Nanostructural haemocompatible coatings for the internal side of artificial blood vessels

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Abstract. The main goal of the work was to elaborate low thrombogenicity of surface inside tube-like elements for cardiovascular system support by combination of low-temperature glow discharge and hydrogel coatings to inhibit blood-clotting cascade activation. A large share of amorphous phase silicon was observed in the microstructure analysis. The crystalline elements were uniformly distributed in the amorphous structure. Combination of low thickness, the proper microstructure and density of the coatings provided a highly flexible nature of the whole system. The blood-material interaction was analyzed in vitro in dynamic conditions by using a designed and fabricated novel blood flow simulator. Coatings deposited by the glow discharge expressed good hemocompatible properties. The use of hydrogel coatings did not reduce coagulation parameter. Hydrogel coatings did not improve the hemocompatibility of the surface modified with carbon based coatings. Modification of surface with hydrogel resulted in further increased risk of hemolysis.

1. Introduction
Non-thrombogenic blood-contacting surfaces and appropriate blood flow characteristics are essential for the clinical application of artificial blood vessels. Similarly, providing various implants in the soft tissue decisively improves the quality of patients’ lives. Tube-like devices (cannulas, connectors, and guiding drains) are currently used in many cardiovascular applications, and their blood-contacting surfaces are often modified with heparin, which protects against blood clots. This modification lowers the thrombogenicity of the tube materials. The main disadvantage of heparinisation is the risk of bleeding. Thus, heparinised blood surfaces could be replaced by biomimetic materials and surfaces (regarding pseudointima formation), which better prevent thrombosis and minimise the bleeding risk [1].

Despite the constant development of materials science dedicated to the development of biomaterials, progress in this area is limited. This is because of the need for an individual approach to the patient. Organs and systems that are being replaced, repaired or supported could can react differently in different people for the introduction of an artificial material. A great body of data [2]
describing the material and biological aspects has been accumulated. Unfortunately, these data are not only scattered throughout different types of publications but are also obtained from experimental and clinical studies that vary greatly in accuracy and precision.

The guidelines of the International Organization for Standardization (ISO) ISO-10933-4 determine the degree of hemobiocompatibility defined as the absence of adverse effects of biomaterials with circulating blood [3]. These effects concern cell activation, damage to blood cells, activation of the coagulation system, changes in the composition of the electrolytes and blood proteins, inflammation and immune responses of the body. In the body, clots are formed in response to damage to the vascular wall. The first reaction in this process is platelet adhesion. On the surface, an enzymatic thrombin complex is formed [4]. Commonly used methods for dynamic testing of whole blood mimic the effects of biomaterials in terms of blood flow. The work, that has been done in the context of the present issue, exhibits aspects of the mechanics and biology to study the impact of the artificial surfaces on blood. The work considered several approaches, i.e., hemocompatible coating design and deposition for the internal side of artificial vessels, preparing protocols for the blood-material interaction analysis and determining the appropriate test performance. Important parameters determined in the analysis of activated platelets are pro-caspatse activating compound-1 (PAC-1), evaluation of platelet activation indicator P-selectin and microparticles procoagulant activity in human plasma, in vitro.

PAC-1 is monoclonal anti-platelet antibody, designated PAC-1, which binds to activated platelets but not to unstimulated platelets [5-9]. It is a pentameric IgM that binds to agonist-stimulated platelets. PAC-1 recognises an epitope on the glycoprotein IIb/IIIa (gpIIb/IIIa, αIIbβ3) complex of activated platelets at or near the platelet fibrinogen receptor.

P-selectin functions as a cell adhesion molecule (CAM) on the surfaces of activated endothelial cells, which line the inner surface of blood vessels, and activated platelets [10-14]. P-selectin belongs to adhesion molecules located in the alpha-granules of platelets and Weibel-Pallade'a bodies of endothelial cells. P-selectin mediates processes related to the blood cell rolling on the endothelial surface connecting blood leukocytes to platelets, endothelial cells and other leukocytes to parts of tissue damage or inflammation.

