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R. MAJOR*#

BIO-INSPIERED BLOOD-CONTACTING MATERIALS ELABORATED FOR THE HEART ASSIST SYSTEM

INSPIROWANE BIOLOGICZNIE MATERIAŁY DO KONTAKTU Z KRWIĄ DEDYKOWANE DLA SYSTEMU WSPOMAGANIA SERCA

I dedicate this work to my Father, who is also my Mentor.
He has enabled me to execute my professional passion.

The paper presents the main achievements of the author on the development of blood contacting materials. The main objective of the work is to elaborate materials dedicated for the heart support systems. Appropriately designed biomaterial surfaces enable fully controlled cellular differentiation, proliferation, and even restoration of the tissue structure on solids. The paper presents two approaches to modify the surface, which can control the life processes of tissue. The first solution considers the topography in the form of cell niches. The main objective of the study is a modified surface of thin films deposited on the polymer substrate constituting the microenvironment for the cells caused by residual stress and optimized stiffness of the surface using the plasma methods. The research hypothesis was the plasma surface modification method generating a controlled contribution of residual stress in the coating affect the surface topography in the form of nano- wrinkles similar to the niches in the tissue environment. Topography and stiffness of the surface coating allows the targeted cellular differentiation. The properly formed surface topography effectively inhibits blood clotting processes. The second solution considers implementation of self-organizing feature of extracellular matrix like coatings and selective cell mobilization. The multiscale analysis and phenomenologic description were performed to experimental research. For this purpose, the deposition method was based on electrostatic interactions in polyelectrolytes. This type of cell-polymer structure imitate the native structures.

Keywords: Surface modification, cell-material interaction, tissue precursors, materials for regenerative medicine

Praca przedstawia najważniejsze osiągniecia autora dotyczące rozwoju materiałów do kontaktu z krwią. Głównym celem prowadzonych prac są materiały o przeznaczeniu w komorach wspomagania serca. Odpowiednio zaprojektowana powierzchnia biomateriału umożliwia w pełni kontrolowane różnicowanie komórkowe, proliferację i nawet odtworzenie struktury tkanki na ciele stałym. W pracy przedstawiono dwa podejścia modyfikacji powierzchniowej, które pozwalają sterować procesami życiowymi tkanki. Pierwszym rozwiązaniem są powłoki o topografii nisz komórkowych. Celem głównym badań jest zmodyfikowana powierzchnia materiałów cienkowarstwowych nałożonych na podłoże polimerowe stanowiąca mikrośrodowisko dla wychwytu i kontrolowanego różnicowania komórek uzyskane przez odpowiedni udział naprężeń własnych i zoptymalizowaną sztywność powierzchniową. Ukierunkowana mikrostrukturą i właściwościami powierzchniowymi monowarstwa hamuje procesy wykrzepiania krwi. Drugie rozwiązanie dotyczy powłok o strukturze macierzy zewnątrzkomórkowej. Celem badań jest wieloskalowa analiza i opis fenomenologiczny samoorganizujących się powłok z funkcją selektywnej mobilizacji komórkowej. Jest to nowoczesne podejście na poziomie badań eksperymentalnych, dotyczące wytworzenia materiałów biologicznie kompozytowych i ich kompleksowej analizy z wykorzystaniem metod inżynierii materiałowej i inżynierii biomedycznej. Do tego celu została zastosowana metoda oparta o oddziaływania elektrostatyczne. Tego typu komórkowo-polimerowa struktura imituje struktury natywne tkanek.

1. Introduction

Heart diseases are one of the most important public health problems in the world, with both the highest rate of morbidity and mortality [1]. Social importance of the problem of the heart disease intensified world efforts of medicine and science in the search for new and highly effective treatments for these diseases. The success of many years of research was the introduction of new technological solutions for the treatment of heart diseases. An excellent progress of pharmacological methods influenced the advanced surgical techniques and remote results of treatment. An important aspect in the development of an effective treatment

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of the heart failure was pulsed external and implantable artificial heart, which became part of the process of heart failure treatment as an effective method for prevention of the critical heart failure. A progress in the field of cardiac support systems was attributed through the use of advanced material solutions. The best solutions, however, to design the appropriate blood contacting material, could be found in nature. One of such solution attempt found in literature describes the blood contacting materials, which are characterized by a strong roughness. An influence of the roughness to the biocompatible implant properties, especially when they are introduced in contact with blood, seems to be negative [2]. The main aim to introduce the rough surfaces was controlled, forced formation clots on the material surface, which adhered strictly to it and did not cause congestion. With time, additional effects of blood cells appeared, similar to inflammatory reactions. Heterogeneous layers containing blood platelets, monocytes (a group of white blood cells), macrophages (tissue cells derived from monocytes which main function is to defend the body), foreign giant cells (big cell formed from the merger epithelial cells), lymphocytes (white form blood cells, belongs to the immune system) and hematopoietic stem cell was finally formed. It was proved that such surfaces effectively inhibited the blood clots. Rough surfaces were dangerous due to the formation of a natural layer which was not always reproducible.

