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Experimental and numerical study of platelets rolling on a von Willebrand factor-coated surface

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ABSTRACT

Blood platelets circulate in the blood and adhere to wounded vessels to initiate coagulation and healing. The first step of this process is the capture of flowing platelets by adhesive molecules located at the wounded vessel wall. In this article, we study the transport of fixed blood platelets in a microfluidic channel coated with von Willebrand factor (vWF), a large multimeric protein expressed by endothelial cells in the vicinity of wounds. We measure the number of platelets adsorbed at the channel surface as a function of both time and space. Experimental results are compared with a new transport model. We show that transverse diffusion is an important feature of our model, while the rolling behaviour of the bounded platelets can be neglected.

1. Introduction

Platelets are small anucleated blood cells that have an essential role in the process of hemostasis. In a wounded vessel, platelets adhere to the wall through ligand–receptor interactions and initiate coagulation. The large multimeric protein von Willebrand factor (vWF) is such a ligand [1]. At high shear rates, vWF unfolds and exposes platelet-binding domains [2,3]. The resulting platelet–vWF bonds are transient, causing platelets to roll along a vWF-expressing wall [4,5]. vWF can also mediate firm adhesion through interaction with activated integrin $\alpha_{IIb}\beta_3$ [6]. Cell rolling is well documented for platelets [7] but also leukocytes [8] and tumour cells [9]. Clinicians need reliable platelet function assays, either for the diagnosis of bleeding disorders [10] or the monitoring of anti-coagulant treatments. Microfluidics is a convenient way to manipulate small blood samples [11] and devices have been developed to assess the effects of anti-platelet treatments with measurements of the concentration platelets bound to the surface of a micro-channel [12–14].

In this article, we study the flow of fixed platelets in a vWF-coated microfluidic chamber. The local concentration of platelets adhering to the wall, C_s, is measured and found to be a decreasing function of the distance x between the channel entrance and the position of observation. This phenomenon, called axial dependency, has been observed in experiments involving whole blood [15]. To the best of our knowledge, although several theoretical and numerical studies focused on the diffusivity of platelets in the transverse direction [16–18] or on the adhesion mechanism [10], no predictions have been made regarding the axial dependency of platelet concentration.

We present a basic model for the transport of blood platelets in the micro-channel. This model involves specific cell–wall interactions and can include advection, diffusion in the transverse direction and cell rolling. The relative importance of the different features of this model is explored through comparison with experimental observations. Physical parameters, such as platelet attachment and detachment rates K_{on} and K_{off}, are used as fitting parameters and we compare their values with experimental measurements or simulated estimations when available.

2. Experimental methods

2.1. Microfluidics

Microchannels are fabricated using standard soft lithography techniques [19] and have a rectangular cross-section of fixed width

Abbreviations: EFS, Etablissement Français du Sang; PBS, phosphate buffer saline; PDMS, polydimethylsiloxane; PFA, paraformaldehyde; SID, shear-induced diffusion; vWF, von Willebrand factor.

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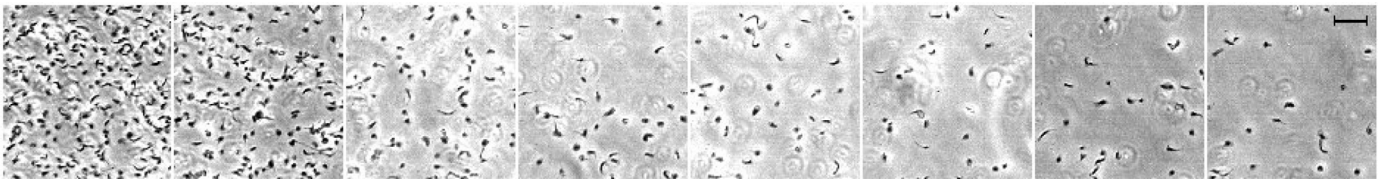

Fig. 1. Photographs of the surface of the micro channel after 95 min experiment. Positions are equally spaced and separated by 1 mm. The first picture is acquired $x =$ 0.6 mm away from the channel entrance. Scale bar represents 20 μm.

$W = 400$ μm and length $L = 4$ cm, and variable height $H \in [14, 63]\,\mu$m. After plasma treatment, channels are sealed on a glass slide, filled with a phosphate buffer saline (PBS, Lonza) solution containing 20 μg mL^{-1} vWF (Wilfactin, LFB Biomedicaments, Les Ulis, France) and incubated overnight at 4 °C. Prior to the experiment, the channel is rinsed with PBS in order to remove unbound vWF.

The cell reservoir is connected to the channel with capillary tubing (internal diameter 228 μm). The homogeneity of the suspension in the entrance reservoir is maintained by gentle agitation. The flow is driven by a pressure control system (Fluigent MFCS-4C). Platelet perfusions are then performed at a wall shear rate compatible with the unfolding of immobilised vWF ($\dot{\gamma} =$ 1400–1800 s^{-1}).

