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Activation and shedding of platelet glycoprotein IIb/IIIa under non-physiological shear stress

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Abstract The purpose of this study was to investigate the influence of non-physiological high shear stress on activation and shedding of platelet GP IIb/IIIa receptors. The healthy donor blood was exposed to three levels of high shear stresses (25, 75, 125 Pa) from the physiological to non-physiological status with three short exposure time (0.05, 0.5, 1.5 s), created by a specific blood shearing system. The activation and shedding of the platelet GPIIb/IIIa were analyzed using flow cytometry and enzyme-linked immunosorbent assay. In addition, platelet P-selectin expression of sheared blood, which is a marker for activated platelets, was also analyzed. The results from the present study showed that the number of activated platelets, as indicated by the surface GPIIb/IIIa activation and P-selectin expression, increased with increasing the shear stress level and exposure time. However, the mean fluorescence of GPIIb/IIIa on the platelet surface, decreased with increasing the shear stress level and exposure time. The reduction of GPIIb/IIIa on the platelet surface was further proved by the reduction of further activated platelet GPIIb/IIIa surface expression induced by ADP and the increase in GPIIb/IIIa concentration in microparticle-free plasma with increasing the applied shear stress and exposure time. It is clear that non-physiological shear stress induce a paradoxical phenomenon, in which both activation and shedding of the GPIIb/IIIa on the platelet surface occur simultaneously. This study may offer a new perspective to explain the reason of both increased thrombosis and bleeding events in patients implanted with high shear blood-contacting medical devices.

Keywords Glycoprotein IIb/IIIa (Integrin α_{IIb}/β_3) · Blood-contacting medical devices · Non-physiological shear stress · Shear-induced hemostatic dysfunction · Shear-induced platelet activation

Introduction

Cardiovascular disease (CVD), lung disease, and chronic renal disease affect millions of Americans [1]. More than one million patients die of these diseases each year. The estimated direct and indirect costs of these diseases are more than $600 billion a year [1, 2]. Blood-contacting medical devices (BCMDs) are frequently used to treat or replace human organs associated with these diseases. Over the years, BCMDs have evolved from simple to more advanced devices for various organs, such as mechanical heart valves (MHVs), hemodialyzers, extracorporeal membrane oxygenation (ECMO), ventricular assist devices (VADs), and respiratory assist devices. While these devices provide clearly medical benefits, unintended consequences often arise. In some cases the use of these devices in patients causes dangerous pathological complications, in

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particular thrombosis and bleeding. These complications have been investigated for many years but still remain the unsolved problems [3–6]. It is known that non-physiological high shear stresses exist in some regions of BCMDs, such as in the blade region of rotary VADs and the hinge area of MHVs, can cause damage to blood. Blood damage manifests as morphologic changes, shortened life span, biochemical functional alterations, and complete rupture of blood cells and proteins [7–13]. All of these lead to alteration of normal blood function. It is well documented in the literature that the non-physiological high shear stress can induce platelet activation.

Platelets are anucleate blood cells that play a critical role in hemostasis. There are many receptors in the membrane of platelets, which are important to maintain platelet function [14, 15]. Shear-induced platelet activation has generally been recognized as the culprit of the increased thrombotic risk in patients with BCMDs although flow stasis and in-biocompatibility of artificial surfaces play an important role in device-related thrombogenesis [8–10, 12]. The activation of the platelet receptors would cause the platelet dysfunction and contribute device thrombosis [3, 7–10]. Many studies have been conducted to identify the level of non-physiological high mechanical shear stress and exposure time as the dominant factors causing platelet activation [8–13, 16–18]. However, few studies on impact of the non-physiological shear stress on platelets have been conducted to examine other aspects of shear-induced platelet dysfunction, which may be associated with bleeding complication.