In our concept of surface modification for cardiac applications, cell niches like structure were proposed. Cell niches can be simulated in different ways. One of the model was to introduce controlled participation of stresses in the coating structure, which was applied to the substrate of polymer implant used for the cardiovascular regeneration. Modification was subjected to the inner side of tube like elements, mainly connecting cannulas of the ventricular assist system. Partial separation of blood caused by mechanical and structural properties of the surface would enable the natural protein layer formation which will finally slowdown the coagulation process. Scaffolds are manufactured from suitable biocompatible materials which will allow disintegration and absorption in the body. Surface modification of polymers using thin layers allows formation of new features of the materials while maintaining or slightly change physical properties of the polymer [3-5]. The main attention of the surface modification of medically used polymers is improving the stability of the material which is in the direct contact with the tissue. Adhesion of layers to the substrate is one of the most important factor classifying materials for the biomedical engineering. In order to reconstruct the niche like structures, the surface wrinkling is regarded. Reducing the overall strain energy in the layer subjected to compressive stress, so-called surface "wrinkling" appears. The suggested explanation considers a common deformation of the substrate surface and the layer [6]. "Wrinkling" is described as the formation of sinusoidal elevations on the surface which do not cause the loss of adhesion to the substrate. The main mechanism of wrinkling is the substrate or subsurface areas of the substrate with the applied layer uplifting. The reason of the folding of the deposited layer comes from the mechanical instability. This can be compared to the problem of instability of the elastic layer on a flexible substrate medium. In this model, both the base layers and the substrate have a biaxial stress. The result of compressive stress relaxation is the formation of the wavy structure on the surface [6-8]. The layer structure of the cluster with adjustable arrangement of folds leads to relaxation and stress compensation through the process of deformation. The effective area of the surface is larger, which promotes the growth of network parameters. The total disappearance of residual stresses is only possible in some areas. The formation of the corrugated surface of the material is the result of induced stresses during growth of coating. A loss of the crystal structure in the subsurface areas affects the lower density of atoms, hence the weaker binding, which results in easier deformation through a greater flexibility and a lower level of stresses. Theoretical considerations of the folding effect of the soft layer-substrate showed that the folding mainly depends on mechanical properties of the substrate and the interfacial area [6].

The latest world's trend nowadays is tissue analogs. Proofs of these statements are presented in publications [9-18]. There are materials similar to the structure of the tissue. The proposed solution concerned mainly the biologically neutral coatings which means with a minimal impact of activation.

Innovative materials described in this manuscript would enable to redesign construction of the ventricular heart assist. They are mainly dedicated to the ventricle assist device (VAD) ReligaHeart (Fig. 1).

Fig. 1. New pneumatic chamber of the cardiac support system ReligaHeart [1]

The tissue analogue concerns an implementation of porous materials, coatings and a surface nano-functionalization. In this part of the work, the idea of a layered structure of an artificial tissue has been taken from the structure of blood vessels. Blood vessels have three layers. The first tunica intima, the thinnest layer, a single layer of simple squamous endothelial cells, the second, tunica media, the thickest layer in arteries, circularly arranged elastic fiber, connective tissue and polysaccharide substances. The tunica media may be rich in vascular smooth muscles, which control the caliber of the vessel. The third tunica adventitia, (the thickest layer in veins) entirely made of the connective tissue [19]. The second and the third layers are separated

by the another thick elastic band called the external elastic lamina. The blood contacting materials were manufactured in the way to mimic the extracellular matrix (ECM) and external elastic lamina. In simple terms, the proposed solution provided the structural support to the cells. The ECM-like structure play an important role in modulating numerous cellular functions including: cell adhesion, migration, proliferation and differentiation [20-23]. For this reason, the use of synthetic materials, like polyelectrolyte was proposed. Polyelectrolyte multilayers (PEMs) were originally developed by Decher [24] and Hong [25] as the surface coatings using the layer-by-layer electrostatic self-assembly technique [26]. Films are built up by alternate dipping of charged substrates into aqueous solutions of positively and negatively charged polyelectrolytes which adsorb onto the substrate surface.

All the mentioned aspects of material science in the surface modification, find the real application in the heart assist system. The paper presents a summary of our previous works carried out within the framework of the development of materials dedicated to a regeneration of the coagulation system.

2. Materials and Methods

2.1. Niche-like structures

Thin coatings were deposited on a substrate made of clinically applicable in the heart assist system, polyvinyl chloride (PCV) in the form of tube. The goal of applying amorphous carbon (a-C:H) and silicon doped amorphous carbon (a-C:H:Si) coatings was to separate the polymer from the tissue environment and stabilize the final hydrogel layer. The detailed deposition parameters are listed in Table 1 [27].

