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Numerical and experimental evaluation of platelet deposition to collagen coated surface at low shear rates

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ABSTRACT

Platelet deposition to collagen-coated surface under low shear conditions was investigated using an experimental model. The flow chamber was created by combining a stationary and a rotational glass plates spaced 50 μm apart. Blood filled into this space was subjected to a simple Couette flow. Both glass plates were covered with albumin to render them anti-thrombogenic. However, one spot 1×1 mm in size was covered with collagen. This spot was where the platelets deposited. The device was mounted on an inverted microscope and the platelet deposition was recorded. Platelets were dyed to render them fluorescent. The blood used was human blood from healthy volunteers. It was subjected to a range of low shear rates (below 700 1/s) to find out how they act on platelet deposition. The results show a characteristic curve with elevated platelet deposition in the range of 150 1/s. For the interpretation of these results a numerical model was developed. It applies the Monte Carlo method to model a random walk of platelets. This diffusive motion was superimposed on the convective motion by the Couette flow. A satisfactory match to the experimental data was achieved.

1. Introduction

Thrombo-embolical events in cardiovascular devices start with the deposition of platelets onto the material of the implant. This is only the beginning of a complex sequence of events, finally leading to a thrombus, which can block the flow through the device or can dislodge. This process has been subject to a large number of publications. Some authors of recent papers model the complex chain of reactions, including as many reactions as possible. Flamm and Diamond (2012) review such models spanning multi-scale phenomena, from the scale of a calcium ion to the diameter of the aorta. Such models may contain 77 reactions with 132 kinetic rate constants and 70 species, or with 28 fluid phase species and complexes and 44 lipid-bound factors and complexes. For the engineer designing a cardiovascular device, a much simpler model is needed. It should work with assumptions that can be checked experimentally. In the search for such a model, the literature was reviewed. The first experimental models did not include numerical analysis; they were devised to observe the generation of a thrombus (Petschek et al., 1968). Ex-vivo blood was directed to flow onto a glass plate. This flow chamber generates a stagnation point flow and a radial outflow. Later Baldauf et al. (1978) and Reininger et al. (1992) used this flow type for further studies. The deposition of platelets could be observed with a dark field reflected light microscope. This model was soon replaced by a parallel flow model, which was more suitable for mathematical analysis (Turitto and Baumgartner, 1975a), and a numerical model was proposed. More models were developed by various authors and reviewed (Stubley et al., 1987). The basic equation for all models is

$$D \times (\partial c/\partial x)_{\text{wall}} = k \times c \tag{1}$$

At the wall, no convective motion is assumed, so the platelet transport is caused by diffusion only. The platelet flux – the left side of Eq. (1) – depends on the platelet diffusivity D and the concentration gradient at the wall. This flux must be equal with the deposition rate of platelets on the surface—the right side of Eq. (1). The deposition rate is assumed to depend on the concentration c of available free platelets and a reaction constant k, a quantity reflecting the likelihood of deposition. The diffusivity of platelets in platelet-rich plasma D_{PRP} is about 1.5×10^{-13} m^2/s. If, however, red blood cells are also present, the diffusivity is much larger, to the order of two magnitudes (Turitto and Baumgartner, 1975b). This is caused by the mixing action of red blood cells as they turn and tumble in a shear flow (Goldsmith, 1972; Goldsmith et al., 1995; Tokarev et al., 2011). Zydney and Colton (1988) have reviewed experiments and models and proposed a diffusivity model for platelets in full blood (Eq. 2) which is still used today. This model assumes a linear dependence of the shear rate γ on the diffusivity. Furthermore, the diffusivity also

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depends on the hematocrit H and the diameter of red blood cells d_{RBC}:

$$D = D_{PRP} \times (1-H) + 0.15 \times \left(d_{RBC}{}^2 \times H/4\right) \times \gamma \times (1-H)^{1.8} \quad (2)$$

