Modification of human polymorphonuclear neutrophilic cell (PMN)-adhesion on biomaterial surfaces by protein preadsorption under static and flow conditions

MIKE OTTO*, BJÖRN WAHN, CHARLES JAMES KIRKPATRICK
Institute of Pathology, Johannes Gutenberg-University of Mainz, Langenbeckstr. 1, D-55101 Mainz, Germany
E-mail: otto@patho-trier.de

Biomaterials induce a specific reaction after implantation in the human body. This reaction depends on the chemical and physico-chemical properties of the material as well as on the site and type of implantation. We have used a dynamic model, the parallel-plate flow-chamber, to examine the interactions of different biomaterials with polymorphonuclear neutrophilic cell (PMN) and how these interactions are influenced by protein preadsorption. Our results clearly show that for hydrophobic materials, glass and PE, which induce a prominent adhesion of PMN, the mixture of albumin and fibrinogen induces the best inhibitory effect. On hydrophilic biomaterial surfaces, untreated TCPS and PC-coated TCPS, reveal only a minor influence of adsorbed proteins on PMN adhesion because of a primary low adhesive surface for PMN and proteins as well. Human citrated plasma leads only to a slight inhibition of PMN adhesion. On the hydrophobic materials, glass and PE, bovine serum albumin (BSA) had the best anti-adhesive potential with respect to PMN. The coating using phosphorylcholine is an excellent surface modification to prevent PMN-adhesion and protein adsorption. The results of our experiments suggest that investigations under static and flow conditions are also needed to determine the influence of protein adsorption on other relevant blood cell populations, for example, platelets and monocytes.

Introduction

Since the beginning of the year 2000 approximately 1 000 000 vascular prosthetic devices have been implanted worldwide into the human body. A vessel calibre of the order of 3 mm, as well as venous flow conditions, represent limitations of the use of biomaterial-based vascular prosthetic devices without permanent anticoagulation. On the other hand, the demand for an adequate prosthetic device for use in the vascular periphery, for example in coronary revascularization, is a major challenge for biomaterial science.

Biomaterials of a broad spectrum of chemical composition form constituents of all implant devices including artificial organs [1] and include synthetic or natural polymers, metals, alloys, ceramics and other avital substances, including devitalized tissues, which induce a specific reaction after implantation in the human body [2]. This reaction depends on the chemical and physico-chemical properties of the material, as well as on the site und type of implantation. The biocompatibility of a biomaterial is not only the absence of direct or indirect cytotoxicity, but also includes the entire field of biosafety and biofunctionality. The International Standard Organisation (ISO) in the directive 10993 [3] described the methods for biological evaluation of medical devices as a basis for the assesment of biocompatibility. Part 4 describes the selection of tests for interaction with blood and part 5 the tests for estimation of cytotoxicity [4, 5]. These directives include the fundamental principles for the *in vitro* evaluation of blood-contacting devices [4]. The testing models should be appropriate systems, which simulate the conditions of blood contact with the devices during clinical application. Important parameters include the duration of contact, sterile environment, as well as flow conditions. In accordance with the recommendations of the ISO our primary interest focussed on the establishment of a reliable *in vitro* panel as a useful screening tool for testing biomaterials for vascular implant devices to evaluate blood/device interactions under reproducible and well-defined flow conditions, as described before [2–5].

Polymorphonuclear neutrophilic cell (PMN) represent an important cell type in the unspecific immune system [6]. They are involved during the early interaction with a

*Author to whom all correspondence should be addressed.

biomaterial [7], and participate in unspecific immunity, thrombosis, healing reaction and material degradation [8]. PMN are blood cells with the ability to phagocytose and degrade foreign materials in the tissues and circulation, including prosthetic vascular devices. During the activation of PMN different chemotactic substances are released which modulate the specific and unspecific immune response as well as the thrombotic reaction, for example mediated by cathepsin G and reactive oxygen species [9], by different proinflammatory cytokines, for example TNFα and IL1β or chemokines, such as IL-8 [10]. On the other hand, the procoagulant situation induces changes of the PMN itself, which can be measured as a decrease of PMN membrane fluidity and an increase of the PMN cytosolic Ca^{2+} content in patients with deep venous thrombosis [11].

The preadsorbed proteins on the biomaterial/blood interface will modify the adhesion of PMN during the initial phase contact. Protein preadsorption induces a significant modification of both PMN adhesion and activation. The surface modification using blood-derived proteins may be useful to reduce the early negative effects of PMN activation. The mobility and concentration of dissolved plasma proteins are so far in excess of those of platelets and leukocytes that the surface of biomaterial devices must become coated with protein before platelets and PMN can reach it [12]. During exposure of blood to an artificial biomaterial surface in biological systems there is a lag period of approximately 1 min before PMN potentially adhere. During this time, depending on many conditions, a deposition layer of plasma proteins varies in its composition.

The aim of this study was to characterize the influence of preadsorption of different proteins on various polymer surfaces on the adhesion of PMN under static as well as under low and high flow conditions to simulate the situation in the arterial and venous peripheral circulation.

For the experiments we used materials with different hydrophilicity to investigate the effect of material surface properties on the protein-dependent PMN-adhesion.