Glycoprotein IIb/IIIa (GPIIb/IIIa, or integrin αIIb/β3) is a major membrane protein complex on the surface of platelets, the most abundant expressed receptor on the platelet surface, about 40,000–80,000 copies per platelet [19]. The complex is formed via calcium-dependent association of two subunits: GPIIb and GPIIIa. There are four binding sites of calcium ion in its structure. It is a receptor for fibrinogen, von Willebrand Factor (vWF), thrombospondin, etc. The platelet GPIIb/IIIa plays vital roles in normal hemostasis and pathological thrombosis [20]. In the resting state of platelets, the GPIIb/IIIa exhibits low-affinity conformation, which can not bind with its plasma ligands. When platelets are activated by a variety of agonists, the inside-out signaling pathway is triggered, finally leading to a conformational change in platelet GPIIb/IIIa receptors. The high-affinity conformational change of platelet GPIIb/IIIa receptors enables a ligand binding site of GPIIb/IIIa becomes accessible to macromolecular ligands, which plays a vital role in platelet stable adhesion and aggregation [21, 22]. Because of the importance of platelet GPIIb/IIIa receptors in hemostasis, it is critical to investigate the effects of non-physiological high shear stress on platelet activation and structural alteration of GPIIb/IIIa receptors to gain insight into non-physiological shear-induced hemostatic dysfunction.

Elevated shear stress can not only induce the activation of platelet, but also induce shedding of platelet receptors. The shedding of platelet receptors, such as GPIbα and GPVI, might provide a mechanism to allow negative regulation of receptors and influence the process of thrombosis and hemostasis [23–25]. For the GPIIb/IIIa, shear-induced activation has been demonstrated by many studies [26–30]. Under pathologic conditions, such as, in stenotic artery, platelets may be exposed to abnormal high shear stress. Shear-induced activation of the GPIIb/IIIa is initiated by the interaction of GPIbα with VWF immobilized to the exposed subendothelium of the injured vessel wall, especially in the atherosclerotic plaque rupture, leading to platelet-surface adhesion. Few studies had been performed to investigate the shear-induced shedding of platelet GPIIb/IIIa receptors by non-physiological high shear stress. Wenger RK et al. [31] noticed the decrease of the amount of the platelet membrane β_3 integrin, which is part of the GP IIb/IIIa complex, during clinical cardiopulmonary bypass. Du et al. [32] and Pfaff et al. [33] reported the proteolytic regulation of β_3 integrin induced by calpain. However, these studies only reported the shedding of the subunit β_3 of the integrin α_{IIb}/β_3 complex. It is still unknown what would be happen to the whole integrin α_{IIb}/β_3 under non-physiological high shear stress conditions and whether the integrin α_{IIb}/β_3 could be shed or not.

In the present study, an in vitro experiment was designed to investigate the effect of the non-physiological high shear stress on the GPIIb/IIIa complex. Blood from healthy donors were exposed to the high shear stress with short exposure time conditions generated by a novel blood-shearing system. The levels of activation and shedding of platelet GPIIb/IIIa were characterized.

Materials and methods

Blood-shearing device

The blood-shearing device used in this experiment is a centrifugal flow-through Couette device, adapted from the CentriMag magnetically levitated blood pump (Thoratec, Pleasanton, CA, USA). The rotor is magnetically suspended with the bearingless motor technology and can rotate between 500 and 5000 rpm. There is a narrow gap with a uniform width of 150 μm and a length of 2.5 mm, created between the housing and the rotor (shown in Fig. 1). The detail design of this device can be found in our previous work [34]. For a fluid with typical viscosity of 0.0036 Pa s, a uniform shear region with the corresponding shear stress between 21 and 212 Pa can be created using

