

## The study of platelet reaction on a-C:H:SiO<sub>x</sub> coatings obtained via plasma enhanced chemical vapor deposition with bipolar bias voltage

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### ABSTRACT

**Aim.** To study platelet adhesion to a-C:H:SiO<sub>x</sub> film on titanium in an *in vitro* experiment to evaluate its antithrombogenic potential.

**Materials and methods.** Thin (less than 1 μm) a-C:H:SiO<sub>x</sub> films were deposited on VT-6 titanium plates with a size of 10 × 10 mm<sup>2</sup> and a thickness of 0.2 mm using a vacuum ion-plasma unit using pulsed bipolar bias. The surface roughness was evaluated according to GOST 2789-73 using an atomic force microscope. The test samples were cultured at 37 °C for 30 min in platelet-rich human blood plasma, prepared for scanning electron microscopy, after which the distribution density of blood plates adhering to the test coating was calculated.

**Results.** With the same roughness index of the studied a-C:H:SiO<sub>x</sub> samples, the film decreased 116 times (in comparison with untreated titanium) the platelet count per 1 mm<sup>2</sup> of the surface.

**Conclusion.** The deposition of a-C:H:SiO<sub>x</sub> thin film on the surface of VT-6 titanium alloy by PACVD method using pulsed bipolar bias significantly reduces the distribution density of platelets in comparison with an untreated metal surface. *In vitro* data suggest a significant antithrombogenic potential of this type of coating on the surface of devices in contact with blood.

**Key words:** human platelet adhesion, *in vitro*, carbonic surface modified by silicon oxides, scanning electron microscopy, atomic force microscopy.

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## Исследование реакции тромбоцитов на а-С:Н:SiO<sub>x</sub> покрытие, полученное методом плазмохимического осаждения с использованием импульсного биполярного смещения

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### РЕЗЮМЕ

**Цель.** Изучить в эксперименте *in vitro* адгезию тромбоцитов к а-С:Н:SiO<sub>x</sub> пленке на титане для оценки ее атромбогенного потенциала.

**Материалы и методы.** Тонкие (менее 1 мкм) а-С:Н:SiO<sub>x</sub> пленки наносили на титановые пластины марки ВТ-6 размером 10 × 10 мм<sup>2</sup> и толщиной 0,2 мм с помощью вакуумной ионно-плазменной установки с использованием импульсного биполярного смещения. Шероховатость поверхности оценивали согласно ГОСТ 2789-73 с помощью атомно-силового микроскопа. Исследуемые образцы культивировали при 37 °С в течение 30 мин в плазме крови человека, обогащенной тромбоцитами, подготавливали для сканирующей электронной микроскопии, после чего подсчитывали плотность распределения кровяных пластинок, адгезирующих к исследуемому покрытию.

**Результаты.** При одинаковом индексе шероховатости исследуемых образцов а-С:Н:SiO<sub>x</sub> пленка в 116 раз снижала (в сравнении с необработанным титаном) количество тромбоцитов на 1 мм<sup>2</sup> поверхности.

**Заключение.** Формирование на поверхности титанового сплава ВТ-6 тонкой пленки состава а-С:Н:SiO<sub>x</sub> методом плазмохимического осаждения с использованием импульсного биполярного смещения значительно снижает плотность распределения тромбоцитов в сравнении с необработанной металлической поверхностью. Полученные *in vitro* данные предполагают существенный атромбогенный потенциал данного вида покрытий на поверхности устройств, контактирующих с кровью.

**Ключевые слова:** адгезия тромбоцитов человека, *in vitro*, углеродная поверхность, модифицированная оксидами кремния, сканирующая электронная микроскопия, атомно-силовая микроскопия.

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## INTRODUCTION

The interaction of implants with the biological environment of the body largely depends on their surface properties, which play a direct role in various post-implantation biological reactions, including the precipitation of various minerals, protein adsorption, cell adhesion and proliferation [1, 2]. In the application to devices (mechanical pumps) and stents for surgical treatment of coronary heart disease, redundancy of inflammatory cell-molecular reactions at the artificial surface/tissue interface increases the risk of thrombus. In this regard, there is renewed interest in surface modification methods for biocompatible artificial materials contributing to their bioinertness [3].

