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Physical properties and haemocompatibility of passive-carbon layer

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Properties

ABSTRACT

Purpose: In the paper physical (surface topography, electrical properties) and antithrombogenic properties of the passive-carbon layer used for enhancing the surface properties of vascular stents made of Cr-Ni-Mo steel have been investigated.

Design/methodology/approach: To characterize the electrical properties of carbon layer the silicon plate was used. The resistivity ρ and relative permittivity of the layer ε_r have been determined on the basis of current-voltage and capacitance-voltage characteristics. In vitro tests of biotolerance evaluation of the passive-carbon layer in blood environment have been carried out in the haemolysis tests (in the direct contact and from the extract) and in the blood clotting tests.

Findings: The results of investigations have shown that deposition process of the passive-carbon layer of dielectric properties on the surface of implants made of Cr-Ni-Mo steel and used in interventional cardiology is an effective way of limiting the reactivity of their surface in blood environment and the blood clotting process in consequence.

Research limitations/implications: Usefulness of the passive-carbon layer for interventional cardiology applications should be verified in in vivo tests.

Originality/value: Modification of physical properties of surface of the metallic biomaterials applied in cardiovascular system by deposition of the passive-carbon layer which has dielectric properties limits the blood clotting process.

Keywords: Electrical properties, Biomaterials, Surface treatment

1. Introduction

Implanting coronary stents is one of the innovative method in treatment of ischemic heart disease. Stents are placed in the narrowed segment of coronary vessel with the use of minimally-invasive techniques (interventional cardiology). It cause enlarging of active cross-section of coronary vessel and permanent setting of the stent in the endothelium tissue of vessel [4-6,10,14, 16-21,23,24,28,30,31].

The process of interaction between blood and surface of implanted stents is not still fully recognized. Problem of haemostasis connected with blood circulatory arrest is one of the negative phenomenon occurring after stent implantation to vessel system. A consequence of blood contact with an artificial implant's surface is protein adsorption, mainly fibrinogen. If the adsorbed fibrinogen undergoes the process of denaturisation the stimulating of activity of the following platelets and plasma blood clotting take place in cascade way. The result of this process is the blood clot forming [3,7,11,13,16,23,26,27,32].

Roughness of the implants surface is one of the main factors influencing the clotting process and reactions of tissues surrounding the implant. Sheth et al. determined in *in vivo* investigations (in pig cardiovascular system) the effects of polishing process of Ni-Ti implants as well Palmaz-Schatz stents made of Cr-Ni-Mo steel. They have stated high reduction in tendency to blood clotting on polished implants surfaces comparing to unpolished ones. Similar investigations on rats and pigs have been conducted by de Scheerder. He obtained significant decrease in occurrence of early thrombosis and reduction in restenosis process when polished stents of Cr-Ni-Mo steel have been implanted [1,25,33]. Results of these investigations are pointing out on the meaningful influence of topography of implants surface on the early and late reactions of vessels that have been stented. It can be stated that assurance the smooth surface of stents made of metallic biomaterials is the fundamental stage in forming of their utility properties. Considering miniaturization of these implants the appropriate smoothness can be achieved by electrochemical treatment. In technology of electrolytic polishing of stents not only the obtained roughness of the surface should be considered. The main factor determining the way of realization of this treatment is the shape of semi-finished product used for stents forming (thin-walled tube, wire). The process of electrochemical treatment should assure the constancy of the geometric features of stent on its whole length. It influences significantly the biomechanical characteristics of stents.

Recently have been published the data explaining the matters of initiation of clotting process which were related to the banding model of solid state body [2,8]. Basing on Gutmann investigations it has been stated that fibrinogen has an electronic structure similar to that of a semiconducting materials. Its energy gap is 1,8 eV. The valence and conduction bands are 0,9 eV below and above the Fermi level, respectively. So the transferring process of protein from its inactive form (fibrinogen) to active (fibrin) could be connected with electrochemical reaction occurring between protein and material surface being in contact with blood. The electrons which are moved from the valence band of fibrinogen to implants material cause the decomposition of the protein. In the consequence the protein undergo transformation into fibrinmonomer and fibrinpeptides. The cross-linking process leads further to forming the irreversible thrombus.