Materials and methods

PMN-Isolation

Freshly obtained citrated human blood was mixed with phosphate-buffered saline (PBS) at a concentration of 20 mM at pH 7.2, in a ratio 1 : 1 (v/v). The blood/PBS-mixture was carefully applied to Ficoll (Histopaque 1.077, Sigma, Germany) in a ratio 1 : 1 (v/v) and centrifuged at 1000 g for 25 min (slowest possible acceleration and deceleration) at 4 °C. The PMN/erythrocyte-phase was removed and mixed with RPMI 1640 (without phenol red, Sigma) in a ratio 1 : 1 (v/v). 45 ml of the mixture were then added to 5 ml Dextran 4% (MG 500.000, Sigma, USA), mixed and incubated at 37 °C for 45 min. The PMN phase was aspirated and washed using PBS followed by centrifugation at 390 g for 5 min. Erythrocyte contamination was minimized by lysis in a buffer containing 9% NH_4Cl, 1% $KHCO_3$ and 0.037% EDTA at pH 7.29. Finally, after centrifugation at 390 g the cell pellets were washed and resuspended in medium RPMI 1640 (Sigma) without phenol red. PMN were counted using a computerized cell counting system (Schärfe, Germany). The PMN concentration was set at 10^6 cells/ml in RPMI 1640.

The analysis of vitality and of the rheological properties of the PMN suspension was completed as previously described [13, 14].

Biomaterials

For static experiments we used 96-well-plates made of standard tissue culture polystyrene (TCPS; type 3072, Falcon, London, UK). E. Campbell (Biocompatibles Ltd., London, UK) performed the covalent binding of Phosphatidylcholine (PC). For the flow experiments we used TCPS of the same quality as used for static experiments.

Polyethylene (PE) from the concerted action EUROBIOMAT-Hemocompatibility, i.e. medical grade according to the regulations of the USP XXI, US-Federal Standard 209B, and DAB 9 were the standard reference in our experiments. Chamber size-adapted biomaterial samples were washed for 10 min with the help of a strong detergent solution (2% RBS 35; Perstop Analytical, Netherlands) in distilled water with ultrasound. After sonification the materials were rinsed in distilled water in order to remove the detergent. To obtain stable conditions in the chamber systems without elastic tension effects, the biomaterial films (PE) were fixed on standard glass slides (Menzel Glas, Germany) using double faced adhesive tape (Hartmann, Germany). For the flow experiments the glass slides without any coating were used as ''positive'' control [13].

Protein preadsorption

For static and dynamic experiments the biomaterials were preadsorbed with fibrinogen (FG), bovine serum albumin (BSA), a mixture of fibrinogen and bovine serum albumin (FG/BSA) or human citrated plasma (CP).

The proteins were used in the following concentrations in PBS (PBS, 20 mM, pH 7.2):

1. Fibrinogen (FG, fraction I, type III, Sigma Germany) – 3 g/l
2. Bovine Serum Albumin (BSA, fraction V) – 43 g/l
3. FG + BSA (FG/BSA) – 3 g/l + 43 g/l
4. Human Citrated Plasma (CP) = platelet poor plasma

The CP was prepared from fresh citrated human blood extracted by venipuncture technique. The blood was centrifuged for 20 min at 600 g and 20 °C. The platelet poor plasma in supernatant was separated from the cell pellet and stored at 4 °C.

For static experiments 250 µl protein solution were added to each well of a 96-well-plate for 240 min on a shaking incubator at 37 °C and a shaking frequency of $35\,min^{-1}$.

After formation of a self-assembling protein layer during this time the protein solutions were removed and