The industrially-scaled equipment for plasma polymerization of nanoparticles (Diener Electronik, Ebhausen, Germany) is based on a cubic chamber, which is pumped to start pressure of 5 Pa by a dry vacuum pump. The PCV substrates are mounted on a vertical rotating cage between square, vertically positioned electrodes. Gases, argon (Ar) and acetylene (C_2H_2) and precursor, hexamethyldisiloxane (HMDSO) purchased in Sigma Aldrich. Gases were introduced into the chamber from the top and their pressure was adjusted before igniting plasma by the pulsed DC discharge (40 kHz frequency). Gas pressure measurements occurred by a Pirani gauge. Thin coating depositions occurred in the "volume polymerization mode" at pressures of around 50 Pa in the capacitive pulsed DC discharge, whereby gas mixtures of 20 Pa hexamethyldisiloxane (HMDSO, 99.5%, Sigma Aldrich, Austria) as liquid precursor and 25 Pa argon (99.999%, Linde Gas, Stadl-Paura, Austria) were applied. In contrast to the commonly used surface polymerization mode, the higher gas pressure (applying of non-reactive Ar as process gas) leads to higher probability of collisions between ionic species in the plasma and chemical reactions, which initiates nanoparticle formation [28,29]. The whole deposition process was performed at temperature of 32°C for controlled precursor vaporization conditions. Temperature of the substrate cage was not changing significantly (<2°C) during nanoparticle deposition, which was measured by temperature indicator strips.

2.2. Porous, extracellular-like coatings

Polyurethane (PU), which is clinically used in the heart assist systems, was optimized to become a tissue analogue precursor. Porous, ECM like coatings were deposited on flat

TABLE 1

The parameters of coatings deposition (sccm-standard cubic centimeter per minute) [37]

	Pre-treatment (for all Versions)	Undercoating (for all Versions)	Top coating		
			Version A	Version B	Version C
Starting pressure	0.15 mbar	0.15 mbar	0.15 mbar	0.15 mbar	0.15 mbar
Gas-flow	6 sccm Ar	HMDSO (counter = 340); 3 sccm O_2	4 sccm C_2H_2	10 sccm C_2H_2	HMDSO (counter = 340); 4 sccm C_2H_2
Coating pressure	0.27 mbar	–	0.60 mbar	1.10 mbar	0.95 mbar
Coating time	3 min	0.5 min	2 min	2 min	2 min

samples, using "layer by layer" (LbL) method, allowing the construction of porous coatings. The designed new material needed to be biocompatible and biologically stable. In all steps of the polyurethane surface modification, the research activity was focused on maintaining the biocompatible properties of all the levels of the material elaboration. Thus, this work was focused on porous coatings and their biological functionalization. The main idea of the design is illustrated in Fig. 2.

Porous, synthetic coatings were created by "layer by layer" (LbL) technique using electrostatic interactions. LbL allows to create a multi-layer tissue- like structure. The technique is based on the sequential adsorption of successive layers of oppositely charged polyelectrolytes. The reproducibility of the coating plays the important role. Therefore self-designed system was built and adopted to the purpose of the cardiovascular implants surface modification [30]. The optimal scaffold should have a self-organizing properties of activating the appropriate surrounding tissues for the repopulation. The solution of tissue mobilization on the surface has been described in one of our previous work [31].