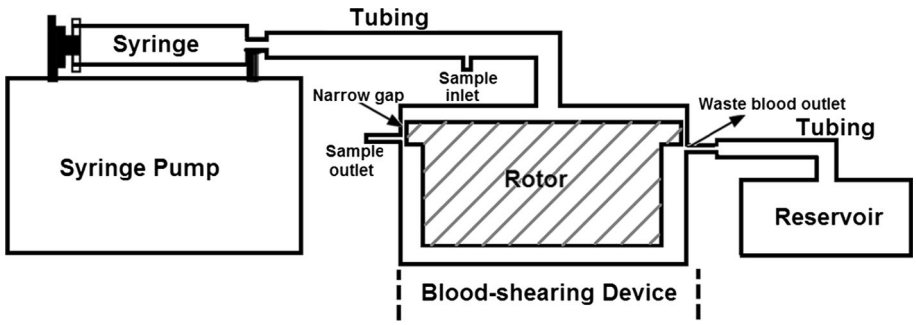


Fig. 1 The schematic diagram of the blood shearing system; it is consisted of a syringe pump used to control the flow rate, a syringe, tubing, blood shearing device (Hemolyzer-L) including 150 μm narrow gap between the inner rotor and the outer housing, blood sample ports, waste blood outlet, and reservoir

this device. As shown in Fig. 1, a syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA) is connected with the blood-shearing device and the whole blood is pressure-driven to pass through the narrow gap between the magnetically suspended rotor and the stationary outer housing in the axial direction, where the blood would expose to the uniform high shear stress. The shear exposure time of blood through the narrow gap is determined by the axial flow rate. The high shear stress and short exposure time can be created by adjusting the spindle rotor speed and the axial flow rate [34].

Experimental procedure

Ten healthy human donors including men and women were recruited to participate in the study. All the donors gave informed consent and did not take aspirin or other anti-platelet medications 10 days prior to the blood donation. The blood collection was carried out in accordance to the protocol approved by the Institutional Review Boards of the University of Maryland and the University of Louisville. Fresh blood (250 ml) drawn from these donors was mixed with anticoagulant acid citrate dextrose (ACD-A) with the volume ratio of 9:1.

Before each experiment, the viscosity of the blood preparation was measured using a semi-micro viscometer (Cannon Instrument Company, State College, PA). The rotational speed of the rotor and the axial flow rate were determined based on the expected shear stresses and exposure time, respectively [34]. In this study, three levels of high shear stresses (25, 75, 125 Pa) were chosen. These levels of shear stresses included the pathological condition (25 Pa) appeared in stenotic vessel and the non-physiological conditions (75 and 125 Pa) encountered within BCMDs [7, 13, 35]. In the patients implanted with VADs, MHVs, or other BCMDs, blood passes these devices quickly and the exposure time of blood cells experiencing high shear stresses is normally short. Thus, three different levels of short exposure times (0.05, 0.5, 1.5 s) were chosen in the present study [7, 34]. The conditions of different shear stresses and exposure time with related flow rates of the syringe pump are listed in Table 1.

During the experiment, a baseline blood sample was collected from the inlet sampling port of the shearing device (shown in Fig. 1) and used as the control for comparison with the sheared blood samples. For each combination of shear stress and exposure time, the sheared blood samples were collected from the outlet sampling port of the blood shearing device. For each condition, the blood shearing experiments were repeated for five times. There was no significant change in the platelet count of the sheared blood samples with the increase of shear stress and exposure time.

Flow cytometric assays of activation and shedding of GPIIb/IIIa and the expression of P-selectin on platelet surface

The activation of the platelet GPIIb/IIIa receptors and expression of platelet activation marker CD62P in the blood samples after exposed to the high shear stress conditions were quantified by flow cytometry. The activation level of the platelet GPIIb/IIIa receptors was determined by fluorescein isothiocyanate (FITC) labeled PAC-1 (BD Bioscience, San Jose, CA). The level of CD62P expression on the platelet surface was determined by phycoerythrin (PE) conjugated anti-CD62P antibody (BioLegend, San Diego, CA). The FITC conjugated IgMk antibody (BioLegend, San Diego, CA) was used to serve as the negative control for activated expression of the platelet GPIIb/IIIa. Briefly, 5 μl whole blood samples were incubated with 25 μl of 10 mM HEPES buffer mixed with

Table 1 The combined experimental shearing conditions

Shear stress (Pa)	Exposure time (s)	Flow rate (ml/min)
25/75/125	0.05	47.76
	0.5	4.776
	1.5	1.592

20 μl PAC-1 antibody and 10 μl CD62P antibody for 30 min at room temperature in the dark. The platelet population was identified by scatter gating [gating on forward scatter (FSC) and side scatter (SSC)] of flow cytometry data (BD Bioscience, San Jose, CA).

