MOTION OF ARTIFICIAL PLATELET IN THE MODEL ARTERIOLE

Hideki Fujita (1), Tetsuya Tsuji (1), Takashi Tabata (1), Shinji Takeoka (2)
Yasuo Ikeda (3) and Kazuo Tanishita (1)

(1) Dept. of System Design Engineering
Keio University
Yokohama, Kanagawa
Japan

(2) Dept. of Polymer Chemistry
Waseda University
Tokyo, Japan

(3) School of Medicine, Keio University
Tokyo, Japan

INTRODUCTION

Platelet transfusion is the most effective therapy for bleeding associated with thrombocytopenia induced in the chemotherapy or surgery. The amount of platelets transfused each year has rapidly grown. To overcome these problems, several approaches have been explored to develop novel human platelet substitutes being capable of long term storage [1]. To estimate the feasibility of clinical application of platelet substitute, we need the knowledge concerning the micro-mechanics of platelet substitutes leading to the hemostatic functions.

Recently Takeoka et al. [2] developed the albumin microspheres conjugated recombinant glycoprotein Ibα (rGPIbα-AMS), which is supposed to be a promising platelet substitute because of their high blood compatibility, high biogradability and long experience in clinical use. The rGPIbα-AMS was interacted with von Willebrand factor (vWF), and enhanced the ristocetin induced platelet aggregation. In the present work, we studied the fluid mechanical behavior of rGPIbα-AMS in the process of hemostasis. As a first step, to evaluate the ability of platelet substitutes, we assessed the motion of platelet substitute in the flow in vitro. We directly observed the motion of artificial platelet toward ligand coating surface in the rectangular channel flow chamber, and the high speed camera with Image Intensifier.

MATERIALS AND METHODS

To detect the small particle with the size down to 200 nm in the high shear rate of 1500 sec^{-1}, we employed the Image Intensifier to amplify the fluorescent, and Argon-ion laser for strong excitation. Moreover to visualize the particles, we used the red cell ghost suspension. Red cell ghost was presented to behave as natural red blood cells [3], and in red cell ghost suspension, the concentration profiles of platelet was similar to that in washed blood cell suspension.

Platelet Substitute

Platelet membrane glycoprotein, rGPIbα conjugated albumin microsphere (rGPIbα-AMS). The diameter could be controlled from about 200 nm to 2 μm and we used the diameter of 240±50 nm and 1900±400 nm. To visualize rGPIbα-AMS, each particle were labeled with fluorescein isothiocyanate, which the excitation and emission wavelength were 495 nm and 520 nm.

Flow Chamber and vWF Coating

To observe the motion of rGPIbα-AMS adhering toward the wall, we used the rectangular channel consisted of two cover glasses and upper and lower chambers as shown in Figure 1. Two cover glasses, 0.2 mm thick, was placed with the interval of 200 μm, and fixed with upper and lower chamber. There was the gap that height and width are both about 200 μm, and we used there as a flow field. The surface coated with vWF is vertical against focal plane, thereby we could observe the motion of the particle toward the vWF coating surface.

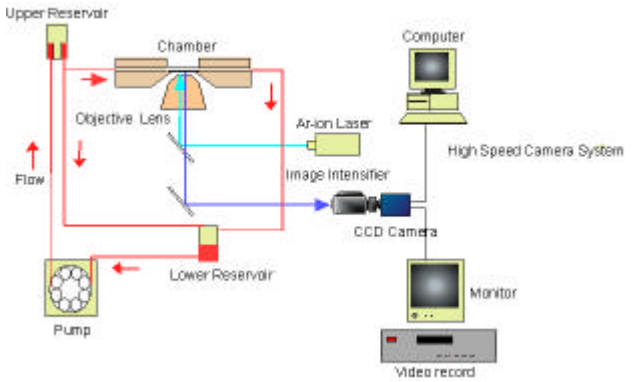

Figure 1. Experimental system to visualize the microscopic motion of artificial platelets flowing in the model arteriole

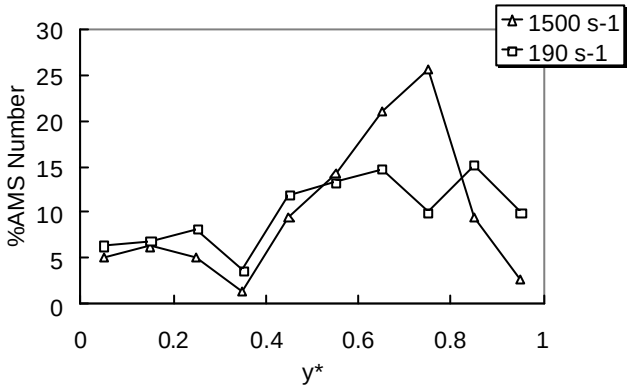

Figure 2. Concentration profile of rGPIbα with varying shear rate for larger particle (size=2 μm)