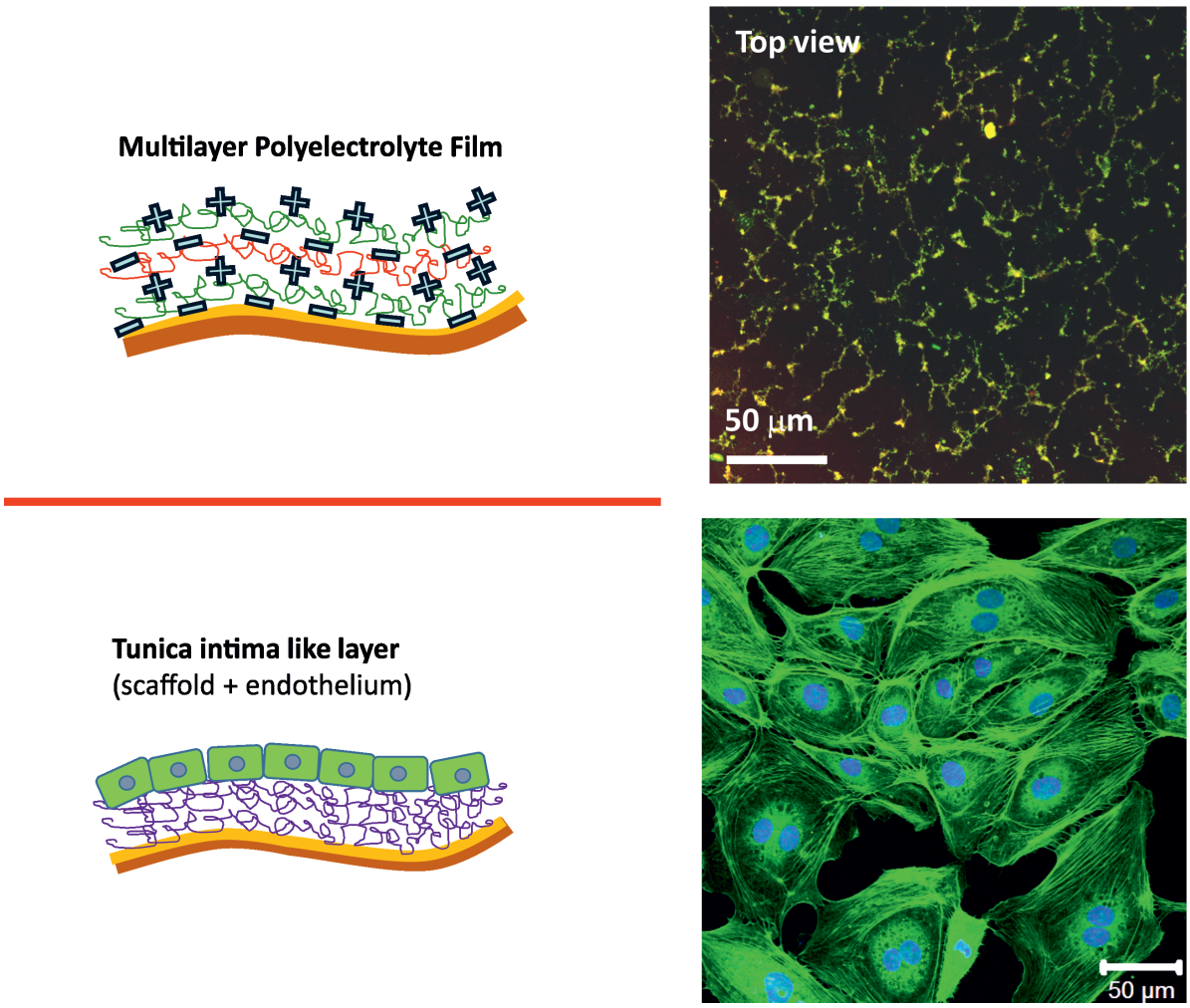

Fig. 2. Synthetic porous coating, type of tissue analogue

In order to ensure a proper adhesion of coatings deposited by the electrostatic method, it was necessary to produce a suitable surface charge. In addition, as a step to prepare the substrate surface for applying the porous coating, the haemocompatibile ultrathin coating were previously deposited. The following samples were prepared: (i) PU with 10-20 nm deposited a-C:H:N coating, (ii) PU with 10-20 nm deposited a-C:H:N coating; with 12 polyelectrolyte (PLL/HA) bilayers, cross-linked and top PLL layer (to reconstitute vascular-like properties the fibronectin was adsorbed under the final HUVEC layer); (iii) PU with 10-20 nm deposited a-C:H:N coating with 12 polyelectrolyte (PLL/HA) bilayers, cross-linked and the top PLL PEG layer; (iv) polystyrene (PS)-reference.

Following information given elsewhere [32, 33], the non-thrombogenic surface for vascular grafts/artificial hearts was prepared in order to consist an intact luminal endothelial cell layer. Porous materials like poly-L-lysine polyethylene glycol and hyaluronic acid (PLL-PEG/HA) in the form of porous coatings were the key issue. In the performed experiments, porous coatings were deposited using the "layer by layer" method using oppositely charged polyelectrolytes. The surface was immobilized by small molecular domains to promote endothelium cells to growth. The PLL-PEG surface was modified using tripeptide Arg-Gly-Asp (RGD) protein sequence that was responsible for binding proteins to cell surfaces. The goal of the RGD incorporation onto the surface was to promote the cell growth on the hydrophilic PEG containing surfaces [32]. In the other papers we suggested the solution enabling the stabilization of the porous coating using silicon nanoparticles [34,35].

2.3. Cell culture

Two cell types were used for these experiments, namely, human umbilical endothelial cells and blood cells. Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza. Each vial had a concentration of 500 000 cells/mL. The cells were stored in liquid nitrogen until use. 100 000-125 000 cells were plated in a 25 cm^2 flask. From each vial, it was possible to prepare 4 or 5 flasks. Cells were re-suspended in an endothelial cell culture basal medium mixed with supplements which ensured the cell growth and survival (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 for 2-3 min into a 37°C water bath. Under

the laminar air flow chamber, a maximum of 1 mL of medium mixes with the bullet kit was added. Everything was then pipetted into a 15 mL Falcon tube and diluted to 4 or 5 mL, to receive 100 000 or 125 000 cells/flask, respectively. The re-suspended cells were taken in the amount of 1 mm from the Falcon tube and introduced into a 25 cm^2 cultivation flask. Then each flask was filled in up to 6 mL of medium with supplements.