For the quantification of the shedding of the platelet GPIIb/IIIa receptors, the mean fluorescence of the GPIIb/IIIa on the platelet surface was characterized. The FITC conjugated anti-CD41 antibody (BioLegend, San Diego, CA) was used to identify the platelet population. The PE conjugated anti-CD41/61 antibody (BioLegend, San Diego, CA) was used to determine the level of the platelet GPIIb/IIIa receptors. PE conjugated IgG2aK antibody (BioLegend, San Diego, CA) was used to serve the negative control for the platelet GPIIb/IIIa. Briefly, 5 μl whole blood samples were incubated with 25 μl of 10 mM HEPES buffer mixed with 5 μl anti-CD41 antibody and 5 μl anti-CD41/61 antibody for 30 min at room temperature in the dark. In parallel, a 5 μl whole blood sample was incubated with 25 μl HEPES buffer mixed with 5 μl anti-CD41 antibody and 5 μl IgG2aK antibody and used as the negative control.

To further confirm the shear-induced shedding of the platelet GPIIb/IIIa receptors, the adenosine diphosphate (ADP) was used to activate those remaining un-activated GPIIb/IIIa receptors which were not activated by the applied shear stress. If the GPIIb/IIIa receptors on the platelet surface were shed by the non-physiological high shear stress, the quantity of the further activated GPIIb/IIIa receptors by ADP would be limited. The procedure for measuring the activation of the platelet GPIIb/IIIa receptors on the platelet surface with the ADP stimulation was the same as the procedure used above for the sheared blood samples. 5 μl ADP (200 μM final concentration) was added into the sheared and baseline blood samples mixed with the antibodies.

After the above labeling steps, paraformaldehyde (PFA, 1 %) in PBS was used to fix the samples for 30 min at 4° in the dark. The flow cytometric data collection of the blood samples was performed with a four color flow cytometer (FACS Calibur, BD Bioscience, San Jose, CA). The data were analyzed offline using the software FCS Express 4.0 (De Novo Software, Los Angeles, CA).