2.2. Biological material

Blood platelets isolated from whole blood are provided by Etablissement Français du Sang (EFS) as the following agreement (CPSL C UNT- 06/EFS/029), prepared as described by Dunois-Lardé and co-workers [20], then fixed with paraformaldehyde (PFA), rinsed and diluted in PBS to reach a concentration of 1.4×10^8 mL^{-1}. The goal of fixation is to preserve as much as possible the structure of platelets but to avoid further metabolic reactions, thus preventing effects such as on-chip activation [21].

2.3. Videomicroscopy

We use a Leica DMI 6000 B inverted microscope, equipped with a 40× magnification dry lens focused on the glass surface of the chip, and a camera (Photron Fastcam SA3) at an acquisition rate of 0.5 fps with a 20 ms shutter to measure the rolling velocity of adherent cells. During the kinetics measurements, only snapshots are acquired. As the motorised stage navigates between several prerecorded positions along the whole channel length in less than 2 min, the experimental error associated with time is $\Delta t = 1$min. We define channel entrance, denoted $x = 0$, as the centre of the 750 μm-wide hole where inlet tubing is inserted. The precision for position is therefore of the order of 300 μm. Images are analysed with ImageJ software, using a routine to automatically count the number of adherent cells in a microscope field. In the case of dense surface coverage, the program fails to separate entangled platelets. Such images are analysed independently by two experimentalists. The discrepancy between their two counts was used to estimate the measurement error on platelet surface concentration $\Delta C_s \sim 20\% C_s$.

3. Experimental observations

Fig. 1 shows photographs of the channel surface after 95 min of perfusion, showing evidence of the axial dependency of platelet adsorption. The exposure time is long, so that only platelets bound to vWF appear sharp. Flowing platelets are not visible.

Fig. 2 illustrates the diversity of behaviours observed at the surface. Positions of surface-adsorbed platelets are shown, with time

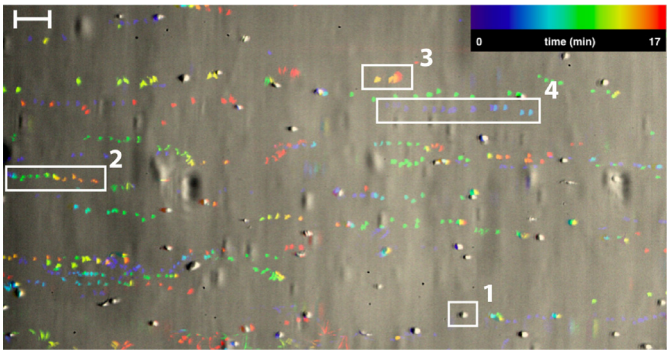

Fig. 2. Superposition of pictures from a video focused on the surface of the microchannel. The colours from blue to red correspond to the time, the scale is 20μm. Both stationary platelets (frame 1) and rolling platelets (frame 2) can thus be observed. One adhesion event is identified in (frame 3) as the first time this platelet is measured is in the middle of the experiment. Likewise a desorption event is seen (frame 4) as the platelet disappears from the surface before the end of the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

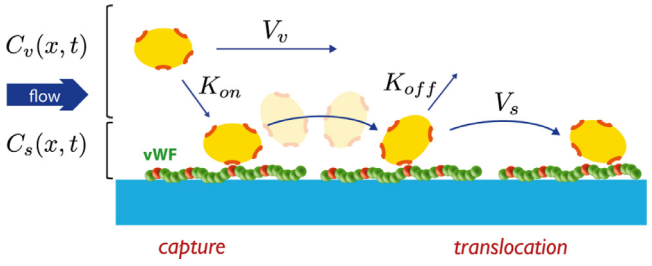

Fig. 3. Interactions of platelets with the vWF-coated wall. Platelets in the volume are advected with a speed V_v and can bind with the vWF with a coefficient K_{on}. Adherent platelets can roll with a speed V_s and detach with a coefficient K_{off}.

colour-coded from purple to red. Some platelets roll along the surface, creating rainbow-like patterns, while others are stationary and appear white. Events of platelet adhesion and desorption from the surface also occur, generating incomplete rainbows.

4. Model

Based on the above observations, we develop a model which describes the transport of blood platelets in the channel. Our goal is here to understand the physical phenomena governing the repartition of platelets between volume and surface along the channel. Three types of dynamical events are involved in the model: adhesion, desorption and rolling.

4.1. Equations

As shown in Fig. 3, we define a small control volume and consider the number of platelets in this volume and on the surface, respectively noted n_v and n_s. The mass balance sketched in Fig. 4

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