ANALYSIS OF WHOLE BLOOD PLATELET TRANSLOCATION ON A VWF-COATED MICROFLUIDIC FLOW CHAMBER

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ABSTRACT

We demonstrate that by tacking and analyzing platelet translocation along a surface of vWF, which is dominated by an initial GPIbα-vWF-A1 interaction, we can detect the effects of ReoPro (abciximab) on the target receptor. These experiments were performed using whole blood, illustrating a potential for clinical application.

KEYWORDS: platelet, von Willebrand factor, blood, abciximab

INTRODUCTION

The first step in the complex process of platelet response to vessel injury involves the interaction between the platelet glycoprotein subunit Ibα (GPIbα) of receptor GPIbIXV and the A1 domain of multimeric protein von Willebrand factor (vWF). Under physiological shear, platelets interact with the surface in an unstable transition state between adhesion and lack of adhesion [1]. The opposing forces involved in this motion are the fluid drag on the particle and transient bonds with the surface. Experiments that flow platlets under controlled shear across surfaces of immobilized vWF can resolve the effects of drugs or mutations on the platelet receptors that bind to vWF, as shown with coated beads [2], cells expressing mutant GPIbα [1], and platelets purified from whole blood. We present data and show results of analysis from whole blood platelet translocation studies using a parallel-plate flow chamber (Figures 1-3) under a controlled arterial shear rate of 1500 s^{-1}, quantitatively demonstrating differences in behaviour of platelet interactions with vWF surfaces when blood is treated with GPIIbIIIa (αIIbβ) receptor antagonist ReoPro (abciximab), compared with untreated blood.

EXPERIMENTAL

To realize the diagnostic potential of blood-flow devices in the clinical setting for evaluation of bleeding and thrombotic disorders, we are developing an assay that characterizes initial platelet interaction(s) with vWF, as encountered in damaged arterial vasculature, prior to the point where platelets begin to interact significantly with each other. Object tracking (Figure 4) has been improved relative to literature reports [3]. Individual platelet trajectories are determined via a position-based probability function. Images are recorded at 25 frames per second; data are only considered from trajectories tracked over at least 10 consecutive frames (Figure 5) Using

to positional uncertainty as a guide, each tracked platelet is determined to be moving or stationary at each point in time; velocity statistics are extracted only from periods of movement.

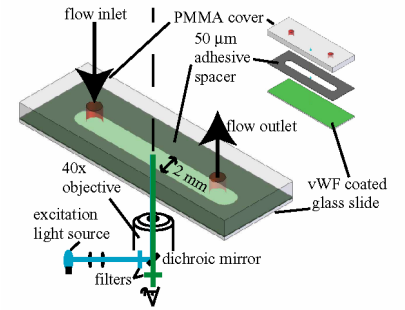

Figure 1. The flow chamber consists of a vWF-coated glass slide, a 50 µm adhesive spacer, and a PMMA cover. Platelets are fluorescently labelled and detected using a standard epifluorescence microscope.

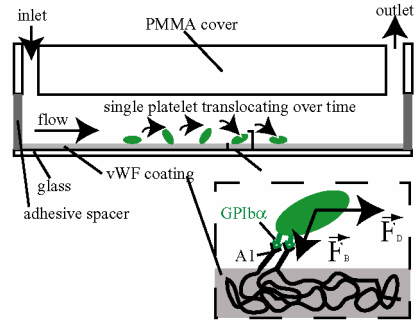

Figure 2. Side view of the device, illustrating a single platelet's interaction with the surface. The sum of the GPIbα-A1 bond forces $\boldsymbol{F}_B$ will oppose the drag force $\boldsymbol{F}_D$. The translocation behavior is a result of the interplay between those forces.

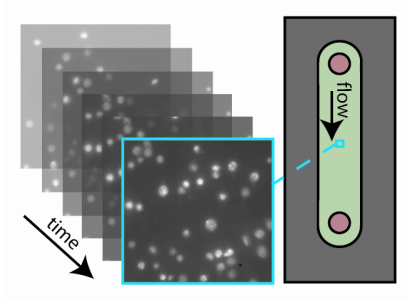

Figure 3. Top view of the device. The view fild is ~50 µm x 50 µm and is recorded at ~25 frames per second.

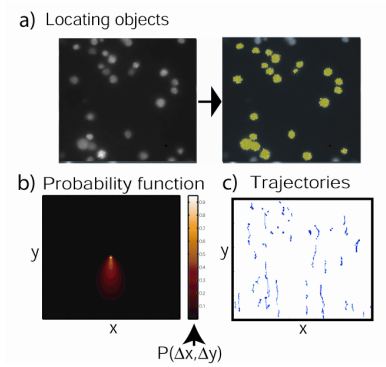

Figure 4. a) the objects were detected, b) objects were linked across separate images using a positionally dependant probability function, and c) trajectories were plotted for comparison.

RESULTS AND DISCUSSION

In the case of ReoPro-treated whole blood, the average platelet velocity during translocation did not change significantly, with values of 7.7 µm/s +/- 1.2 µm/s for normal blood and 8.9 µm/s +/- 2.2 µm/s for ReoPro-treated blood. However, the rela-

tive amount of time in the mobile versus stationary state, measured at 8.2% +/- 1.7% for normal blood and 13.5% +/- 2.6% for ReoPro-treated blood, shows clear separation, indicating a possible diagnostic measure to characterize platelet dysfunction in interaction with vascular matrix protein vWF and the effects of platelet drug treatment under fluid shear (Figure 6)

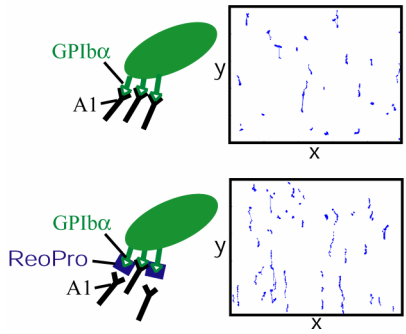

Figure 5. Results: Differences in translocation behaviour between ReoPro-treated and untreated blood are clear from the trajectory plots. There appears to be more overall motion with Reopro present.

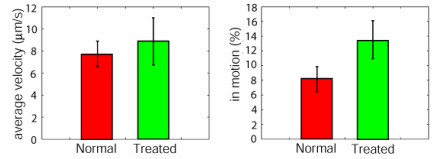

Figure 6. Results: Six seperate experiments on normal whole blood were compared with four experiments using whole blood treated with reoPro. Each experiment lasted for 20 seconds. Despite the clear differences shown in figure 5, a significant change was not detected in the average velocity of the moving platelets. Rather, the percent of time spent in motion showed clear differences.

CONCLUSIONS

Platelet response to vessel injury is complex and can be altered or disrupted in a number of ways. These results demonstrate measurement of platelet behaviour dominated by initial GPIbα-vWF-A1 interaction, characterize mobile and stationary kinetics of platelet interactions, identify the complexity of contributing factors involved in platelet adhesion events mediated by additional platelet receptor interactions, and measure the effects of clinical drug therapies on platelet interactions under haemodynamic conditions of vascular fluidic shear.

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