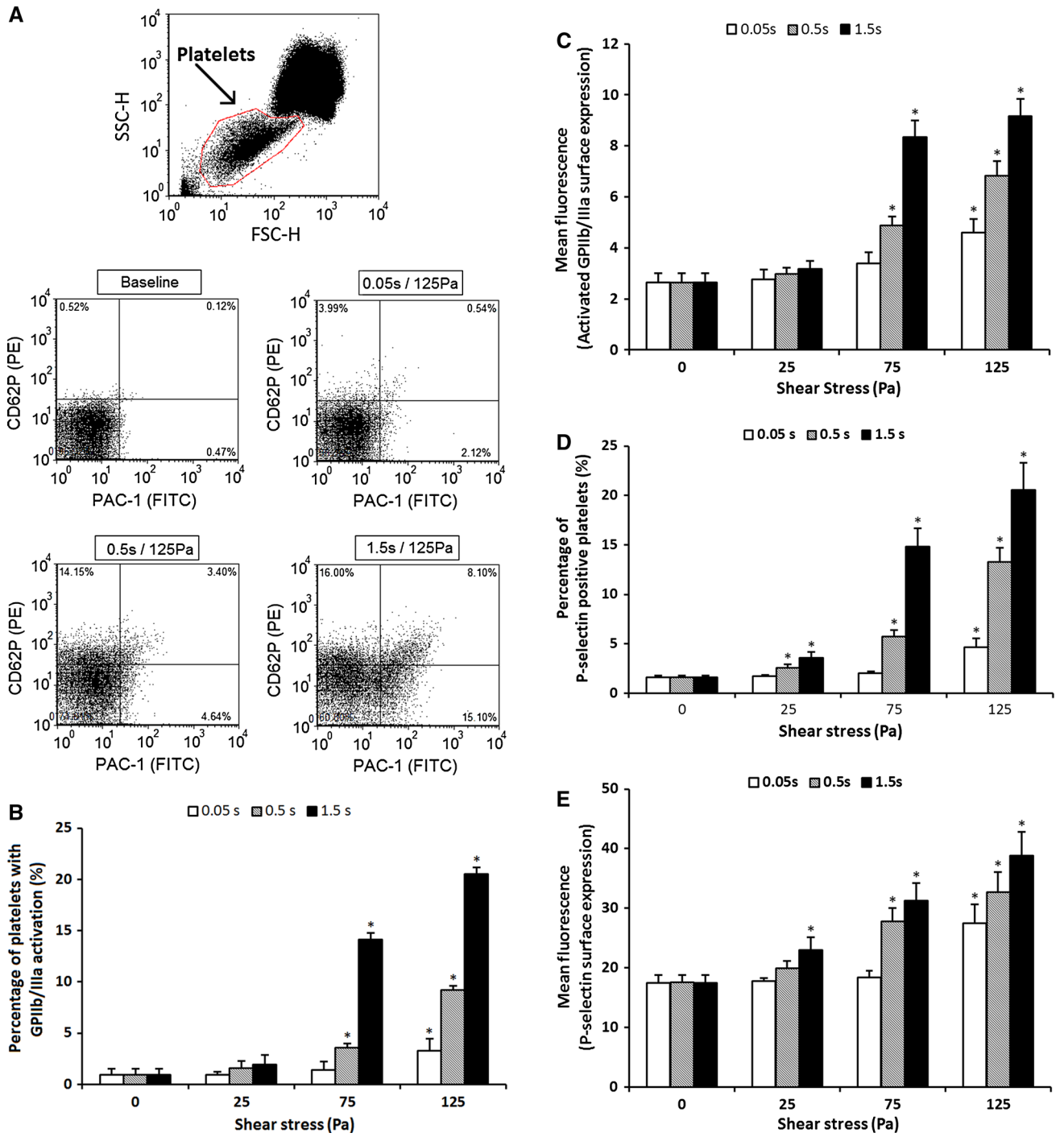
Enzyme-linked immunosorbent assay (ELISA) of GPIIb/IIIa concentration in microparticle-free plasma

To further confirm the non-physiological shear-induced shedding of the platelet GPIIb/IIIa, the concentration of GPIIb/IIIa in microparticle-free plasma was measured. Aliquots of blood samples were centrifuged at $160\times g$ for 15 min at room temperature to obtain supernate. The supernate was centrifuged at 14,000 rpm for 15 min at 4 °C to get the cell-free plasma. The cell-free plasma was centrifuged at $20{,}000\times g$ for 30 min at 4 °C to get the microparticle-free plasma [24]. A human GPIIb/IIIa enzyme-linked immunosorbent assay (ELISA) kit (Assaypro LLC, MO, USA) was used to quantify the concentration of GPIIb/IIIa in microparticle-free plasma. According to the kit instruction, 50 μl of microparticle-free plasma was added into each well of a 96-well plate and incubated for 2 h. After washing the 96-well plate for five times, 50 μl of biotinylated human GPIIb/IIIa antibody was added into each well and incubated for 1 h. After the second washing, 50 μl of Streptavidin-Peroxidase (SP) Conjugate was added into each well and incubated for 30 min. After the third washing, 50 μl of Chromogen Substrate was added into each well and incubated for 12 min. In the last step, 50 μl of Stop Solution was added into each well. The 96-well plate was read at 450 nm immediately using Synergy Mx Multi-Mode Reader (Bio-Tek, VT, USA).