From the analysis conducted above is resulting the advisability of physical properties modification of the surface of implantation materials. The surface treatment by forming the layer of high corrosion resistance and semiconducting or dielectric properties of the surface of implants applied in cardiovascular system can effectively reduce the transferring of electrons from fibrinogen valence band. Such layer can be effective in limiting the process of blood clotting occurring in a consequence of the contact with the surface of implanted stent.

2. Methods

The evaluation of antithrombogenic properties of the passive-carbon layer formed on the surface of coronary stents made of the Cr-Ni-Mo steel has been performed in the work. The layer was deposited in a multi-stage process. The process consisted of electropolishing, chemical passivation and forming of the carbon layer by RF PACVD method [16,21-23]. The usefulness of the proposed layer enhancing the quality of implants surface applied in interventional cardiology has been evaluated on the basis of surface topography investigations as well electric properties and blood interaction tests.

2.1. Surface topography investigations

Taking into consideration the requirements concerning the surface smoothness of coronary stents, in the work the investigations of topography of the passive and the passive-carbon layer have been performed with the use of atomic force microscope (AFM) type Nanscope E, Digital Instruments. It is possible to obtain in a single measurement the high resolution image of specimen surface, from the area of 16 x 16 µm. Investigations were carried out on the specimens in the form of sections of Cr-Ni-Mo steel wire, which is used to forming the stents of “coil” type [16, 21-23]. Observations were performed in 10 different places of each specimen.

2.2. Electrical properties investigations

To perform the electrical properties investigations the carbon layer has been deposited on the silicon plate of resistivityρ= 0,005- 0,02 Ωcm. On the basis of the measurements conducted by elypsometric method it has been stated that its thickness was 248 nm. In the next stage the process of vaporization of aluminium contacts of diameter d = 1 mm on the silicon plate surface has been performed. The structure of condensers formed in that way made possible to determine the current-voltage and capacitance-voltage characteristics – Fig. 1.

The current-voltage characteristic was determined with the use of measurement set which is shown on Fig. 2. The resistivity of the layer was determined from the relationship (1) basing on the current-voltage characteristic (I-U):

$$\rho_{U=const} = \frac{U \cdot S}{I \cdot X} \tag{1}$$

where:
U – voltage, V,
S – area of the aluminium contact, mm^2,
I – current intensity, A,
X – layer thickness, mm.

The measurement of capacitance-voltage characteristics (C-U) has been realized by high frequency method with the use of the measurement set presented in Fig. 3. HP 4061 device enabled to perform the capacity measurements. Measurements were realized at frequency of 1 MHz, both in direction from inversion to accumulation and from accumulation to inversion. On that basis relative permittivity of the layer ε_r has been determined according to the following relationship:

$$\varepsilon_r = \frac{C_{\max} \cdot X}{\varepsilon_o \cdot S} \tag{2}$$

where:
C_{max} – maximum condenser capacity, F,
X –layer thickness, nm,
ε_o – permittivity of free space (8,85 · 10^{-12} F/m),
S – area of the aluminium contact, mm^2.

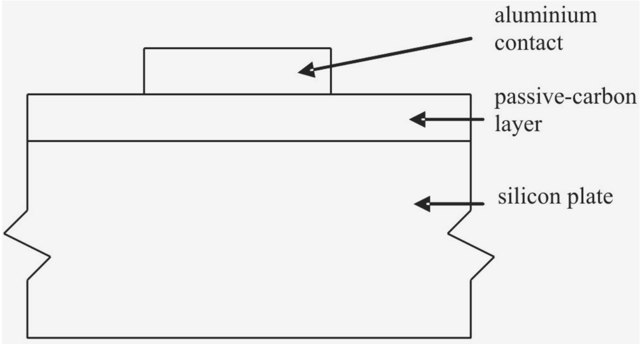

Fig. 1. Section of the tested MIS structure (metal – insulator – semiconductor)

