15]. Macrophages preferentially accumulate on rough surface in vitro [16]. A correlation between a decreasing number of interfacial ED1 positive macrophages and increasing surface roughness was found after one week, but not after 6 and 12 weeks. In present study, the surface roughness increasing after argon ions bombardment did not lead to the significant increasing of adhesive potential of phagocyte cells in vitro tests. On the contrary, the changing of surface free energy, polar part parameters, and shift to more hydrophilic region can effect on the increasing of adhesive potential, phagocytic and metabolic activity of cells after electron irradiation process.

15.4 Conclusions

The deposition process carried out with simultaneous bombardment by argon ions and post-treatment by electron irradiation were resulted in structural changes of the growing films. The change of structural parameters leads to changing of coatings surface characteristics.

The roughness parameters were significantly increased in the case of Ta_2O_5 coatings deposited with simultaneous bombardment by argon ions. In contrast, the post-treatment by electron beam was not resulted in the principal change of roughness parameters. The structure of electron beam irradiated coatings was similar to as-deposited Ta_2O_5 coatings.

Tantalum pentoxide ceramic coatings deposited with simultaneous bombardment possess the minimal values of SFE. The Ta_2O_5 coatings demonstrate more hydrophobic properties after surface treatment by argon ions. On the contrary, properties of tantalum pentoxide ceramic coatings shift in the more hydrophilic region and values of the surface free energy increase for the coated substrates after electron beam irradiation post-treatment.

It was shown that the argon ions bombardment during tantalum pentoxide coating deposition process reduces adhesive and proliferative potential of antigen-presenting cells. On the contrary, electron irradiation process led to the stimulation of adhesive potential, being a primary link in providing functional (phagocytic and metabolic) activity of these cells on the Ta_2O_5 coated surfaces. The change of surface free energy, polar part parameters, and shift to more hydrophilic region can lead to the increasing of functional activity of cells of monocytic-phagocytic system after electron irradiation process.

Results demonstrate that surface modification can affect the adhesive potential of immune cells that is important for further clinical applications.

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References

1. T.A. Blaine, J. Bone Jt. Surg. **78-A**(8), 1181 (1996)
2. C.M. Takebe, A. Champagne, A. Diener, B. Nebe, F. Luthen, P. Becker, U. Beck, H.G. Neumann, J. Rychly, Biomaterials **26**(4), 383 (2005)
3. M. Bartneck, K.H. Heffels, Y. Pan, M. Bovi, G. Zwadlo-Klarwasser, J. Groll, Biomaterials **33**, 4136 (2012)
4. C.A. Janeway, R. Medzhitov, Annu. Rev. Immunol. **20**(1), 197 (2002)
5. P. Thomsen, C. Gretzer, Curr. Opin. Solid State Mater. Sci. **5**, 163 (2001)
6. C.R. Jenney, J.M. Anderson, J. Biomed. Mater. Res. **49**, 435 (2000)
7. S. MacLauchlan, E.A. Skokos, N. Meznarich, D.H. Zhu, S. Raoof, J.M. Shipley, R.M. Senior, S. Offenbacher, K. Ishibashi, L.F. Cooper, J. Biomed. Mater. Res. **64 A**(2), 207 (2003)
8. S. Yakovin, S. Dudin, A. Zykov, V. Farenik, Problems At. Sci. Technol. Ser. "Plasma Physics" **71**(1), 152 (2011)
9. A. Zykova, V. Safonov, A. Goltsev, T. Dubrava, I. Rossokha, N. Donkov, S. Yakovin, D. Kolesnikov, I. Goncharov, J. Phys: Conf. Ser. **700**, 012027 (2016)
10. A. Zykova, V. Safonov, A. Goltsev, T. Dubrava, I. Rossokha, J. Smolik, R. Rogovska, S. Yakovin, D. Kolesnikov, I. Sudzhanskaya, I. Goncharov, Surf. Coat. Technol. **301**, 114 (2016)
11. N. Donkov, E. Mateev, A. Zykova, V. Safonov, D. Kolesnikov, I. Goncharov, I. Sudzhanskaya, S. Yakovin, J. Phys: Conf. Ser. **558**, 012036 (2014)
12. N.J. Hallab, K.J. Bundy, K. O'Connor, R.L. Moses, J.J. Jacobs, Tissue Eng. **7**(1), 55 (2001)
13. K. Anselme, P. Linez, M. Bigerelle, D. Le Maguer, A. Le Maguer, P. Hardouin, H.F. Hildebrand, I. Alain, J.M. Leroy, Biomaterials **21**, 1567 (2000)

14. A.S. Shanbhag, J.J. Jacobs, J. Black, J.O. Galante, T.T. Glant, J. Biomed. Mater. Res. **28**, 81 (1994)
15. M. Werthen, A. Sellborn, M. Kalltorp, H. Elwing, P. Thomsen, Biomaterials **8**, 827 (2001)
16. A. Rich, A.K. Harris, J. Cell Sci. **50**, 1 (1981)