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# Relation between platelet adhesion and shear stress around micro stenotic channel

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## Abstract

In the consideration of pathological thrombosis and the atherosclerosis progression, hemodynamic features and platelet activation play important roles. In our previous studies, the probability of platelet adhesion in the microchannel was increased under high-hematocrit conditions. The present study aimed to investigate the interactions between hemodynamic properties and platelet adhesion around stenosed channel. After passing through the narrow stenotic channels with different stenotic width (10 μm), the platelets can be activated, and then activated platelets were adhered on the downstream of the stenosis. The velocity field and shear rate around the stenotic channel were measured by using particle image velocimetry (PIV) measurements. Due to the high shear stress in the 10-μm-wide stenosis, adhesion of platelets with a 3D circulating motion was significantly observed at the posterior end of the stenosis. In high hematocrit conditions, frequent particle collision contribute to the promotion of platelet activation and adhesion, even when the shear rate is relatively low. This finding indicates that relatively high viscosity with frequent particle collisions can contribute to the promotion of activated platelet adhesion under relatively low-shear conditions.

## 1 Introduction

Blood is a concentrated suspension of elements, including red blood cells (RBCs), leukocytes, platelets, and protein macromolecules, in plasma. platelets are the principal components that arrest the bleeding caused by an injury to the vascular wall. In addition to its role in normal hemostasis, platelet adhesion plays an important role in pathological thrombosis (Massberg, Brand et al. 2002). A multi-step adhesion process between the platelet receptors and adhesive ligands can stabilize the cells to withstand the mechanical forces generated by blood flow (Dopheide, Maxwell et al. 2002). This adhesion mechanism is regulated by various factors, such as subendothelial matrix proteins, biochemical activators, and hemodynamic features. When platelets pass through a narrowed vessel, they may be subjected to different hydrodynamic forces, depending on the sites of the stenosed vessels. This variation in the hydrodynamic force, caused by arterial stenosis, contributes to platelet activation and thrombus growth (Kroll, Hellums et al. 1996).

To estimate the platelet functions, platelet adhesion was estimated without labeling the platelets by using a correlation map that visualizes the decorrelation of the flow stream in our previous studies. without labeling the platelets by using a correlation map that visualizes the decorrelation of the flow stream (Jung and Yeom 2017, Yeom, Kim et al. 2017). In addition to the platelet adhesion, the blood viscosity was simultaneously measured based on the pressure ratio between the blood sample and a reference fluid in an H-shaped channel composed of two parallel side channels and a bridge channel (Yeom, Park et al. 2016). Using this microfluidic system, we observed that, under high-hematocrit conditions, the probability of platelet adhesion increased and these adhered platelets were also well sustained. However, the effects of the shear stress on platelet adhesion were not investigated in the previous study. In the present study, we investigated the relation between shear stress and platelet adhesion around stenosed channels.

## 2 Materials and method

### Fabrication of stenosed channels

PDMS microfluidic channels with a depth of 50 μm were fabricated by using soft lithography and deep reactive-ion etching. Three stenosed channels had different stenosis severities of 90, 95, and 99 % with the same stenosis length. In all stenosed channels, the input and output channel widths were 1000 μm. PDMS prepolymer (Sylgard 184; Dow Corning, USA) was mixed with a curing reagent at a mass ratio of 10:1. The mixture was poured onto silicon molds that were placed into a vacuum chamber for 1 h to remove any trapped air bubbles. Subsequently, the PDMS was cured at 80°C for 3 h. The PDMS block was then peeled from the molds. The channel inlets and outlet were made using a 1-mm diameter punch. Oxygen-plasma treatment (CUTE, Femto Science, Korea) was then applied for 90 s at 40 W. Finally, the microfluidic device was prepared by bonding the PDMS block to a glass substrate.

### Experimental setup

The flows in the stenosed channels were observed through an optical microscope (Olympus BX51; Olympus Co., Ltd., Tokyo, Japan) with a 4× or 10× objective lens. The samples were injected into the microfluidic channels at a rate of 1 mL/h by a syringe pump (neMESYS, Centoni GmbH, Germany). Four frames of the flow in the microfluidic device were consecutively captured by a high-speed camera (Phantom VEO710L, Vision Research Inc., Wayne, NJ, USA) at 5000 fps (frame per second) when the trigger signal from the delay generator (model 555, BNC, USA) was input to the camera. The time interval between each trigger signal was 1 s.

### Blood sample preparation

Human red blood cells (RBCs) and platelet rich plasma (PRP) were obtained from the Red Cross blood bank (Busan, Korea). The RBCs were washed with a phosphate-buffered saline (PBS) solution (pH 7.4, Bio Solution, Korea). Three blood samples with different hematocrits (0, 30, and 50%) were prepared by discretely mixing the stored RBCs and PRP. During all experiments, the PBS solution was used as the reference fluid to prevent bursting of the RBC membranes due to differential osmotic pressure. For this case study, all experiments were repeated three times using different samples. All experimental procedures were performed in accordance with the approved guidelines of Pusan National University's Ethics Committee.

### Micro-particle image velocimetry (PIV) technique

The captured optical images of the blood flows were cropped to 960 × 512 pixels prior to applying the PIV algorithm to each image pair. To enhance the signal-to-noise ratio in the cross-correlation map, digital image-processing techniques were applied to the cropped images. A binary mask was applied to the stenosis area to improve the measurement accuracy near the wall region. The detailed procedure and image-processing techniques are described in our previous studies (Yeom, Kang et al. 2014, Yeom, Nam et al. 2014). For all experiments, the size of each interrogation window was 64×8 pixels with a 50% overlap. The obtained velocity vectors were filtered using a 3 × 3 median kernel. The representative velocity field was obtained from the ensemble average of approximately 1500 vector files.