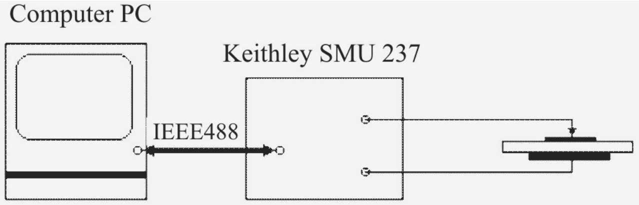

Fig. 2. Scheme of the measuring system of the current-voltage characteristics

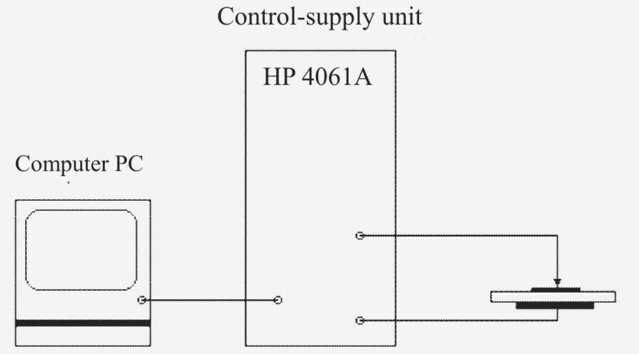

Fig. 3. Scheme of the measuring system of the capacitance-voltage characteristics

2.3. Investigations of interaction with blood

Investigations of biomaterial interaction with blood have been performed in *in vitro* conditions according to the PN-ISO 10993 standard [1].Such tests are required for implants exposed to blood environment longer then 30 days. These investigations have been performed for the specimens of Cr-Ni-Mo steel of electrolytically polished and passivated surfaces, and electrolytically polished, passivated and coated with carbon layer ones.

In the presented work haemolysis and blood clothing tests were conducted. The specimens used for haemolysis investigations were prepared in shape of cylinder with the diameter of d = 10 mm and height of h = 30 mm (required minimal mass of the specimen was 15 g). For blood clothing tests the specimen in cylinder form of diameter of d = 10 mm and height of h = 25 mm (required minimal total area of the specimen was 8 cm^2) were used.

Investigation of interactions of specimens with blood in *in vitro* tests was carried out basing on the haemolysis test in the direct contact and from the extract, as well as on the coagulation test, according to recommendations of the ISO 10993-4 standard [2]. The haemolysis test in the direct contact consisted in determining the haemoglobin level in blood plasma, reflecting the extent of the erythrocytes' cell membrane damage in contact with the examined specimens. The specimens were placed in test-tubes to which the citrated blood was added. The test-tubes were incubated for 60 min at the temperature of 37±0,5^{o}C in the Heraeus B20 thermostat. After incubation the blood was removed to the next test-tube and centrifugalized for 10 min at the 500xG rate. Centrifugation separates the cells that are free of damage and the cellular lysis. Lysated cells were left in the solution at the bottom of the test-tube. The extent of the erythrocytes' lysis and release of the haemoglobin caused by the investigated material was determined using the Schimadzu UV/VIS spectrophotometer, UV 2101 PC type, at the 540 nm wavelength.

The purpose of the haemolysis tests from the extract was determination of the haemoglobin level in blood plasma which reflects the extent of the erythrocytes' cell membrane damage in contact with the extract from the examined material. The specimens were placed in the 0,9% solution of NaCl at the temperature of 70^{o}C for 24 hours to prepare the extract. Then the extract was placed in the test-tubes, to which the citrated blood was added. Next, the test-tubes were incubated for 60 min at the temperature of 37^{o}C in the Heraeus B20 thermostat. After incubation the blood was removed to the next test-tube and centrifugalized for 10 min at the 500xG rate. Centrifugation separates the cells that are free of damage and the cellular lysis. Lysated cells were left in the solution at the bottom of the test-tube. The extent of the erythrocytes' lysis and release the haemoglobin caused by the investigated material was determined using the Schimadzu UV/VIS spectrophotometer, UV 2101 PC type, at the 540 nm wavelength.

The citrated blood plasma was used in coagulation tests. The plasma was subjected statically to the activity of the investigated material. After the specified contact duration time with the investigated surface the following determinations were made:

- determination of the prothrombin time (PT),
- determination of the partial thromboplastine time (PTT).

