Microfluidic measurement for blood flow and platelet adhesion around a stenotic channel: Effects of tile size on the detection of platelet adhesion in a correlation map

Sung Yong Jung¹ and Eunseop Yeom^{2,a)}

¹Department of Mechanical Engineering, Chosun University, Gwangju, South Korea

²School of Mechanical Engineering, Pusan National University, Busan 46241, South Korea

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Platelet aggregation affects the surrounding blood flow and usually occurs where a blood vessel is narrowed as a result of atherosclerosis. The relationship between blood flow and platelet aggregation is not yet fully understood. This study proposes a microfluidic method to measure the velocity and platelet aggregation simultaneously by combining the micro-particle image velocimetry technique and a correlation mapping method. The blood flow and platelet adhesion procedure in a stenotic micro-channel with 90% severity were observed for a relatively long period of 4 min. In order to investigate the effect of tile size on the detection of platelet adhesion, 2D correlation coefficients were evaluated with binary images obtained by manual labeling and the correlation mapping method with different sizes of the square tile ranging from 3 to 50 pixels. The maximum 2D correlation coefficient occurred with the optimum tile size of 5×5 pixels. Since the blood flow and platelet aggregation are mutually influenced by each other, blood flow and platelet adhesion were continuously varied. When there was no platelet adhesion ($t=0$ min), typical blood flow is observed. The blood flow passes through the whole channel smoothly, and jet-like flow occurs in the post-stenosis region. However, the flow pattern changes when platelet adhesion starts at the stenosis apex and after the stenosis. These adhesions induce narrow high velocity regions to become wider over a range of area from upstream to downstream of the stenosis. Separated jet-like flows with two high velocity regions are also created. The changes in flow patterns may alter the patterns of platelet adhesion. As the area of the plate adhesion increases, the platelets plug the micro-channel and there is only a small amount of blood flow, finally. The microfluidic method could provide new insights for better understanding of the interactions between platelet aggregation and blood flow in various physiological conditions. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4982605>]

I. INTRODUCTION

Atherosclerosis, caused by the accumulation of lipoproteins, is a major cause of circulatory vascular diseases which are leading causes of death. Variations in hemodynamic conditions such as reduced and oscillatory wall shear stress (WSS) are reported to be closely related to the formation and progression of atherosclerosis.^{1,2} Platelet aggregation usually occurs where a blood vessel is narrowed as a result of atherosclerosis. This stenosis leads to changes in hemodynamic parameters such as pressure, flow, and shear stress. It is well known that the effects of hemodynamic forces and cellular interactions significantly impact thrombosis.³

Numerous studies have been conducted to identify the relation between hemodynamic features and thrombus formation. Banerjee *et al.*⁴ investigated that thrombus formation occurs

^{a)}E-mail: esyeom@pusan.ac.kr. Fax: +82-51-514-5236.

in the recirculation zone of the post-stenosis region due to the relatively low WSS. Nesbitt *et al.*⁵ found that the shear gradient caused by flow acceleration and deceleration regulates platelet aggregation during thrombosis in a stenosed vessel. For severe stenosis, Reynolds shear stress is also as significant as laminar shear stress for blood cell interactions, which are closely related to platelet adhesion.^{6,7}

Ha and Lee⁸ demonstrated that platelet aggregation in a stenotic channel occurs where the momentum of the jet and the recirculation flow are balanced. It was reported that platelet aggregation is not a simple response to shear and force but a complicated response to the cumulative effects of the entire shear history experienced by platelets.^{9,10} The relation between blood flow and thrombus formation has been widely investigated, but it is not yet fully understood.

Several microfluidic systems have been utilized to measure platelet function, in which the platelet aggregation is estimated by labelling platelets under sudden shear gradient conditions.^{11–15} Although various blood flow conditions give rise to platelet activation, aggregation, and adhesion, most previous studies observed the aggregation under specific flow conditions. To investigate the effects of various flow conditions on the platelet aggregation, it is essential to measure the velocity information of the flow. However, as far as we surveyed, the studies investigating both the platelet aggregation and the velocity information of surrounding flows are lacking.

Various studies have measured the characteristics of blood flow through a stenosed channel,^{16–19} but only the velocity information of the flow around the stenosis was measured without measuring the platelet aggregation. In addition, in these studies with a microchannel, diluted blood with low hematocrit was employed to measure the motion of red blood cells (RBCs) to investigate the effect of local contraction on the hemodynamic characteristics. Ha and Lee⁸ measured the velocity information of whole blood flow with platelet aggregation in a stenotic microchannel. However, experiments for measuring aggregation and velocity information were separately performed because different optics tools were used for each measurement. In addition, platelet aggregation was occasionally monitored and the region where the platelet aggregation occurs was manually determined.