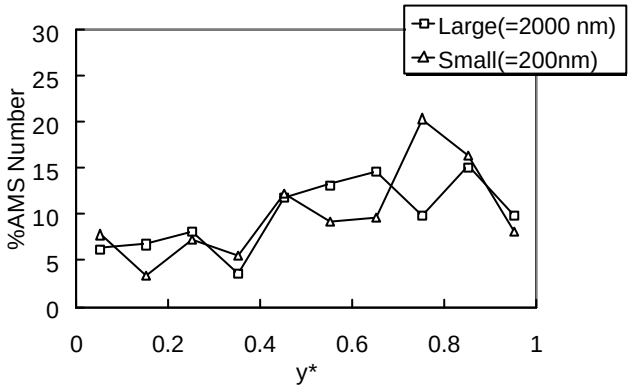

Figure 3. Effect of particle size on the concentration profile with the shear rate of 190 sec^{-1}

RESULTS AND DISCUSSION

Concentration Profile of AMS Particles Varying with Wall Shear Stress

The half width of flow duct was divided into 10 segments with the thickness of 10 μm, where the number of particles was counted from 150 images. Thereby the concentration profile was determined, which is shown in Figure 2 and 3. The abscissa is the distance from the center axis divided by the half width; namely y*=0 and y*=1 correspond to the center axis and the wall surface respectively.

Figure 2 shows the concentration profiles of large rGPIbα-AMS at the higher and lower shear rate. We should note that the maximum concentration appeared at y*=0.85 at the wall shear rate of 1500 s^{-1}, and the concentration is excess near the wall, which was observed for the profile of natural platelets. Similarly, the profile for the lower shear rate 190 s^{-1} tends to increase near the wall, although the peak concentration is half of that of higher shear rate and the ratio of peak to center concentration was only 2.4.

Figure 3 shows the concentration profile at the lower shear rate for larger and smaller particles. There is no significant difference between the size of particles and both of them tend to increase with the increase of y*. It is interesting to see that the maximum concentration appeared near the wall for all of the cases and this tendency demonstrates well that the motion of AMS particles simulates the natural platelets.

Drift Angle Φ of the Moving Particles

We measured the velocity vectors by means of particle tracking method. Since the particle motion occurs in the stochastic manner and we need the parameter to identify the stochastic process of particle motion. Thus we defined the drift angle Φ with the axial and drift velocity components by the following.

$\Phi = \tan^{-1}$ (Vdrift / Vaxial)

Figure 4 shows the histogram of drift angle Φ of large rGPIbα-AMS flowing in the rectangular channel at 190 s^{-1}. The shape of histogram indicates the statistical characteristics of particle motion, namely the peaked shape indicates the less lateral motion and widely spread shape means the strong back and forward motion in the y-direction. Near the wall, the back and forward motion in the lateral direction becomes intensified and this feature is advantageous to adhesive process of artificial platelet. In previous study, we measured the drift velocity of liposome flowing in the rectangular channel with inactivated wall, and the drift velocity was the highest near the wall too.

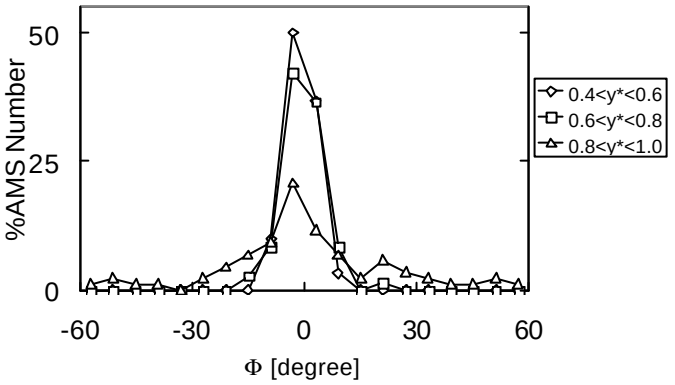

Figure 4. Histogram of drift angle of particle motion in the flow. We should note the profile tends to be flat near the wall.

ACKNOWLEDGEMENTS

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