1. Introduction

Cardiovascular disease is one of the greatest threats to public health. In Poland about a million people suffers from heart problems. Artificial heart transplant allows the patient to wait for the transplant. It can also allow for regeneration of one’s own heart or in the future, replace the natural heart permanently [1]. Polish achievements rank in the world leaders. Domestic demand can be assessed from several hundred to several thousand. Polish family includes extracorporeal cardiac prostheses, heart pulse prosthesis ReligaHeart® EXT, partially implantable heart assist pulsed pump ReligaHeart® IMPL, partly implantable centrifugal pump and a completely implantable cardiac support pump ReligaHeart® TOTAL.

The paper presents the development of methods of surface modification dedicated to cardiac support system. Implementation of the topic of materials in contact with the blood began with the preliminary researches to long-term national project, “Polish Artificial Heart”. As part of the ongoing tasks the polymer surface modification was prepared and elaborated. Thin, metallic coatings were optimized to improve blood flow and minimize the blood clotting effect as well as inhibit the immune response.

A progress in the field of cardiac support systems is attributed through the use of advanced material solutions. The best solutions, however, to design the appropriate blood contacting material, could be found in nature. The paper presents new biomimetic solutions for the construction of cardiac support chamber. Depending on the place in the construction of the pump, various problems associated with the direct influence of artificial devices for life processes of the surrounding tissue occur.

The project “Polish Artificial Heart” created new problems, which resulted in new projects of the portfolio of projects concerning materials in contact with blood. The first milestone was the development of metallic coatings based on titanium, carbon, and nitrogen with the elastic effect allowing to withstand the strain of the polymer substrate. In the next step of the surface modification the concept evolved to the structure of the cell niches. These structures have influence on the controlled differentiation and cellular activation. They are adjusted for the effective capturing progenitor cells from the bloodstream.

Simultaneously, for the pneumatic systems the structure of the extracellular matrix-like are developed based on synthetic materials in the form of thin coating. The multilayer polyelectrolyte films (PEMs) seem to be promising coatings to simulate the structure and behavior of the extracellular matrix. PEMs constructed through Layer by Layer deposition of oppositely charged polymers have become powerful tools for tailoring biointerfaces. They are interesting for biomedical applications as thin coatings on artificial cardiovascular prostheses or as coverage for biosensor electrodes. The optic transparency, easy deposition and quantitative response character of polyelectrolyte multilayer enhance the applicability of nanoparticle array based nanosensors in chemical and biological sensing. Finally to improve its bioavailability the polyelectrolyte complex nanoparticle was proposed as the drug livery carriers [2-
2. Materials and Methods

2.1. PVD thin film deposition

The deposition of the titanium and carbon based coatings was performed by physical vapor deposition (PVD) (for a detailed description see [5]). Before deposition, the substrates were cleaned ultrasonically. The vacuum chamber was pumped down to at least $4 \times 10^{-3}$ Pa. Titanium coatings were grown in argon atmosphere. Carbon based coatings in acetylene flow conditions. Growth of all coatings in different thicknesses from 5 nm to 0.5 μm was achieved at room temperature (25 °C) with less than 5 °C heating during deposition in plasma. Magnetron sputtering was performed from highly unbalanced rectangular sputter targets (electrographite, 99.8% purity) in Ar atmosphere (5x10^-3 mbar). The introduction of micro-intrinsic stresses generally influence the macro-intrinsic stresses of the substrate-coating compound. The system always tries to reduce the overall strain energy: If high adhesion of the deposited film prevents delamination and buckling, this leads to surface deformation by wrinkling for deformable compound materials with huge difference of the elastic moduli [6].

2.2. Porous, synthetic coatings

The next stage in the concept of the functional surface fabrication was the deposition of the porous layer [7, 8]. Scheme images of the architecture of the functional layers are shown in Fig 1. The porous coating were deposited as 12 bi-layers of polyelectrolyte, poly-L-lysine (PLL)/ Hyaluronic acid (HA), stabilized with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfo succinimide (NHS). The film coated substrate was put in contact with the EDC–sulfo-NHS solution for 12 hours. The final coating on the top was PLL. Porous coatings were a key part of the surface optimization and they were deposited with the “Layer by Layer” method using oppositely charged polyelectrolytes. The last phase of surface functionalisation was the adsorption of the corresponding protein for endothelial cells. The characteristic protein of the extracellular matrix, fibronectin, was adsorbed to the surface. The fibronectin was prepared with a final concentration of 50 mg/mL [9-13].

