

Polish Artificial Heart – new coatings, technology, diagnostics

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Abstract. Since 1991, the Foundation for Cardiac Surgery Development in Zabrze has been implementing research on the artificial heart. In 1995, an artificial ventricle, POLVAD, was implanted to a patient, and in 1998, the prototype of a clinical controller, POLPDU-401, was created. A further development of the studies on an implantable artificial heart requires an integrated approach and an application of advanced methods of materials sciences in order to develop new materials suitable for the contact with blood, as well as to apply a multilateral biomedical diagnostics in hydrodynamic conditions.

The estimation of the cell–material interaction plays an important role in the biomaterial design. An analysis of the influence of the carbon content in titanium nitride on the biological and biophysical properties of biomaterial coatings was studied. The cell-material reactions were considered in dynamic and static conditions. Three groups of materials were under examinations – titanium nitride (TiN), as well as titanium carbonitride with a low and high carbon content – of which the best properties were observed for TiN. We found a strong influence of the stoichiometry of TiN (atomic ratio of Ti/N) on the biocompatibility. A non-stoichiometric TiN could have a negative influence on the surrounding tissue.

Key words: pulsed laser deposition, microstructure, cell adhesion.

1. Introduction

The genesis of the research on the artificial heart in Poland corresponds in time with the development of this field of studies around the world. A direct stimulus for the search of the artificial heart was the first successful heart transplantation performed by prof. Zbigniew Religa in 1985, in a clinic in Zabrze. Three years earlier, in 1982, the Jarvik artificial pneumatic heart was implanted for the first time in the USA. Ready-for use artificial hearts were also developed by the Japanese. The very high costs of those pioneer devices made it impossible to apply them in Poland. From the beginning of the 70s, the studies on a pneumatic artificial heart were also conducted at the Purkiny's University in Brno, the Czech Republic, in cooperation with the team from Zabrze. In 1986, for the first time in Poland, the left ventricle was supported by the artificial ventricle BRNO-VAD. In 1998, the first full implantation of the first artificial heart BRNO-TAH was performed. The Czech artificial heart was implanted four more times, out of which one was successful - it supported the patient's heart for four weeks until the transplantation. In 1989, in Zabrze, the Russian artificial heart POISK VII was implanted for the first time. This prosthesis was implanted four times. In spite of the high quality of the Czech and Russian technical solutions, neither of those prosthesis were introduced into the regular production of medical equipment. With the lack of foreign prosthesis, the team in Zabrze began to develop the Polish artificial heart and the extracorporeal ventricle support. The first research project was implemented under the direction of prof. Religa in 1988. Since 1991, the studies on the artificial heart have been performed at the Foundation for Cardiac Surgery

Development in Zabrze. In 1991–1993, the Foundation, in cooperation with the PLASTMED technological company and the Medical Academy in Śląsk, performed research and implementation works on the Polish artificial heart POLTAH, the Polish heart support ventricle POLVAD (Fig. 1), as well as the controller for the pneumatic heart prosthesis POLPDU. The effect of those works was the first experimental implantation of the artificial ventricle POLVAD in 1995, and in 1997 – of the first artificial heart POLTAH. In 1998, the prototype of the clinical controller POLPDU-401 was created. So far, the system of the extracorporeal heart support POLCAS, consisting of the extracorporeal heart support ventricle POLVAD-MEV and the controller of the pulsatory heart prosthesis POLPDU-401, has been applied in the treatment of over 200 patients.

The studies on the new artificial heart have been implemented for 15 years at the Foundation for Cardiac Surgery Development in Zabrze, in the field of advanced designs of implantable heart prosthesis, as well as in the search of modern technologies of materials sciences, mainly in cooperation with the Medical Academy in Śląsk, Institute of Metallurgy and Materials Sciences of the Polish Academy of Sciences in Krakow, Poland, and the Laser Centre Leoben in Austria. The effects of these studies are among others:

- a model of an implantable electrohydraulic prosthesis for the left ventricle support POLHIVAD – awarded by the Golden Medal at the International Fair of New Technologies in London;
- a model of an implantable pulsatory heart support ventricle, developed with the application of an elastic nanolayer of titanium nitride, applied by the method of laser ablation