The prothrombin time is the main screening test revealing abnormalities in the extrinsic clotting mechanism. The extrinsic clotting mechanism occurs when the blood flowing from the blood vessels gets into contact with the damaged tissues. The basis of this test is the optimum concentration of calcium ions and excess of thromboplastine. Prothrombin and ancillary factors are measured in this test. The blood coagulation time depends on the concentration of prothrombin, factor V, and factor X, in the presence of the tissue thromboplastine. The citrated blood plasma was added to the test-tubes and placed in the MCL 2 Instrumentation Laboratory coagulometer incubator at the temperature of 37±0,5^{o}C. Next, the PT reagent and calcium chloride were added to each test-tube. Calcium chloride causes releasing of the anticoagulant and allows the blood clot formation process to proceed. Time duration to development of the thrombus was determined using the coagulometer. Determination of the prothrombin time for each specimen was carried out in relation to the negative control test.

Determination of the partial thromboplastine time (PTT) features the main screening test revealing abnormalities in the intrinsic clotting mechanism. The intrinsic clotting mechanism is revealed by the blood contact with materials or compounds with the negative charge. This test determines the time duration of coagulation of the recalcinated citrated blood plasma under the influence of adding of the partial thromboplastine. The citrated blood plasma was added to the test-tubes, which were placed in the coagulometer incubator at the temperature of 22±0,5°C for 60 minutes. Next the PTT reagent was added for 3 minutes. Further, the calcium chloride solution was added to each test-tube. Calcium chloride causes releasing of the anticoagulant and allows the blood clot formation process to proceed. Time duration to development of the thrombus was determined using the coagulometer. Determination of the partial thromboplastine time for each specimen was carried out in relation to the negative control test.

3. Results

3.1. Results of surface topography investigations

Observations with the use of atomic force microscope (AFM) showed the different surface topography of passive and passive-carbon layers deposited on surface of wire intended for coronary stents forming – Figs. 4 and 5.

The electrolytically polished and passivated surface characterized the parallel arrangement of layer crystals. It was a result of the scratches induced by the drawing process and which weren't removed during electrolytic polishing of the wire surface – Fig.4. Characteristic and cyclic unevenness on the layer profile reflected their presence.

The additionally carried out analysis of surface layer development showed that the maximum values of unevenness along the analysed lines were in the range of 58,5-83,5 nm. The measurements that were realised also let to determine the roughness of surface layer. It has been stated that the values of arithmetical average of profile deviation from the mean line were in the range of R_a = 8,5-14,8 nm.

The process of carbon layer deposition on polished and passivated wire changed the topography of surface. Investigations have shown on the specimens' surface the presence of continuous layer consisting of crystals closely adhered one to another – Fig. 5. In that case it hasn't been stated the arrangement of layer crystals characteristic for passivated specimen which reflects the topography of wire surface induced by drawing process. The development analysis of the surface layer has shown that the maximum values of unevenness along the analysed lines were in the range of 75-93 nm. The roughness of the surface has values from the range of 16,5 nm-20,3 nm – Figs. 6 and 7.