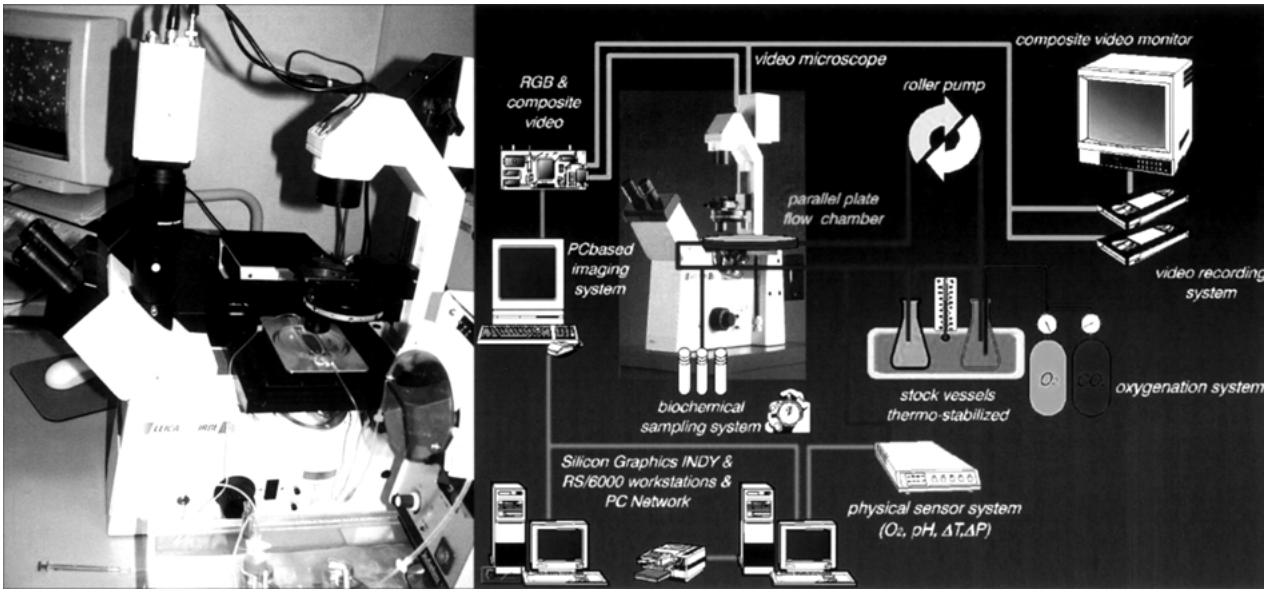

Figure 1 Flow chamber on the temperature controlled microscope table (left); schematic diagram of the flow chamber system (right).

the plates were washed twice using PBS. The plates were then immediately used for the adhesion experiments.

For dynamic experiments the prepared slides were incubated with the protein solutions in Quadriperm-dishes (Coulter, USA). The dishes were filled with 5 ml protein solution for 240 min at 37 °C in a shaking incubator with a shaking frequency of 35 min^{-1}. After preadsorption the slides were washed twice in PBS and stored for a maximum of 12 h in a humid chamber for use in the flow experiments.

Static experiments

For static experiments each preadsorbed or control well was allowed to interact with 200 µl of PMN-suspension. The wells were incubated for 30 min at 37 °C. After incubation the cell suspension was removed and each well was carefully washed with PBS two times. After washing the PMN were fixed using 1% formaldehyde for 2 h. After fixation the wells were washed with PBS, followed by naphtol-ASD-chloroacetatesterase histochemistry (SOP Institute of Pathology, University of Mainz). After the histochemical reaction all wells were filled with glycerine gelatine (Sigma, Germany). The number of adherent PMN per mm^2 was determined using a conventional light microscope Leica DMRB (Leica, Germany) with a JVC CCD-camera (TK-1381, JVC Japan). All images were digitized using a Hauppauge frame grabber board in a PC with MS Windows 2000 and the imaging software ImageTools 1.27.

Flow chamber system

The flow chamber system consisted of three basic modules: the parallel plate flow chamber, the flow loop and the image analysis system. The flow chamber, consisting of a polycarbonate-based frame, contained two parallel plates, one polycarbonate surface and an opposite frame that consists of the glass slide with seeded endothelial cells. The exposition area was 637 mm^2 with a chamber height of 100 µm. The rheological properties of this parallel plate flow chamber were analyzed previously [13, 14]. The flow loop (Figs. 1 and 2) consisted of a computer-controled roller pump (Ismatec, Germany) and sterile silicon tubes which were changed after each experiment. The tubes formed a complete circuit with a temperature-controled reservoir, which consists of a Teflon tube.

The system temperature was thermostatically controlled at 37 °C. The system pressure was adjusted to 1 cm H_2O. The chamber was observed by the use of an inverse microscope IRBE (Leica, Germany) with a scanning table, which held the chamber in a fixed position (Fig. 2). Continuous observation was achieved

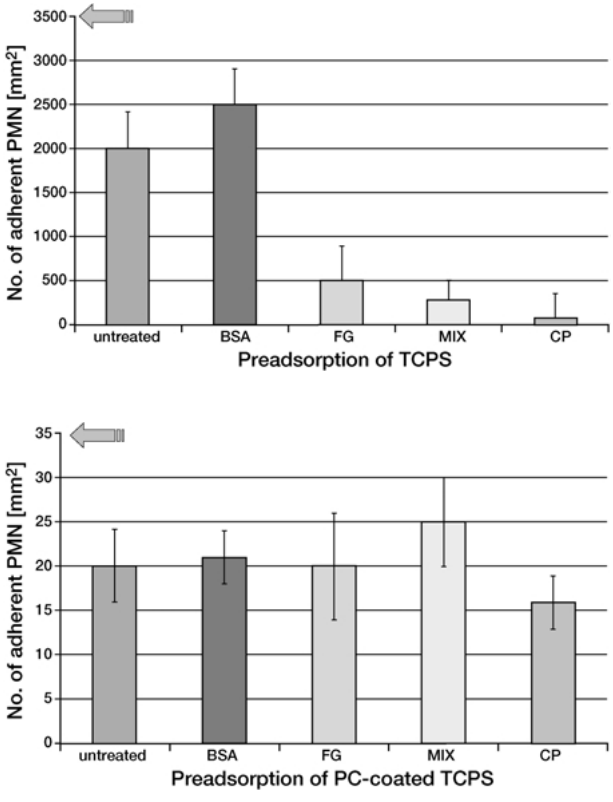

Figure 2 PMN-adhesion on protein-preadsorbed TCPS surfaces as well on PC-precoated TCPS-surfaces. Note the marked difference in the ordinate scale between both graphs.

using a three-chip CCD-camera (SONY, Japan). Every minute an image (jpeg-format, 15% compression rate) was obtained using a MiroVideo PCTV-frame grabber board (Pinnacle, Germany) using Webcam32 software version 6.0 (Surveyor, USA).