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on a thermodegradable polymer. The model was awarded by the prestigious Grand Prix at the XXIII World Fair for Innovation, Research and New Technologies EUREKA in Brussels;

- a model of the full system of the long-term heart support prosthesis, based on an implantable artificial heart with the portable controller POLPDU-501. The model of the system was awarded by the Golden Medal at the Concours Lepine International Inventions Fair in Paris. The long-term commission programme “Polish Artificial Heart” was established by the resolution of the Government in 2007, and its implementation began in the field of the first two initiatives: “The developments of technologies in materials sciences, surface engineering and bioengineering in the field of the artificial heart” and “The development of designs of the clinical heart prosthesis system”.



Fig. 1. Model of term heart support POLVAD-IMPL

The remaining three initiatives implemented within the frames of the programme are: “The development of metrological, information and tele-information technologies in the field of the artificial heart” and “The implementation of the cardiac insufficiency treatment methods involving the Polish heart prosthesis in the clinical application”, as well as “The creation of a highly specialized research-technology platform for complex research and development works in the field of the artificial heart”.

The coordinator of the programme is the Foundation for Cardiac Surgery Development in Zabrze.

Over the past 30 years, much effort in the research of biomaterials has been directed toward the development of materials that are inert to human blood and do not react with platelets or coagulation factors [1]. A number of often conflicting approaches have been developed. There is still no consensus about the advantageous hydrophilic or hydrophobic behaviour of a surface. This lack of agreement is largely due to both the incomplete understanding of the biological pathways leading to material failure and the designers’ inability to fully evaluate the blood material failure and the blood material responses. In the development of the materials with a lower thrombogenicity, researchers have primarily focused their

efforts on modifying the surfaces. This approach is reasonable, since it is only the surface chemistry of the material that should dictate its biological responses and the mechanical characteristics of the material are primarily dictated by the bulk chemistry. Despite the successes in reducing the protein and the cellular deposits on some materials, a truly nonthrombogenic surface does not exist.

Experimental and theoretical work has been dedicated to the cell adhesion from three very different perspectives [2]: (i) From the biological point of view, the understanding of the molecular mechanisms is of central importance, which means the understanding of when a cell adheres, rolls, or slides on passive or reactive substrates. Many functions performed by living cells depend on these properties. (ii) From the physical-chemical point of view, bio-adhesion involves cells, a solid substrate, and a liquid medium. The relevant properties of a micro organism are the hydrophobicity and the charge of the cell surface, cell size, and possession. A lot of experimental and theoretical work has been dedicated to cell adhesion [3]. (iii) From the physical point of view, even the passive (non-reactive) response of the cells to an external force offers new phenomena that find no equivalent in more traditional materials. This is the case in the bio-adhesion phenomena, where the discrete nature of the contact regions, the weak or non-covalent bonding of the cell to the substrate, and the multi-component variety of the cytoplasmic membrane or of its extra cellular matrix (ECM) are the origin of contact forces. Measuring the strength of these bonds is a major challenge in cellular biology, since it allows for the identification of the different factors at work in the adhesion phenomena.

Biomaterials such as: titanium (Ti), and stoichiometric titanium nitride (TiN) as well as titanium carbo-nitride (Ti(C,N)), seem to be good candidates for future blood-contact applications [1]. These materials were under examination in this work and they were deposited as thin films by the hybrid technique to examine the influence of the deposited material on the cell behaviour [4]. Much attention was put to the diagnostics of the deposited coating in order to establish the optimal technological parameters from the physicochemical parameters of the biomaterials. The kinetics energy of the evaporated particles was controlled by an application of a variation of different reactive and non-reactive atmospheres during the deposition. The conventional TEM and the high resolution transmission electron microscopy (HREM) was used to reveal the structure dependence on a specific atmosphere in the reactive chamber. The biomaterial examinations were performed in static conditions with fibroblast cells line and then subjected to the dynamical test to observe the cell detachment kinetics. For a given cell, the detachment occurs for the critical stress values, caused by the applied hydrodynamic pressure above a threshold, which depends on the cell size and the physicochemical properties of the substrate, but it is not affected by the depolymerization of the actin and tubulin cytoskeleton. The tests revealed differences in behaviour, depending on the applied coating material [5]. The strongest cell-biomaterial interaction was observed for the carbon-based materials, as compared to titanium and titanium nitride.