The present study measures velocity information and platelet aggregation simultaneously by combining the micro-particle image velocimetry (PIV) technique and the correlation mapping method. The effect of tile size on the detection efficiency of platelet adhesion was examined by comparing the 2D correlation coefficients between the results obtained by manual labeling and the correlation mapping method with various window sizes. Finally, the temporal variations in blood flow and platelet adhesion in a stenotic micro-channel were observed using this method.

II. MATERIALS AND METHODS

A. Fabrication of the microfluidic device

A PDMS (polydimethylsiloxane) microfluidic device with a depth of 50 μm was fabricated by using soft lithography and deep reactive-ion etching. The stenosed channel has a severity of 90% (from 1000 μm to 100 μm) with a contraction–expansion angle of 45°. 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, and trapped air bubbles were removed in a vacuum chamber for 1 h. Subsequently, PDMS was cured at 80 °C for 3 h. The PDMS block was peeled off from the molds. The inlet and outlets of the channels were made by a puncher with 1-mm diameter. Oxygen-plasma treatment (CUTE, Femto Science, Korea) was then applied for 90 s at 40 W with a flow rate of 30 sccm and base pressure of 0.923 Torr. The microfluidic device was finally prepared by bonding the PDMS block to a glass substrate. Prior to bonding, PDMS devices were cleaned with ethanol and then incubated in a 5 mg/mL BSA (bovine serum albumin) solution for 1 h to prevent adhesion of blood components. To prepare the collagen films on glass substrates, collagen type I (from rat tails; Invitrogen, Carlsbad, CA, USA) was diluted to be a final concentration of 0.32 mg/mL using phosphate buffered saline (PBS; pH 7.4, Bio Solution, Korea) solution. The mixture was incubated on the glass substrates overnight with the previously prepared methyl-terminated glass overnight at 37 °C.^{20,21}

B. Blood preparation

Male Sprague-Dawley (SD) rats (14 to 15-weeks old) were anesthetized with an intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood samples were collected through an abdominal aortic puncture and anticoagulated using heparin (100 IU/mL). The collected blood samples were directly used. All procedures performed on animal models were approved by the Animal Care and Ethics Committee of the Pusan National University, and the methods were carried out in accordance with the approved guidelines.

C. Experimental setup

As the contraction–expansion angle of the stenosis becomes steep, the platelet aggregation is accentuated.¹⁰ Considering the measurement time, a gentle slope (about 45°) was adopted. The flow rate was set to be 1 mL/h to produce about the shear rates of 1800 s^{-1} at the upstream of channel (supplementary material, Fig. 1), considering the shear rates of small arteries/arterioles.¹⁵ As the severity of stenosed channel increases, high hydrodynamic drag force induces rolling or translocation motions of adhered platelets. The behaviors can affect adhesion process of

FIG. 1. Proposed microfluidic-based method to measure blood flow and platelet adhesion. (a) Schematic of the measurement system composed of a microscope equipped with a CMOS camera and stenotic microchannel. The stenosed channel has 90% severity (from 1000 μm to 100 μm) with a contraction–expansion angle of 45° . The flow rate of the syringe pump is fixed at 1 mL/h. (b) Data processing procedure of velocity field measurements. A micro-particle image velocimetry (PIV) technique was applied to six consecutive images acquired at a high frame rate (5000 fps) to measure velocity fields. By adjusting the trigger signals, the image acquisition is repeated at 1 s intervals for a total of 4 min. (c) Procedure to calculate the area of platelet adhesion. A correlation map in the squared tile is obtained to quantify the area of platelet adhesion. The adhered platelets can be determined by converting the correlation map into a binary image with a thresholding technique. The tile size was varied from 3 to 50 pixels to investigate the effect of tile size on the detection of platelet adhesion.

platelets. After conducting preliminary experiments, the stenotic micro-channel with 90% severity was determined based on time required for the platelet adhesion and measurable flow range.

