MEASUREMENT OF VENOUS THROMBUS FORMATION AND MASS TRANSFER EFFECTS IN THE INITIAL STAGE USING MICROCHANNEL FLOW

Yusuke Yamamoto[1], Kazuya Tatsumi[1], Hitoshi Shirouzu[1], Hideo Hirakata[2], Naoko Sugita[1], Kyo Inoue[1], and Kazuyoshi Nakabe[1]

[1]Kyoto University, JAPAN, and [2]Kyoto City Hospital, JAPAN

ABSTRACT

In this study, the process of venous thrombus formation, particularly the initial stage of fibrin network formation, was measured and analyzed using a microfluidic platform. The results showed that the platelet activation and mass transfer from the platelets has a strong correlation with the fibrin network formation and pattern.

KEYWORDS: Venous thrombus, Mass diffusion, Fibrin network, Microchannel

INTRODUCTION

Venous thrombus is a significant issue in the blood circulatory system that can lead to serious symptoms such as deep venous thrombosis and pulmonary embolism (PE) which has large number of mortality [1]. However, compared to arterial thrombus, the mechanism, formation process, and structure of a venous thrombus have not yet been well understood [2,3]. We have fabricated a microchannel, modeling the vain, with an area exposing the collagen layer to the blood flow, thus representing a condition that the wall of vein is injured (Fig. 1). When a vessel wall is wounded, platelets aggregate at the site of injury and are activated through various reactions. In this process, fibrin fibers are formed from platelets and increase the volume by capturing other blood cells. We measured the fibrin pattern growing from the activated platelets adhered to the vessel wall. The pattern was compared with the apparent mass diffusion from the platelets to see the correlation between the two phenomena.

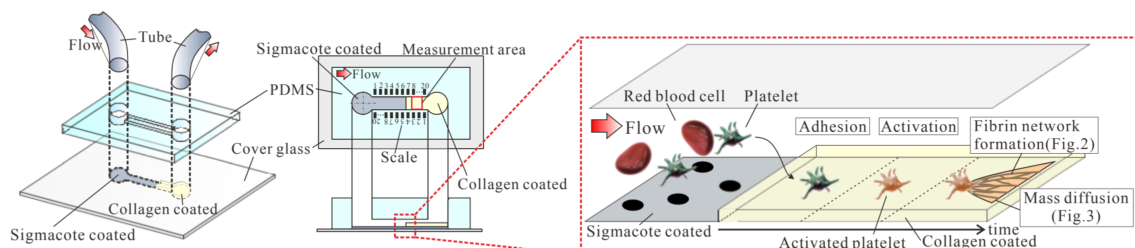

Figure 1: Schematics of the microchannel adjusted to the blood flow condition in vein. A part of the channel wall is coated by collagen and the platelets adhere to this region and then activates. To avoid stimulating platelets by glass (to prevent intrinsic coagulation), whole channel wall is coated by sigmacote.

EXPERIMENTAL

The microchannel was made of cover glass and PDMS (polydimethylsiloxane) and is shown in Fig. 1. Healthy human blood, into which fibrinogens fluorescently labeled by Fibrinogen-Alexa Fluor 546 (Thermo Fisher Scientific, F13192) were mixed, were supplied to the channel at the flow rate of 1.3μL/min. The wall shear rate under this condition corresponds to that in the vein. The fluorescence image of the fibrin and the particle motion were measured using a high-sensitivity camera. The particle velocities and positions were analyzed by the motion analyze software (Library, Move-tr/2D ver 7.90) to measure the apparent diffusion coefficients.

RESULTS AND DISCUSSION

Figure 2 shows the visualization of the activated platelet and fibrin network in the initial ((a), (b)) and developed ((c), (d)) stages. Figure 2(d) shows the fibrin pattern in the well (stationary fluid with zero flow velocity). As time progresses in Figures (a)-(c), fibrin fibers appear from the platelets and mainly expand downstream in the microchannel flow. While the fibrin network observed in the well

shows an isotropic pattern, the fibrin network in the microchannel flow is significantly influenced by the flow and aligned along the flow direction.