The results presented in this work concern the problems of materials science at the intersection of engineering and medicine and precisely, the surface characterization and the cell-material interaction of thin films in the detachment assays.

The aim of the work is to describe the detachment kinetics of fibroblast cells line with different biocompatible materials. These materials were deposited as thin films, allowing only the surface to be modified and not the bulk of the material.

2. Materials and methods of examinations

2.1. Preparation of the surfaces. A development of the surface modification with the use of thin film materials was performed by magnetron sputtering in direct current (DC), unbalanced mode. Titanium (medical grade titanium for titanium and titanium nitride coatings) and carbon targets (for Ti(C,N)) were used to deposit about 10–20 nm thick films on polyurethane substrates at room temperature in argon atmosphere (Ti) or nitrogen-argon atmosphere (TiN). To ensure the homogenous film thickness over the entire coated surfaces, the substrates were rotated during the deposition at the speed of 5.4 cm s^{-1} through the plasma plumes. A detailed description of the deposition arrangement is given elsewhere [4].

3. Material examinations

3.1. Thermal effect. Deposition process could have the significant influence on the material properties. It is crucial aspect in the case of biomaterials designing procedure. The temperature and the atmosphere seem to be the basic parameters which determine the proper structure. The temperature effect was analyzed as the Heat Flow in the function of the applied temperature (Fig. 2). The performed investigations involved a small glass transformation on the level of 110 deg. All the visible effects probably come from the substrate. The following materials which were applied as the surface modification exhibited various reactions:

- Ti, Ti(C,N), TiO, DLC+Si caused an additional endothermic effect in the lower temperature area
- TiN, DLC, DLC+Ti influenced the additional endothermic effect but the change of the temperature of the glass transformation is visible as well.

3.2. Surface analysis. The surface analysis was performed using a scanning electron microscopy (E-SEM) and a scanning acoustic microscopy (SAM).

The proper surface structure and the lack of cracks is strongly correlated with the appropriate mechanism of the thin film growth [5]. The coating deposited under the late mechanism of the thin film growth is brittle and exhibits the cracks on its surface [4].

Scanning electron microscopy E-SEM. In the E-SEM examinations of TiN, Ti(C,N) (Fig. 3), it was stated that these films have the proper thickness. The visible scratches come from the substrate. The titanium coating was too thick. It caused numerous cracks, which could influence the platelet activation process. Thus, the thrombus formation could be blurred by the effect coming from the scratches. An optimization of the deposition parameters leads to the limiting of the thickness of the film to the primary mechanism of the deposition [5].

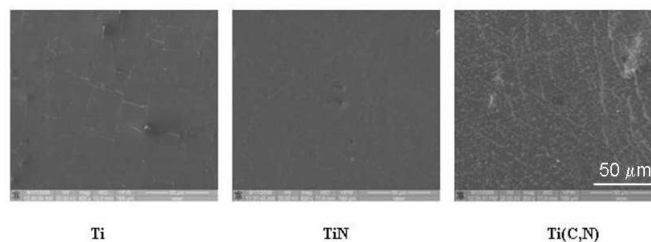
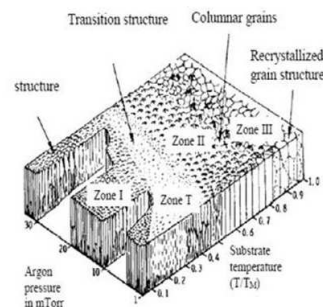


Fig. 3. E-SEM examinations of the deposited coatings with the Thornton diagram (Ref. 5)

Confocal Laser Scanning Microscopy CLSM. The Confocal Laser Scanning Microscopy (CLSM) is the technique which is based on an optical microscopy, but the light comes from the laser. It is conventionally used for advanced fluorescent observations. In this case, it was used with regard to the different contrast.