2.3. Thin film characterization

Basic studies of the thin film the microstructure of the coatings were performed by using high-resolution transmission electron microscopy (TEM, TECNAI &2 F20-TWIN (200 kV)) and sample preparation based on focused ion beam. For analyzing the compound surface compliance. Surface topography images were observed using atomic force microscopy.

Biocompatibility was considered in aspect of blood-material interaction. International Organisation for Standardisation (ISO) developed a guidance on testing medical materials that have contact with circulating blood [14]. The guidelines does not provide the exact test methods or evaluation criteria. Instead, a list of various applicable references is suggested. The most abundant blood cells are erythrocytes. 40% total blood volume is occupied by erythrocytes. These cells are also the most rigid ones and prone to rupture which can occur in the interaction with mechanical devices. Blood platelets are roughly twenty times less abundant and platelet diameter is only the one fifth of erythrocytes. Blood platelets are critical for vascular homeostasis, as they easily activate in contact with the exposed components of the vessel wall. The primary hemostatic function of platelets could lead to thrombosis.

For endothelialisation analysis, human umbilical endothelial cells (HUVECs) were collected for the experiment to assess the surface impact on endothelialisation. Approximately, 100000–125000 cells were plated in a 25 cm² flask. From each vial, it was possible to prepare 4 or 5 flasks. Cells were resuspended in an endothelial cell culture basal medium mixed with cell growth and survival supplements (bullet kit growth mixture purchased from Lonza, including cell growth promoting serum, vitamins, and antibiotics). Before adding cells, the medium was warmed in a 37°C water bath. Cells were taken from the liquid nitrogen container and placed into a 37 °C water bath for 2–3 min. Under the laminar airflow chamber, a maximum of 1 mL of medium mixed with the added bullet kit. Everything was then pipetted into a 15 mL Falcon tube and diluted to 4 or 5 mL to receive 100000 or 125000 cells/flask, respectively. The resuspended cells were taken in the amount of 1 mm from the Falcon tube and introduced into a 25 cm² cultivation flask. Then, each flask was filled up to 6 mL of medium with supplements.

3. Results and discussion

3.1. Thin films

In the first stages we were focused on the surface modification of the clinically used polyurethane. The work considered thin titanium and carbon based thin coatings. Considering thin film deposition in room temperature one should take into account the mechanism of the thin film nucleation from the gas phase. Thin coating with the elastic effect were deposited on the polyurethane substrate Fig. 2. The advantage of the process was the ability of the thin film growth in room temperature. Polymer substrate would not withstand
the high temperature. The further advantage was lack of the substrate degradation, high adhesion and biocompatibility. High adhesion was confirmed by cross section transmission electron microscopy, bright field mode and tribological analysis Fig. 2.

![Fig. 2. Bright field mode analysis using transmission electron microscopy of the thin coating microstructure from the cross section](image)

A classification for hemocompatibility of tested materials was constructed using functions, where percentage of platelets remaining after the shear stress was presented as a function of: platelets aggregates, platelet-monocyte aggregates, platelet percentage positive for activation marker P-selectin (Fig. 3). TiN and Ti(C,N), these two materials substantially activated platelet markers but had the lowest generation of thrombotic activity related to microparticles.

![Fig. 3. Blood-material interaction, a.) platelets aggregates, b.) platelet-monocyte aggregates, c.) platelet percentage positive for activation marker P-selectin d.) platelet percentage positive for activation marker IIb/IIIa](image)

### 3.2. Niche-like structures

Transition electron microscopy (TEM) observations were performed on a cross-section of the a-C:H 100 nm coating deposited on the polymer. The folds have a size characterized in the micrometre scale. No cracks in the surface were observed (Fig. 4).

Selected area electron diffraction pattern demonstrated the presence of an amorphous structure of the coating. This is important because of the possibility of transferring large values of residual stress. Accumulation of stress in the amorphous structure can cause wrinkling without the danger of surface cracks appearing.

Taking into consideration both activation of platelets and blood during dynamic testing and adhesion of blood cells to artificial surfaces, the analysis enables accurate assessment of blood-material interactions. Activation of platelets and their aggregation in blood collected after dynamic testing and the adhesion of active platelets and leukocytes to surfaces were analysed separately. Analysis of the quality of blood through the techniques of flow cytometry is presented in Fig. 5a and Fig. 5b. Fig. 6 presents the results of the platelet aggregate formation on the materials subjected to analysis. The study focused on two types of aggregates, small and large. Small platelet aggregates were defined as two plates, while large aggregates were defined as more than two plates.

