Migration distance-based platelet function analysis in a microfluidic system

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Aggregation and adhesion of platelets to the vascular wall are shear-dependent processes that play critical roles in hemostasis and thrombosis at vascular injury sites. In this study, we designed a simple and rapid assay of platelet aggregation and adhesion in a microfluidic system. A shearing mechanism using a rotating stirrer provided adjustable shear rate and shearing time and induced platelet activation. When sheared blood was driven through the microchannel under vacuum pressure, shear-activated platelets adhered to a collagen-coated surface, causing blood flow to significantly slow and eventually stop. To measure platelet adhesion and aggregation, the migration distance (MD) of blood through the microchannel was monitored. As the microstirrer speed increased, MD initially decreased exponentially but then increased beyond a critical rpm. For platelet-excluded blood samples, there were no changes in MD with increasing stirrer speed. These findings imply that the stirrer provided sufficiently high shear to activate platelets and that blood MD is a potentially valuable index for measuring the shear-dependence of platelet activation. Our microfluidic system is quick and simple, while providing a precise assay to measure the effects of shear on platelet aggregation and adhesion.
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I. INTRODUCTION

Platelets in the blood circulate in discoid form until activated, at which time shape changes occur that include formation of filopodia and lamellipodia. This leads to platelet aggregation and adhesion, both of which play critical roles in hemostasis and thrombosis at sites of vascular injury. Platelet aggregation and adhesion occur through complex signaling mechanisms associated with vascular subendothelial matrix proteins (i.e., collagen),^{1,2} biochemical activators in blood (i.e., thrombin, adenosine diphosphate (ADP), and von Willebrand factor [vWF]),^{3–5} and hemodynamic factors (i.e., shear stress and shear rate).^{6–8} Among these factors, shear stress influences all processes directly or indirectly. For instances, shear stress activates platelets leading to adhesion on endothelium and aggregation.⁹ Therefore should be accounted for in any *in vitro* platelet analysis. This has long presented a challenge in the development of new testing methods for examining platelet function.

Recently introduced methods and devices can successfully study platelet functions using either whole blood or platelet-rich plasma with or without the use of agonists.^{10–17} Light-transmission aggregometry has been widely used as the gold standard to identify and diagnose defects in platelet function.^{12,13} However, it requires platelet-rich plasma, a time-consuming test procedure, and large sample volumes. Platelet aggregation can be measured in anticoagulated whole blood using electrical impedance. In fact, impedance aggregometry is conceptually measuring the integrin $\alpha\text{IIb}\beta 3$ (GPIIb/IIIa)-dependent platelet-to-platelet aggregation. The impedance

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aggregometry still requires a fairly high sample volume, and the assay is still time consuming and expensive.^{18,19} Meanwhile, VerifyNow (Accumetrics, San Diego, CA, USA) is a turbidimetric, point-of-care device based on integrin $\alpha\text{IIb}\beta 3$ (GPIIb/IIIa)-dependent platelet aggregation, which is augmented by the presence of fibrinogen-coated beads.^{11,14}

A shear-induced platelet function analyzer (PFA-100, Siemens, Germany) has been introduced.^{10,15–17} This device lets anti-coagulated whole blood flow through a narrow hole ($d = 150\ \mu\text{m}$) punched in membranes coated with collagen and either ADP or epinephrine. Before passing through the membrane, the blood sample is passed through a capillary ($d = 200\ \mu\text{m}$) at a high shear rate ($\gamma > 5000\ \text{s}^{-1}$) to activate the platelets as well as vWFs. With this device, activated platelets tend to aggregate together and adhere to the surface of the aperture, which eventually becomes blocked. The time required for blood to completely occlude the aperture is referred to as the closure time. The flow conditions in this device simulate the *in vivo* arterial environment in terms of vascular (collagen), biochemical (ADP or epinephrine), and hemodynamic (high shear rate) factors.

Compared to the conventional bleeding time assay, the previously mentioned device and methods have shown significant improvements in reproducibility and allow quick and easy operation. Owing to these advancements, platelet function assays are becoming more common in clinics. However, further improvements are needed. For instance, PFA-100 uses a once-through flow system with non-uniform shear rate distributions that might not completely activate platelets. Furthermore, this method is also dependent on vWF function and hematocrits, and 2-step serial tests for different agonists (collagen-ADP and collagen-epinephrine) require double the cost and test time.^{10,15–17} Above all, this test requires a large blood volume (i.e., $800\ \mu\text{l}$ for PFA-100), which cannot be obtained from a simple finger stick without vein puncture.

