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A modular *in vitro* flow model to analyse blood-surface interactions under physiological conditions

Abstract: Newly developed materials for blood-contacting devices need to undergo hemocompatibility testing to prove compliance with clinical requirements. However, many current *in vitro* models disregard the influence of flow conditions and blood exchange as it occurs *in vivo*. Here, we present a flow model which allows testing of blood-surface interactions under more physiological conditions. This modular platform consists of a triple-pump-chip and a microchannel-chip with a customizable surface. Flow conditions can be adjusted individually within the physiological range. A performance test with whole blood confirmed the hemocompatibility of our modular platform. Hemolysis was negligible, inflammation and hemostasis parameters were comparable to those detected in a previously established quasi-static whole blood screening chamber. The steady supply of fresh blood avoids secondary effects by non-physiological accumulation of activation products. Experiments with three subsequently tested biomaterials showed results similar to literature and our own experience. The reported results suggest that our developed flow model allows the evaluation of blood-contacting materials under physiological flow conditions. By adjusting the occurring wall shear stress, the model can be adapted for selected test conditions.

Keywords: hemocompatibility, *in vitro* flow model, blood-surface interactions

https://doi.org/10.1515/cdbme-2021-2044

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1 Motivation

The rapidly growing need for cardiovascular implants and other blood-contacting devices necessitates the development of new hemocompatible materials. In order to prove compliance with clinical requirements, newly developed materials need to undergo hemocompatibility tests. Though ISO standard 10993-4 provides a guideline with specific testing categories, it does not define a specific measurement setup. This absence of standardization has led to a great variety of testing methods with individual benefits and disadvantages. Static and agitated blood incubation models usually offer a simple setup allowing rapid screening of numerous samples. However, the validity of the results is compromised by the negligence of flow-dependent effects like shear-induced platelet activation and thrombus formation. Various blood flow chambers address this challenge and analyse hemocompatibility under more realistic conditions. The majority of them allows the adjustment of wall shear stress in the range of 1 to 70 dyn/cm², representing flow conditions from large veins to small capillaries. [1,2]

Nevertheless, most described blood flow chambers do not consider the physiological exchange and continued replacement of blood. Such steady pool systems impede kinetic analysis and can cause exhaustion of source factors and accumulation of activation products. This may lead to the cross-activation of pathways to a non-physiological extent. Here, we describe a dynamic blood flow model to address that challenge, enabling a constant blood supply from a reservoir. The continuous blood exchange avoids disadvantages of steady pool systems, providing adjustable shear forces in a physiological range and allowing continuous sampling for the kinetic analysis of activation processes.

2 Model Setup

2.1 Microfluidic setup

The microfluidic setup (see Figure 1) is divided into two units, a pumping and a blood stream unit. Both units are connected within the triple-pump-chip, which accordingly belongs to both. The pumping unit consisting of the control unit "MPScontrol" (1), the damper "MPShemodyn" (2) and the triple-pump-chip (4) was already described previously [3]. In brief, the triple-pump-chip is made of three independent channels, each with a cascade of an inlet valve, a pump chamber, and an outlet valve. Through frequent membrane deflection by pressured air, the pump chambers get charged and discharged with fluid. This initializes a directed pulsatile volume flow and draws the liquid from the blood bag. The frequency of this process is regulated by the "MPScontrol", while the "MPShemodyn" module adjusts the hemodynamic pumping behavior (velocity curves and wall shear stress).