At a distance from the surface, basic laws of fluid mechanics determine the transport of platelets. Platelet deposition at the wall is thought to be limited by only one surface property: the surface availability. The concept of surface availability (Turitto and Baumgartner, 1975a) is that already-adhered platelets form a monolayer on the surface. They occupy a site and exclude oncoming platelets from adhering. It is assumed that this surface availability attains zero when the surface is completely covered. This is reflected in Eq. (3) for the reaction constant. The surface coverage is defined by the surface density of already-adhered platelets $M(x, t)$ in relation to the surface density of full coverage M_∞. In this way, a time dependence of platelet deposition is achieved.

$$k = k_0 \times (1-(M(x,t)/M_\infty)) \quad (3)$$

The reaction constant k_0 has been a subject of discussion by various authors. Some state that it is constant. However, comparison with experiments lead other authors to the assumption that the reaction constant increases with the shear rate (David et al., 2001), either linearly or in a more complex way (Sorensen, 2002; Weller, 2008).

The many discussions and open questions have lead to the work presented in this paper, which consists of an experimental model and a numerical model. The most elementary viscous flow has been selected – the plane Couette flow – and, in addition, the model is limited only to the initial phase of platelet deposition. In this way, the question of surface availability is excluded. The complex cascade of thrombus generation is truncated to its first phase.

2. Materials and methods

2.1. Experimental model

The experimental model is designed to simulate the initial phase of thrombus generation in the human body. If in a blood vessel the endothelial layer is locally disrupted, collagen fibers are exposed. Platelets carried by the bloodstream become activated upon contact with collagen and deposit on its surface. This is considered the initial phase of the coagulation system. The experimental model is designed to create the simplest conditions: stationary blood flow, constant low (<1000 1/s) shear rate which does not activate platelets (Hellums, 1994), anti-thrombogenic surface throughout the device, collagen exposed in a small area, freshly-drawn citrated human blood with non-activated platelets. A flow with a constant shear rate is approached by a parallel plate design (Watanabe et al., 2011). Fig. 1 shows the experimental model: a circular stationary glass plate 40 mm in diameter and an upper rotational plate 35 mm in diameter are spaced 50 μm apart. A set of 50 μm gap for each experiment is done with a precision of the depth of sharpness of the used microscope. The resulting error of the gap set was 4.6 μm. This space is filled with blood and the Couette flow is created when the upper plate is rotated. Both plates are microscopic circular glass coverslips of 175 μm thickness for best optical conditions. The model is positioned in an inverted microscope (Fluovert FU, Leica AG, Germany). The coverslip glass plates are covered with albumin (Carl Roth GmbH & Co KG, Karlsruhe, Germany). This albumin layer makes the glass anti-thrombogenic and thus non-activated platelets will not deposit on the glass. To induce platelet deposition, a spot of collagen (Horm, Nycomed Austria GmbH, Austria) with a diameter of 1000 μm is applied at a radius of 7.5 mm on the lower stationary coverslip. The field of view of the microscope is directed onto this spot. A shear rate between zero and 700 1/s can be generated at this spot. Blood was drawn from the cubital vein of voluntary healthy male donors, which gave informed consent on the work. For each run, two samples each of 9 ml of blood were drawn into vacuum tubes ("Vacutainer") containing 1 ml of anticoagulant (0.106 mol/L sodium citrate) (Sarstedt AG, Nürmbrecht, Germany). The first sample was discarded to exclude platelets that were activated by venipuncture. For fluorescent labeling of the platelets, 0.01 g of Quinacrine dihydrochloride (Sigma Aldrich Chemie GmbH, Steinheim, Germany) was dissolved into 50 g of phosphate-buffered solution (Invitrogen, UK). Then 100 μL of that solution was added to the blood sample. After gentle shaking, the sample was protected from light and kept at room temperature. The samples were used within three hours after venipuncture. Each sample was divided into six portions allowing performing up to six runs. Prior to each run about 100 μL of blood was put onto the lower coverslip. The upper coverslip was carefully lowered avoiding the generation of air bubbles. Through the lower stationary plate light with 355 to 425 nm wavelength illuminated the blood. Illuminated platelets emit light at 496 nm. The upper coverslip was set in motion, and after focusing on the stationary coverslip plane the recording began. The process of deposition was recorded with a video camera (UI-2230-M, Imaging Development Systems GmbH, Germany) with a resolution of 1024×768 pixels and a frame rate of 20 fps and covering a field of view of 480×360 μm^2. Once deposited on the plate, platelets remained fixed and were easily distinguishable from the moving platelets. Camera images were processed and resulted in surface coverage by the platelets as a function of time and shear rate. The duration of each run was 60 s. After this time, platelets start to form large aggregates that create wakes affecting the probability of adhesion, a phenomenon which is not a part of this study.