One widely discussed solution to the problem of deterministic biocompatibility in the bioinertness/bioactivity range is the application of thin diamond-like carbon coatings (diamond-like coating, DLC). Since the beginning of the 2000s, it has been shown that DLC films are bioinert, resistant to mechanical stress and corrosion, and not cytotoxic to monocytes/macrophages, fibroblasts, and osteoblasts [4]. Owing to the optimal ratio of sp<sup>3</sup>/sp<sup>2</sup> hybridized carbon atoms, they have good hemocompatibility [5, 6]. In the last 5 years, due to some dissatisfaction with the results of biomedical testing of DLC coatings, publications have been accumulating on their physicochemical modification (in particular, silicon and its oxides), which improves the consumer properties of a-C:H:SiO<sub>x</sub> surfaces on medical materials and products [7].

On the basis of the Institute of High Current Electronics of the Siberian Branch of the Russian Academy of Sciences (IHCE SB RAS), a new approach has been developed for plasma enhanced chemical vapor deposition of a-C:H:SiO<sub>x</sub> films on the internal surfaces and moving parts of auxiliary circulatory devices, based on the use of pulsed bipolar substrate bias.

The aim of the work was to study in an *in vitro* experiment the adhesion of platelets to a-C:H:SiO<sub>x</sub> film on titanium to evaluate its antithrombogenic potential.

## MATERIALS AND METHODS

VT-6 titanium plates with a size of 10 × 10 mm and a thickness of 0.2 mm coated with a thin a-C:H:-

SiO<sub>x</sub> film (less than 1 μm thick) were used for the research (five «T2» samples). Titanium samples without a-C:H:SiO<sub>x</sub> coating were used as control samples (five «T1» samples). The film was deposited on a vacuum ion-plasma installation with the technological deposition parameters described in Grenadyorov [8].

The standard deviation of the  $R_q$  profile according to GOST 25142-82 was determined according to GOST 2789-73 using an atomic force microscope (AFM) Solver P47 (NT-MDT, Russia) with an area of 5 square micrometers.

To perform the platelet adhesion test from the blood of a healthy adult male donor (intended for blood transfusion), 50 ml of platelet-rich plasma was obtained by centrifugation and separation of blood cells [9, 10].

The resulting plasma was diluted with 0.9% sodium chloride in a ratio of 1:1. The tested samples were immersed in the platelet suspension and incubated at 37 °C for 30 minutes. Then, the samples were washed with distilled water to remove nonadherent cells. The platelets remained on the surface were fixed with a 2% glutaraldehyde solution at room temperature for 1 hour and dried at 37 °C.

The samples were coated with a 20 nm thick chromium layer in an argon atmosphere at an ion current of 6 mA and a pressure of 0.1 mm Hg using the Q150T ES setup (Quorum Technologies, UK) and were subjected to scanning electron microscopy using a Mira3 microscope (Tescan, Czech Republic). On each sample, the number of adherent platelets in 20 random fields of view was calculated according to the principles of morphometry [11].

Statistical processing was performed using the software Statistica10.0 software (StatSoft, USA). The normality of the distribution was checked using the Shapiro-Wilk criterion with subsequent assessment of the equality of variances according to the Leven criterion. In the case when the distribution in the experimental groups was normal, and the intergroup equality of variances was observed, further processing was carried out using the method of parametric statistics, the Newman-Coles test. For a distribution other than normal and non-compliance of the intergroup equality of variances, the methods of nonparametric statistics

were used via the Kruskal-Wallis test. Results are presented as mean ( $M$ ) and standard error of the mean ( $m$ ). Differences between groups were considered significant at  $p < 0.05$ .

## RESULTS

The results of atomic force microscopy showed a certain smoothing of the surface roughness of the VT-6 titanium alloy after the formation of the a-C:H:SiO<sub>x</sub> film (Fig. 1). However, differences in the roughness index  $R_q$  did not reach statistical differences (Table 1). The data obtained correspond to the results published previously [12].

In the biological part of the study, the use of whole blood plasma enriched with platelets led to the formation of their microconglomerates and crystallization of dissolved salts on the surface of the samples (Fig. 2), which made it difficult to count the number of individual cells. Dilution of plasma with an isotonic solution of sodium chloride in a ratio of 1 : 1, followed by washing the samples with a solution of distilled water, allowed us to obtain images available for morphometric analysis (Fig. 3).

Platelet counts showed that a-C:H:SiO<sub>x</sub> coating on a titanium substrate dramatically reduced their surface adhesion (Table 1).

