Journal of Achievements in Materials and Manufacturing Engineering
VOLUME 17
ISSUE 1-2
July-August
2006

Investigations of antithrombogenic properties of passive-carbon layer

Z. Paszenda [a,*], J. Tyrlik-Held [a], W. Jurkiewicz [b]
[a] Division of Biomedical Engineering,
Institute of Engineering Materials and Biomaterials, Silesian University of Technology,
ul. Konarskiego 18a, 44-100 Gliwice, Poland
[b] DRG MedTek, ul. Wita Stwosza 24, 02-661 Warszawa, Poland
* Corresponding author: E-mail address: zbigniew.paszenda@polsl.pl

Received 15.03.2006; accepted in revised form 30.04.2006

Properties

ABSTRACT

Purpose: The main purpose of the paper was evaluation of antithrombogenic properties of the passive-carbon layer used for enhancing the surface properties of vascular stents made of Cr-Ni-Mo steel.
Design/methodology/approach: In vitro tests of biotolerance evaluation of the passive-carbon layer in blood environment have been carried out on the basis of haemolysis tests (in the direct contact and from the extract) and blood clotting tests.
Findings: The carried out investigations have shown that deposition process of the passive-carbon layer which has dielectric properties on the surface of implants made of Cr-Ni-Mo steel and used in interventional cardiology is an effective way in limiting the reactivity of their surface in blood environment and the blood clotting process in consequence.
Research limitations/implications: The carried out investigations should be completed with biotolerance in vivo investigations.
Originality/value: Modification of physical properties of surface of the metallic biomaterials applied in cardiovascular system by deposition of the passive-carbon layer of dielectric properties limits the blood clotting process.
Keywords: Electrical properties; Biomaterials; Surface treatment

1. Introduction

Innovative progress in treatment methods of ischemic heart disease with the use of minimally-invasive techniques (interventional cardiology) leads to working out the new shape of implants–coronary stents. These implants, placed in the narrowed segment of coronary vessel, enlarge its active cross-section [1-14].

One of the negative phenomena occurring after stent implantation to the vessel system is haemostasis (blood circulatory arrest). The process of interaction between blood and implantation materials is not still fully recognized. It is generally assumed that as a consequence of blood contact with an artificial implant's surface the protein adsorption (mainly fibrinogen) is occurring. In case the adsorbed fibrinogen undergoes the process of denaturisation the stimulating activity of the following platelets and plasma blood clotting factors take place in cascade way. It leads in consequence to the blood clot forming [1, 2, 5, 15-25].

Data explaining the matters of initiation of clotting process basing on the banding model of solid state body have been published recently [26-28]. It has been stated on the basis of Gutmann investigations 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 [29]. 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. The consequence is the protein decomposition into fibrinmonomer and fibrinpeptides. Further the cross-linking process leads to forming the irreversible thrombus.

Advisability of physical properties modification of the surface of implantation materials by their surface treatment is resulting from the analysis conducted above. 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. It can be an effective method for limiting the process of blood clotting as a consequence of the contact with the surface of implanted stent.

2. Methods

In the work the evaluation of antithrombogenic properties of the passive-carbon layer formed in order to enhance the quality of surface of coronary stents made of the Cr-Ni-Mo steel has been performed. The deposition of that layer is a multi-stage process. It contains electropolishing, chemical passivation and forming of the carbon layer by RF PACVD method [1, 4-6]. The usefulness of that layer proposed for implants applied in interventional cardiology has been evaluated on the basis of investigations of electric properties and interaction with blood.

2.1. Electrical properties investigations

Carbon layer has been deposited on the surface of silicon plate of resistivity ρ = 0,005÷ 0,02 Ωcm in order to determine its electrical properties. On the basis of measurements conducted by elypsometric method it has been stated that its thickness was 248 nm. Further 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.

