Thromboresistant properties of nanostructured tantalum coatings on the stainless steel surfaces

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Abstract. The nanostructured coatings of tantalum and its compounds on the stainless steel surfaces were investigated by atomic force microscopy. Their hydrophilic properties were studied by sessile drop and atomic force spectroscopy methods. The thromboresistant properties of samples were analysed in vitro by incubation of the samples in the rich platelets plasma of patients with acute coronary syndrome. The effect of surface properties on the thromboresistant properties was analysed.

1. Introduction

The development of metal stents is one of the main advances in the interventional cardiology. The requirements for stents are quite strong: high flexibility, plasticity, strength and rigidity, X-ray contrast, and biocompatibility. They should not be destroyed by surrounding tissue, induce tapping of electrolyte balance, toxicity, allergical, carcinogenic and immune reactions and thrombosis, disorders of cellular elements of blood, denaturation of plasma protein and enzymes of blood. For this purpose, the stent surfaces are covered with various types of ceramic coatings to prevent changing their properties. The biogrowth and thrombosis processes can occur on the surface of implants. For modern stents, both polymeric and metallic materials (steel or titanium and chromium alloys) are used. To improve materials performance, their surface is modified by different coatings [1]. One of promising coatings is tantalum, due to its good biocompatibility, mechanical strength, corrosion resistance, and radiopacity [2,3].

Modern methods of research, such as NMR-spectroscopy, scanning microscopy, and others allow receiving the data on the surface properties of materials [1-6]. Atomic force microscopy (AFM) gives the versatile characterization of the surface and at the same time can simultaneously examine and quantify different surface phenomena, such as roughness [7], adhesion [8,9], and morphological features after different treatment [10]. The study of mechanical properties of tantalum films and evaluation of their operational properties is an important task, because the surface of stents is often subjected to chemical and mechanical effects.

The work is aimed at studying the aggregation properties of thrombocytes before and after incubation with nanostructured coatings of tantalum and its compounds on the stainless steel substrates using AFM.
2. Experimental
The coatings were deposited using Direct Current planar magnetron sputtering. Before experiment, steel substrates of the 316 LSS were cleaned in an ultrasonic bath by the standard method and by ion beam of medium energies of ion source in crossed field of the Radical type in argon atmosphere (pressure is \(5 \times 10^{-4}\) Torr, ion acceleration voltage – 3 kW, ion source current – 100 mA) during 5 min. Then stainless steel substrates were put into the camera, pumped to the vacuum less than \(10^{-5}\) Torr, and Ta, Ta\(_2\)O\(_5\), TaN, and TaON coatings were deposited on them. The distance between the magnetron and the sample was near 30 cm. The feature of this system is additional oxygen activation by discharge plasma source induction. Oxygen was puffed through plasma source for activation. For TaN deposition, only nitrogen was puffed without argon. The deposition parameters are presented in Table 1.

The surface microstructure was investigated by AFM method on NT-206 device (Belarus) with CSC 38 cantilevers (Mikromasch, Estonia). The stiffness of cantilevers was 0.03 N/m according to the manufacturer's passport, the radius of curvature was less than 10 nm. The adhesion characteristics were studied on Dimension FastScan (Bruker, USA) in PeakForce QNM regime with CSG10_SS cantilevers (Mikromasch, Estonia) with the curvature radius of 1.14 nm and stiffness of 0.365 N/m. Roughness values were determined by AFM method. The adhesion force was determined on the base of force-distance curves. The surface free energy at the interface between the probe and the sample was calculated by the formula for elastic adhesive contacts of the parabolic profile probe.

The hydrophilic properties were investigated by sessile drop method on the device DSA 100 E (KRUSS, Germany) by three test-liquids: water, glycerol, and diiodomethane. Then the free surface energy was calculated by the Jirifalko-Hood-Fawkes equation [11].

To determine the thromboresistant properties, the samples of steel with tantalum oxynitride coatings were cleaned with ethanol (\(w_{mass} = 96\%)\) and kept in platelet rich plasma during 15 min, obtained from the stabilized sodium citrate venous blood of patients with acute coronary syndrome. Then the samples were cleaned with phosphate buffer from non-adherent cell and dried in air. After that the optical microscopy (MICRO 200, Belarus) and atomic force microscopy (NT-206, Belarus) measurements were performed. The aggregation degree was estimated according to three morphological classes: (a) few activated (spherical and with pseudopodia); (b) highly activated (completely flattened); (c) aggregates. This classification was proposed in the article [12].