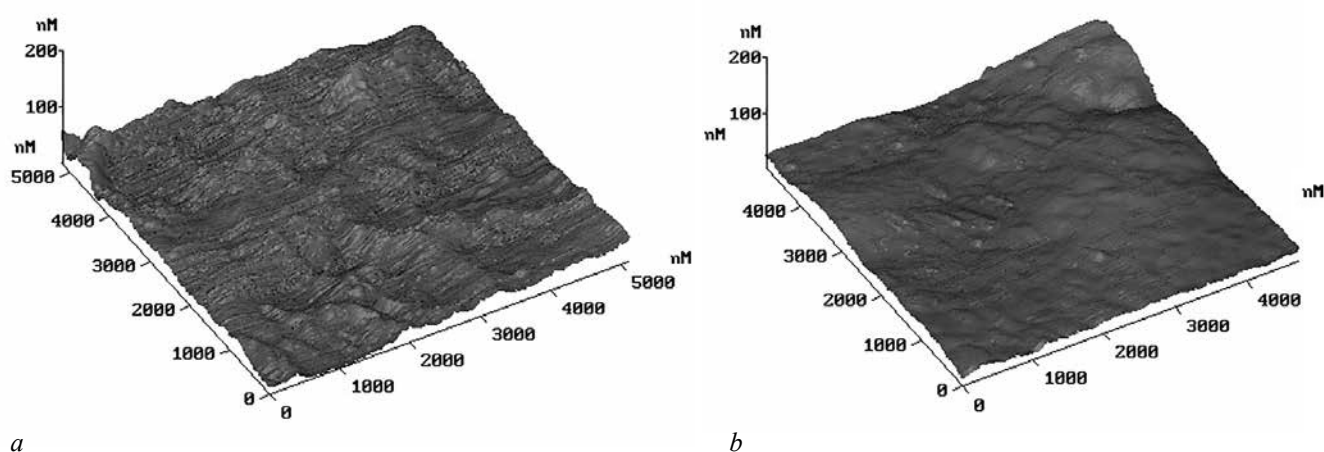


Fig. 1. AFM images of the surface morphology of titanium (a) and titanium coated with a-C:H:SiO<sub>x</sub> film (b)

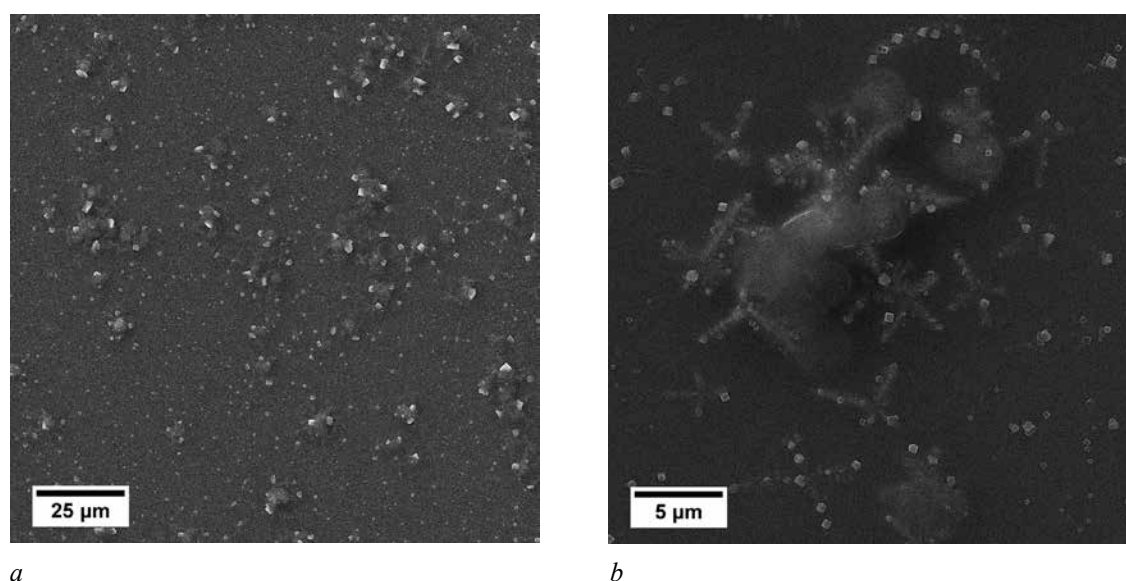


Fig. 2. SEM image of platelet conglomerates and salt crystals on the surface of a VT-6 titanium sample. Scale bar 25 and 5  $\mu\text{m}$