2.4. Haemocompatibility test of flat samples

130 µL blood volume was used for each shear stress test and the aliquot of blood was gently mixed for 60 s on the rotational wheel (10 rpm) to prevent sedimentation of blood cells before each of replicates. The details were described by Sanak et al [36]. 5 µL aliquots of blood were gently mixed with fluorochrome-conjugated monoclonal antibodies: 5 µL FITC-PAC-1, 5 µL PE-CD62P, 4 µL PerCP-CD61 in phosphate- buffered saline (PBS) containing 0.2 % bovine serum albumin and 2 mM calcium chloride (final volume 35 µL). The blood collected from above the surface was analyzed by a flow cytometry and the active blood elements attached on the surface were investigated using confocal microscopy (CLSM; Carl Zeiss, Exciter 5).

2.5. Validation of the blood flow chamber enabling the analysis of tube like elements

A dynamic test on blood was designed and manufactured (Fig. 3).

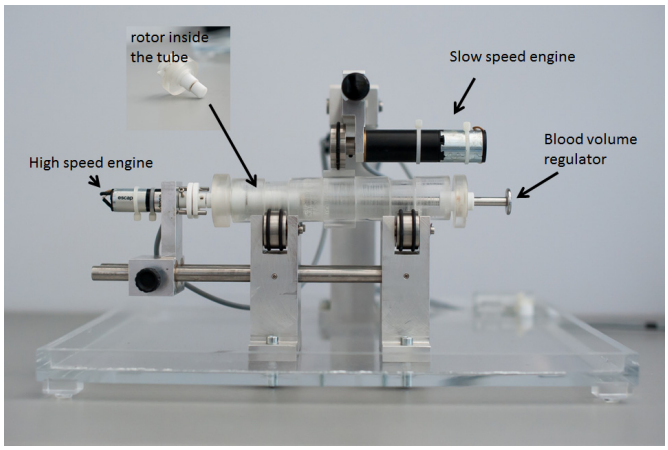

Fig. 3. Self elaborated and validated to ISO 10993 blood flow chamber

The original layout of the test was dedicated to analyse the dynamic haemocompatibility of coatings applied on the internal parts of the tube-like elements. The system was prepared in accordance with the ISO-10933-4 standard. Both the concept and the design were based on assumptions of the commercially available dynamic test on blood (Impact-R). The Impact-R test is clinically used to evaluate the quality of blood flow in hydrodynamic conditions. Important parameters that have to be transferred from the Impact-R test to the aortic flow simulator are following: the surface roughness of the rotor, the distance between the analysed surface and the rotor, the rotor rotational rate and the duration of the single experiment. The newly designed testing apparatus was equipped with two motors that were responsible for two different and important aspects resulting from the features of blood, i.e. the low rotation motor was introduced to prevent sedimentation of blood before the test and the high rotation motor was applied to simulate the arterial flow conditions in the internal (luminal) side of the tube. The imitated blood flow caused shear stresses between the blood cells and the analysed luminal surface, modified with the fabricated thin coatings. The dedicated control unit allowed a precise control of the rotation rate, yielding shear stresses in the range from 0 to 100 Pa. The operational and hydrodynamic parameters, such as the rotation rate and inner diameter of the tube, were recorded by the developed software. The rotational rate was automatically recorded on the computer connected to the device.

Effects of shear forces on inner surfaces of the tubular elements were studied. The number of active platelets and leukocytes on the surface and in the blood collected above the tested samples were evaluated after exposure to flow.

The protocol for analysing blood-material interactions was prepared according to the ISO-10933-4 standard. The test procedure using the aortic flow simulator allows studying the degree of platelet activation and the amount of circulating monocyte-platelet aggregates in the blood taken from above the tested material. For this purpose, the corresponding antibodies were used to communicate with the cellular membrane protein receptors. Measurements of active platelets were performed by analysing the expression of PAC-1 and P-selectin antigens bound to the platelet fibrinogen receptor and the expression of active markers. An analysis focused on the activated platelets was performed. A mixture of antibodies and adenosine di-phosphate (ADP) solutions were prepared with different concentrations to perform the positive control (Table 2) [27].

TABLE 2

Antibodies composition [37]

Name	Composition	Application
A/M	4 µL CD61-PerCP, 5 µL CD62P, 5 µL PAC-1	Estimate platelet activation upon contact with the material (+control)/ Estimate of platelet activation by ADP
ADP1	ADP 0,4mM	Positive control
ADP2	ADP 40mM	Negative control
B	4 µL CD61-PerCP, 4 µL CD62-FITC	Estimate of the amount monocyte-platelet aggregates

The tube-like element having a diameter of 7 mm and a length of approximately 6 cm was placed inside the flow chamber. The active volume was determined using an appropriate holder. The tube-like element was filled with 2 ml of blood for each specimen. Before performing the actual test, blood was gently mixed for 2 min. Then, the test began automatically, being controlled by the software.