Recently, microfluidic systems have been adopted for shear-dependent platelet function.^{19–26} In order to activate platelets, most of the studies adopted once-through flow system consisting of single or multi-channels. However, a pressure-driven microfluidic system may not provide homogeneous shear stresses over a cross-sectional area due to parabolic velocity profile. Thus, the location-dependent, non-uniform shear stress profile would cause non-homogeneous activation of platelets, which results in poor reproducibility of platelet function tests.

In this study, we developed a microfluidic system that provides a simple and rapid assay for platelet aggregation and adhesion and can be used to measure migration distance (MD) of blood flow. As a proof of principle, we demonstrated shear-dependent platelet aggregation and adhesion by measuring the difference in MD of blood samples in a 2-channel microchip. However, adopting multi-channels in a microchip can perform multiple platelet function assays in a single test, which can reduce required cost and time. Also, other essential features of the present design are minimum sample volume which can be obtained from a simple finger stick without vein puncture. The system performance was evaluated and optimized at room temperature and vacuum pressure using varying agonist concentrations and stirrer rotation speeds.

II. MATERIALS AND METHODS

A. Samples and materials

This study was approved by the Human Use Ethical Committee at Korea University Guro Hospital. Venous blood samples were obtained from five healthy male volunteers, aged between 25 and 35 yr. Blood was collected in sodium citrate and K_2EDTA tubes (BD Vacutainer Systems, Franklin Lakes, NJ, USA) by venipuncture. Complete blood count (CBC) was measured using an automated blood analyzer (Beckman Coulter, Miami, FL, USA). The whole tests were conducted within 2.5 h from sample collection. $25\ \mu\text{l}$ of blood was pipetted into a microchamber of the microfluidic system. For platelet stimulation, the ADP (Sigma-Aldrich, Ontario, Canada), which was coated on the surface of the microchamber, was dissolved at a final concentration of $0.05\ \text{mg/ml}$ during stirrer rotation. Collagen type I (BD biosciences, MA, USA) was then coated around the entrance to the microchannel. In order to remove platelets, blood

was centrifuged at 150 *g* for 12 min and buffy coat layer was removed. Remaining blood was used for negative control.

B. Microfluidic system and operation

Figure 1 shows the schematics of the microfluidic device and the working principle for the platelet function assay. The device enables activation of platelets at adjustable shear rates and allows adhesion of the activated platelets at the microchannel entrances. The system consists of a micro-chamber coated with ADP, entrance microchannels (90 μm in height and 0.8 mm in width) coated with collagen, the main microchannel section (90 μm in height and 1.6 mm in width), a solenoid valve, and a syringe pump. The ADP-coated microchamber ($\phi = 8$ mm, $H = 0.5$ mm) stores 24 μl of blood and includes a stirrer (6.2 mm $\times$ 0.25 mm $\times$ 2 mm) that can be remotely rotated by a magnet mounted on a rotor. When the stirrer rotates, the ADP coated on the bottom surface of the sample chamber is dissolved and mixed with a blood sample.

It is worthy to note the design of the stirrer we used. The center of stirrer is thicker (0.45 mm) than the both ends (0.25 mm) and so that the stirrer is cone-shape. Thus, the rotating stirrer in a chamber simulates cone-and-plate rheometry, which allows the application of well-defined fluid shear on blood.²⁷ When a cone rotates at low speeds, the shear rate in the rheometry is uniform throughout the chamber. In the present rheometry with cone angle 2θ (1.47 rad or 84°) rotated at a constant angular velocity Ω , the applied shear rate $\dot{\gamma}$ is given by $\Omega/\tan \theta$. The applied shear stress for Newtonian fluids is proportional to the shear rate according to $\tau = \mu\dot{\gamma}$, where μ is medium viscosity. Even though blood yields shear-dependent non-Newtonian behavior, blood viscosity at high-shear rates ($>1000 \text{ s}^{-1}$) yields Newtonian characteristic, which is independent of shear rate. As described below, applied shear rates corresponding to rotating speeds of stirrer range of 900–4500 s^{-1} , which are sufficiently high enough to yield Newtonian behavior of blood viscosity.