Figure 4 shows CLSM examinations of the deposited coatings with the Thornton diagram [6]. The cracks in the Ti coating are also visible but additional defects in the TiN were observed. The ideal surface was found for titanium-carbonitride.

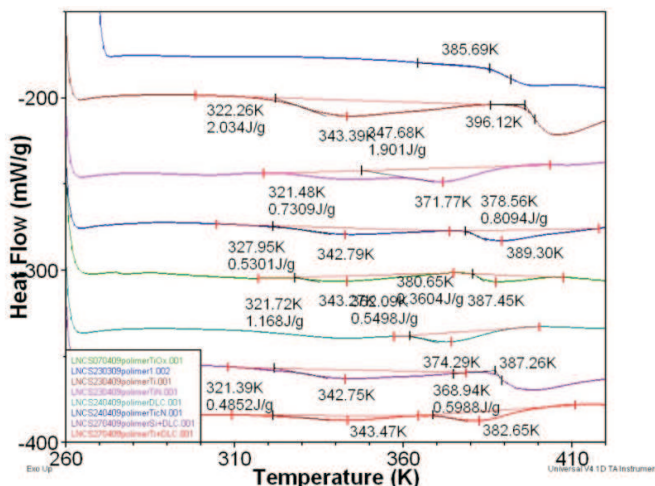


Fig. 2. Heat Flow in the function of the applied temperature in calorimetric measurements

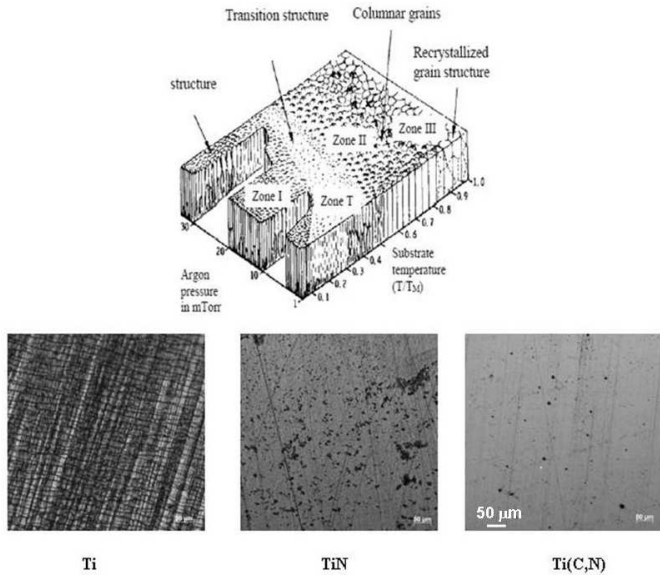


Fig. 4. CLSM examinations of the deposited coatings with the Thornton diagram (Ref. 6)

Scanning Acoustic Microscopy SAM. The Scanning Acoustic Microscopy (SAM) is a technique which enables to investigate whether the material has been uniformly or non-uniformly deposited [6–9]. The only problem which was observed was associated with Ti. The other coating materials were deposited properly. In the case of Ti(C,N), a visible effect of the residual stress concentration was seen by the isolines appearance (Fig. 5).

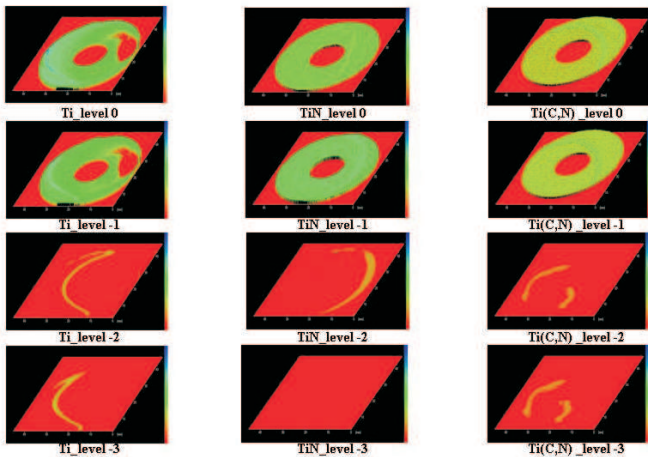


Fig. 5. SAM examinations of the deposited coatings on different thickness levels, starting from the surface to bottom of coating at thickness step of about 7 nm; thickness of coating about 20 nm

3.3. Microstructure analysis. The intended target of the work was to elaborate materials which would not change the properties of the PU substrate but also effectively separate it from the tissue.