Microparticles (MPs) are small membrane vesicles that are released by activated or apoptotic cells. Microparticles from various origins are present in plasma. They are generated by the activation of blood cells (platelets, endothelium, leucocytes, monocytes). According to their origin, these microparticles can expose a procoagulant phospholipids surface and thrombogenic proteins such as Tissue Factor. Cell membrane microparticles are also called microbubbles. Their size is 0.2-2.0 microns. Microparticles are released into the plasma membrane of all cellular components of the blood. MP release from eukaryotic cells is a normal physiological process that occurs during maturation and aging of the cell [15, 16]. Increased release of MP can occur upon activation of cells, for example, by binding to complement components or immune complexes; cytokines; chemokines; stress factors such as temperature, change in the osmotic pressure, tension generated during flow in vessels; or under the influence of the factors such as improperly prepared material introduced directly into the bloodstream leading to apoptosis. It is now known that increased numbers of MPs are detected in the plasma of patients in the course of many diseases (e.g., deep vein thrombosis, cardiovascular diseases, diabetes and various types of infections, as well as in patients supported with artificial organs).

The release of cytoplasmic granules and platelet mechanical failure results in platelet microparticles. These include fragments of cell membranes in the form of circulating bubbles having a diameter on the scale of fractions of micrometres. Phosphatidylserine-rich phospholipids appear on the surface of microparticles as a result of the enzymatic activity. This phenomenon is a characteristic marker of the functional importance of the microparticles [17, 18]. In addition to the expression of phosphatidylserine, platelet microparticles retain all of the markers that are characteristic of the platelets, such as P-selectin and CD61. Phospholipids of the microparticles form a platform on which the tissue factor complex and the activated coagulation factor X complex are formed with the
participation of calcium ions. The complex is known as the prothrombinase complex. As the consequence of prothrombinase complex formation, thrombin is generated. Thrombin is an enzyme causing fibrin precipitation and clot formation. Clinical studies confirmed that the concentration of circulating microparticles in the healthy organism is less than 10 nM of phosphatidylserine [16,17]. The research hypothesis of the described work concerns the surface modification by the biopassive hydrogel coating stabilised by the physical vapour deposited thin films can effectively influence the minimization of the coagulation processes.

2. Materials and methods

2.1. Thin coating preparation

The surfaces of the clinically used polyurethane AR/LT was modified in two stages. A first phase considered hemocompatible coating, based on carbon deposited by magnetron sputtering. These coatings were supposed to protect the substrate, improve hemocompatibility and stabilize final hydrogel coating. The hydrogel coatings were deposited by dip coating. They were supposed to be the most effective surface for the direct contact with blood.

2.1.1. Headers and footers

The applied coating setup for inner surfaces of tubes is basically comparable to gas discharge lamps (Figure 1). However, the glass tube is replaced by a polycarbonate (PC) polymer tube of 12 mm inner diameter and 30 cm length, which was itself substrate and vacuum chamber together. For easier characterization of the deposited films, flat PC and (100) silicon substrates were placed inside in the center of this tube for some experiments. The setup (Diener Elektronik GmbH, Nagold, Germany), described elsewhere [4] (Figure 1), is afterwards closed on both sides with vacuum flanges, acting as electrodes (cathode and anode). After the evacuation of the tube to 10 Pa base pressure by a fine vacuum pump through the mesh anode, the process gases are delivered to the polymer tube through the mesh cathode.

Figure 1. Applied setup for film deposition in DC discharge inside tubes, whereby the tube wall is the barrier between vacuum and atmosphere (a) schematics (b) image taken during.
The following gas atmospheres were chosen to obtain different types of film materials, consisting always of a base and a top layer. As base layer, a SiO2 coating of about 20 nm thickness was obtained by using 80 Pa HMDSO (hexamethyl disiloxane, Sigma Aldrich, 99.7%) and 20 Pa O2 gas mixtures. This layer provides the required adhesion on the tube substrates for the following top layer deposition, where 3 types of different C (and Si) containing plasmapolymers were applied:

- A: 60 Pa C2H2
- B: 110 Pa C2H2
- C: 35 Pa HMDSO + 60 Pa C2H2

The plasma modification and coating starts in the pulsed 535 Hz DC discharge between the cathode and the anode with a duty cycle of 15% (= percentage of active plasma discharge in pulsed plasma). The supplied voltage signal has a rectangular shape with slightly decreasing voltage in the end of the discharge. The control of the gas supply (O2, C2H2) occurs by gas mass flow meters, the control of liquid HMDSO vaporization by temperature control (25 ± 1°C) and a liquid gas flow meter. Pressure measurement was performed by a Pirani cell and correlated to the applied gas and the precursor vapor types. This pressure was used for the control of the deposition process, allowing a reproducibility of film thickness of ±8%. A reproducible modification with nearly constant chemical film composition and contact angles over the tube length is reached in the so called “positive column” of the discharge, which covers > 90% of the discharge length for 50 cm long tubes [19, 20].