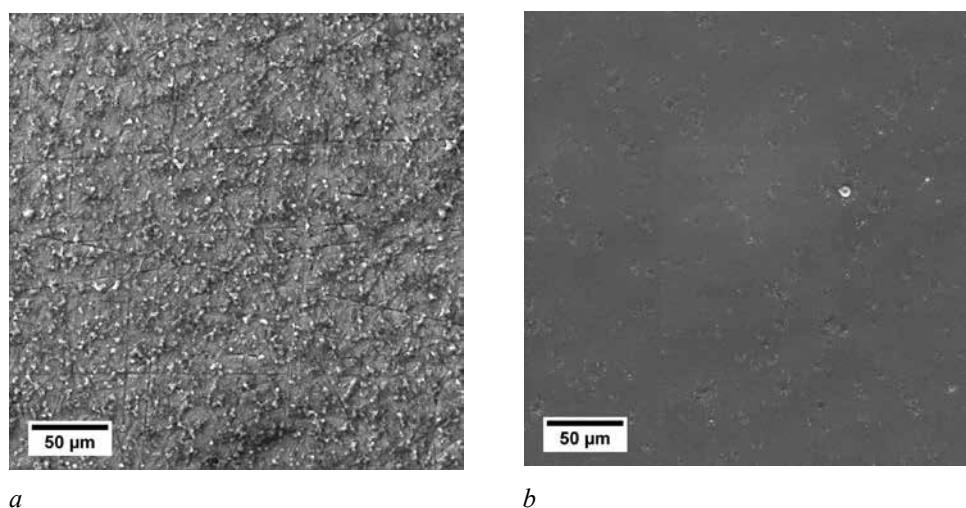


Fig. 3. SEM images of the surface of a titanium sample T1 (a) and T2 with deposited a-C:H:SiO<sub>x</sub> film (b) after a platelet adhesion test. Scale bar 50 μm

Table 1

The content of adherent platelets in the studied samples according to scanning electron microscopy, $M \pm m$			
Group marking	Group name, $n = 5$	The average number of cells per surface area 250 μm <sup>2</sup>	The surface roughness index of the samples, $R^a$ , nm
T1	VT-6 titanium without film deposition	$290 \pm 72$ $n_1 = 20$	$7.9 \pm 0.8$
T2	VT-6 titanium with a-C:H:SiO <sub>x</sub> film deposition	$2.5 \pm 1.8$ $n_1 = 20$ $<0.001$	$7.2 \pm 0.7$

Note:  $n$  is the number of samples studied in each group;  $n_1$  is the number of studied fields of view on each sample.

In the T2 group (coated titanium), the number of blood platelets was 116 times less than that on the surface of the samples from the T1 group (without plasma-chemical treatment). It should be emphasized that there are no statistically significant differences in the roughness index of the studied surfaces (Table 1), since the relief of implants and other medical devices has significant biological significance.

## DISCUSSION

Studies of DLC coatings modified with silicon and its oxides have focused mainly on the study of their physicochemical properties [13]. Thus, the formation of Si–C bonds significantly increases the adhesion of the coating to substrates while maintaining the high tribological characteristics of DLC films [12]. At the same time, the physicochemical processes of improving the hemocompatibility of materials due to both DLC [14] and Si-DLC films remain in the hypothesis area. Due to the identical and insignificant roughness of the studied samples (Table 1), required for products in contact with blood, from the

whole variety of biologically active physicochemical factors (surface energy, phase and elemental composition, solubility, presence of biologically active (medicinal) molecules in the composition of the surface) [15] the charge (zeta ( $\xi$ ) potential) of the surface can come to the fore in determining hemocompatibility. Sawyer et al. [16] suggested that the anticoagulant properties of implantable materials can give an electrostatic charge to their surface. Ikada et al. [17] hypothesized that in biological fluids there is a relationship between the surface  $\xi$  potential and the anticoagulant properties of the surface of medical devices. Indeed, the introduction of silicon into the composition of thin films significantly changes their electrical and biological characteristics [18].

In this regard, the *in vitro* established antithrombogenicity of a-C:H:SiO<sub>x</sub> film on titanium is a valuable consumer property for devices and products contacted with blood, and requires further study of the electrokinetic and other physicochemical characteristics of its biological inertness.



## CONCLUSION

The formation on the surface of the VT-6 titanium alloy of a thin a-C:H:SiO<sub>x</sub> film obtained by plasma-chemical deposition using pulsed bipolar displacement of the substrate reduces the distribution density of human platelets by more than 100 times in comparison with an untreated metal surface. *In vitro* data suggest a significant antithrombogenic potential of a-C:H:SiO<sub>x</sub> coatings on the surface of blood contact devices.

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## Authors contribution

Zhulkov M.O. – carrying out of the research, processing of the obtained data, drafting of the article.  
 Grenadyorov A.S. – deposition of coatings, carrying out of the research, drafting of the article.  
 Korneev D.S. – carrying out of the research, processing of the obtained data.  
 Agaeva H.A. – carrying out of the research, drafting of the article.  
 Chernyavsky A.M. – analysis of the obtained data, editing of the article.  
 Khlusov I.A. – analysis of the obtained data, editing of the article.

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