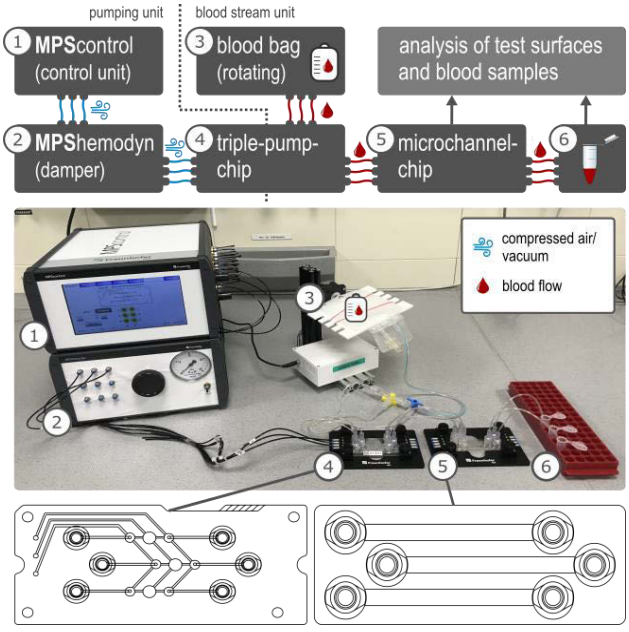

Figure 1: Experimental setup of the microfluidic setup below: 1) "MPScontrol", 2) "MPShemodyn", 3) blood reservoir and 180° rotator; 4) triple-pump-chip; 5) microchannel-chip; 6) Eppendorf tubes for blood collection.

The blood stream unit consists of the blood reservoir (3), the triple-pump-chip (4), the microchannel-chip (5), and the collection tubes (6). The entire blood stream unit is placed in an incubator (37 °C, 5 % CO_2) to ensure physiological temperature and stability of blood gases and pH. All components are interconnected via Luer connection tubes (B. Braun SE, Germany). Directly after the blood reservoir, the blood stream is divided into three independent courses by three-way stopcocks (Fresenius Kabi, Germany). The triple-pump-chip draws the blood from the blood bag and passes it to the microchannel-chip, the core component of the modular flow model. The chip is made of four layers: (A) the upper layer with Luer-Lock connections for in- and outlet (Makrofol® DE 1-1 000000, Covestro, Germany), (B+C) the middle layers, which determine the shape of the channels (B: Makrofol® DE 1-1 000000; C: 3M™ Adhesive Transfer Tape 94714 LE, 3M, Germany), and (D) the bottom layer with the customizable test material. The design of the channels is adaptable, here we used dimensions of 3 mm x 40 mm (channel area) and 0.25 mm (channel height). After the contact with the test material, the blood was collected in Eppendorf tubes for further analysis. The adhesion of blood cells on the test surface can be analyzed by microscopy of the microchannel-chip.

2.2 Conditions of dynamic blood flow

We characterized the dynamic flow conditions within the microchannel-chip by micro-particle-imaging velocimetry (µPIV), as described elsewhere [4]. Briefly summarized, a Zeiss Primovert inverted microscope (Zeiss, Germany) coupled with a high-speed camera (Baumer HXC40, Baumer Optronic, Germany) was used to track the motion of 10 µm CML latex beads (4 % w/v, 10 µm, Life Technologies, Germany). To mimic the viscoelastic shear thinning behavior of blood, a mixture of water, glycerin, and xanthan gum [5] was applied as base fluid during µPIV measurements. The measured maximum velocity (v_{max}), the channel height (h), and the viscosity of whole blood ($\eta = 3.5$ cP) were used to calculate the wall shear stress (τ) at the test surface (see eq 1) [6].

$$\boldsymbol{\tau = \frac{6 * \eta * v_{max}}{h}} \qquad (1)$$

In Figure 2, we show the corresponding results for a pumping frequency of 6 bpm. This frequency corresponds to a cycle time of 10 s with a shift of the pump chambers every 2.5 s. The peak wall shear stress can be varied by adjusting the signal actuating the triple-pump-chip with the pneumatic damper "MPShemodyn". This offers the possibility to independently vary flow conditions within the system mimicking hemodynamic conditions as they occur in different parts of the vascular system. Here, we show the corresponding wall shear stress with three different damping volumes, ranging from 6.7 to 34.0 dyn/cm².