Table 1. The parameters of the magnetron sputtering for the coatings of tantalum and its compounds deposited on the steel substrates.

| Type of coating | \(P_A\) (Torr) | \(U_m\) (V) | \(I_m\) (A) | Deposition time (min) | \(Q\) (cm\(^3\)/min) |
|----------------|---------------|-------------|-------------|----------------------|----------------------|
| Ta             | \(8 \times 10^{-4}\) | 495         | 6.6         | 30                   | -                    |
| Ta\(_2\)O\(_5\) | \(8 \times 10^{-4}\) | 500         | 6.4         | 20                   | 25 (O\(_2\))         |
| TaN            | \(9 \times 10^{-4}\) | 800         | 3.4         | 60                   | 95 (N\(_2\))         |
| TaON           | \(11 \times 10^{-4}\) (general) | 620         | 4.0         | 30                   | 55 (O\(_2\))         |

3. Results and discussion
3.1. Microstructure and properties of tantalum derivatives coatings on the steel surface
Study of coatings structure of the tantalum and its compounds on the stainless steel 316 LSS by AFM showed that the polished steel surface had a cellular structure [13]. Cells were the surface areas rising above the main matrix. These parts might be phases with dopants, being harder than the rest matrix. Most of cells (90% of the total amount) have a diameter of 117 nm; the size of other formation is 61 nm. The AFM image of the initial steel and steel with the oxynitride tantalum coating is presented in Figure 1.
After the deposition of tantalum coating on the steel surface a cellular structure preserved (Fig. 1b), the values of Ra and Rq in 4x4 μm² scan increased, and in 7x7 μm² scan decreased, which indicates the increase in surface uniformity. This microstructure is rather typical for PVD coatings on the polished substrates [14]. The average cell size was 86.5 nm. The cellular surface of the tantalum coatings had more pronounced edges that bound the cells in comparison with the initial microstructure of steel. They formed even on the smallest cells and their height increased. Thereby, they played the role of crystallization centres, on which the tantalum atoms and clusters were initially fixed during magnetron sputtering. In the structure of tantalum oxide, the cells were not detected; the nature of the surface changed from a cellular structure to an islet. The height of the islands was 5.5 nm, and the size was from 100 to 500 nm. For the tantalum oxide coatings with the increase in the scanning area (Fig. 1c), the surface roughness Ra did not change, indicating the uniformity and equability of the applied coating.

For TaN (Fig. 1d) and TaON (Fig. 1e) coatings deposited onto the steel, the cellular structure also preserved. It should be noted that the presented protruding grains demonstrate predominant growth on the cells ledge. The cellular microstructure of TaON is significantly different from TaN. In this case, the cells were almost 2 times larger than those before deposition on the substrate. Rq values increased up to 11.4 in the case of TaN and up to 11.9 nm for TaON. The cells diameter for TaN was 96.3 nm and for TaON was 178.5 nm. The roughness values after tantalum coating deposition are presented in Table 2.

Table 2. The roughness values (Ra, Rq) of tantalum coatings on steel surface.

| Type of sample | Ra, nm | Rq, nm |
|---------------|--------|--------|
|               | 4 x 4  | 7 x 7  | 4 x 4  | 7 x 7  |
| Steel         | 3.8    | 7.4    | 4.8    | 9.0    |
| Ta            | 4.9    | 6.2    | 6.2    | 8.3    |
| Ta₂O₅         | 4.2    | 4.4    | 5.4    | 7.6    |
| TaN           | 9.5    | 9.4    | 11.8   | 11.9   |
| TaON          | 5.6    | 8.7    | 6.9    | 11.4   |
After the deposition of nanostructured tantalum coatings on the steel surface, as was shown in the article [15], all types of films, to a greater extent tantalum oxynitride, increased the hydrophobic properties of the surface, the contact angle increased from 75 to 94°. The tantalum and tantalum nitride coatings had the same hydrophilic surface properties, since they were characterized by the contact angle of 85°, and by the specific surface energies of 0.1 mJ/m², which was two times less than those for tantalum oxynitride coatings. The polar component of tantalum oxides surfaces was significantly reduced, and according to the data determined by two methods, they were characterized by pronounced hydrophobic properties.

3.2. Thromboresistant properties of the nanostructured tantalum and its derivatives coatings

It was shown, that on all of the researched samples platelets were adhered and aggregated to a greater or lesser extent, while their morphological characteristics significantly changed as compared with the initial state.

After incubating the samples of initial steel in platelet plasma, a large number of aggregates formed on the steel samples (Figs. 2a, 3a), while the diameter of an individual cell was 600-800 nm, which indicated about the exit of granules and the formation of clusters in the form of aggregates. From 2 to 5 platelets formed aggregates. We consider that process provided on the steel surface can be classified as the third class of platelet aggregation, described above.