To see if the previously mentioned pattern can be attributed to the mass diffusion of the activator released from the platelets, each fibrin fiber formed downstream of the platelets was measured and the area was presented by the 95% inclusive area. This area was derived using the apparent diffusion coefficient obtained from the probability density distribution of the spanwise displacement of the fibrin growth in 5s and is shown in Fig. 3(a). Mass diffused downstream of the platelet was measured in blood and water flows using micro-particles and is shown in Fig. 3(b). Comparing the fibrin network pattern and diffusion area, a fair correlation can be observed. The reason why the fibrin area shows larger value than the diffusion area can be attributed to the blood cells attached to the channel bottom wall which could interfere the flow and change the direction and amplitude.

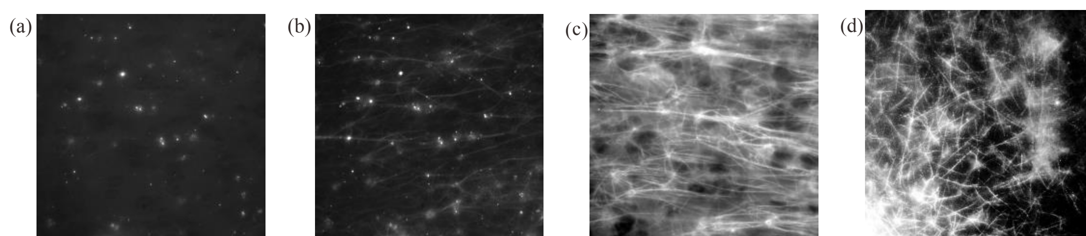

Figure 2: Visualization of the fibrin network and platelet at different stage. (a) and (b) show the results of elapse time of (a) 10min (b) 12min, and (c) and (d) show the developed stage. (a)~(c) are measured in the microchannel (flow direction in these figures is left to right) and (d) is measured in the well (in stationary fluid).

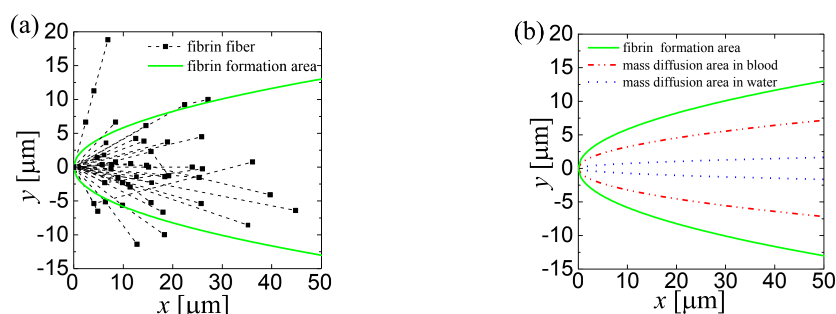

Figure 3: (a) the distributions of fibrin fibers formed from each platelet (the platelet is located at the coordinate origin). The dash lines depict the fibrin fibers and the square symbols are the edge of the fibrin measured every 5s. The solid line represent 95% inclusive area obtained by the apparent diffusion rate. (b) the area of mass diffusion with the released source at the coordinate origin measured by the particle motions in the water and blood flows are compared with the result of Fig. (a).

CONCLUSION

The fibrin network growing from the platelet could be visualized clearly in the microchannel. The diffusion area of the mass assumed to be originated from the platelets showed a fair correlation with the direction of the fibrin fibers growing, which indicated the possibility of some relationship between activators released from the platelets and the pattern of the fibrin network.

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CONTACT

* Kazuya Tatsumi; phone: +81-75-383-3606; tatsumi@me.kyoto-u.ac.jp