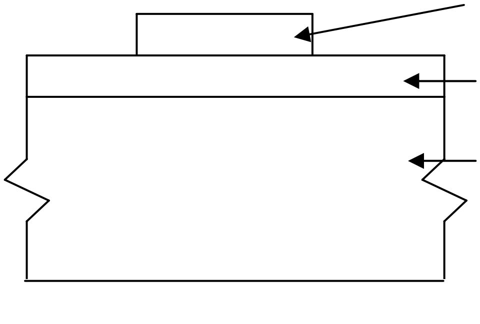

Fig. 1. Section of the tested MIS structure (metal – insulator – semiconductor)

Determination of current-voltage characteristic (I-U) defines the resistivity of the layer from the following relationship:

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

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. 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 ($8{,}85 \cdot 10^{-12}$ F/m),
S – area of the aluminium contact, mm^2.

2.2. Investigations of interaction with blood

For the implants exposed to the blood environment longer then 30 days the investigations of their interactions with blood in *in vitro* conditions are required according to the PN-ISO 10993 standard [30]. In the work these investigations have been performed for the specimens of Cr-Ni-Mo steel with electrolytically polished and passivated surfaces, and electrolytically polished, passivated and coated with carbon layer ones.

In particular the following tests were conducted [31]:

- haemolysis tests in the direct contact and from the extract,
- blood clotting tests.

The specimens for the tests 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) – haemolysis tests,
- cylinder with the diameter of d = 10 mm and height of h = 25 mm (required minimal total area of the specimen was 8 cm^2) – blood clotting tests.

The haemolysis tests in the direct contact consisted of determination of haemoglobin level in the plasma, showing the damage degree of the cell membrane of red blood cells in contact with the specimens investigated. The degree of lysis of the red blood cells and release of haemoglobin induced by contact with investigated material has been evaluated by the use of the Schimadzu spectrophotometer UV/VIS, type UV 2101 PC, at the 540 nm wavelength.

The haemolysis tests from the extract consisted of determination of haemoglobin level in the plasma, showing the damage degree of the cell membrane of red blood cells in contact with the extract from the investigated material. In order to prepare the extract the specimens were immersed in 0,9% NaCl solution at temperature 70±0,5°C during 24 h. The extract prepared in such way was placed in test-tube with citrated blood. Next the test-tubes were incubated during 1h at temperature 37±0,5°C in Heraeus dryer type B20. Further investigations were realized in the same way as in the case of haemolysis tests in the direct contact.

In blood clotting tests the citrated plasma was used. The plasma was statically influenced by the investigated material.

After the specified time of contact with investigated surface the prothrombin time (PT) and of partial thromboplastin time (PTT) have been marked out. Time PT is the main test which allows detecting the abnormalities in clotting of exogenous system, and time PTT of endogenous system.

3. Results

3.1. Results of electrical properties investigations

In electrical properties investigations the current-voltage and capacitance-voltage characteristics were determined – fig. 2 and 3. In 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. Its height is equal to thickness of dielectric layer and its base area is the area of contact formed on that layer. On the basis of the current-voltage characteristic and the relationship (1) it has been stated that resistivity of the carbon layer is in the range of $\rho = 1 \div 5 \times 10^8\ \Omega cm$.

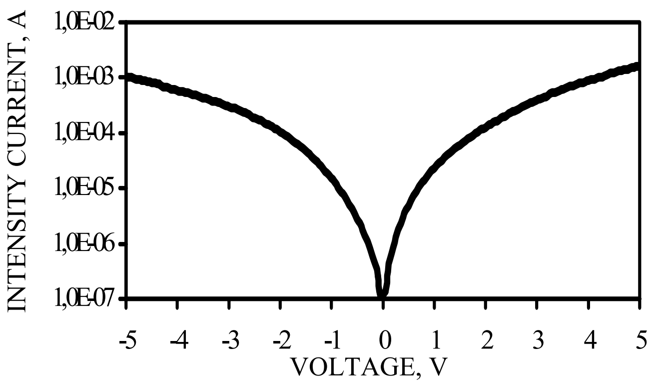

Fig. 2. Current-voltage characteristic of the MIS condenser

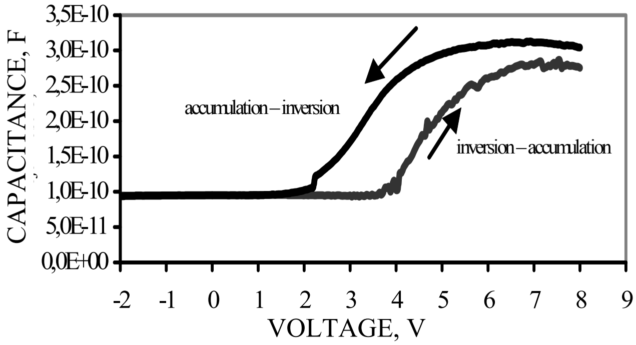

Fig. 3. Capacitance-voltage characteristic of the MIS condenser

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

3.2. Results of interaction with blood investigations

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

The carried out 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 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.