Coatings based on amorphous carbon better enhance the efficiency of endothelial monolayer formation.

![Fig. 4. Electron microscopy analysis of the folded surface. a.) SEM top view analysis b.) TEM cross section microstructure](image)

![Fig. 5. Analysis of the quality of blood through the techniques of flow cytometry. a.) platelet percentage positive for activation marker P-selectin b.) platelet percentage positive for activation marker IIb/IIIa](image)
3.3. Porous, extracellular matrix-like coatings

Synthetic porous coating made it possible to create a suitable environment to rise endothelial cells [15]. Fig. 7 shows the top view of the properly prepared synthetic coating. The surface of the synthetic coating was covered by the endothelial monolayer is shown in Fig. 8. Porous synthetic coating were designed cum certa propositum to simulate the native structure of the tissue. The creation of appropriate and optimal conditions for cell accumulation led to restore the properties of the luminal side of the blood vessel.

The quantities of platelet aggregates PLT AGG% (CD61 +) as a function of PLT% (CD 61+) the materials were classified according to the capacity to form aggregates (Fig. 9). Materials favorable aggregation should be eliminated from further analysis. Materials in the design of tissue analogs showed very good properties. Low potential to aggregate was observed.

Analysis of blood were performed using the dynamic test. After the test the endothelial cell layer was mechanically removed (Fig. 10). Based on the preliminary microscopic examination we found the pro-angiogenic potential of synthetic coatings. Under the layer of endothelial cells vessel-like structures were seen. This demonstrates the probability of additional natural layer of extracellular matrix formation and then network of vessels. Vascular network beneath the cell is very important. Properly shaped vessel can promote the formation of appropriate tissue.

Fig. 6. Platelet aggregate formation on the materials subjected to analysis

Fig. 7. CLSM analysis of the top view of the properly prepared synthetic coating.

Fig. 8. CLSM analysis of endothelial monolayer formed on porous coating. Green- Alexa fluor marked actine cytoskeleton, blue- DAPI marked cellular nuclei

Fig. 9. Material classification- the quantities of platelet aggregates PLT AGG% (CD61 +) as a function of PLT% (CD 61+)

Fig. 10. Proof of the pro-angiogenic potential of synthetic coatings. Vessel like structures observed under endothelial monolayer
The properly formed endothelial tissue would effectively inhibit clot formation and would simultaneously remain for long time.

Synthetic coatings have very good properties for docking proteins, they expressed also pro-angiogenic properties, which favors the formation of the targeted tissue. Under physiological conditions polyelectrolyte coating are unstable. At this stage of research the method described in section 3.1 was used. In this case it was not applied as thin coating. PVD was used to introduce the nanoparticles into the structure of the synthetic thin, porous coating (Fig. 11). This resulted in a stabilization of the material and significant reduction in coagulation as well as immune response parameters. This has been proven by the following functions: platelets contribution [% of all objects] (Fig. 12a), aggregates contribution [% of all objects] (Fig. 12b), leukocytes contribution [% of all objects] (Fig. 12c), surface coverage [%] (Fig. 12d). The final surface modification considered nano and microspheres formation in the structure. The micelle-like shape pockets would allow to introduce anti clot as well as immunosuppressive drugs (Fig. 13). The feature of release would be used to control the drug release with time.

Materials which have an application in medicine should be designed according to their exact implantation starting from experimental research up to clinical development. The structure of the material in the form of structure of the tissue seems to be most appropriate. Natural is the most bio compatible material. The paper presents three significant milestones that have been achieved. An effective coating based on titanium was designed and adjusted as a polymer-blood barrier. The ceramic coating expressed the elastic properties by application of a suitable mechanism of nucleation from the gas phase. Another milestone was associated with reproducing the surfaces of micro- and nano-environment for progenitor cells captured from the flowing blood. Suitable surface topography allowed the uptake and controlled cell differentiation. The surfaces in the form of wrinkles restored the structure of cell niches. Folding of the surface was possible by application of modern techniques based on physical vapor deposition. The surface folded without the risk of delamination. Another milestone was the modification of the surface in the form of porous, synthetic structures. This layer was the most similar considering the structure and features to the extracellular matrix. The problem is leaks with its stability. It has been shown that the introduction of nanoparticles of titanium and silicon in its structure improves the stability.

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