The morphometrical analysis was performed on a PC Pentium 750 with Windows 2000 using Image Tools 1.27 (University of Texas, Health Science Center, San Antonio, USA). Additionally, the whole experiment was recorded using a conventional video recorder (Grundig, Germany). The adhesion process of the PMN was observed over a period of 30 min under venous and arterial flow conditions (shear stress of 0.16 and 1.0 Pa, respectively). The numbers of adherent cells were counted every minute using the IT software.

Statistics

All statistical evaluations were made using the STATLETS software package version 2.01 from StatPoint LLC (Englewood Cliffs, NJ, USA) that is available on www.statpoint.com. For all datasets the following statistical tests were made: *F*-test, Students *t*-test, Mann–Whitney–Wilcoxon test and the Kolmogorov–Smirnov test. For all tests the confidence level was set to 95%.

Results

PMN and rheological properties of PMN solution

At the start as well as at the end of the experiment no more than 3% of the PMN showed an incorporation of toluidine blue as a marker of cell death [13].

The analysis of the viscosimetrical properties of the cell culture medium without any PMN gave a viscosity of 0.7363 mPa (standard deviation 0.0501). The viscosity of the PMN suspension demonstrates a viscosity of 0.7483 mPa (standard deviation 0.0488).

The analysis of the relationship between shear stress (τ) and shear rate (S) indicated the typical linear correlation. The correlation between the fluid's apparent viscosity (η) and the shear rate (S) gave a constant ratio. These two profiles were typical for ''Newtonian fluids''.

These results are comparable to our previous results [13, 14].

Static experiments

The results of the static experiments derive from a threefold repetition of the experiments.

The repetition demonstrates a clear reproducibility of the results for all used biomaterial surfaces. The aberration of the results never exceeded the 10% mark for a separate measurement.

PMN adhesion on TCPS and PC-coated TCPS

During the static experiment the numbers of adherent PMN were determined as 2000 PMN/mm^2 on untreated TCPS surfaces (Fig. 2). On BSA preadsorbed TCPS-surfaces we found an insignificantly higher level of PMN adhesion (2500 PMN/mm^2). Preadsorbed fibrinogen (450 PMN/mm^2), BSA/fibrinogen-mixture (300 PMN/mm^2) and human citrated plasma (100 PMN/mm^2) induced a significant ($p < 0.01$) decrease in adhesion of PMN.

The adhesion experiments on PC-precoated surfaces showed comparable results for all proteins. The number of adherent PMN on native PC-coated surface reached only 20 PMN/mm^2 (Fig. 2). The measurements for FG, BSA, MIX and CP gave no significant difference ($p > 0.05$) from the native PC-surface. The results did not exceed a value of 25 PMN/mm^2 for all experiments.

On the basis of these results we found the following ranking for PMN-adhesion under static conditions:

$$\text{BSA} = \text{untreated glass} > \text{FG} > \text{MIX} > \text{CP}$$

In comparison to the native TCPS or preadsorbed-TCPS surfaces to the PC-precoated surfaces, all results showed a significant decrease in PMN adhesion ($p < 0.001$) on the PC-surfaces.

Flow experiments

The results of the flow experiments derive from a threefold repetition of the experiments. The estimation of the standard deviation resulted in a maximal deviation of 10% for all experiments.

PMN adhesion on glass surfaces (positive control) under flow conditions

On the glass surface we found the maximum of PMN adherence after the surface contact with no prior protein preadsorption protocol. After 30 min under venous flow conditions ($\tau = 0.16$ Pa) the number of adherent PMN was 10119 PMN/mm^2 (Fig. 3). The CP-preadsorption led to an insignificant ($p > 0.05$) decrease of adherent PMN with a value of 9405 PMN/mm^2. The preadsorption of FG induced a significant reduction of adherent PMN to 8643 PMN/mm^2 ($\tau = 0.16$ Pa, $p < 0.05$). On glass surfaces with BSA-preadsorption we found 7881 PMN/mm^2 at the end of the experiment using venous flow. The smallest amount of adherent PMN was measured on surfaces that were preadsorbed using Mix (BSA/FG) with 553 PMN/mm^2.

On this basis we had the following ranking for venous flow ($\tau = 0.16$ Pa): Untreated glass = CP > FG > BSA > MIX

Under arterial flow conditions ($\tau = 1.0$ Pa) we found a significant decrease of adherent PMN in comparison to the venous flow on native glass ($p < 0.05$) with 8000 PMN/mm^2 (Fig. 3). The CP-precoated glass gave a value of 4667 PMN/mm^2, which was statistically significantly ($p < 0.05$) fewer compared to native glass as well as CP-precoated glass under venous flow. The BSA-preadsorbed glass showed 3381 PMN/mm^2 adherent PMN, followed by FG-preadsorbed surfaces with 1214 PMN/mm^2 and mix-preadsorbed surfaces with 136 PMN/mm^2. All these results were statistically significantly ($p < 0.05$) lower than the adsorption on native glass under arterial flow and also fewer in comparison to the results of the experiments with venous flow conditions.