Rotating speed of the stirrer, which is carefully controlled by the motor, ranges from 700 to 3500 rpm (0.2–1 rad/s). Because the shear rate induced by the rotating stirrer is similar to that from cone-and-plate, calculated shear rates are 900–4500 s^{-1} .²⁷ Since the applied shear rates were quite high, whole blood viscosity becomes independent of shear rates and yields

FIG. 1. (a) Schematics of a microfluidic system consisting of a stirring microchamber and microchannel. (b) Platelets are sheared and activated by the rotating stirrer. (c) Activated platelets aggregated and adhered on the collagen-coated surface at the microchannel entrance.

constant value (3.54 mPa-s) at 900–4500 s⁻¹. Thus, the corresponding shear stresses are 3.2–15.9 Pa. Because of the characteristics of a rotating stirrer, the generated shear rates are intermittently applied on platelets. Therefore, the stirring time is extended to 3 min so that all platelets are fully exposed to each shear rate and activated, as shown in Fig. 1(b). The detailed design of this microfluidic system can be found elsewhere.²⁸

We examined the effect of stirring time (1, 2, 3, and 5 min) on platelet aggregation and cellular morphology for various rotational speeds. At a fixed rotational speed (2800 rpm or 3600 s⁻¹), any morphological change was not observed regardless of stirring time and maximum platelet activation corresponding to minimum migration distance, was observed. At the fixed rotational speed (2800 rpm), shear-induced platelet activation increased with stirring time and reached asymptote at $t = 3$ min. Thus, the present study carefully chose the duration of stirring as 3 min, which did not show any changes in cell morphology at an optimum rotational speed (2800 rpm).

After stirring for 3 min, the blood samples were driven by a pressure gradient when the solenoid valve was opened and a specified vacuum pressure in the syringe pump was applied to the sample in the microchamber. A large dead volume chamber (25 ml) was connected to the syringe pump so that there was no detectable change in the vacuum pressure ($\Delta p = 2.3$ kPa) during the testing period. Under a constant pressure gradient, blood began to flow through the microchannel, overcoming the flow resistance from the increased contact area between blood and microchannel. In the entrance region, the bottom surface was coated with collagen, a strong agonist, to promote adhesion of platelets. The entrance channel had a height of 0.18 mm and width of 0.7 mm, with a circular chamber having a diameter of 1.4 mm. While flowing through the collagen-coated microchannel, activated platelets tended to aggregate and adhere to the surface with the assistance of activated vWFs, as shown in Fig. 1(c). The adhesion and aggregation of platelets on the bottom surface of the microchannel would have resulted in the gradual reduction of the microchannel cross-sectional area and eventual stoppage of blood flow.

After flowing through the collagen-coated section, blood entered the main section of the microchannel. As the length of the blood sample entered the microchannel increases with time, the flow resistance is getting increased and finally the blood flow in a microchannel stops. Then, the distance from entrance to the final stop position of blood sample is defined as MD. A similar concept, which is distance-based measurement for analytical device, was recently introduced.²⁹ The main microchannel was 0.18 mm tall, 1.0 mm wide, and 135 mm long, with two 180° turns. Depending on the degree of platelet activation, the MD of blood was expected to vary. The length of a microchannel was carefully determined to locate MDs of highly-sheared blood sample for healthy controls at 50%–60% of an entire microchannel length. Thus, MD for a zero-sheared blood sample was chosen as a reference value for normalization. The normalized MD was referred to as migration ratio (MR). Owing to the normalization, MR would be independent of hematocrits, number of platelets, and other rheological properties of blood sample such as viscosity, erythrocyte deformability, and aggregation.

The MD through the microchannel was determined from images captured every 0.1 s by a CCD camera. The image processing was automatically conducted with NI LabView Vision[®] software. In each image, a migration distance was precisely measured with counting the number of pixels along a centerline of microchannel. A few hundred images were analyzed by PC, and the MDs were determined within several seconds. Furthermore, the migration velocities were analyzed with comparing two migration distances for a fixed time interval.