2.6. Microparticle analysis

The ZYMUPHEN MP-Activity kit is proposed for the measurement of the in vitro microparticle procoagulant activity in human plasma using automated or manual methods [37]. The diluted assayed plasma sample supplemented with calcium, Factor Xa and thrombin inhibitors was introduced into one of the microplate wells coated with streptavidin and biotinylated annexin V and then incubated. Following a washing step, the Factor Xa-Va mixture containing calcium and purified prothrombin was introduced. Microparticles bind to annexin V and expose the phospholipid surface, thus allowing FXa-FVa, in the presence of calcium, to activate prothrombin into thrombin. Therefore, the phospholipid concentration is the limiting factor. There is a direct relationship between the phospholipid concentration and the amount of thrombin generation, which is measured via its specific activity on the thrombin substrate. The blood plasma was collected through a frank venipuncture on 0.109 M (or 0.129 M) citrate anticoagulant. The plasma supernatant was rapidly decanted (within 2 hours) following a 15 min centrifugation (specified in terms of relative centrifugal force expressed in units of gravity) at 1 500 g, and room temperature. The plasma supernatant was then again rapidly centrifuged for 2 min at relative centrifugal force 13 000 g and room temperature. The plasma was obtained by collecting the supernatant, avoiding contact with the platelet pellet. The plasma was tested within 4 hours or stored frozen at –80°C or below for up to 6 months and thawed for 15 min at 37°C prior to use.

3. Results

3.1. Niche like structures

The coating in the form of cell niches was designed to separate protein from blood and subsequently influence the process of cell differentiation. When exposed to physiological-like conditions, blood components are activated, which may result in platelet and leukocyte adhesion to the biomaterial surface. This process is preceded by coagulation of blood above the surface. Blood coagulation, followed by increasing platelet aggregate density in blood could initiate the thrombus formation. Physiological shear stress promotes all of these processes and induces the immune response of the organism. Shear forces can also destroy platelets, resulting in the microparticle formation.

The cell density on a surface is a relevant parameter determining blood-material interactions. Surfaces are classified basing on the deposition parameters (Table 1 [27]): 4 sccm C_2H_2 and coating pressure 0.60 mbar; 10 sccm C_2H_2, coating pressure 1.10 mbar; HMDSO 4 sccm C_2H_2, coating pressure 0.95 mbar. Results of the number of active blood components in particular leukocytes and platelets are shown in Fig. 4.

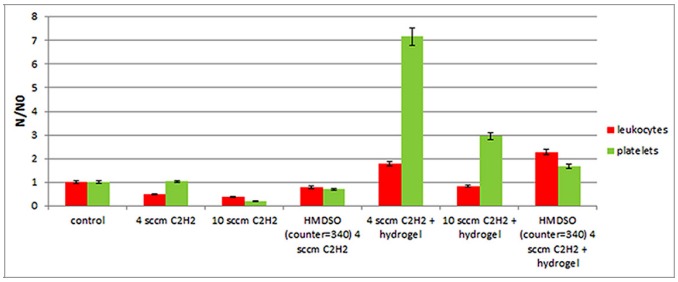

Fig. 4. The relative amount of activated blood cell counts in the function of the coating design

For this case, the active platelets and leukocytes were studied. As a control substrate, the tubular element currently used for the regeneration of the circulatory system was used. A comparison was made between the control substrate and the tubular implants covered with haemocompatible coatings. N_0 is the number of active blood elements on the surface of the control tube substrate, and N is the number of active blood elements on the surface of the tube substrate with modified luminal side. Among all coatings, the smallest number of platelets and leukocytes were observed for sample 10 sccm C_2H_2, coating pressure 1.10 mbar. The decreased immune response and decreased activation of the coagulation cascade were also visible. The detailed analysis of this material is presented elsewhere [27].

Microparticle concentrations in blood after testing were measured (Fig. 5).

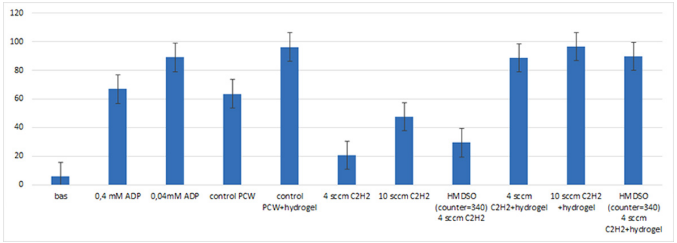

Fig. 5. Microparticles concentration on the function of the coating design

Results revealed a decreased microparticle content in the blood collected above the surfaces of the considered coatings. The highest concentration of membrane fragments was observed for the coating which was deposited with parameters 10 sccm C_2H_2, coating pressure 1.10 mbar.

A tendency to the mesenchyme origin cell interaction was performed on HUVEC cells. Results are presented in Fig. 6 a-c.