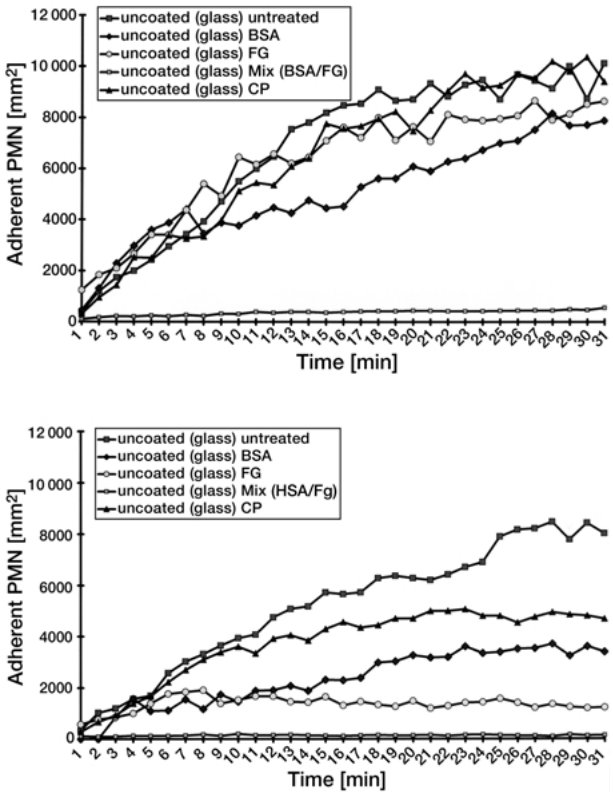

Figure 3 Adhesion of PMN on glass surfaces with/without protein preadsorption under venous ($\tau = 0.16$ Pa; top diagram) or arterial ($\tau = 1$ Pa; bottom diagram) flow conditions.

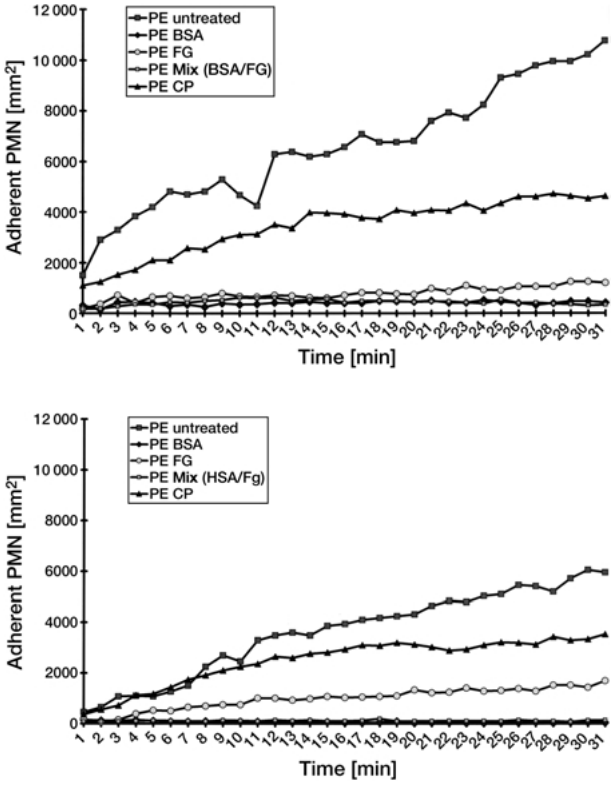

Figure 4 Adhesion of PMN on PE surfaces with/without protein preadsorption under venous ($\tau = 0.16$ Pa; top diagram) or arterial ($\tau = 1$ Pa; bottom diagram) flow conditions.

On this basis we achieved the following ranking order for arterial flow ($\tau = 1.0$ Pa): untreated glass > CP > BSA > FG > MIX

For venous as well arterial flow conditions a relative steady state of PMN adhesion was found after 20 min of interaction.

PMN adhesion on PE surfaces under flow conditions

All flow experiments demonstrated a relative numerical steady state of PMN-adhesion after 20 min of experiment duration.

Under venous conditions ($\tau = 0.16$ Pa) we found the highest PMN adhesion on untreated PE-surfaces with a value of 10762 PMN/mm^2 (Fig. 4). The CP-preadsorption resulted in a significant ($p < 0.05$) decrease of PMN-adhesion to 5952 PMN/mm^2. The FG-protocol induced a PMN adhesion with 1190 PMN/mm^2 and BSA-preadsorption an adhesion of 413 PMN/mm^2, also significantly ($p < 0.05$) reduced in comparison to native PE. The preadsorption of Mix (BSA/FG) induced the strongest reduction of PMN-adhesion with only 328 PMN/mm^2.