C. Device fabrication

As shown in Fig. 2(a), a polydimethylsiloxane (PDMS) microfluidic channels was fabricated on a replica mold, which was fabricated on a silicon wafer through photolithography by using an SU-8 negative photoresist (MicroChem, Newton, MA). The base and curing agent of PDMS were mixed, degassed in a vacuum chamber, and cured in an oven (80 °C for 1 h). The device consisting of the PDMS microchannel and the glass slide was bonded using a standard process, which can be found elsewhere.^{20–23} Briefly, the PDMS microchannel and the glass

FIG. 2. (a) A photograph of the microfluidic chip with blood migrating through the microchannels. (b) Normalized migration of blood samples exposed to various shear rates. (c) Migration velocities at various shear rates.

slide with the nickel wire were treated with oxygen plasma at 250 W and 80 mTorr (CUTE, Femto Science Co., Korea) for 50 s. After oxygen plasma treatment, the PDMS slab and the glass slide with the nickel wire were dipped in high-purity ethanol (99.9%) to prevent instant, irreversible bonding and to allow smooth movement between each layer during alignment of the microchannel and the nickel wire. The residual ethanol remaining in the microchannel was dried on a hot plate at 80 °C for 90 min for irreversible bonding. Finally, holes on both sides of the PDMS microchannel were blocked with epoxy glue.

ADP and collagen were coated on specified positions of glass substrate before assembly, respectively. For ADP coating, a PDMS circular well ($\phi = 8$ mm, $H = 5$ mm) was prepared on the glass. 4 μ l of an ADP solution (22 mM) was filled in the well and incubated for 24 h at room temperature. Then, the PDMS well was removed. For collagen coating, a PDMS square well (10 mm $\times$ 10 mm) was also prepared on the glass and fill a collagen solution in the well. The collagen solution was prepared with dissolving collagen 500 μ g/ml in 0.02 M acetic acid. The collagen solution was filtered through 5 μ m pore filter and 0.45 μ m pore filter, respectively.

Then, 1 ml of the collagen solution was poured into the square PDMS well and incubated for 24 h at room temperature. Then, the PDMS well was removed and the collagen-coated glass was washed with phosphate buffer solution (PBS).

D. Flow cytometric analysis for platelet activation

In order to verify the shear-induced platelet activation (SIPA), we adopted a flow cytometric analysis with monitoring expression of CD62-PE and CD41FITC antibody solution (BD Bioscience, CA). For immunological staining of whole blood after agonist stimulation, whole blood (5 μ l) was immediately added to a polypropylene tube, containing 50 μ l of staining buffer (PBS with 1% bovine serum albumin (BSA)) and a saturated antibody (5 μ l of 6.25 μ g/ml CD62P-PE). After a 20 min-incubation in the presence of the antibody at room temperature, 2 ml of 0.5% paraformaldehyde was added to fix the samples for an additional 20 min. Within 4 h, the samples were analyzed using a flow cytometer (Coulter EPICS XL-MCL flow cytometer equipped with a 488 nm laser, Beckman Coulter, USA), and 20 000 events were acquired without prior washing and centrifugation, to minimize artifactual platelet activation. Platelets were identified in a sideward scatter/forward scatter dot plot, and the gate for indicating CD62P positivity was arbitrarily set to include 1% of platelets treated with phycoerythrin (PE)-conjugated control antibody. To compensate for the nonspecific immunofluorescence, the percentage of CD62P positive platelets was obtained after subtracting the percentage of positive platelets, when the antibody was replaced by the isotype-matched PE-conjugated immunoglobulin controls for CD62P (isotype IgG1).

III. RESULTS AND DISCUSSION

Fig. 2(b) shows MR results for blood exposed to various shear rates between 0 and 4500 s^{-1} . The case of no shear recorded the shortest time (30 s) to reach the end of the microchannel. However, with increasing shear rate, MD decreased. For example, at a fixed time of $t = 30$ s, the MR for $\gamma = 900 \text{ s}^{-1}$ reached 89.6%, whereas for $\gamma = 3600 \text{ s}^{-1}$, MR was only 65.2%. It is noteworthy that the shear-activated samples did not reach the end of the main microchannel but instead stopped somewhere in the middle of the microchannel. The migration velocities at various shear rates as a function of time are shown in Fig. 2(c). The velocities decreased exponentially with time. For $\gamma = 0 \text{ s}^{-1}$, migration velocity did not decrease to zero when blood reached the end of the microchannel. Migrations at other shear rates stopped in the middle of the microchannel, and the corresponding velocities became zero.