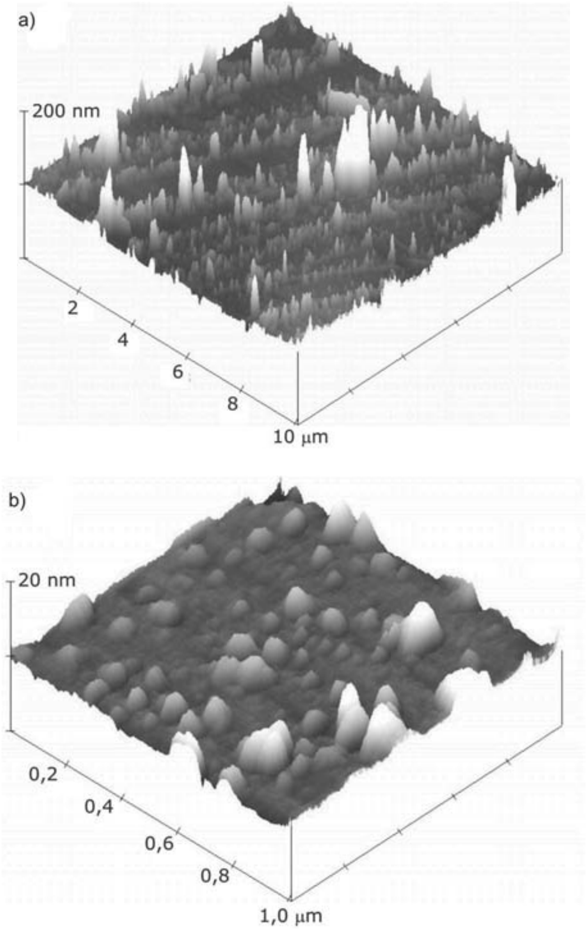

Fig. 4. Topography of the passive layer: a) area 10x10 µm, b) area 1x1 µm

3.2. Results of electrical properties investigations

The current-voltage and capacitance-voltage characteristics were determined to define the electrical properties of carbon layer – Figs. 8 and 9. In the measurements of resistivity ρ of the carbon layer it has been assumed that the current which is passing through condensers is passing through the cylinder. The height of cylinder is equal to thickness of dielectric layer and its base area is the area of contact formed on that layer. It has been stated on the basis of the current-voltage characteristic and the relationship (1) that resistivity of the carbon layer varied in the range of ρ = 1-5 x 10^8 Ωcm.

Further the relative permittivity ε_r of carbon layer has been determined by the measurements of maximum capacity of condenser in accumulation state. The performed analysis of capacitance-voltage characteristics showed that capacity value was in the range of C_{max} = 230-313 pF. Therefore, taking into account relationship (2), it has been stated the permittivity of layer varied in the range of ε_r = 8, 2-11, 1.

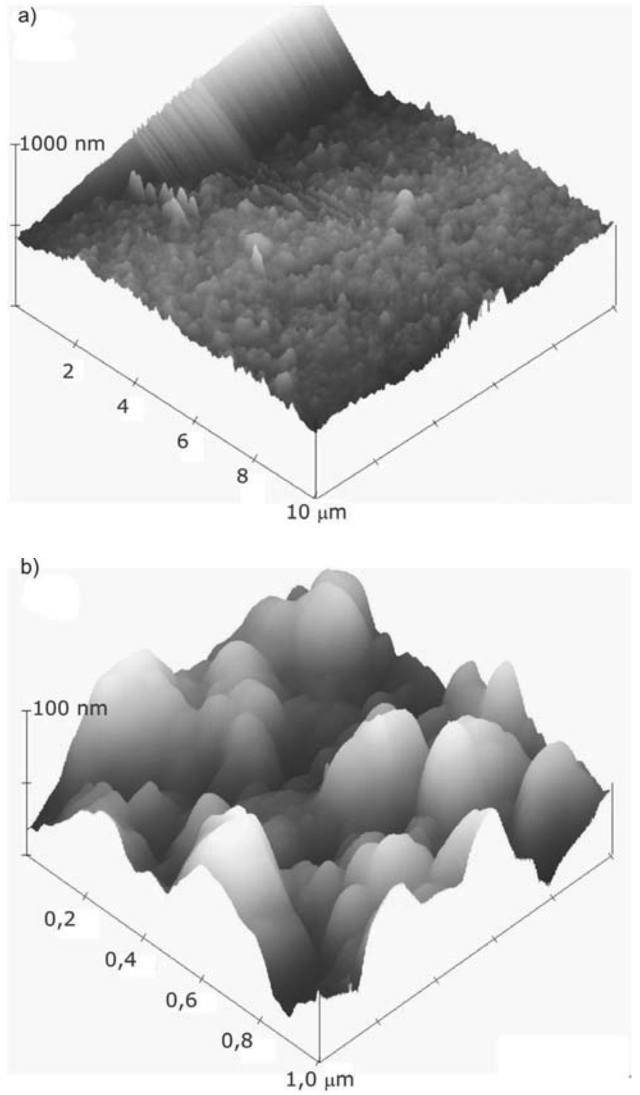

Fig. 5. Topography of the passive-carbon layer: a) area 10x10 µm, b) area 1x1 µm

3.3. Results of interaction with blood investigations

The haemolysis degree determined in the direct contact for the electrolytically polished and passivated specimens was 1,54% and for electrolytically polished, passivated and coated with the carbon layer was 1,23%. Both of this values didn't exceed 3,0%, which is the value obtained in the positive control test. The haemolysis degree obtained from the extract for the electrolytically polished and passivated specimens gave the result of 0, 53% and for electrolytically polished, passivated and coated with the carbon layer, the value of 0,89% was achieved. In that case the results also didn't exceed the value obtained in positive control test, which was 1,2%.