Thin coatings were deposited on a substrate made of clinically applicable in heart assist system, polyvinyl chloride (PCV). 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 stabilise the final hydrogel layer. The detailed deposition parameters are listed in Table 1.

| Parameter         | Pre-treatment (for all Versions) | Undercoating (for all Versions) | Top coating Version A | Top coating Version B | Top coating 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 O2| 4 sccm C2H2           | 10 sccm C2H2          | HMDSO (counter=340); 4 sccm C2H2 |
| 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                 |

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 (Ar, C2H2) and precursors (hexamethyldisiloxane HMDSO purchased in Sigma Aldrich), are introduced in the chamber from the top and their pressure is adjusted before igniting plasma by the pulsed DC discharge (40 kHz frequency). Gas pressure measurements occurred by a Pirani gauge. Thin coating deposition 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 the ionic species in the plasma and chemical reactions, which initiates nanoparticle formation [19, 20]. The whole deposition process was performed at a temperature of 32 °C for controlled precursor vaporization conditions. The temperature of the substrate cage was not changing significantly (<2°C) during nanoparticle deposition, which was measured by temperature indicator strips.

2.1.2. Biopassive hydrogel coatings
The best way for the final surface modification are hydrophilic passive coatings [21-23]. Biopassive surfaces are characterized by low interfacial energy, which results in a decreased number of surface-adsorbed proteins and surface-adhered cells. In addition, the strength of interaction between the protein and the surface is small, which limits the conformational changes occurring in the protein molecules and enables maintenance of their biological activity. The final hydrogel coatings were prepared from polyvinylpyrrolidone (PVP) macro-molecules, which were bonded to polyurethane (PU) substrate, previously coated with carbon based thin films. The coatings was designed to improve the surface hemocompatibility of blood-contacting medical devices. The hydrogel coatings were deposited by dip coating. The hydrogel coating preparation on the luminal side of the tube-like element made of polyvinylchloride. The coating was carried out in 2 stages using dip coating. Two steps meant in this case two immersion in two different solutions. The exact concentrations of the reagents used in the preparation of the hydrogel coatings are listed in table 2. Each process was preceded by flushing in a 5% aqueous alcohol solution and then with water. They were dried to constant weight at 40 °C. In the first stage, the covered tubes were immersed in a solution of hexane containing 5% ethylene glycol dimethacrylate (EGDMA) and 3% cumene hydroperoxide (CHP) for 5 min at 25 °C. In a second step, the samples were placed in an aqueous solution containing 0.1% FeCl2, 1% ascorbic acid (AA) and 5% polyvinylpyrrolidone (PVP) for 15 min at 25 °C. PVP is highly hydrophilic, biocompatible polymer with low cytotoxicity. Therefore, this material is appropriate for many medical applications such as an artificial pancreas, wound dressings, artificial skin, and cardiovascular devices [24-27]. The tubes were immersed in an aqueous polymer solution containing 0.1% sodium dodecyl sulphate (SDS) and were placed on a shaker for 5 min to remove unbound PVP. The samples were washed in water then transferred to phosphate buffered saline (PBS) and incubated at 37 °C overnight. The films were chemically cross-linked by ethylene glycol dimethacrylate (EGDMA).
Table 2. The exact concentrations of reagents used in the preparation of hydrogel coatings. The process consists of two steps: the first step involves reagent diffusion to the interface; during the second step macroradical formation along with their recombination occurs.

| Steps in hydrogel layer formation by dip coating | Chemical compound of solution | Content % |
|-------------------------------------------------|-----------------------------|-----------|
| STEP 1                                           | PVP                         | 5.0       |
| Reagents diffusion to interphase                 | FeCl2                       | 0.1       |
|                                                | AA                          | 1.0       |
| STEP 2                                           | CHP                         | 3.0       |
| Radical polymerization                           | EGDMA                       | 5.0       |