As shown in Fig. 1(a), the microfluidic device was mounted on an optical microscope (Nikon, Tokyo, Japan) with a 4× objective lens (NA of 0.1). Blood samples were supplied to the microfluidic device using a syringe pump (neMESYS, Centoni GmbH, Germany) with a 1 mL plastic syringe (BD; Becton Dickinson, Franklin Lakes, USA). When a trigger signal, generated from a delay generator (model 555, BNC, USA), is inputted to a high-speed complementary metal-oxide semiconductor (CMOS) camera (FASTCAM SA 1.1, Photron Ltd., San Diego, USA), six consecutive images of blood flow are acquired at a frame rate of 5000 frames per second (fps). The time interval between two trigger signals is 1 s. All experiments were conducted at the room temperature of 25 °C.

D. Micro-PIV technique

Before applying the cross-correlation PIV algorithm to each image pair, the captured optical images of blood flow were cropped into the images of 1000 × 200 pixels. The images were also processed using digital image-processing techniques to enhance the signal-to-noise ratio of the cross-correlation map. A binary mask was applied to the processed flow images for improvement of the measurement accuracy near the wall region. The detailed procedure and image-processing techniques are described in our previous studies.^{22,23} The size of each interrogation window was 64 × 8 pixels with 50% overlapping for all experimental conditions. The obtained velocity vectors were filtered using a 3 × 3 median kernel.

E. Analysis of flow characteristics

Based on our previous study,²⁴ the measured velocity information can be postulated to represent the mean velocity of particles in the whole depth because of the relatively large depth of correlation ($\delta_C = 310 \mu\text{m}$). This quantity expresses the depth over which particles contribute to the cross-correlation analysis, and it is larger than the channel depth under the present experiment condition.

The flow rate of blood (Q_{Blood}) in the microchannel can be estimated using the following equation:

$$Q_{\text{Blood}} = \int_0^W U(x) dx \times H, \quad (1)$$

where $U(x)$ is the velocity profile at a specific lateral position (x) and W and H indicate the width and depth of the microchannel, respectively.

The Reynolds number (Re) is calculated using the following equation:

$$\text{Re} = \frac{4Q}{\nu P}, \quad (2)$$

where P is the perimeter of the channel, Q is the flow rate, and ν is the kinematic viscosity of the blood sample. In the present study, the kinematic viscosity of rat blood sample is $3.2 \times 10^{-6} \text{ m}^2/\text{s}$ for the Re estimations.

F. Image processing techniques for labeling platelet adhesion

The correlation mapping method was applied to flow images to distinguish adhered platelets from flowing blood. Each image was divided into small tiles ($m \times n$), and the 2D correlation coefficient (R) of two tiles is calculated using the following equation:

$$R = \frac{\sum_{i,j} (A(i,j) - \bar{A})(B(i,j) - \bar{B})}{\sqrt{\left(\sum_{i,j} (A(i,j) - \bar{A})^2\right) \left(\sum_{i,j} (B(i,j) - \bar{B})^2\right)}}, \quad (3)$$

where A and B represent the tiles of two consecutive images, (i, j) is the pixel coordinates of the tile, and $\bar{A}$ and $\bar{B}$ are the mean values of tiles A and B , respectively. After labeling the centroid of the tile with R squared, the labeling process is repeated for all captured images. The correlation maps are converted into binary images using a thresholding method with an optimal value determined by Otsu's algorithm.²⁵ The area of the adhered platelets is estimated by counting the number of nonzero pixels in the binary image.

G. Manual labeling of area for adhered platelets

The binary image obtained through the correlation mapping was compared with the manually labeled binary images to evaluate the detection accuracy. The flow image acquired at $t = 2.5$ min was used for this evaluation. The CS3 Photoshop software (Adobe Systems Inc, San Jose, CA) was used for manual labeling analysis. After monitoring flow images around $t = 2.5$ min, areas considered as adhered platelets were selected using the Quick Selection Tool. Selected regions were labeled as white in the binary image. To improve detection accuracy, the manually labeled binary image was carried out by one operator in three times.

III. MICROFLUIDIC METHOD TO MEASURE BLOOD FLOW AND PLATELET ADHESION SIMULTANEOUSLY

As shown in Fig. 1(a), the experimental setup was composed of a microscope with a CMOS camera and a microfluidic device with a stenotic channel. Blood samples were delivered into the microfluidic device using a syringe pump with a flow rate of 1 mL/h. In order to investigate the procedure of platelet aggregation with velocity information for a relatively long period of 4 min, a predetermined number of images were saved when a camera received a trigger signals from the delay generator. In this study, time interval between every two trigger signals is 1 s and six consecutive images were acquired for each trigger signal at a frame rate of 5000 frames per second (fps). For the purpose of minimizing the measurement error, five instantaneous velocity fields obtained by six consecutive images were averaged.