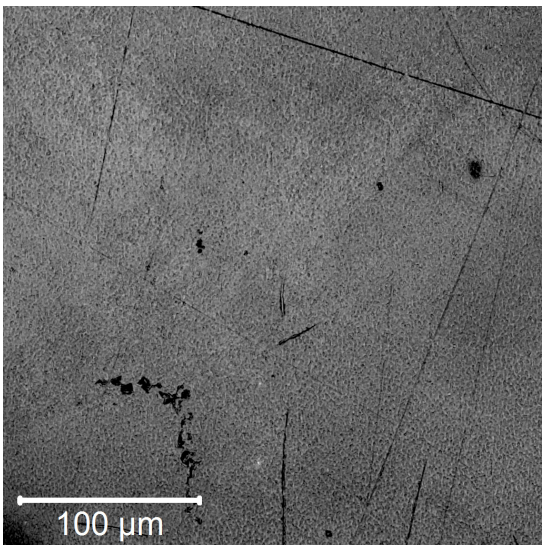

Fig. 6a. A tendency to the mesenchyme origin cell interaction – surface topography

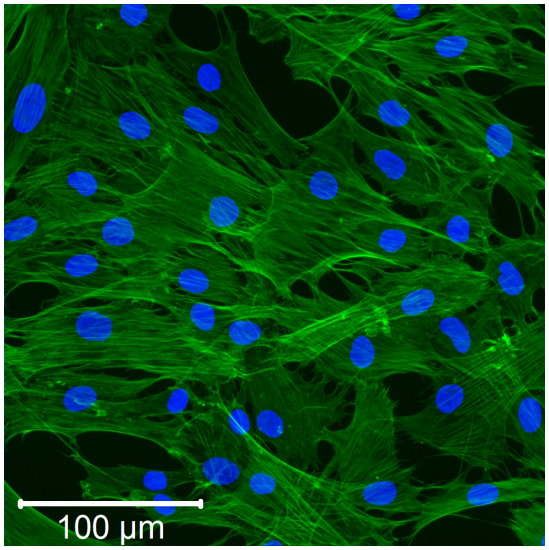

Fig. 6b. A tendency to the mesenchyme origin cell interaction – HUVEC cell proliferation

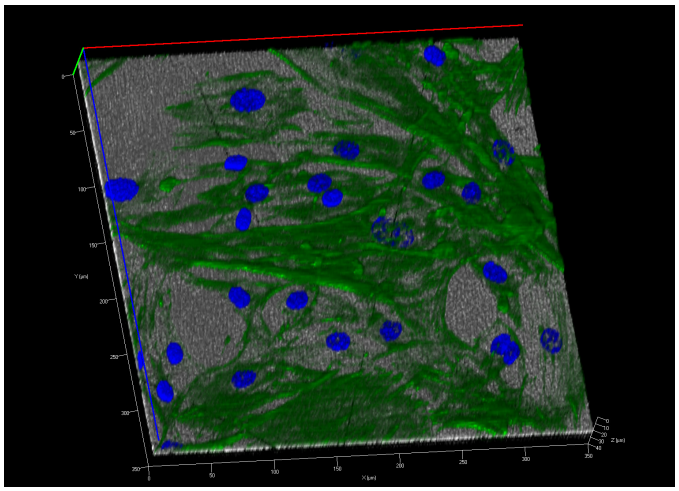

Fig. 6c. A tendency to the mesenchyme origin cell interaction – three dimentional analysis of the HUVEC cell proliferation on PEEK substrate

3.2. Porous, extracellular matrix-like coatings

A group of porous materials, particularly selected synthetic polyelectrolyte coatings was investigated to optimize probability of the natural endothelial layer formation to inhibit the coagulation process. HUVEC cells were deposited on the surface of the selected, porous, extracellular-like material. Cells were generally cultured 3-4 days before being used in the haemo-compatibility tests. Fig. 7 presents the appropriate confluence which was formed before the dynamic blood-material interaction.

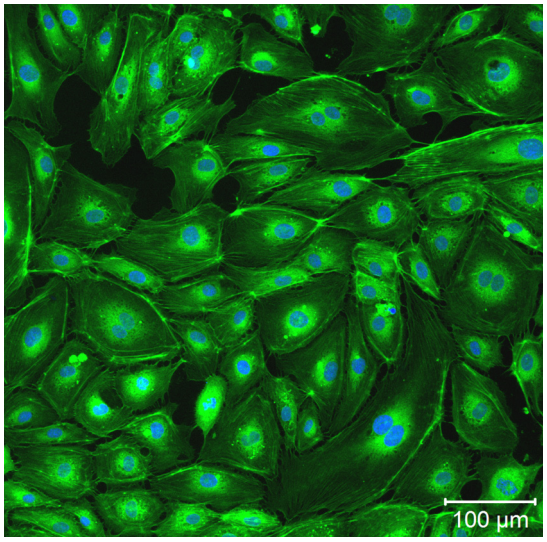

Fig. 7. The appropriate confluence of HUVEC formed before dynamic blood-material interaction