In tests concerning the influence on the intrinsic mechanism of blood clotting it has been stated that the obtained value of partial thromboplastine time (PTT) for the electrolytically polished and passivated specimens was 29 sec, whereas 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 of electrical properties were realized with the method often applied in testing of that kind of layers. The carried out measurements determined the resistivity ($\rho = 1 \div 5 \times 10^8\ \Omega cm$) and the permittivity ($\varepsilon_r = 8,2 \div 11,1$) of the carbon layer deposited on the substrate of silicon plate. These values are situated in the range typical for layers of DLC type [1].

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

The antithrombogenic properties of the passive-carbon layer were confirmed in *in vitro* tests. Biological investigations of materials remaining in contact with blood environment are specified in PN-EN ISO 10993 standard [29]. The obtained test results of the influence of the specimens' surfaces with the deposited coatings on the extrinsic- and intrinsic pathway blood clotting mechanisms should be evaluated as very advantageous.

The worked out investigations have shown that the carbon coating of dielectric properties (ρ = 0,7x10^{-4} Ωcm) deposited on the implants' surface made of Cr-Ni-Mo steel applied in interventional cardiology is an effective way of limiting the reactivity of their surface in blood environment. It reduces positively the blood clotting process in consequence.

References

[1] Z. Paszenda, Forming of physico-chemical properties of coronary stents made of Cr-Ni-Mo steel applied in interventional cardiology. Wydawnictwo Politechniki Śląskiej, Gliwice, 2005, (in Polish).

[2] Z. Paszenda, Issues of metal materiale used for implants in interventional cardiology. Engineering of Biomaterials, 21 (2002) 3-9.

[3] Z. Paszenda, J. Tyrlik-Held, Corrosion resistance investigations of coronary stents made of Cr-Ni-Mo steel. Proceedings of the 10th International Scientific Conference AMME' 2001, Gliwice-Zakopane, 2001, 453-460

[4] Z. Paszenda Z, J. Tyrlik-Held, Z. Nawrat, J. Zak and K. Wilczek, Corrosion resistance investigations of coronary stents with regard to specificity of coronary vessels system. Engineering of Biomaterials, 34 (2004) 26-33.

[5] Z. Paszenda, J. Tyrlik-Held, Z. Nawrat, J. Zak, J. Wilczek, Usefulness of passive-carbon layer for implants applied in interventional cardiology. Journal of Materials Processing Technology, 157-158C (2004) 399-404.

[6] Z. Paszenda, J. Tyrlik-Held, W. Chrzanowski, J. Lelatko, Structure investigations of passive-carbon layer on coronary stents of Cr-Ni-Mo steel. Engineering of Biomaterials, 46 (2005) 6-8.

[7] P. de Feyter, D. Foley, Coronary stent implantation: a panacea for the interventional cardiologist? European Heart Journal, 21 (2000) 1719-1726.

[8] P. de Feyter, The quest for the ideal stent. European Heart Journal, 22 (2001) 1766-1768.

[9] W. Walke, Z. Paszenda, J. Filipiak, Experimental and numerical biomechanical analysis of vascular stent. Proceedings of the 13th International Scientific Conference AMME'2005, Wisła, 2005, 699-702.

[10] W. Walke, Z. Paszenda, J. Filipiak, Experimental and numerical biomechanical analysis of vascular stent. Journal of Materials Processing Technology, 164 (2005) 1263-1268.

[11] A. Violaris, Y. Ozaki, P. Serruys P, Endovascular stents – a break through technology, future challenges. International Journal of Cardiac Imaging, 13 (1997) 3-13.