The velocity field and area of platelet adhesion were individually achieved using the same images, as shown in Figs. 1(b) and 1(c). For each analysis, images were divided into small regions called as interrogation windows and tiles for the velocity and platelet area measurements, respectively. In the PIV analysis, the size of the interrogation windows was fixed and the velocity fields were obtained by applying the cross-correlation PIV algorithm. To increase the maximum measurable velocity, relatively elongated interrogation window ($k \times l$ pixels) was used. High shear force caused by stenosis can initiate platelet aggregation. Collagen was coated on the glass substrate of the stenosed channel, so that platelets can easily adhere to the micro-channel. The size of the squared tile was determined after looking at the effect of the tile size on the detection of platelet adhesion by varying the size from 3 to 50 pixels. The correlation map was obtained by calculating the 2D correlation coefficients in tiles of consecutive images with time intervals of 1 s and then converted the image into a binary image.

This estimation method has several advantages. First, the biophysical properties, including flow velocity and platelet adhesion, can be measured simultaneously. Second, labeling of platelets or injection of artificial particles is not required. This method can be used to monitor the temporal variations of hemodynamics and platelet adhesion in a relatively long period, which means that the mutual influences between platelet adhesion and blood flow can be investigated in detail under various physiological conditions.

IV. RESULTS AND DISCUSSION

A. Changes in flow images due to platelet adhesion

Figure 2 shows the original and binary flow images at various times ($t=0, 0.5, 1, 2$, and 4 min). The region of platelet adhesion is shown as a relatively bright spot in the original flow images. Adhered platelets are initially observed at the end of the stenotic apex and near the shear layer of a jet-like flow at $t=0.5$ min. The area of platelet adhesion is increased as times go on after the initiation of platelet adhesion. The region of platelet adhesion is obviously widened at the stenotic apex and post-stenosis after $t=2$ min. Platelet adhesion is also formed at upstream of the stenosis region. Due to the platelet adhesion around the stenosed channel, flow passage is blocked as times goes on. This kind of increase in fluidic resistance leads to a reduction in the flow rate despite a constant injection flow rate of the syringe pump.

As shown in the right side of Fig. 2, binary images were obtained using Otsu's algorithm without applying correlation mapping. Although the platelet adhesion is observed after 0.5 min in the original images, the adhered platelets were detected after 2 min in the binary images. The regions of platelet adhesion with relatively dark image intensity are diminished in binary images. Therefore, the detecting area of platelet adhesion in the binary images is much smaller than that in the original images. It is known that the performance of global thresholding techniques such as Otsu's algorithm is limited by many factors, including small object size, small mean difference, large variances of the object and the background intensities, and large amounts of noise.²⁶ In order to accurately distinguish an area of adhered platelets in the present study, each original image was divided into small tiles and their correlation coefficients for consecutive images were mapped. Then, the thresholding procedure based on Otsu's algorithm was employed to the correlation map.²⁷ The relative correlation coefficient depends on the tile size, which strongly influences the results. Thus, it is needed to examine the effects of tile size on the detection of the adhered area.

B. Micro-PIV measurement

In the general micro-PIV technique, fluorescent particles are used as flow tracers.²⁸ However, the use of artificial particles in blood flow gives rise to the problems related to the particle-particle and particle-blood protein interactions. RBCs have also been used as blood flow tracers instead of artificial particles in the micro-PIV experiments.^{29,30} RBCs are larger

FIG. 2. Changes of flow images at $t=0, 0.5, 1, 2$, and 4 min. Left and right images are original flow images and corresponding binary images, respectively. Binary images were obtained using Otsu's algorithm without applying correlation mapping.

than artificial particles. In PIV analysis, this larger size brings about the increase of the depth of correlation which results in the underestimation of measured velocities. However, it is reported that the results with RBCs and artificial particles are nearly identical for relatively low magnifications.³¹ As shown in [supplementary material Fig. 2](#), velocity results obtained by seeding RBCs or the tracer particles (0.52 μm mean diameter; Thermo Scientific) are quite similar between them. The measurement accuracy of flow rate using RBCs can be verified by comparing the flow rate measured by the tracer particles. Significant statistical difference is not observed between them. Therefore, RBCs were used as tracer particles in the present study.