## 3 Results and discussion

### Flows around stenosed channels

Tracer particles (0.52 μm mean diameter; Thermo Scientific) were used to accurately investigate the flows around the stenosed channels with different severities (90, 95, and 99 %). Prior to the experiment, the tracer particles were washed twice in 1% bovine serum albumin (BSA, Sigma-Aldrich), and then they were suspended in the PBS at a final concentration of $5 \times 10^9$ microspheres/mL. **Figure 1** shows the velocity

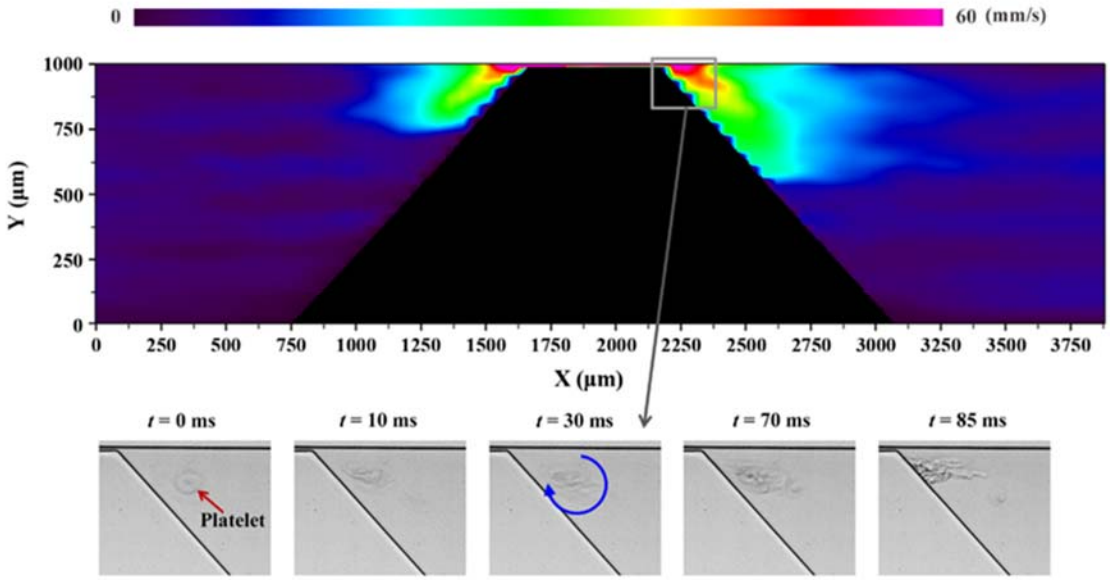


**Figure 1**. Ensemble-averaged contour map of the velocity magnitude for the stenotic width of 10 μm. Progress of platelet adhesion after the stenosed channel with increasing time.

fields around the stenosed channels with stenotic width of 10 μm under a constant flow rate of 1 mL/h; for ease of understanding, a contour map of the velocity magnitude is included. For all channels, the main flow stream was accelerated as the particles passed through the stenosis apex. In the downstream region of the stenosis, this jet-like flow decelerated due to the channel area expansion. As expected, the degree of flow acceleration around the stenosis apex was greater in the 50-μm-wide stenotic channel than in the 100-μm-wide stenotic channel. However, the region of flow acceleration was relatively small in the 10-μm-wide stenotic channel; due to the very narrow stenotic width, the tracer particles easily accumulated at the anterior end. This type of accumulation results in the reduction of flow rate. From the measured velocity information, the distribution of the shear rate around stenotic channel can be determined. The flow acceleration around the stenosis apex can cause a high shear rate near the wall region.

### Effect of hematocrit on platelet adhesion and blood viscosity

Bottom images of **Fig. 1** show the procedure of platelet adhesion around the posterior end of the 10-μm-wide stenosis. Platelets were activated by the flow acceleration through the stenosis apex and then flowed downstream of the channel. Due to the high severity of the stenosed channel, a recirculation zone was induced in the post-stenosis region. In this recirculation zone, 2D movement of platelets with the variation of diffraction pattern was observed. From the results, it was inferred that there is a 3D circulating motion of the activated platelets because the diffraction pattern is determined by the location of particle in the object focal plane (Luo, Yang et al. 2006). As the time proceeded, the discoid platelet was changed into an elongated aggregation of platelets. After the activated platelets aggregated, they slightly tethered to the collagen film on the bottom of the channel. Then, firm cell adhesion occurred around the posterior end of the stenosis. Platelets with RBCs easily adhered around the stenosed channel. This flow blockage made it difficult to monitor the behaviors of platelets in the blood samples with hematocrit levels of 30 or 50 %.

## 4 Conclusion

In the present study, flow information, such as the velocity fields and shear rates around stenotic channels and viscosity, was estimated by using PIV measurements and through simulation. As the flow passed through the stenosis apex, flow acceleration with a jet-like flow was observed. Then, the main stream

decelerated downstream of the stenosis due to the expansion of the channel area. After passing through the narrow stenotic apex, the platelets were activated, and then these platelets adhered downstream of the stenosis. The platelet behavior after passing through the stenoses differed depending on the distribution of the blood samples' shear stresses around the stenosed channels. Due to the high shear stress in the 10-μm-wide stenosis, significant adhesion of platelets was observed at the posterior end of the stenosis. During platelet adhesion, a 3D circulating motion of the platelets occurred. This finding indicates that relatively high viscosity with frequent particle collisions can contribute to the promotion of activated platelet adhesion under relatively low-shear conditions.

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