Fig. 2 **a** Typical FSC/SSC scatter plot for identifying platelet population and typical fluorescence scatter plots for identifying activated platelets indicated by both the activated GPIIb/IIIa receptors and surface expression of CD62P in the baseline blood and three sheared blood samples (0.05 s/125 Pa, 0.5 s/125 Pa, and 1.5 s/125 Pa). The platelets population was identified by scatter gating [gating on forward scatter (FSC) and side scatter (SSC)]. The percentage of platelets with activated GP IIb/IIIa expression was determined by FITC fluorescence (PAC-1) and the percentage of platelets with CD62p expression was determined by PE fluorescence (anti-CD62P). **b** The percentages of the activated platelets indicated by the activated GP IIb/IIIa surface expression (FITC labeled PAC-1) in total platelets in the baseline and sheared blood samples under the three levels of shear stresses (25, 75, 125 Pa) for the three exposure times (0.05, 0.5, and 1.5 s.) ($n = 5$). **c** The quantification of the mean fluorescence of the activated GP IIb/IIIa surface expression indicated by FITC labeled PAC-1 in the baseline and sheared samples under the three levels of shear stresses (25, 75, 125 Pa) for the three exposure times (0.05, 0.5, and 1.5 s.) ($n = 5$). **d** The percentages of the activated platelets indicated by the P-selectin surface expression (PE conjugated anti-CD62P) in total platelets in the baseline and sheared blood samples under the three levels of shear stresses (25, 75, 125 Pa) for the three exposure times (0.05, 0.5, and 1.5 s.) ($n = 5$). **e** The quantification of the mean fluorescence of the CD62p surface expression (indicated by PE conjugated anti-CD62P) in the baseline and sheared blood samples under the three levels of shear stresses (25, 75, 125 Pa) for the three exposure times (0.05, 0.5, and 1.5 s.) ($n = 5$)

Statistical analysis

The data are presented as mean ± SE (standard error) and statistically analyzed using SPSS statistical software (Statistical Package for Social Sciences for windows, release 18.0; SPSS Inc., Chicago, IL, USA). Statistical differences were determined using Student's t test. Statistical significance was assigned at $p < 0.05$.



Results

The dependence of platelet activation on the levels of applied shear stress and exposure time was characterized by examining the number of activated platelets indicated by two commonly used markers (PAC-1 binding and surface CD62p expression) and the mean fluorescence intensity of the total platelet population. Figure 2a shows a typical FSC/SSC scatter plot for identifying platelet population and typical fluorescence scatter plots for identifying activated platelets indicated by both the activated GPIIb/IIIa receptors and surface expression of CD62P in the baseline blood and three sheared blood samples (0.05 s/125 Pa, 0.5 s/125 Pa, and 1.5 s/125 Pa). As shown in the Figure, the expression of the activated GP IIb/IIIa and CD62P increased with increasing the exposure time at the shear stress level of 125 Pa. Figures 2b and c show the percentage of activated platelets as indicated by the

activated GPIIb/IIIa expression (FITC labeled PAC-1) and the mean channel fluorescence (FITC) of the total platelet population, respectively. Both these two flow cytometric parameters increased with increasing the applied shear stress and exposure time. The increases in the percentage of activated platelets and the mean fluorescence of the total platelet population become significant ($p < 0.05$) for both the exposure time of 0.5 and 1.5 s when the shear stresses were 75 and 125 Pa compared with those of the baseline blood. Figures 2d and e show the percentage of activated platelets as indicated by the CD62P expression (PE conjugated anti-CD62P) and the mean channel fluorescence (PE) of the total platelet population. As shown in these two plots, both the two flow cytometric parameters also increased with increasing the applied shear stress and exposure time, respectively, in the sheared blood samples compared with those of the baseline blood.

The above results indicated that even the exposure time was very short (0.05 s), the platelets became activated by the non-physiological shear stress if the applied shear stress level exceeded 75 Pa. Overall, the number of activated platelets, as indicated by the expression of the activated GPIIb/IIIa and P-selectin, increased with increasing the shear stress level and exposure time.