The transmission electron microscopy TEM and the high resolution electron microscopy HREM was used to study the microstructure of the thin foils prepared from the film cross-section, using the focused ion beam (FIB) technique. The ex-

aminations focused on the study of the adhesion of the coating to the substrate and thus, the quality of the bio-material.

The microstructure of Ti presents columnar character which proves the late mechanism of the thin film growth (Fig. 6). It could result in the stiffness increment of the PU-coating system which automatically would reject such material from the clinical use.

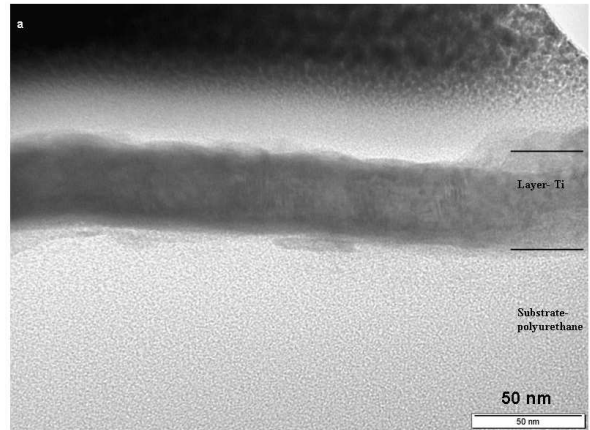


Fig. 6. TEM microstructure of layer cross-section of Ti

Cross-section in the bright field mode of the TiN and Ti(C,N) microstructure, deposited on PU, were similar and exhibit elastic properties of the coating (Figs. 7, 8). It was possible because the proper mechanism of the film growth and close to amorphous structure.

The STEM picture revealed the so called Z-contrast (phase contrast). These pictures were obtained with the use of the detector HAADSTEM – *high angle angular dark field STEM*. The more scattered are the electrons, the brighter the picture. The chemical line analysis was carried out by means of the EDS technique (Energy Dispersive Spectroscopy) across the thickness (Fig. 9).

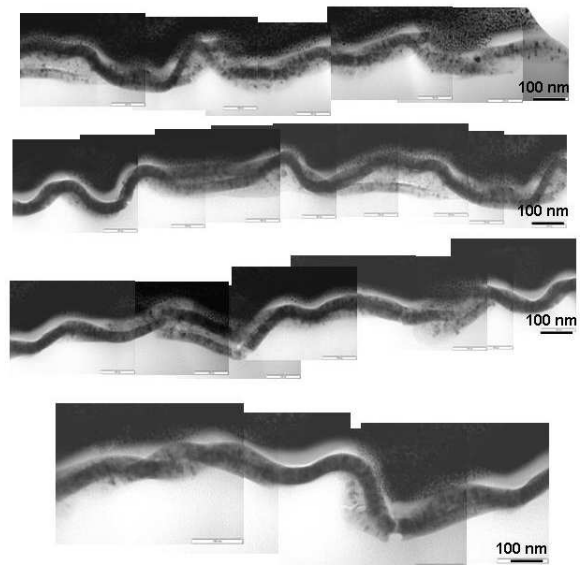


Fig. 7. TEM bright field of TiN/PU coating, cross-section, sets of pictures along the coating