On this basis we had the following ranking for venous flow ($\tau = 0.16$ Pa):

$$\text{untreated PE} > \text{CP} > \text{FG} > \text{BSA} > \text{MIX}$$

The arterial flow conditions ($\tau = 1.0$ Pa; Fig. 4) gave the highest adhesion level on the native material (5952 PMN/mm^2), which is a significant ($p < 0.05$) reduction in comparison to the venous data. CP-preadsorption induces a significant decrease of PMN-adhesion (3524 PMN/mm^2) in comparison to the native biomaterial surface. This level was also significantly reduced ($p < 0.05$) in comparison to the venous flow. The preadsorption of FG (1690 PMN/mm^2) demonstrated an insignificant ($p > 0.05$) increase of the PMN-adhesion in comparison to venous flow conditions, whereas in comparison to the native PE the FG-treatment induced a significant ($p < 0.05$) reduction of PMN-adhesion.

The BSA (40 PMN/mm^2) and Mix-layers (139 PMN/mm^2) demonstrated under arterial conditions a significant ($p < 0.05$) reduction in comparison to the venous flow, as well in comparison to the static experiments.

On this basis we had the following ranking for arterial flow ($\tau = 1.0$ Pa):

$$\text{untreated PE} > \text{CP} > \text{FG} > \text{MIX} > \text{BSA}$$

This adhesion levels under venous and arterial flow range between the levels of the glass-experiments, with higher adhesion levels, and the TCPS-experiments, with lower adhesion levels.

PMN adhesion on TCPS surfaces underflow conditions

The adhesion of PMN on TCPS was significantly reduced for all preadsorbed surfaces in comparison to the glass surfaces (Fig. 5).

The native TCPS gave under venous flow a mean PMN adhesion with 653 PMN/mm^2. On BSA-preadsorbed TCPS we found 787 PMN/mm^2 under venous flow at the end of the experiment, but this was an insignificant difference ($p > 0.05$) in comparison to native TCPS. FG-

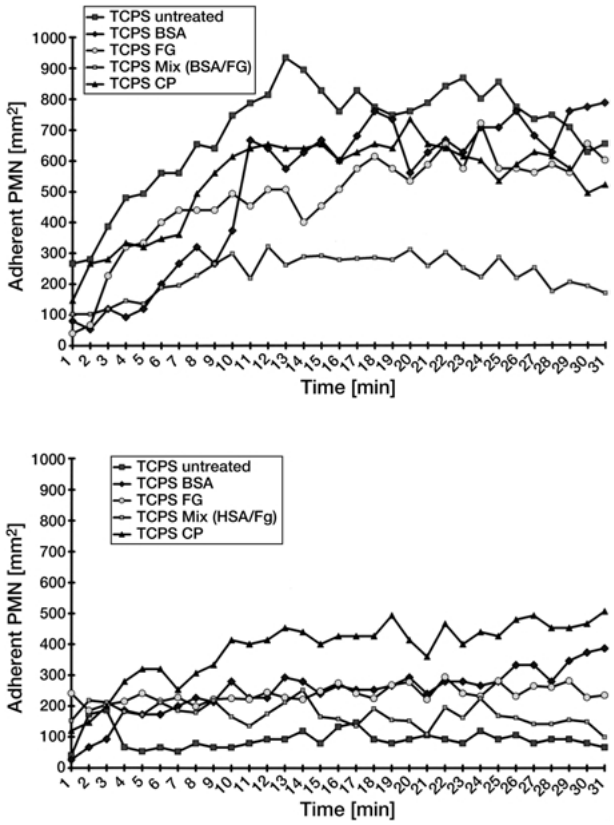

Figure 5 Adhesion of PMN on TCPS surfaces with/without protein preadsorption under venous ($\tau = 0.16$ Pa; top diagram) or arterial ($\tau = 1$ Pa; bottom diagram) flow conditions.

preadsorption (600 PMN/mm^2) as well as CP-preadsorption (520 PMN/mm^2) induces an insignificant ($p > 0.05$) reduction of PNM-adhesion on the TCPS surface. Only the mix (BSA/FG) gave a significant ($p < 0.05$) reduction of PMN adhesion with a final level of 169 PMN/mm^2.

Thus, the ranking on TCPS for venous flow ($\tau = 0.16$ Pa) is as follows:

$$\text{BSA} = \text{TCPS} = \text{FG} > \text{CP} > \text{MIX}$$

The arterial flow conditions ($\tau = 1.0$ Pa) induced on native TCPS an adherence of 67 PMN/mm^2. The protein-preadsorption of the protein MIX (99 PMN/mm^2) gave an insignificant ($p > 0.05$) increase of PMN-adhesion in comparison to native TCPS. On the other hand all other protein-preadsorption protocols using FG (235 PMN/mm^2), BSA (387 PMN/mm^2) and CP (507 PMN/mm^2) induced a statistically significant promotion of PMN-adhesion on the material surface.

The comparisons of PMN adherence under venous and arterial flow show a significant reduction under arterial flow conditions for all preadsorption protocols with the exception of CP-preadsorption (venous 520 PMN/mm^2; arterial 507 PMN/mm^2; Fig. 5).

The relative steady state level was reached for all experiments after 20 min of experimental duration.

Summarizing, the ranking on TCPS under arterial flow conditions ($\tau = 1.0$ Pa) was:

$$\text{CP} > \text{BSA} > \text{FG} > \text{MIX} > \text{untreated TCPS}$$

PMN adhesion on PC-coated TCPS under flow conditions

On PC-coated TCPS-surfaces we found no adherent PMN under flow conditions, independent of protein preadsorption or shear stress.