[12] A.Colombo, G. Stankovic, J. Moses, Selection of coronary stent. Journal of the American College of Cardiology, 6 (2002) 1021-1033.

[13] C. Pepine, D. Holmes, P. Block, J. Brinker, D. Mark, Ch. Mullins, S. Nissen et al, Coronary artery stents. Journal of the American College of Cardiology, 28 3 (1996) 782-794.

[14] J. Gunn, D. Cumberland, Stent coatings and local drug delivery. European Heart Journal, 20 (1999) 1693-1700.

[15] J. Lahann, D. Klee, H. Thelen, H. Bienert, D. Vorverk, H. Hocker, Improvement of haemocompatibility of metallic stents by polymer coating. Journal of Materials Science: Materials in Medicine, 10 (1999) 443-448.

[16] T. Peng, P. Gibula, K. Yao, M. Goosen, Role of polymers in improving the results of stenting in coronary arteries. Biomaterials 17 (1996) 685-694.

[17] I. Verweire, E. Schacht, B. Qiang, K. Wang, I. de Scheerder, Evaluation of fluorinated polymers as coronary stent coating. Journal of Materials Science: Materials in Medicine, 11 (2000) 207-212.

[18] R. Hoffmann, G. Mintz, Coronary in stent restenosis – predictors, treatment and prevention. European Heart Journal, 21 (2000) 1739-1749.

[19] N. Weber, H. Wendel, G. Ziemer, Hemocompatibility of heparin-coated surfaces and the role of selective plasma protein adsorption. Biomaterials, 23 (2002) 429-439.

[20] G. Michenatzis, N. Katsala, Y. Missirlis Y, Comparison of haemocompatibility improvement of four polymeric biomaterials by two heparinization techniques. Biomaterials, 24 (2003) 677-688.

[21] I. de Scheerder, K. Wang, K. Wilczek, Experimental study of thrombogenicity and foreign body reaction induced by heparin-coated coronary stents. Circulation, 95 (1997) 1549-1553.

[22] P. Serruys, B. van Hout, H. Bonnier, V. Legrand, E. Garcia, C. Macaya, E. Sousa, W. van der Giessen, Randomised comparison of implantation of heparin-coated stents with angioplasty in selected patients with coronary artery disease (Benestent II). The Lancet, 352 (1998) 673-681.

[23] N. Chronos, C. Markou, J. Kocsis, G. Lianos, S. Hanson, Surface heparinization profoundly decreases acute thrombosis on Crown and Mini-Crown stents in the baboon arteriovenous shunt model. Journal of the American College of Cardiology, 2 (1998) 1163-77.

[24] K. Christensen, R. Larsson, H. Emanuelsson, G. Elgue, A. Larsson, Heparin coating of the stent graft – effects on platelets, coagulation and complement activation. Biomaterials, 22 (2001) 349÷355.

[25] H. Zhao, J. van Humbeeck, Electrochemical polishing of 316L stainless steel slotted tube coronary stents. Journal of Materials Science: Materials in Medicine, 13 (2002) 911-916.

[26] N. Huang, P. Yang, X. Cheng, Y. Leng, X. Zheng. et al, Blood compatibility of amorphous titanium oxide films synthesized by ion beam enhanced deposition. Biomaterials, 19 (1998) 771-776.

[27] J. Y. Chen, Y. X. Leng, X. B. Tian, L. P. Wang, N. Huang, P. K. Chu, P. Yang, Antithrombotic investigation of surface energy and optical bandgap and hemocompatibility mechanism of $Ti(Ta^{+5})O_2$ thin films. Biomaterials, 23 (2002) 2545-2552.

[28] N. Huang, P. Yang, Y. Leng, J. Chen, H. Sun, J. Wang J. et al, Hemocompatibility of titanium oxide films. Biomaterials, 24 (2003) 2177-2187.

[29] F. Gutmann, H. Keyzer, Modern bioelectrochemistry. Plenum Press, New York 1986.

[30] ISO 10993-1997, Biological evaluation of medical devices.

[31] ISO 10993-4-1997, Biological evaluation of medical devices – Part 4. Selection of tests for interactions with blood.