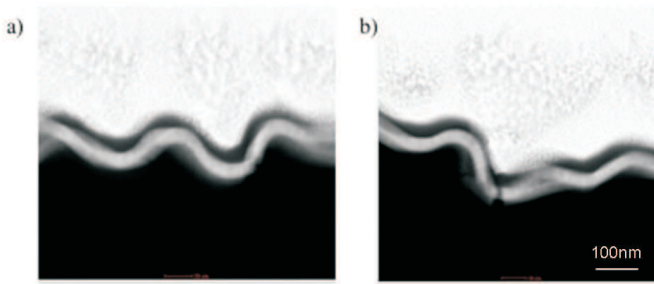


Fig. 8. STEM microstructure of TiN/PU; cross-section

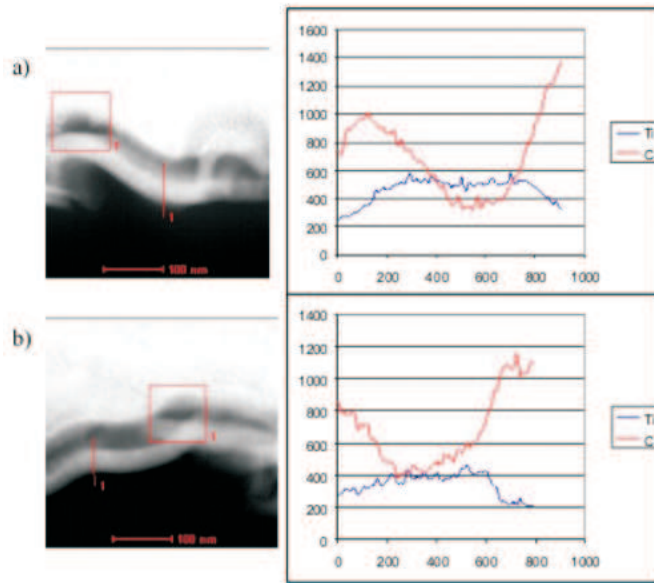


Fig. 9. TEM/EDS line chemical analysis of the Ti(C,N)/PU coating

4. Preparation of the cell culture

4.1. Cell culture. For the study of the cell–material interactions, the mouse fibroblast cells line L929 from the ATCC collections were used. The cells were cultured in “Medium 199” supplemented with 10% FCS (Fetal Calf Serum) and with antibiotics streptomycin and penicillin (Sigma) at 37°C with 5% CO₂ in a humidified incubator (Haereus). The media were changed every 2 days. The cells were cultured in 25 cm² culture flasks. The media were changed every 2 days. When the L929 cells in the culture had grown to 75% confluency, the cells were ready to use for the cytotoxicity tests.

4.2. Seeding procedure. Before the tests, the samples were subjected to the sterilization process using ethylene oxide. Afterwards, they were ventilated for 2 weeks to remove the rest of ethylene oxide. Before seeding, the cells were harvested from the culture dishes using 0.05% Trypsin/EDTA solutions and resuspended in the culture medium (Medium 199 supplemented with 10% FCS, 1% L- glutamine) to the concentration of 5×10^6 cells/ml.

The tested materials were cut into squares and placed in 24 wall culture dishes. To ensure a complete contact between the samples and the wall, the samples were pressed with plastic rings. Then the pellet of the resuspended cells were plated

on the material surface with the density of the cells of around 10 000 cells/well. After 30 min. of incubations, the culture media were completed to a total volume of about 3 ml/well. An empty well with fibroblast cells was used for control purposes. The materials with the cells and the control were cultured at 37°C with 5% CO₂ in a complete medium (Medium 199 supplemented with 10% FCS, 1% L- glutamine) for a total of 24 h.

4.3. Fluorescent microscopy. In order to observe the changes in the cell viability and morphology after the cell material interactions (attachment), we applied a fluorescent microscopy. The viability of the cells was analysed with the use of the Live/Dead dye Fluoresceine Diacetate (FDA) and the Propidium Iodide (PI). The viable cells stained green with FDA and the non-viable cells stained red with PI.

5. Cell-material interaction

5.1. Cell-material interaction under hydrodynamic conditions (Radial detachment test). The radial shear flow test was used to determine the efficiency and the kinetics of the cell detachment from the plain, as well as the modified surfaces as a function of the applied shear stress [2, 3, 10]. The investigated materials were considered in the particular conditions described above. Depending on the material, a different exposition time was necessary. The results of the cell rate detachment under the applied shear stress are presented in Fig. 10.