Since ADP is a well-known agonist inducing platelet aggregation,³⁰ a critical question is raised whether the present migration results in Fig. 2 were due to ADP-induced platelet aggregation or shear-induced platelet aggregation. Thus, we compared MR and velocity between blood samples with and without ADP-agonist at zero shear rate (Figs. 2(b) and 2(c)). The migration velocity for the sample with ADP (open circle) was slightly slower than that for without ADP (solid circle) but much faster than those for sheared sample with ADP, as shown in Fig. 2(c). These results apparently indicate that the addition of ADP causes platelet aggregation even without shear,³⁰ which results in increase of blood viscosity. However, the blood sample without ADP at zero-shear showed almost the same degree of migration (98.1%) compared to that with ADP at zero-shear (100%) at $t = 30$ s (Fig. 2(b)). These results suggest that the effect of the ADP-induced platelet aggregation on the present analysis of MR was negligibly small compared to that of shear-induced platelet aggregation. Thus, the MR in the present analysis could be regarded as characteristic of shear-induced platelet aggregation.

Precise analysis of Fig. 2 requires determination of a reference time at which to compare migration results. One option is the time required by a no-shear sample to reach the end of the microchannel. In this case, the reference time is 30 s, and the normalized MD ratio is indicated as MR_{30} . Another choice is to compare MD when the sample stops flowing, which is the MD at $t = \infty$ and a corresponding ratio of MR_{∞} . MR_{30} and MR_{∞} are compared in Fig. 3. Interestingly, there was no significant difference between the 2 curves except for the absolute values. Furthermore, both yielded low coefficients of variation ($<3\%$). Because it was quite

FIG. 3. Comparison of migration ratios normalized for migration distance at a fixed time ($t = 30$ s, MR_{30}) and stopped position ($t = \text{final}$, MR_{∞}). Both of MR showed minimum at 3600 s^{-1} (or $\tau = 12.7 \text{ Pa}$). Blood with platelets removed (negative control) does not show any shear-dependence. The coefficients of variation were less than 3.5%.

simple to determine MR_{∞} by analyzing a photograph at $t = \infty$, we used MR_{∞} as the representative index for this study.

Both MR curves in Fig. 3 clearly demonstrate the shear-dependence of platelet activation and adhesion via the MD through the microchannel. As the shear rate increased, MR rapidly decreased until it reached an asymptotic value between 1800 and 3600 s^{-1} . These results were confirmed in the previous studies^{31,32} that the percentage of platelet activation was proportional to the applied shear rates. In other words, the higher shear rate is applied, the more platelets are activated and aggregated. Thus, we confirmed that the magnitude of shear rates is proportional to the extent of platelet activation, which is also directly proportional to flow resistance through a collagen-coated microchannel due to platelet aggregation and adhesion.

Furthermore, the previous study reported minimal platelet activation below a threshold shear rate ($\sim 2200 \text{ s}^{-1}$). However, our study did not show the minimum threshold shear rate to activate platelets. In Fig. 3, MR reached 87% even at a very low shear rate (900 s^{-1}). It is worthy to note that we adopted a stepwise, pulsating shear using a rotating stirrer, whereas most of previous studies did steady rotating shear in a cone-plate rheometry. A previous study reported that sinusoidal pulsating shear resulted in significantly increased platelet aggregation compared to continuous shearing except at low shear stress ($< 10 \text{ dyne/cm}^2$).³³ This increasing trend showed positive correlations with both stress amplitude and pulse frequency. However, another study reported that the time-varying shear with low frequency was less effective than the steady shear.³¹ Comparing these previous studies for sinusoidal pulsating shear, the present time-varying shear adopted stepwise-pulsating shear, which frequency ranges $15\text{--}58 \text{ Hz}$ ($900\text{--}3500 \text{ rpm}$). The present pulsating frequencies, which are much higher than those of the previous studies, resulted in sufficient platelet activation, as shown in Fig. 3. However, it is still required to conduct comparative study of platelet activation between steady shear and pulsating shear with various frequencies.

Meanwhile, previous studies also found that fluid shear stress controls platelet activation rather than shear rate.^{31,32} Addition of viscous medium such as 1.5% dextran (molecular weight 2×10^6) increases suspending medium viscosity (from 1.1 cP to 2.2 cP) and doubles the shear stress without changing the rotating shear rate in whole blood sample. Subsequently, this led significant increase (3.3 folds) in platelet activation.³¹ Thus, the use of shear stress rather than shear rate would be highly recommended for analyzing SIPA. Also, the incubation of platelets with dextran alone for 3 min did not induce platelet activation. Thus, adding dextran in whole blood should be considered in a future study.