Discussion

The interaction between biomaterials and PMN is an important biological response during the interaction of the human body and implanted devices. Currently used implants induce an activation of PMN [15, 16]. For vascular prostheses the interaction with platelets with secondary induction of thrombosis is one important feature of this interaction [11, 17–19]. Besides this reaction, which is typical for vascular implants, all other implants interact with PMN's, which are rapidly recruited by the implants followed by macrophages and lymphocytes [15]. During the acute inflammatory response to implanted medical devices, human PMN's release oxidative and hydrolytic activities, which may ultimately contribute to biodegradation [8], and thus influence the biofunctionality of the implant.

During the last decade many protocols for biomaterial surface modification were used to reduce the interaction between biomaterials and PMN [20, 21]. For vascular implant devices as well as for other extracorporally used biomaterials, such as hemodialysis filters etc., the inhibition of PMN activation is an important step in improving hemocompatibility [13, 20]. The first element in the interaction between these biomaterials and the blood is the adsorption of plasma proteins with establishment of self-assembling protein layers [12], with then influence the properties of the biomaterials [12, 18, 19, 22–26].

We have used a dynamic model, the parallel-plate flow-chamber, to examine the interactions of different biomaterials with PMN and how these interactions are influenced by protein adsorption. The static experiments suggest that, with the exception of BSA, all preadsorbed proteins lead to a significant decrease of PMN adhesion on TCPS. BSA does not influence the PMN adhesion on the TCPS-surface. This interpretation is validated by the measurement of a significant amount of adsorbed BSA (unpublished data) using the Micro-Biuret-Assay [27].

Coating of the TCPS-surface with PC induces a massive decrease, of the order of 100-fold, of the number of adherent PMN. These results are comparable with the results from Campbell [21], which were found for platelet adhesion and activation by measurement of the GPIb and P-selectin expression on PC-coated surfaces after contact with platelets. The results of the preadsorption experiments suggest that the different proteins do not influence the direct effect of PC-coating. Our results from measurements (unpublished data) of the protein adsorption using Micro-BSA or DELFA-assay [27] support the interpretation that this effect is based on a significant inhibition of protein adsorption [21]. The DELFA-assay uses directly enzyme labeled fibrinogen for the determination of fibrinogen adsorption.

The analysis of PMN-adhesion under venous flow conditions gave the highest amount of adherent PMN on native glass, a result that confirms our findings in

previous experiments [13]. Arterial flow leads in comparison to venous flow to a significant reduction of adherent PMN, a phenomenon that has also been observed for other biomaterials [13]. The ranking of the effect of material preadsorption differs in comparison to the results of static experiments. Whereas under static conditions BSA shows no inhibition of PMN adhesion under venous and arterial flow we were able to demonstrate a significant but only mild reduction of PMN adhesion. This reduction of PMN adhesion under venous flow correlates with the results of many other authors [16–18, 28]. The difference between static and dynamic conditions may be an effect of the basic material employed, for example, glass versus TCPS (static) with their different degrees of hydrophilicity. The significant inhibition of PMN-adherence by fibrinogen, which was demonstrated under static as well under venous and arterial flow conditions, appears to be a dominant effect [23].

The human citrated plasma (CP) shows under static conditions the most prominent anti-adherent effect on the PMN. By contrast, under flow conditions we found only a slight or insignificant inhibitory effect. This important contrast might be induced by a small amount of residual citrate in the static samples, which induces a reduction of free calcium. The reduction of calcium ions could possibly lead to a reduction of PMN activation, motility and adhesion [29]. During the flow experiment the distribution volume for the residual complex-inducers exceeds the volume during static experiments by orders of magnitude.

The mixture of BSA and FG in physiological concentrations demonstrated the best inhibitory effect on PMN adhesion. These results are consistent for static and dynamic conditions and show that CP differs intensively from the mixture of BSA/FG. The difference could be induced by many other adsorbed proteins which are included in the self assembled CP-layer.

The effect of shear stress shows a typical effect on PMN adhesion. Under arterial flow conditions the amount of adherent PMN was minimally reduced to 50% compared to venous conditions. This effect has been described by multiple authors and is based on fluid dynamics [13, 30].

The experiments using PE as material for PMN adhesion show in comparison to the other materials that the PMN do not adhere as well as on glass, but nevertheless stronger than on the TCPS surface. This may be an effect of the hydrophilicity of the materials. The running order of the experimentally used materials on the basis of their contact angles from hydrophilic to hydrophobic is PC–TCPS–PE–glass. On this basis our results reinforce the results from other authors that adhesion of PMN as well of platelets was higher on hydrophobia surfaces in comparison to hydrophilic surfaces [19, 22–25]. The preadsorption of CP induces only a small reduction of PMN-adhesion under flow conditions. The minimal influence of plasma on PMN-adhesion was described by measurement of the super-oxide-production by Katz *et al*. [16]. These authors had demonstrated that a preadsorption of ePTFE with plasma shows no significant effect on the oxidative burst released by PMN. The comparison of the results of static and venous flow experiments for PE-surfaces preadsorbed with CP indicate a significant increase under flow conditions (100 vs. 5952 PMN/mm^2). This effect may also be a result of the Ca^{2+}-dependent adhesion of the PNM's, which is possibly inhibited by the Ca^{2+}-binding-effect of sodium citrate [29] under static conditions, as described for the glass samples.