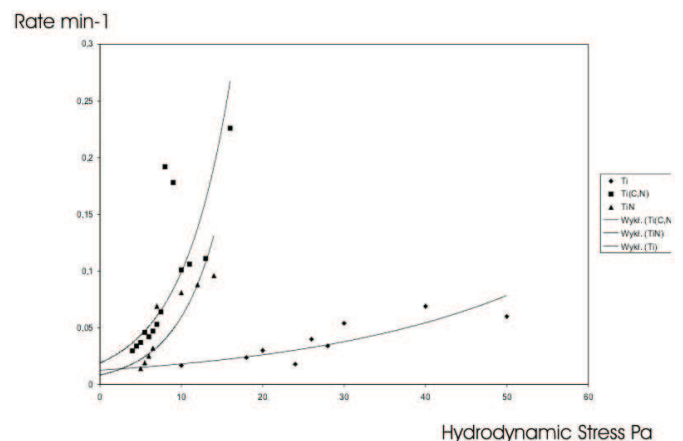


Fig. 10. Cell rate detachment under the applied shear stress

TiN exhibits a low cell material interaction. The detachment rate for titanium carbonitride with a low carbon content was over estimation, thus it was not presented in the diagram. The values of the threshold stress confirm the observations of the cell distribution. The lowest value 3 Pa typical for TiN, the highest for TiC_xN_y(highC) with a high carbon content 7 Pa. The function extrapolation for the 0 Pa hydrodynamic stress revealed a spontaneous cell detachment – rate 0.02 [1/min⁻¹] for Ti(C,N) and 0.008 [1/min⁻¹] for TiN [2, 3, 10].

The cell detachment test gives an overview of the cell behavior and the shear stress which could appear between

the cell and the biomaterial. The biomaterial design requires biophysical tests, as well as biocompatible tests which would illustrate the biological response of the biomaterial to the biological cell. In this case, the tests are performed in static conditions.

5.2. Cell-material interaction in static conditions (biocompatibility testing). The presented chapter describes the biocompatible tests performed on the investigated materials in static conditions. Two important aspects of the biomaterial design were considered – the stoichiometry and the phase composition modification with an introduction of carbon. Another cell type was selected for the experiments. To measure the biocompatibility, fibroblasts were applied.

The stoichiometric titanium nitride is known as a biocompatible material. The lack of stoichiometry could result in a negative influence on the surrounding tissue. In order to examine this problem, both groups, the biocompatible and the non-biocompatible titanium nitride, were studied. Figure 11 presents the result of the biocompatibility tests of the example material. For this purpose titanium nitride (TiN) was considered.

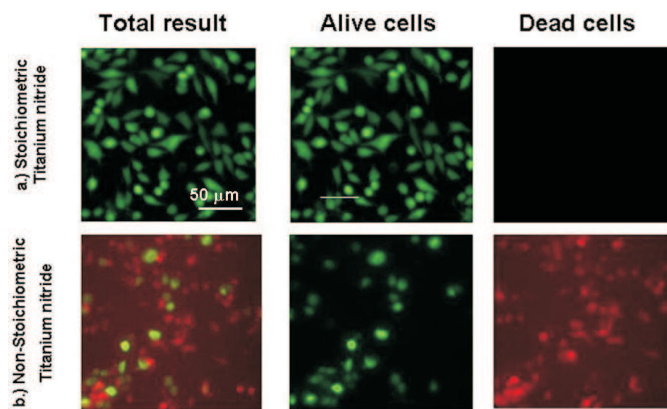


Fig. 11. Material biocompatibility: a) Cells reaction on stoichiometric titanium nitride; b) Cells reaction on non-stoichiometric titanium nitride

The experiments revealed that the cells exhibit a high biocompatibility on the surface of the fully stoichiometric TiN (Fig. 11a). There was a visible growth in the cells and a spread across the surface and no influence on the cell death was detected. The cells revealed individual focal adhesions.

The cell function is also related to the cell shape [1]. The cells grow when spread, die when fully retracted, and differentiate into capillary tubes if maintained at a moderate degree of extension, while also forming cell-cell contacts (e.g., on thin micro patterned lines).

On the non-stoichiometric titanium nitride, the cells demonstrated round shapes, typical for the cell death (Fig. 11b). Over 50% of the deposited cells were directed to the death path. The remaining live cells did not spread on the surface.

