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Analysis of Microscopic Motion and Adhesion of Artificial Platelets

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1. Introduction

Blood platelets are important constituents of blood which permit the hemostasis function, keep the integrity of vessels and prevent blood loss. They are needed for transfusion during surgery but also in coagulation diseases like thrombocytopenia. However they have a short storage period, are prone to be easily infected by viruses and in the mean time, the demand is endlessly growing. So the development of artificial platelets is an important objective for future research.

A candidate for substitutive platelets is recombinant glycoprotein Ibα-conjugated latex beads (rGPIbα-LB). GPIbα is found on natural platelets and can bind to von Willebrand Factor (vWF), a protein found on vessel subendothelium, and thus allows aggregation of platelets and formation of blood clot, especially under high shear stress. The presence of red blood cells (RBCs) in vessels also plays a major role in hemostasis process: as RBCs accumulate in the center of the flow stream, platelets are pushed toward the wall, improving the availability of platelets in case of wall damage. This phenomenon is called near wall excess (NWE) and was shown to depend on hematocrit and shear rate in previous studies[1] .The dynamics of NWE is still unclear, and the role of RBCs has still to be elucidated. The study of motion and adhesion properties of rGPIbα-LB and their interactions with RBCs would allow us to have a better understanding of coagulation process and to validate rGPIbα-LB as an artificial platelets candidate. The development of new informatics tools will allow optimizing tracking and analyzing of data.

2. Materials and Methods

In this study, we used fluorescent rGPIbα-LB developed by Prof. Takeoka from Waseda University as platelet substitutes flowing in a rectangular flow channel observed through an inverted microscope illuminated with an Ar-Ion laser (fig.1). As RBCs' hemoglobin absorbs most of fluorescence, we prepared red cell ghosts according to Dodge's method[2] to be used for flow experiments. Flow chamber is constituted from two plastic cover slips on top and bottom, and two aluminium plates for lateral walls, forming a 200μm wide and 400μm high path. Aluminium plates were coated with vWF so rGPIbα-LB can adhere on them. To appreciate the particles trajectories, we used a travelling stage which made the flow chamber move in an opposite direction with the flow, allowing us to observe the trajectories of rGPIbα-LB in the channel in a Lagrangian point of view. This method permits to separate convective and diffusion components of the trajectories and thus to obtain specific characteristics of trajectories. Fluorescent images were detected with a CCD camera and we recorded the movies on computer.

We developed a Matlab program to extract trajectories from the movies and analyze data from a great number of frames with few manual interventions.

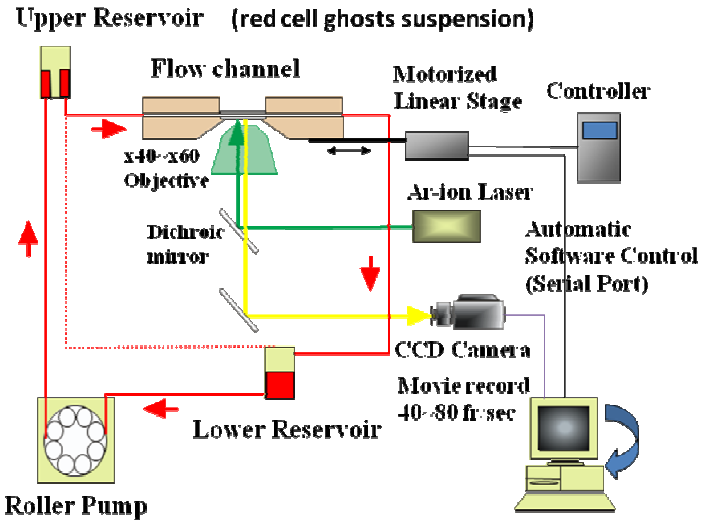

fig.1: Experimental device

Red arrows represent the RBC ghosts and LB suspension flow direction. A peristatic pump brings the fluid into the upper reservoir and flow rate is chosen by adjusting the upper reservoir's height, for different shear rate values

3. Results

We manage to create an algorithm able to detect precisely particles positions and compute trajectory data

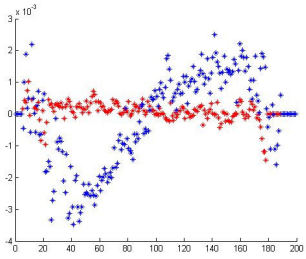

Fig2. : Comparision of trajectorygradient between 0% and 40% hematocrit

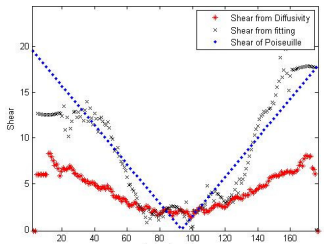

fig.3: Theoretical shear against experimental shear

The effect of hematocrit on platelets trajectories has been confirmed on the new data, the software giving us expected behaviour. Computed shear rate is coherent with theory, with a blunted velocity profile compared with Poiseuille flow, and higher wall shear rate. As we verified that the software was able to track particles efficiently, we will next verify influence of high shear rate, and apparition of adhesion

4. Bibliography

(1) Yeh C and Eckstein EC, Transient lateral transport of platelet-sized particles in flowing blood suspensions, *Biophys J*, 1994, 66, 1706-1716.

(2) J. T. Dodge, C. Mitchell and D. J. Hanahan, The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes, *Arch Biochem Biophys* 100(1963), 119-130.