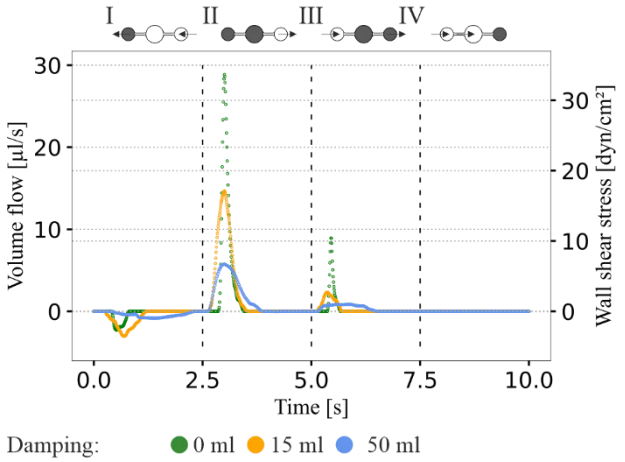

Figure 2: Volume flow and corresponding wall shear stress in the microchannel-chip measured by micro-particle-image velocimetry (µPIV) at 0, 15 and 50 ml pneumatic damping; I) – IV) Behavior of the pump deflection with charged (white) and discharged (grey) pump chambers.

3 Performance test

3.1 Methods

The system was tested concerning its general performance and its ability to differentiate the blood compatibility of different materials. Venous blood was freshly drawn from two ABO-compatible healthy voluntary donors after informed consent. The blood was immediately anticoagulated with 5 µM hirudin and subsequently pooled. The performance test was conducted for 2 hours with a pumping speed of 6 BPM, corresponding to a volume flow of 32.4 µl/min. The maximum wall shear stress on the test surface were adjusted to a maximum of 17 ± 5 dyn/cm², representing physiological conditions in coronary stents [7]. Hemocompatibility was assessed according to hemolysis (Drabkin's Reagent, Sigma-Aldrich, USA), pH stability and blood gases (blood gas analyser, Medizintechnik Hadler und Braun, Germany), activation of the complement cascade (fragment C5a measured by enzyme-linked immunosorbent assay, DRG Instruments GmbH, Germany), cytokine MIP1-β (ProcartaPlex Immunoassay, Thermofisher Scientific, USA), and the leukocyte activation marker CD11b (flow cytometry, LSRFortessa™, BD Biosciences, Germany) [8], as well as the adhesion of leukocytes and platelets to the test surface (fluorescence labeling with DiOC6 and DAPI). Data are presented as mean ± standard deviation of 3 replicates if not stated otherwise. Statistical analysis was performed by one-way analysis of variance (ANOVA) and subsequent Holm-Sidak multiple comparisons.

3.2 Hemocompatibility of the flow model

In vitro flow models require blood transport minimizing traumatic effects on cells during the experiment. Hemolysis in the developed flow system was found to be negligible (0.09 ± 0.08%), confirming the absence of blood damage by the pumping process. Blood gases and pH remained within the expected values. Inflammation, assessed by granulocyte CD11b expression, was comparable to the incubation in the well-established quasi-static incubation chamber [9] (~40% compared to LPS-activated blood). Based on these data, we conclude the flow model to be hemocompatible over the examined time period.

3.3 Blood-surface interactions

The performance of the flow model was further assessed with three well-characterized biomaterials: (1) glass as hydrophilic reference with negative surface charge, (2) fluoropolymer Teflon AF™ (DuPont, Germany) as hydrophobic material with no ionizable surface groups, and (3) polyether-based aliphatic thermoplastic polyurethane Tecoflex™ SG-80A (Lubrizol, USA) as clinically approved reference material.