As presented in Fig. 11 the stoichiometric titanium nitride exhibits good biocompatible properties. When design-

ing a new biomaterial, a phase composition modification by means of carbon was considered. A comparison of the biocompatible results for the stoichiometric titanium nitride, as well as for the titanium carbonitride with a low and high carbon content is presented in Fig. 12.

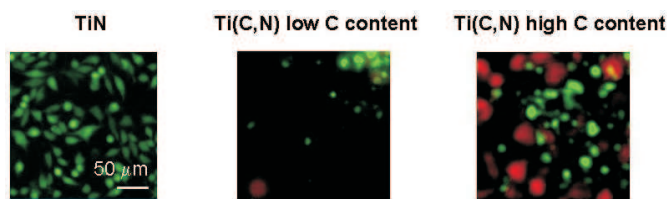


Fig. 12. Biocompatibility of titanium nitride, titanium carbonitride with low and high carbon content

6. Discussion

A development of novel, low thrombogenic materials is of central importance. The presented work focused on the processing of the coating with an improved biocompatibility. The microstructure examinations by means of highly specialized methods tested both the quality of the surface morphology from the point of view of its homogeneity and the coating adhesion to the polymer substrate on the cross section.

The intended target of the work was to elaborate materials which would not change the properties of the PU substrate but also effectively separate it from the tissue. The polymer exhibits the change in stiffness in the long term clinical application thus the need of the surface modification.

Optimisation of deposition parameter has been established from the point of view of chemical composition and surface morphology as well as satisfactory adhesion. Coatings have been fabricated on thermo-sensitive polymer substrate by application of hybrid method. It was necessary to investigate the thermal effect which could influence in the physical-mechanical parameters changes. It was found that polyurethane is thermally stable. The first effect was observed over 100 deg.C. One can conclude that the process of layer deposition does not influence the polymer degradation. As the argument against is that the additional endothermic effect appeared and the temperature ranges of the glass transformation. This aspect is still under consideration.

A measurement of the strength of the bonds between the biomaterials and the cells is a major challenge in cellular biology, since it allows for the identification of the different species in the adhesion phenomena, thus the cell-material interaction was under examination by means of the dynamic test of the cell detachment. The biocompatibility was studied in the static test. As model cells, fibroblasts were taken under consideration. It was observed that it is highly important to focus on the proper phase composition and the stoichiometry of the material coating. The biocompatibility tests revealed the negative effect of the loss of stoichiometry, which was shown especially for titanium nitride.

The cell- material interaction was investigated using cell detachment essay. The cell detachment test gives the overview about the cell behavior and shear stress which could appear

between the cell and the biomaterial. The lowest cell material interaction was found for stoichiometric titanium nitride. There is still opened question which is pointed out by the investigators over the world. Should we deal with low cell material interaction or rather consider their strong influence on the substrate? When the problem concerns the thrombus effects elimination of the answer is simple- no interaction. When one would like to elaborate more sophisticated materials then the other biological phenomena should be analyzed and the answer is not so simple. Biomaterial design requires the biophysical tests as well as biocompatible tests which would illustrate the biological response of the biomaterial into the biological cell. In this case the tests are performed in static conditions. Stoichiometric titanium nitride is known as biocompatible material. The lack of stoichiometry could result in a negative influence on the surrounding tissue which was proved by the fluorescence observations. Cell – material interaction in static conditions revealed stoichiometric titanium nitride as biocompatible material and this behaviour was damage by non-stoichiometry

7. Conclusions

The performed examination allows to conclude:

- Titanium and carbon based coatings have been fabricated on thermo-sensitive polymer substrate by application of hybrid method due to the RT crystallization.
 - Deposition parameter optimisation has been established from the point of view of chemical composition and surface morphology as well as satisfactory adhesion.
 - Application of the radial detachment test determines the efficiency of the cell detachment as a function of the applied shear stress in order to investigate cell-material interaction.
 - Titanium nitride exhibits low cell–material interaction which increased with the carbon introduction into the Ti(C,N) phase.
- Cell-material interaction in static conditions revealed stoichiometric titanium nitride as biocompatible material and this behavior was damaged by non-stoichiometry.

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