Figure 3(a) shows velocity fields superimposed on the corresponding flow images at different times ($t=0, 1$, and 4 min). When no platelets are adhered ($t=0$ min), the blood flow passes through the whole stenosis channel smoothly without any flow separation. Jet-like flow is observed after the stenosis region. This is similar to the flow patterns observed in a previous study, in which the stenosis channel had 85% severity at low Re ($\text{Re} < 5.4$).⁸ The velocity magnitude increases as the flow approaches the stenotic apex, whereas the blood flow is decelerated in the downstream region of the stenosis due to the reduction and extension of the flow channel area.

The inset in Fig. 3(b) indicates the streamwise velocity profile in the initial state ($t=0$ min) without any platelet adhesion in the far downstream region from the stenosis apex, which is indicated by a box in Fig. 3(a). The velocities are averaged from several streamwise positions with the same position in the channel width direction. The velocity profile has a typical pattern of flows in a rectangular microchannel, which has flat velocity vectors in the middle region.²⁴ This flatness results from the low aspect ratio of the microchannel ($H/W=0.05$). At $t=0$ min, the measured flow rate is about 1 mL/h, which coincides well with the input value of the flow rate from the syringe pump.

Small regions with platelet adhesion are notably observed after $t=1$ min. As aforementioned, those regions are represented as bright spots in flow images. Platelet activated by the flow acceleration around the stenosis apex is primarily adhered on the collagen film on the glass substrate of the end of the stenotic apex. In the initial stages of platelet adhesion, the platelets slightly tether to the collagen film. Since the tethered platelets are subjected to hydrodynamic drag force, their rolling or translocation phenomena along the flow stream can be observed.²⁰ Therefore, some of the platelet aggregates were detached from the stenosis apex and reattached in the post-stenosis region. After the specific periods of stationary adhesion (>10 s), firm cell adhesion occurs by inducing the development of elongated membrane extensions at the trailing edge of the platelets.³² This stabilized platelet tethering is associated with shear deceleration after peak shear.⁵ The firmly adhered platelets lead to the rapid development of stable platelet adhesion because this accelerates clustering platelets and sustaining of cell adhesion.¹⁵ The platelet adhesion around the stenosis apex induces a broad region of high acceleration. In the post-stenosis

FIG. 3. Flows in the stenotic microchannel. (a) Instantaneous velocity fields superimposed on the corresponding flow images at $t=0, 1$, and 4 min. (b) Temporal variation of the measured flow rate estimated by Eq. (1). Inset: streamwise velocity profile from the region indicated by a box at $t=0$ min.

region, adhered platelets create separated jet-like flows with two high velocity regions. The area of the attached platelet aggregates increases with the lapse time due to the active interaction between the platelets adhered to the collagen surface and the jet flow. At $t = 4$ min, the channel is almost blocked by platelet aggregates after the stenosis. The jet-like flow disappears in the post-stenosis region, and high velocity is observed in only the center of the stenotic region

Figure 3(b) shows the variation of the flow rate calculated using Eq. (1). The measured flow rate is gradually decreased after 2 min due to the increase of fluidic resistance caused by the accumulation of platelets in the microchannel. Maximum pressure for this syringe pump with 1 mL syringe is relatively low about 87 psi. The occlusion caused by the platelet adhesion significantly accentuates the fluidic resistance. When the pressure drop for inducing flow is higher compared to the maximum pressure of the pump, the flow rate can be reduced due to relatively low deriving pressure. At $t = 4$ min, the measured flow rate is decreased to about 0.1 mL/h because the channel is almost plugged by the platelets. The corresponding Re is varied from 0.169 to 0.017. The area of adhered platelets is extended further downstream by two times the channel width from the posterior end of the stenosis apex ($t = 4$ min).

C. Effect of tile size on detection of platelet adhesion

In order to investigate the effect of tile size on the detection of platelet adhesion, the detection efficiencies were compared by varying the size of the square tiles from 3 to 50 pixels. The calculated correlation maps with the different window size and corresponding binary images are presented in Fig. 4. With the smallest tile size, the wrong regions are detected as adhered platelets due to noise near the edge of the microchannel, in which light intensity fluctuation could occur. This measurement error disappears, but several regions are undetected when the tile size is larger than 3×3 . As the tile size increases, the neighboring wall and flow channel are included together in one detecting tile. Therefore, smaller detection spots with fewer adhered platelets than the tile size can be underestimated. In addition, some overestimation is observed where some walls are regarded as platelet adhesion regions because platelet adhesion occurs near the walls. Accordingly, geometrical parameters such as the stenotic channel width and inclination angle in the binary images can be distorted after the correlation mapping process.