2.2. Microstructural analysis
The microstructure of the deposited coatings was characterised using a TECNAI G2 FEG (200kV) transmission electron microscope (TEM) equipped with a field emission gun (FEG). Cross-sectional analysis of the microstructure was performed for the samples with the crystallised thin buffer titanium carbonitride coating. Thin foils for TEM observation were prepared directly from the location of interest using the focused ion beam (FIB) technique. A detailed description of the TEM microstructure analysis is presented in [28]. A Quanta 200 3D DualBeam was used for FIB preparation. Phase analysis was performed using electron diffraction patterns and was confirmed through identification of high-resolution images (HR-TEM).

2.3. Validation of the blood flow chamber
As part of this work, a dynamic test on blood was designed and manufactured (Figure 2). The original layout of the test was dedicated to analysing the dynamic haemocompatibility of the coating applied on the internal parts of the tube-like elements. The system was prepared in accordance with the ISO-10933-4 standard. The concept and the design were based on assumptions of the commercially available dynamic test on blood (Impact-R) [29]. 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 the surface roughness of the rotor, the distance between the analysed surface and the rotor, the rotor rotational speed and the duration of the single experiment [30, 31].
Table 3 shows the parameters of the individual elements of a commercial assay and their implementation to the aortic flow simulator.

| Impact-R                  | Aortic flow analyzer |
|---------------------------|----------------------|
| The shape of the sample   | Flat disc d=14.4 mm   |
| The shape of the rotor    | Conical               |
| The rotor speed           | 720 rpm               |
| The rotor drive           | The rotor rotates in the magnetic field |
| Time of analysis          | 2 min                 |
|                           | Tube, internal diameter 7 mm |
|                           | Flat                  |
|                           | 72 rpm                |
|                           | The rotor attached to an electric motor |
|                           | 20 min                |

The newly designed testing apparatus was equipped with two motors that were responsible for two different and important aspects resulting from the features of the blood. The low rotation motor was introduced to prevent sedimentation of the blood before the test. The high rotation motor was applied to simulate arterial flow conditions in the internal (luminal) side of the tube. This flow caused shear stress between the blood cells and the analysed luminal surface, which was modified with the thin coatings. The dedicated control unit allowed precise control of the rotation rate, yielding shear stresses in the range of 0 to 100 Pa. The operational and hydrodynamic parameters, such as the rotation rate and inner diameter of the tube, were recorded by developed software (the rotational rate was automatically recorded on a computer connected to device).
The effect of the shear forces on inner surfaces of the tubular elements was 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 test 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. The analysis focused on the activated platelets. A mixture of antibodies and adenosine diphosphate (ADP) solutions were prepared with different concentrations to perform the positive control (Table 4).

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, the blood was gently mixed for 2 min. Then, the test began automatically, as controlled by the software. The applied shear stress can be expressed according to equation [32]:

$$\tau = \frac{M}{2\pi R^2 l}$$  \hspace{1cm} (1)

where $M$ is the torque input by the engine ($M=2.7$ dyne-cm), $R$ is the radius (cm; $R_a \leq R \leq R_b$; $R_a$-radius of rotor; $R_b$-radius of tube) and $l$ is the effective length of the rotor (cm).

The values of shear stress decreased along the radius (Figure 3).

The shear rate can be calculated by using equation (2).

$$\gamma = \frac{\tau}{\eta}$$  \hspace{1cm} (2)

where $\eta$ is the viscosity of blood in 37 °C.

| Name | Composition | Application |
|------|-------------|-------------|
| M    | CD61-PerCP, CD62P, PAC-1 | Estimate platelet activation upon contact with the material (+ control) |
| A    | CD61-PerCP, CD62P, PAC-1 | Estimate of platelet activation by ADP |
| ADP 1 | ADP 0.4mM | Positive control |
| ADP 2 | ADP 40mM | Negative control |
| B    | CD61-PerCP, CD62-FITC | Estimate of the amount monocyte-platelet aggregates |
2.3.1. Blood-material interaction analysis

The blood used in this test was collected from a healthy male volunteer. Aliquots of blood (0.4 L) were activated with adenosine diphosphate (ADP, 20 μM final concentration) for 5 minutes. These aliquots served as the positive controls that were prepared to simulate conditions of maximum platelet activation. Blood samples stored under static conditions were used as a negative control (bas). The blood collected from above the sample was mixed with the antibodies CD61-PerCP, CD62P, and PAC-1 to estimate the platelet activation upon contact with the material. The platelet activation was analysed using flow cytometry.