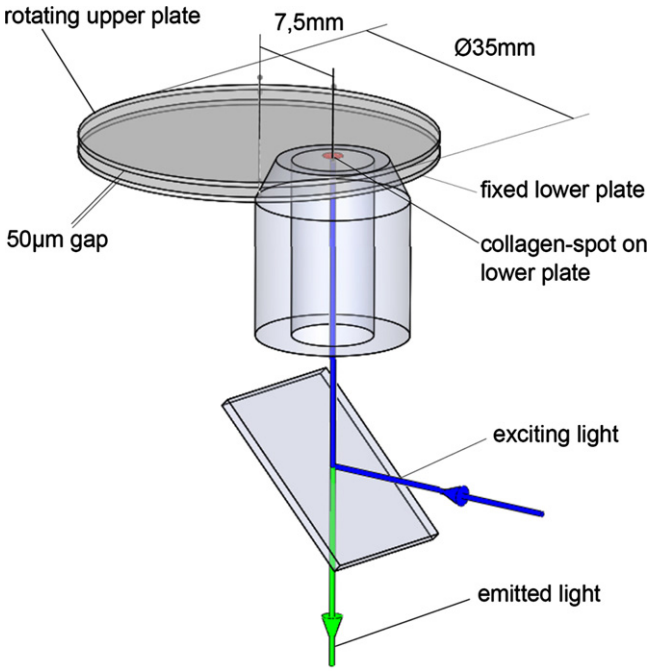

Fig. 1. Couette flow model with two parallel plates (coverslips). Coverslips were used because of their superior optical qualities. The lower coverslip is fixed, while the upper rotates. Blood fills the 50 μm gap between the coverslips. A small spot 1 mm in diameter on the lower coverslip is covered with collagen. The platelets are dyed fluorescent and glow when illuminated by excitatory light.

2.2. Numerical model

The objective of the numerical model is to reproduce and to interpret the results of the experiments. The numerical model follows the concept of Virchow (1856), who concluded that three parameters were responsible for thrombus generation: the quality of the blood, the quality of the vessel wall and the quality of the flow. He stated in hypothesis that these three players interact and this became known as Virchow's triad. In this numerical model, only the initial short phase of platelet deposition is considered. The highly complex system of coagulation with its many stages of platelet activation and the fibrinogen–fibrin system are left out. The numerical model consists of the following elements:

a) Shear flow field: In the experimental model, the flow field is the simplest and is characterized by an approximately homogeneous shear rate in the field of view. For the numerical model, a rectangular box is considered, which represents a small part of the Couette flow field. This rectangular box has a height of 50 μm, a width of 1000 μm and a length of 1200 μm (Fig. 2). Its base plane is defined as a wall at which the platelets deposit. The flow enters the box through the front plane. At the inflow side of the box new platelets are inserted at each time step. The number of platelets inserted at each time step is proportional to the flow in the respective velocity. It is thus proportional to time, distance z to the base plane and shear rate to achieve a homogeneous concentration. Platelets which leave the box through its lateral or upper sides during a time step are reflected inwards. For this simple flow and geometry the mirror reflection does not make notable difference. For more complicated flows, however, more appropriate periodic boundary conditions should be incorporated. Platelets close to the bottom can deposit if certain conditions are met according to our model. The flow has no vertical velocity component and