According to the data of optical microscopy and AFM, a large number of pseudopodia are established on Ta coating. According to the AFM data, the granules with a diameter of 0.5 μm are presented; individual cells are spread over the surface of the samples. A large number of pseudopodia form a dense network (Figs. 2b, 3b). The thickness of the lamellas in the cross section is 400 nm. A reversible aggregation process is observed (class 2). These processes are due to hydrophilic properties of tantalum coating surfaces. The tantalum and TaN coatings (Figs. 2d, 3d) are characterized by similar properties. On the nanostructured surface of tantalum nitride, the degree of platelet aggregation is weak. In this case, platelets are stuck on the protrusions surface. According to the Table 2, the highest values of Rq = 11.6 nm were obtained for tantalum nitride. Platelets are unevenly adhered on the surface. As a rule, they are observed in hollows. The platelets behaviour can be classified as class 1.

Figure 2. Optical images of steel surfaces with different coatings after the platelets aggregation: (a) initial steel; (b) tantalum; (c) tantalum oxide; (d) tantalum nitride; (e) tantalum oxynitride.
In the same way, the individual platelets sank on the surface areas with a high roughness on the oxynitride coating surface (Figs. 2e, 3e). But for this type of surface the dispersion component of free surface energy significantly increases, which leads to the aggregation incremented. The number of aggregates is less compared to the tantalum coating. A dense network of pseudopodia is established. According to the classification, oxynitride coating surface can be classified as the second group.

On the surface of nanostructured tantalum oxide films, the adherent platelets are unevenly distributed, the platelets are weakly activated, spherical, and the number of pseudopodia is minimal (class 1). A large number of aggregates on these samples are not identified (Fig. 2c, 3c).

4. Conclusion
Thus, the type, nature of the material, and its hydrophilic properties are defined the thromboresistant properties of coatings. On the surfaces with hydrophilic properties (stainless steel), irreversible aggregation is provided. An increase in the hydrophobicity of the surface (tantalum nitride) should to increase thrombotic resistance, but additional adsorption of blood plasma proteins on the surface of the material is possible.

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References
[1] Papirov I I, Shkuropatenko V A, Shkurov V S and Pikalov A I 2010 Medical Stent Materials (Kharkov: NSC KIPT) p 40
[2] Sidorenko E S 2005 Bull. RUDN Univ. 1 109-11
[3] Orlovsky P I, Gritsenko V V, Yukhnev A D, Evdokimov S V and Gavrilov V I 2007 Artificial heart valves (Moscow) p 448
[4] Volova T G, Volova T G, Shishatskaya E I and Mironov P V 2009 Materials for medicine, cell and tissue engineering (Krasnoyarsk: IPK SFU) p 116

[5] Sevastyanova V I 1999 Biocompatibility (Moscow) p 368

[6] Donkov N, Walkowicz J, Zavaleev V, Zykova A, Safonov V, Dudin S and Yakovin S 2018 Phys.: Conf. Ser. 992 012034

[7] Kuznetsova T A, Zubar T I, Lapitskaya V A, Sudzilouskaya K A, Chizhik S A, Didenko A L, Svetlichny V M, Vylegzhanyina M E, Kudryavtsev V V and Sukhanova T E 2017 IOP Conf. Ser.: Mater. Sci. Eng. 256 012022

[8] Kuznetsova T A, Lapitskaya V A, Chizhik S A, Uglov V V, Shymanski V I and Kvasov N T 2018 IOP Conf. Ser.: Mater. Sci. Eng. 443 012018

[9] Lapitskaya V A, Kuznetsova T A, Chizhik S A, Sudzilouskaya K A, Kotov D A, Nikitiuk S A and Zaparozhchanka Y V 2018 IOP Conf. Ser.: Mater. Sci. Eng. 443 012019

[10] Lapitskaya V A, Kuznetsova T A, Chizhik S A and Rogachev A A 2018 IOP Conf. Ser.: Mater. Sci. Eng. 443 012020

[11] Kloubek J 1992 Adv. Colloid Interface Sci. 38 99-142

[12] German E A, Younes R A, Khudoshin A K, Gabrielyan N I and Sevostyanov V I 2012 Bull. Transplantol. Artif. Org. 1 66-71

[13] Melnikova G B, Petrovskaya A S, Kuznetsova T A, Chizhik S A, Zykova A, Safonov V and Yakovin S 2019 Int. J. Nanosci. 18 1940078

[14] Warcholinski B, Kuznetsova T A, Gilewicz A, Zubar T I, Lapitskaya V A, Chizhik S A, Komarov A I, Komarova V I, Kuprin A S, Ovcharenko V D and Goltvyantysya V S 2018 J. Mater. Eng. Perf. 27 3940-50

[15] Petrovskaya A S, Lapitskaya V A, Melnikova G B, Kuznetsova T A, Chizhik S A, Zykova A V and Safonov V I 2019 J. Phys.: Conf. Ser. 1281 012061