PAC-1 recognises an epitope on the glycoprotein IIb/IIIa (gpIIb/IIIa, αIIbβ3) complex of activated platelets at the platelet fibrinogen receptor. PAC-1 is a receptor for fibrinogen [33] and von Willebrand factor and occurs in platelet activation. P-selectin (P-SEL) is a cell adhesion molecule (CAM) that accumulates in thrombocyte granules of unactivated platelets. When there platelet activation occurs, rapid translocation of P-selectin from granules to the cellular membrane occurs [34, 35]. P-SEL promotes platelet aggregation by binding platelets with other platelets and with fibrin.

The inner surfaces of the tube-like elements were imaged using confocal microscopy (CLSM; Carl Zeiss, Exciter 5). Before image acquisition, the materials were divided into 10x15 mm sections. Cells adhered to material surfaces were fixed and permeabilised for 10 min with 4% paraformaldehyde (PFA). After washing the samples three times for 5 min with PBS, they were incubated with anti-human CD62P P-selectin antibody conjugated with fluorescein isothiocyanate (FITC), which is a dye that marks platelets with the P-selectin active receptor. After 30 minutes of incubation, the samples were washed three times with PBS and were covered by the human monoclonal antibody CD45 PE conjugated with Texas Red for 10 minutes. This secondary antibody marks active leukocytes on the material surfaces. Finally, the samples were rinsed three times in PBS and were immediately imaged. The cell densities on the surfaces were measured using the “colocalisation” tool of the microscope software (Carl Zeiss Exciter 5 equipped with ZEN 2008). Colocalization analysis is done on basis considering pixel by pixel analysis.

2.3.2. Microparticle analysis

The ZYMUPHEN MP-Activity kit was proposed for the measurement of the in vitro microparticle procoagulant activity in human plasma using automated or manual methods [36-38].

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. When present in the tested sample, 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. 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 at 1,500 g and room temperature. The plasma supernatant was then again rapidly centrifuged for 2 min at 13,000 g and room temperature. The plasma was obtained by collecting the supernatant, avoiding any 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 and discussion

3.1. Microstructure

Microstructural characterisation was performed on the cross-sections of the coatings in bright field observation mode (Figure 4). The phase analysis was performed using selected area electron diffraction patterns (SAED).

![Figure 4. TEM microstructure of the ceramic coating directly deposited on the polymer substrate with discernible two different phases; crystalline and amorphous](image)

The microstructural characterisation confirmed the high elastic properties of the coating. The nanocrystalline characteristic of the coating was confirmed by TEM BF imaging at high magnifications and high-resolution observations (HRTEM) (Figure 5). The crystallite diameter according to the bright field imaging was approximately 11 nm. The high-resolution TEM analysis of the crystalline areas observed in bright field mode showed crystallite agglomerates. The actual crystallite diameter was 7 nm (Figure 4). The high-resolution analysis showed the presence of crystallised elements in the amorphous matrix, which was associated with the initial mechanism of thin film nucleation from the gas phase. Two-dimensional thin film nucleation allows unique mechanical properties, which are unusual for ceramic coatings. The coating that had elastic properties was directly applied to the polymer. Crystalline elements were uniformly arranged along the coating (Figure 5). To fabricate biologically functional coatings, a final top hydrogel layer was applied to the surface of the partially amorphous layer, introducing functional molecular groups. Crystalline components irregularly distributed in the hydrogel were observed.
3.2. Blood-material interactions in the dynamic condition
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 the blood could initiate 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 microparticle formation.