The 2D correlation coefficient was examined for quantitative investigation of the tile size effect, as shown in Fig. 5(a). The coefficient is calculated using binary images obtained from

FIG. 4. Comparison of correlation maps and binary images with various window sizes. Left and right images are correlation maps with different window size and corresponding binary images, respectively. A flow image acquired at $t = 2.5$ min is used for the comparison, and the top image on the right side indicates manually labeled platelets.

FIG. 5. Quantitative evaluation of the window size effect. (a) 2D correlation coefficient is calculated from manually labeled binary images and binary images using correlation mapping with different tile sizes. (b) Width of stenotic channel is measured to investigate the degree of structural distortion.

the manual labeling method and the proposed technique based on correlation mapping. A higher 2D correlation coefficient means that the final image obtained is more consistent with the manually labeled binary image. Therefore, the tile size with the highest 2D correlation coefficient is the optimum size, assuming that manual labeling accurately indicates the actual platelet adhesion.

The highest 2D correlation coefficient occurs with the optimum tile size of 5×5 (Fig. 5(a) inset). The coefficient continuously decreases as the tile size increases. The reduction of the correlation coefficient results from the more significant effects of noise near the channel edge, diminishment of small adhered area, and the over-estimation of adherence in the detection region.

The use of a large calculation tile is accompanied by distortion of the geometrical structure information. The distortion was investigated by measuring the width of the stenotic channel, as shown in Fig. 5(b). When the tile size was 3×3 , the exact width was obtained, whereas the measured width was smaller than the real width, except when using the smallest tile size. The distortion becomes more severe as the tile size increases, and the width of the stenotic channel is measured as zero when the size is larger than 25×25 . Although the measured width ($95 \mu\text{m}$) is slightly smaller than the real value when the tile size is optimal (5×5), the distortion is acceptable when considering the detection accuracy of platelet adhesion.

D. Variation of adhered platelet area

Figures 6 shows graphical and quantitative changes in the amount of platelet adhesion obtained with the proposed method using a tile size of 5×5 . At $t = 0$ (without any adhesion), the measured area of platelet adhesion is about 0.05 mm^2 , which indicates a degree of measurement accuracy. A sudden shear acceleration due to stenosis can initiate platelet activation,

FIG. 6. Changes of platelet adhesion under flow. (a) Correlation maps and corresponding binary images at various times ($t = 0.5, 1, 2$, and 4 min). (b) Temporal variation of adhered area. The window size for obtaining these results is 5×5 , which is the optimum value.

regardless of biochemical triggers. Thus, platelets activated by passing through the narrow stenosed channel adhere to the channel downstream.^{5,8}

The process of platelet adhesion can be divided into two stages in accordance with the adhesion growth rate. The adhered area gradually increases until 2 min and reaches 0.2 mm^2 . In this initial stage, the flow rate remains almost constant, but the flow pattern varies. As shown in Fig. 3(a), separated jet-like flows with more shear layer regions occur. Platelet adhesion can occur near these shear layers. Therefore, the changes in flow patterns in the initial stage give rise to the change in the rate of platelet adhesion. The area rapidly increases after 2 min, at which the measured flow rate begins to decrease. The adhesion area finally approaches 0.6 mm^2 at $t=4\text{ min}$. At this time, micro-channel is almost blocked by adhered platelets, and the flow rate is significantly reduced to 10% of the initial flow rate.

V. CONCLUSION

This study has proposed a microfluidic method for the simultaneous measurement of the velocity field and platelet adhesion using the same images. The velocity field information and platelet adhesion were measured by the micro-PIV technique and calculating the correlation maps with a threshold method, respectively. The blood flow and platelet adhesion process in a stenotic micro-channel with 90% severity were investigated for a period of 4 min.

To demonstrate the effect of tile size on the detection accuracy of platelet adhesion, 2D correlation coefficients were evaluated with a manually determined binary image. The highest 2D correlation coefficient was detected with the optimal tile size of 5×5 . The changes of the flow and adhesion are highly interactive. Without any platelet adhesion, a typical flow pattern is observed in a stenotic micro-channel. However, the flow pattern is changed when platelet adhesion is initiated. The alteration of flows changes the rate of platelet adhesion. As the area of plate adhesion increases, the micro-channel is almost blocked and there is a small amount of blood flow. This microfluidic method could be a useful tool for studying the mutual influences between platelet aggregation and blood flow.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for Fig. 1 for rough variations of shear rate depending on the stenosis severity in the microchannel under the flow rate of 1 mL/h. Supplementary Fig. 2 shows comparison between flow information obtained by seeding RBCs and tracer particles.

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