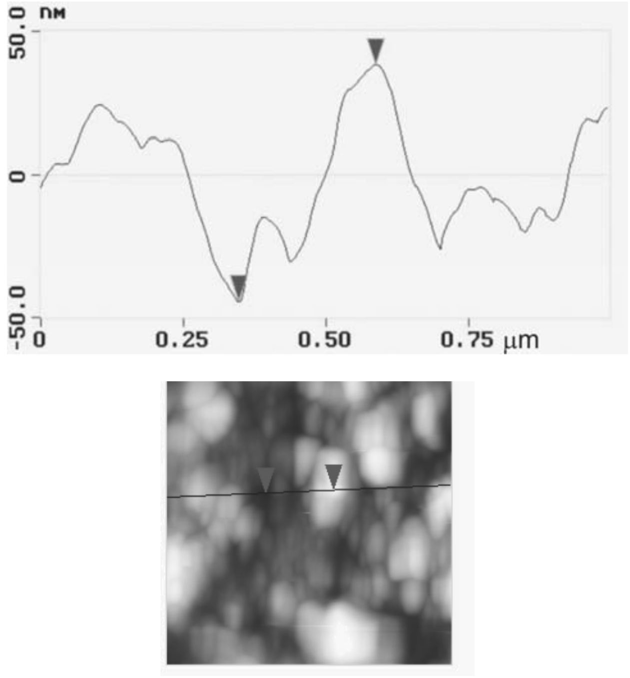

Fig. 6. Linear analysis of surface development of passive-carbon layer

Image Statistics

Img.	Z range	182,29 nm
Img.	Mean	-0,00002 nm
Img.	Raw mean	-0,00002 nm
Img.	Rms (Rq)	23,16 nm
Img.	Ra	18,53 nm