Analysis of the whole blood samples revealed the highest inflammation response for blood from the glass microchannel. The release of MIP-1 β was significantly higher compared to Teflon AF™ and medical-grade polymer Tecoflex™. Differences in C5a release displayed the same tendency but could not be statistically confirmed (see Figure 3A+B). These results correspond to data from literature [2,10] and our own experiences, where glass is commonly applied as activating control during hemocompatibility assessment.

Analysis of cell adhesion to the test surfaces revealed an increased platelet activation on all surfaces compared to static blood incubation. This behavior may be attributed to the shear-induced platelet activation. Hereby, the initial adhesion of platelets is primarily induced by the shear-dependent interaction of the platelet receptor GPIbα with the A1 domain of von Willebrand factor, a glycoprotein typically adsorbing to the material immediately after initial blood contact. [11] This results in platelet activation, associated flattening and pseudopod formation. When comparing all three test surfaces with each other, we observed the formation of neutrophil extracellular traps (NETs) on Teflon AF™ (see Figure 3C). NETs are a function of the innate immune system, originating from neutrophilic granulocytes, which release reticular structures of decondensed DNA, histones and enzymes to trap foreign objects. It has been described previously that biomaterial-induced NET formation occurs preferably at hydrophobic surfaces (as Teflon AF™) with significantly

increased platelet activation and thrombin generation [10]. The performance test confirmed the applicability of this flow model to differentiate blood-surface interactions between the evaluated materials. The single pass process allows easy blood sampling at defined time points, the analysis of activation kinetics, and avoids feedback effects of activation products.

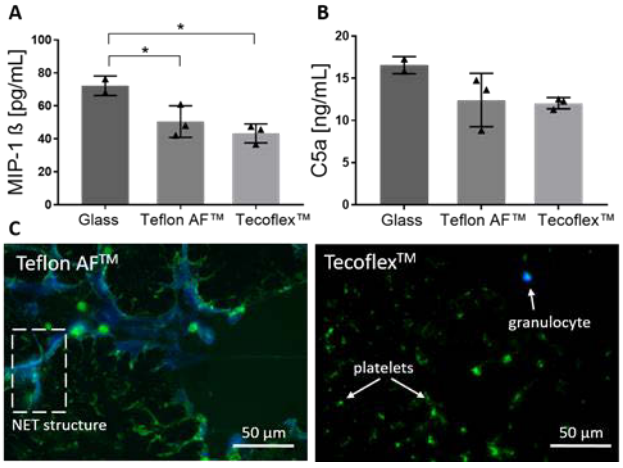

Figure 3: Release of (A) MIP-1 β and (B) C5a in analysed blood samples for glass (n=2), Teflon AF™ and Tecoflex™, statistical significance: * p<0.05; (C) Test surfaces Teflon AF™ and Tecoflex™ after blood incubation, leukocytes stained with DAPI (blue), platelets stained with DiOC6 (green)

4 Conclusion

This work describes a newly developed *in vitro* flow model to analyse blood-surface interactions under physiological flow conditions. We demonstrated its gentle pumping process as well as its applicability to evaluate the hemocompatibility of different test surfaces. In contrast to existing flow chambers, the model is characterized by its unidirectional blood flow with steady supply of blood from a reservoir. This single-pass system avoids the depletion of source factors and accumulation of activation products, thus preventing cross-activation of pathways to a non-physiological extent. The modular setup of the flow model allows the simple insertion of various planar test surfaces as well as an easy adaption of individual shear conditions. Specific flow conditions can be adjusted by modifying the geometry of the microchannel-chip.

Author Statement

The authors would like to express great appreciation to the Federal Ministry of Education and Research (project "Zwanzig20 RESPONSE – Partnerschaft für Innovation in der Implantattechnologie") as well as to the Free State of Saxony and the European Union EFRE (SAB projects "HERMES" and "TRIOMED") for their financial support. The authors state no conflict of interest. The use of whole blood in the study was approved by the ethics committee of the Sächsische Landesärztekammer. Informed consent has been obtained from all individuals included in this study.

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