Results are presented as the amount of platelet aggregates formed versus the tested materials. Among the platelet aggregates, the small (2 platelets) and the big one (more than 2 platelets) were taken under consideration. The negative control, the base line (bas) was determined; PS-polystyrene reference material; Ti(C,N)-layer of a-C:H:Ti:N deposited on the polyurethane substrate; PLL+HUVEC-porous 12 bilayers coating PLL/HA deposited on the polyurethane substrate with a-C:H:Ti:N thin coating deposited in the initial stage, cross-linked with EDC and NHS. As the final step PLL was deposited and HUVEC monolayer was formed in the incubation conditions; PEG+RGD+HUVEC-porous 12 bi-layer coating PLL/HA deposited on the polyurethane substrate with a-C:H:Ti:N thin coating deposited in the initial stage, cross-linked with EDC and NHS. As the final step PEG was deposited. The small molecular domains were immobilised to activate mezenchymal origin cells to grow. Finally HUVEC monolayer was formed; ADP- it was positive control. Results are presented in (Fig. 8).

The graph summarizes the haemocompatibile properties of a-C: H:Ti:N, pure polyelectrolyte, and the coating with the endothelium monolayer on the top. All solutions presented the appropriate blood-material interactions in the dynamic conditions. The detailed analysis of this part of work is presented elsewhere [23].

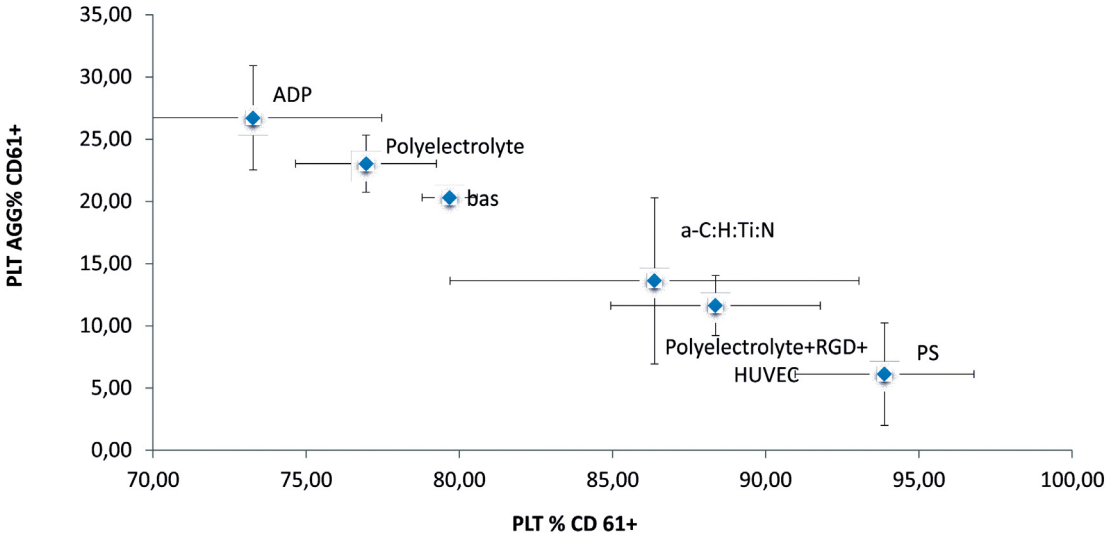

Fig. 8. Platelet aggregate in the function of number of platelets

4. Concluding remarks

The surface functionalization dedicated to blood contacting purposes is a challenging problem for the regenerative medicine. The idea is promising, but there are many difficulties involved in the biomimetic surface design. Structures found in nature seem to be most helpful in decreasing the pain of patients suffering from the heart or circulatory system dysfunction. The coating systems presented in this work attempted to create the surface functionalisation for endothelial cell cultivation.

The surface modification was focused on the haemocompatibility, antibacterial properties and the ability to generate the appropriate surface charge. All these functions were achieved by one type of material, which was applied as the thin coating directly deposited onto the polyurethane substrate. Conventionally used elements with antibacterial properties do not prevent the clot formation. Furthermore, the surface charge played a crucial role in the surface porosity, which was the next step in the novel biomaterial surface design. As described, the surface porosity provided the proper environment for the endothelial cell seeding. We have been able to design, propose, and prepare the appropriate pre-treatment of the surface using the coating of a few nanometers thick. It was observed that coatings in the thickness range of 5-30 nm demonstrated elastic properties. The adjusted coatings were finally deposited with the so-called initial model of thin film nucleation. An idea for the next biomaterial design step was found in nature, as well. The luminal side of blood vessels is covered with endothelial cells. This layer is in the direct contact with the bloodstream and under normal physiological conditions is able to effectively inhibit activation of the coagulation system. This study evaluates the performance of an alternative in vitro dynamic test for haemocompatibility. They showed that surface functionalization with the porous extracellular-like structure decreased the number of activated platelets and aggregates in comparison to non-porous structures and the endothelial cells on the surface. Preliminary studies were carried out under the attempt to create microenvironment for cells in the form of wells, made by laser ablation [28] and they are in the form of wrinkles [29].

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