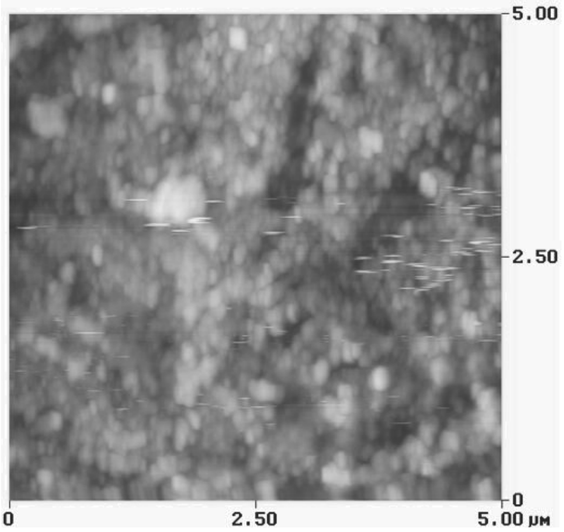

Fig. 7. Roughness analysis of passive-carbon layer

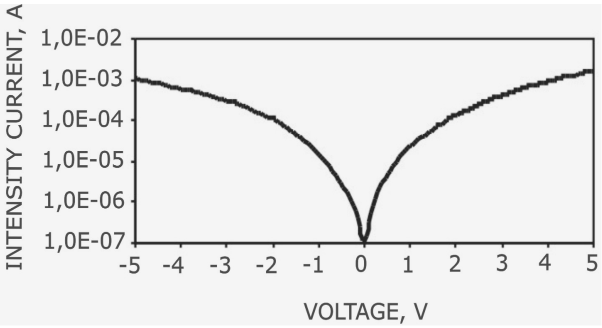

Fig. 8. Current-voltage characteristic of the MIS condenser

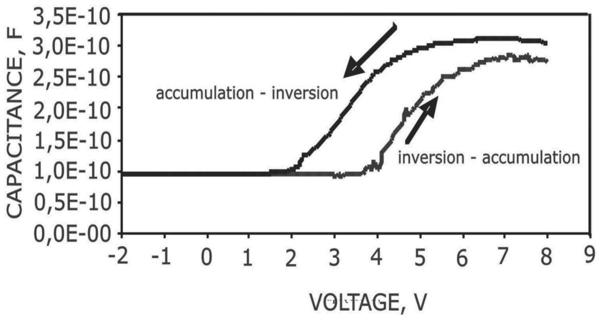

Fig. 9. Capacitance-voltage characteristic of the MIS condenser

The investigations concerning the influence of deposited layer on exo- and endogenous mechanism of blood clotting showed that for electrolytically polished and passivated specimens the value of prothrombin time (PT) was 11,7 sec and respectively for specimens electrolytically polished, passivated and coated with carbon layer the value was 12,1 sec. The obtained results differ only slightly from the control test value, which was equal to 11,5 sec.

It has been stated in tests concerning the influence on the intrinsic mechanism of blood clotting that the value of partial thromboplastine time (PTT) for the electrolytically polished and passivated specimens was 29 sec. For the specimens additionally coated with the carbon layer the value of 31,5 sec was obtained. Measurements carried out for the control test revealed that the PTT time value was 28 sec.

4. Conclusions

Investigations for the evaluation of usefulness of passive-carbon layer for applying on the implants used in interventional cardiology have been carrying out in the Institute of Engineering Materials and Biomaterials of Silesian University of Technology since a few years. In initial considerations, for the clinically justified form of the coronary stent, the biomechanical analysis of the stent-coronary vessel system was carried out, taking into consideration both the implantation process as well as the usage process. Stresses and radial displacements for possible geometrical features of the wire the stent was made of, were calculated with the use of the finite element method. The analysis was the base for considerations of selection of mechanical properties of the metallic biomaterial [12,16].

To ensure the required physico-chemical properties of the stent surface, the processes of the electrolytic polishing, the chemical passivation, and the carbon layer deposition were worked out. Both the passive and the passive-carbon films were tested in detail *in vitro*. The pitting corrosion resistance of the stents was tested, taking into consideration the forming technique of the finished stent, its implantation and the varying functional loadings. The tests showed that the layers deposited on the stent surface ensure the corrosion resistance. The nanocrystalline structure of the layers doesn’t initiate unfavourable processes of decohesion and corrosion of the stents [14-16,20-23].

Investigations presented in the paper were mainly focused on the evaluation of antithrombogenic properties of the passive-carbon layer. Therefore they constitute a continuation and supplementation of the earlier research.

Investigations that were realized with the use of atomic force microscope showed the continuity of passive-carbon layer deposited on the surface of coil stents made of Cr-Ni-Mo steel. The layer had low roughness (R_a = 16,5-20,3 nm). High smoothness of stents surface with the deposited layer undoubtedly influences the limitation of the process of blood clotting.
Measurements of electrical properties were realized by the method often applied in testing of that kind of layers. The carried out measurements determined the resistivity (ρ = 1-5 x 10^8 Ωcm)and the permittivity. ε_r = 8,2-11,1) of the carbon layer deposited on the substrate of silicon plate. These values are situated in the range typical for layers of DLC type [16].

The obtained results of electrical properties of carbon layer should be considered as preliminary ones. Application of the silicon plate as a substrate material without possibility of realization of electrolytic polishing and passivation process on its surface influenced undoubtedly as well the chemical composition of constituted layer as its properties. But finally in spite of that the obtained results of tests should be regarded positively. Carbon layer is limiting the blood clotting mechanism.

The carried out *in vitro* tests confirmed the antithrombogenic properties of the passive-carbon layer. The results obtained in the tests showed that the influence of the specimens’ surfaces with the deposited coatings on the extrinsic- and intrinsic clotting mechanism should be evaluated as very advantageous.

The carried out investigations were pointed out that the carbon coating of dielectric properties and of high surface smoothness deposited on the implants’ surface made of Cr-Ni-Mo steel (ρ = 0,7x10^{-4} Ωcm) is an effective way of limiting the reactivity of their surface in blood environment. This layer reduces positively the blood clotting process in consequence, what is the significant problem for the biomaterials applied in interventional cardiology.

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