The FG-experiments demonstrate an intermediate reduction of PMN-adhesion on the PE-surfaces, a result that was described for other materials [23].

The best inhibition of PMN-adhesion was observed following the adsorption of BSA and MIX. For BSA Katz *et al*. [16] also found an inhibitory effect of albumin on PMN adhesion to ePTFE. The higher adhesion level of PMN on BSA-preadsorbed surfaces in comparison to the Mix-preadsorption is insignificant. With respect to venous flow experiments the mixture of fibrinogen and BSA (MIX) elicited the best inhibitory activity on PMN-adhesion. The results of the PE-adsorption experiments correspond to the results on the preadsorbed glass, in that CP has markedly different properties than the mixture of BSA/FG.

Under venous flow conditions after 30 min in comparison with the static experiments we observed on TCPS-surfaces significantly lower levels of PMN adhesion ($p < 0.05$) for native TCPS (2000 vs. 653 PMN/mm^2), for BSA preadsorbed TCPS (2500 vs. 787 PMN/mm^2) and for mix (300 vs. 169 PMN/mm^2). The reduction of PMN-adhesion may be an effect of the reduced contact time between PMN, which is induced by the flow properties of the PMN-suspension [27, 30]. Additionally, the stability of PMN-adhesion under flow conditions will be affected by the vessel wall shear stress [31].

FG-preadsorption on TCPS leads to an insignificantly higher level of adherent PMN in comparison to the static experiment (450 vs. 600 PMN/mm^2). The cause of this behavior is unclear and might be an effect of reduced adsorption of FG on TCPS because of the hydrophilicity of the material [32]. During the course of the experiment a desorption or protein exchange between the FG-preadsorbed TCPS and the cell culture media may play only an ancillary role. Dekker *et al*. show that a self-assembled FG-layer on TCPS shows a reduction of FG-content to approximately 60% after 6 h of incubation using a cell culture medium containing 20% human serum [26].

A comparison of the results of static and venous experiments for CP-preadsorbed TCPS gave a significant increase under shear stress (100 vs. 520 PMN/mm^2). This effect may also be related to the Ca^{2+}-dependent adhesion of the PNMs, which is possibly inhibited by the sodium citrate [29, 20]. The analysis of PMN-adhesion under arterial flow conditions demonstrates a significant reduction of PMN-adhesion for all preadsorption protocols including native TCPS in comparison to venous conditions and static experiments as well.

For CP-preadsorbed TCPS-surfaces we could not demonstrate a significant reduction of adherent PMN under arterial flow in comparison to venous flow (507 vs. 520 PMN/mm^2), although the increase of PMN-adhesion in comparison to static conditions was apparent (507 vs. 100 PMN/mm^2). This increase of

PMN-adhesion may be an effect of the Ca^{2+}-ion concentration [29, 20], as discussed previously.

The experiments using PC-coated TCPS demonstrate on native materials, that is without protein preadsorption, that under venous as well arterial conditions no cells could adhere. These results underline the theory that a hydrophilic surface reduces the adhesion of PMN significantly [19, 22–25]. In comparison to the static experiments we found a reduction of adherent PMN to the zero-level. This may be an effect of the instability of PMN-adhesion on the PC-coated surfaces. The PC-coated specimens did not show any changes in their PMN-adhesion during our flow experiments. The cause of this behavior is the very low amount of protein adsorption on these surfaces. Campbell *et al*. [21] demonstrate a reduction of FG-adsorption on PC-coated surfaces (PVC and Polycarbonate) to levels near the lowest detection limit. Our results using DELFA [27] reveal that in comparison to untreated TCPS the FG-adsorption decreases to 0.1% (approx. detection level of test; unpublished data).

Conclusions

Our results clearly show that for hydrophobic materials, glass and PE, which induce a prominent adhesion of PMN, the mixture of albumin and fibrinogen induces the best inhibitory effect. On hydrophilic biomaterial surfaces, untreated TCPS and PC-coated TCPS, reveal only a minor influence of adsorbed proteins on PMN adhesion because of a primary low adhesive surface for PMN and proteins as well.

Human citrated plasma leads only to a slight inhibition of PMN adhesion. It is possible that the contained sodium citrate may inhibit PMN adhesion by its ion-binding capacity. On the hydrophobic materials, glass and PE, BSA had the best anti-adhesive potential with respect to PMN. The use of a dynamic system for the determination of PMN-adhesion is from our point of view absolutely essential for the evaluation of biomaterials for vascular prosthesis, and is in accordance with the international standards [2, 4]. The coating using phosphorylcholine is an excellent surface modification to prevent PMN-adhesion and protein-adsorption but might be inappropriate for implants or implant components, which require cell adhesion or tissue integration. The results of our experiments suggest that investigations under static and flow conditions are also needed to determine the influence of protein adsorption on other relevant blood cell populations, for example, platelets and monocytes.

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