Even though both theories potentially explain the shear dependence of platelets, further investigation is required to directly measure the shear-dependent platelet activation. Figure 4

FIG. 4. Measurements of activated platelets at various shear rate with monitoring the expression of CD62-PE and CD41FITC antibody solution. The shear rate was induced by a rotating stirrer in a microchamber and activated platelets were quantified (with Coulter EPICS XL-MCL flow cytometer equipped with a 488 nm laser, Beckman Coulter, USA). The shearing time was 3 min at each shear rate. The coefficients of variation were less than 4.6%.

shows platelet activation induced by the rotating stirrer in a microchamber with varying shear rate. With monitoring the expression of CD62-PE and CD41FITC antibody solution (BD Bioscience, CA), we counted the number of cells which express with both solutions (C2 in Fig. 4(a)). As shear rate increases, the fraction of activated platelets increases, shows the maximum at $\gamma = 3600 \text{ s}^{-1}$ and tends to decrease (Fig. 4(b)). These results are exactly coincident with those of MR as shown in Fig. 3. Thus, these results demonstrated the effectiveness of shear-activation of platelets with the present design of the rotational stirrer in a microchamber.

It is worth noting that the MR at $\gamma = 4500 \text{ s}^{-1}$ was larger than that at $\gamma = 3600 \text{ s}^{-1}$. This result might be caused by rheological alterations of blood cells, including red blood cells (RBCs) and platelets. First, we examined morphological changes in erythrocytes with varying shear rates (Fig. 5). There were no apparent changes in erythrocyte morphology at shear rates of 900 s^{-1} and 3600 s^{-1} . However, the highest shear rate (4500 s^{-1}) in the present study caused apparent morphological changes in erythrocytes and erythrocytes were highly aggregated. Also, additional experiments were conducted to examine and compare their rheological characteristics between two shear rates (3600 s^{-1} and 4500 s^{-1}) using a microfluidic hemorheometer (RheoScan-AnD300, Rheomeditech., Seoul, Korea). Deformability was apparently enhanced with shear rate and these results were consistent with a recent report of shear-induced improvement of erythrocyte deformability.³⁴ Furthermore, erythrocyte aggregation at higher shear rate (4500 s^{-1}) was also apparently increased compared to that at lower shear and these results were also consistent with our microscopic observation.

Considering these rheological changes in erythrocytes, there would be alteration in fluidity of blood samples. First, larger the aggregated cells, the larger lateral migration toward center-line of flow and subsequently this would cause the Fåhræus–Lindqvist effect in microchannel. Thus, enhanced deformability and aggregated erythrocytes associated high shear would result in longer migration distance while binding force between platelets and collagen surface was saturated at high shear. The speculation should be further validated with proper experimental set-up in a future study.

FIG. 5. Morphological changes of erythrocytes with varying shear rates at fixed shearing time (3 min). (a) Shear rate of 900 s^{-1} (b) 3600 s^{-1} and (c) 4500 s^{-1} .

IV. CONCLUSION

We developed a simple microfluidic system that provides an assay of shear-induced platelet activation, aggregation, and adhesion. The MD of blood through a microchannel was used as a measure of platelet function in response to mechanical shearing. MD showed a strong dependence on applied shear, reaching a minimum at 3600 s^{-1} , and then increasing beyond the threshold shear rate. Because only the platelets responded to mechanical shear, the platelet-removed blood sample showed the same results as the no-shear blood sample. The shearing mechanism of our device, a rotating stirrer in a microchamber, provided sufficiently high and homogeneous activation of platelets such that MR reproducibility was excellent ($<3.5\%$). Our data were captured using a CCD camera followed by image processing within 1 min. Our findings indicate that the present microfluidic system would facilitate the quick and simple but precise assay of shear-dependent platelet aggregation and adhesion. As a proof of principle, we demonstrated an assay of platelet function for a fixed agonist (collagen-ADP). However, as we have used multiple channels on the chip, the device can perform multi-assays at once with additional agonists (i.e., collagen-epinephrine). Therefore, this device is useful as a diagnostic tool for investigating shear-dependent platelet function with individual blood samples.

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