The impact of the applied shear stress on the surface GPIIb/IIIa receptors was examined by quantifying the GPIIb/IIIa expression (activated and un-activated) on the platelet surface. Figure 3a shows typical fluorescence histograms of platelets for the GPIIb/IIIa expression (PE conjugated anti-CD41/61) in the baseline blood and sheared blood samples under the three combinations of shear stress and exposure time (125 Pa/0.05 s, 125 Pa/0.5 s, and 125 Pa/1.5 s). There was a noticeable decrease in the GPIIb/IIIa expression in platelets of the sheared blood samples when the exposure time was increased to 0.5 s, as indicated by the shift of the histogram peak to the weak fluorescence end (left) and the increase in the platelets with weak fluorescence intensities. Figure 3b shows the quantification of the platelet GPIIb/IIIa expression (PE mean fluorescence) in the baseline and sheared blood samples under the three levels of shear stresses (25, 75, 125 Pa) for the three exposure times (0.05, 0.5, and 1.5 s). The mean fluorescence, indicating the platelet GPIIb/IIIa expression, decreased in all the sheared blood samples. This decrease suggested that there was a reduction in the number of copies of the platelet GPIIb/IIIa receptors after the blood experienced the non-physiological shear stress. The reduction of the platelet GPIIb/IIIa receptors increased with increasing the shear stress level and exposure time.

To verify whether the reduction in the mean fluorescence intensity of the GPIIb/IIIa was associated with the reduction in the number of copies of the platelet GPIIb/IIIa receptors in the sheared blood samples, ADP was used to

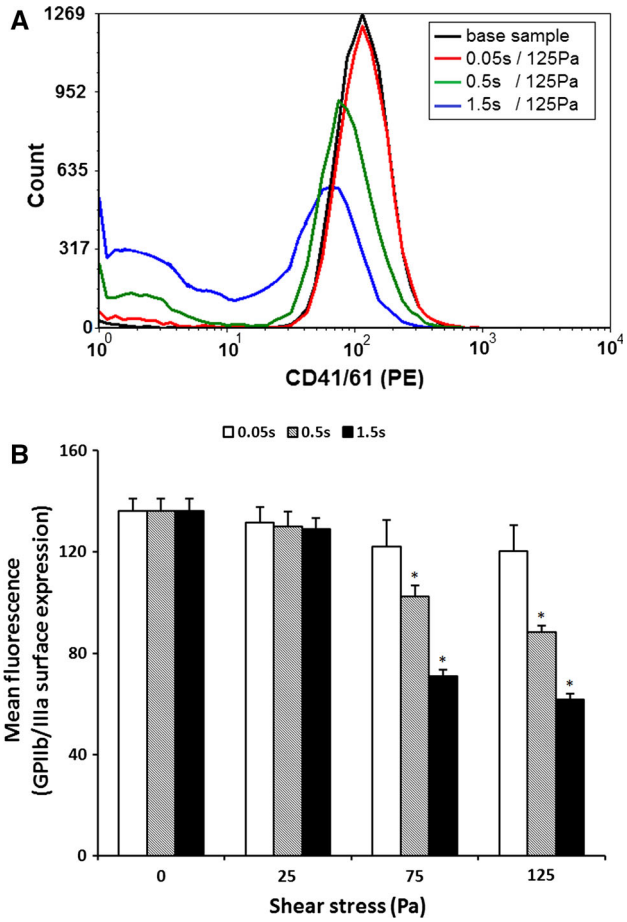


Fig. 3 **a** The typical fluorescence histograms of the GP IIb/IIIa surface expression (indicated by PE conjugated anti-CD41/61) in the baseline blood and three sheared blood samples under the shear stress of 125 Pa for the three exposure time (0.05, 0.5, and 1.5 s). **b** The quantification of the platelet GPIIb/IIIa expression (PE mean fluorescence) in the baseline and sheared blood samples under the three levels of shear stresses (25, 75, 125 Pa) for the three exposure times (0.05, 0.5, and 1.5 s.) ($n = 5$)

stimulate the sheared blood samples to induce full activation of available GPIIb/IIIa receptors. Figure 4a shows typical fluorescence histograms of the activated GPIIb/IIIa expression (indicated by FITC labeled PAC-1) of platelets in the baseline and sheared blood samples after ADP stimulation under the three combinations of shear stress and exposure time (125 Pa/0.05 s, 125 Pa/0.5 s, and 125 Pa/1.5 s). There was a significant shift in the activated GPIIb/IIIa expression in platelets of the sheared blood samples when the exposure time was increased to 0.5 s. The population of platelets with a low fluorescence intensity increased with increasing the exposure time. Figure 4b exhibits the quantification of the activated GPIIb/IIIa expression in the platelets (FITC labeled PAC-1) in the baseline and sheared blood samples after ADP stimulation under the three levels of shear stresses for 0.05, 0.5, and 1.5 s. Compared with the baseline sample, the activated