3.2.1. Blood cell response to shear forces and material contact
The cell density on a surface is a relevant parameter determining blood-material interactions. The results of the number of active blood components in particular leukocytes and platelets attached to the surfaces, designated as option “A”, “B”, or “C” (Table 1), are shown in figure 6. The figure shows a qualitative (N/N0) analysis of the impact of blood cells on the tested surfaces. For this case, subjected active platelets and leukocytes were studied. As the control substrate, the tubular element currently used for regeneration of the circulatory system was used. The comparison was made between the control substrate and tubular implants coated with haemocompatible coatings. N0 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 the coatings designated as “A”, “B”, and “C” (Table 1), the smallest number of platelets and leukocytes were observed for sample “B”; therefore, sample “B” appears to be the best solution. Decreased the immune response and decreased activation of the coagulation cascade was visible for sample “B”. Conversion of the immune response instead of activation of the coagulation system was observed. For variant “C”, the number of active leukocytes and platelets increased. The predomination of the immune response over activation was retained.
The relative amount of activated blood cell counts in the function of the coating design.

The final modification of the surfaces involved hydrogel coatings. The final surface modification caused increased activation of platelets and leukocytes (Figure 6). The layers with hydrogel coatings showed worse response in dynamic contact with blood compared with the coatings “A”, “B”, and “C” without hydrogel coatings. Comparing option “B” without a hydrogel layer with the coated option “B” with the hydrogel, a change in the response of the cellular components of blood can be clearly observed. The activation analysis of circulating blood above the analysed surfaces was performed using a flow cytometer. Platelet activation of the PAC-1 active receptor above the surface of option “A”, “B”, and “C” is shown in figure 7. The average amount (MNF) of active platelets with active PAC-1 and P-SEL receptors and an indicator of platelet activation (IPA) were measured. In unactivated endothelial cells, P-selectin is stored in granules called Weibel-Palade bodies, and α-granules are stored in unactivated platelets. Other names for P-selectin include CD62P, granule membrane protein 140 (GMP-140), and platelet activation-dependent granule to external membrane protein (PADGEM). The expression level of CD62 was specified as the binding parameter to determine the ratio of platelet activation. This analysis indicated that the IPA is determined as a percentage (%). The value of P-SEL MNF was the smallest for the sample designated as version “B” among all of the tested coatings. The MNF value for PAC-1 for this coating was the greatest. A hydrogel layer applied on the inner side of the tubular element resulted in a constant level MNF and IPA for PAC-1 receptor (Figure 7), regardless of the coating. The second receptor, whose activity was analysed using flow cytometry, was P-selectin (P-SEL). The result of the analysis of P-SEL shows a similar dependence to the analysis of PAC-1. A hydrogel layer applied on the inner side of the tubular element resulted in a constant level MNF and IPA of the receptor P-SEL (Figure 7), regardless of the coating.

![Figure 6](image6.png)

**Figure 6.** The relative amount of activated blood cell counts in the function of the coating design.

3.2.2. **Microparticle formation**

The microparticle concentrations in the blood after testing were measured (Figure 8). The results show decreased microparticle content in the blood collected above the modified surfaces of “A”, “B”, and
“C” compared with the control sample (control PCV). The highest concentration of membrane fragments was observed for the coating “B”. For the materials with hydrogels, the microparticle concentration was higher compared with the coatings “A”, “B”, and “C”. This result indicates high influence of the hydrogel layer on the platelet destruction process.

4. Conclusions

- The ceramic coatings showed elastic properties. This result was due to the amorphous structure with uniformly distributed crystalline particles. The elastic properties of the ceramic coating depended on the appropriate mechanism of thin film nucleation from the gas phase. Coatings that are utilised for coating implants should not change their mechanical properties and would improve biological properties. This property has been achieved in the present study.
- The coating “B” (110 Pa C2H2) comprising the greatest amount of carbon in the structure, showed high haemocompatible properties. Based on the dynamic analysis of the blood, decreased coagulation processes and immune response were observed compared with “A” (60 Pa C2H2), and “C” (35 Pa HMDSO + 60 Pa C2H2). It is apparent from the carbon content in the structure. As shown in table 1, The coating indicated as “A” has the highest carbon content. The deposition process was performed in the strongest flow of acetylene. Hydrogel coatings are highly hydrophobic. On the surface, an additional layer of water is created, which protects the surface against aggregation processes. Hydrogel coatings are also very highly liquid absorbent, which affects the swelling of the material. In the dynamic tests on the blood, strong haemolysis was revealed, therefore it was found to abandon the last modification of the surface which was hydrogel coating.
- Based on extensive investigations, thin coating were selected to modify the internal parts of the tubular elements for applications in regenerative cardiac surgery.

5. References

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