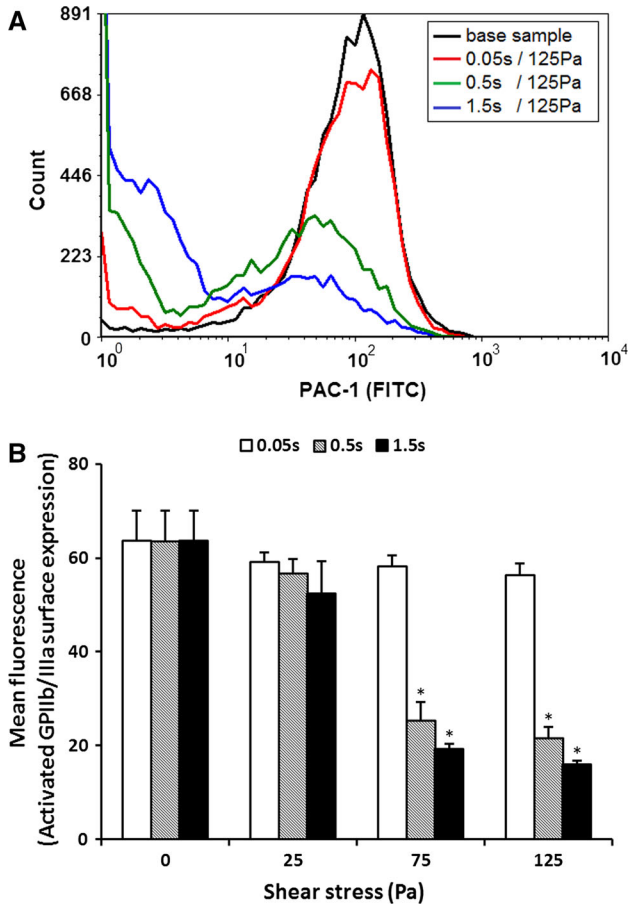


Fig. 4 **a** Typical fluorescence histograms of the activated GPIIb/IIIa expression (indicated by FITC labeled PAC-1) of platelets in the baseline and sheared blood samples after ADP stimulation under the shear stress for 125 Pa for the three exposure times (0.05, 0.5 s, and 1.5 s). **b** The quantification of the mean fluorescence of the activated platelet GP IIb/IIIa surface expression (indicated by FITC labeled PAC-1) after ADP stimulation in the baseline and sheared blood samples under the three levels of shear stresses (25, 75, 125 Pa) for the three exposure times (0.05, 0.5, and 1.5 s.)

GPIIb/IIIa expression either induced early by the non-physiological shear stress or late by ADP decreased in the platelets of the sheared blood samples. The reduction in the activated GPIIb/IIIa expression increased with increasing the shear stress and exposure time, which indicated that the available copies of the GPIIb/IIIa receptors for ADP stimulation were reduced after the blood experienced the non-physiological shear stress.

Comparing Fig. 4b with 2c, it can be seen that the ADP stimulation caused a significant increase of the activated GPIIb/IIIa expression [mean fluorescence from 2.5 to 65 (arbitrary unit)] in the platelets in the baseline blood sample. However, such a big increase in the activated GPIIb/IIIa expression in the platelets in the sheared blood samples under the high shear stress for a longer exposure time could not be induced by the ADP stimulation. The reason should be that GPIIb/IIIa receptors on the platelet surface were shed by the non-physiological high shear stress and the copies of the remaining GPIIb/IIIa were less compared to the baseline blood sample. These results further confirmed the reduction of the GPIIb/IIIa receptors on the platelet surface under the non-physiological high shear stress conditions.

To examine whether the reduction in the GPIIb/IIIa expression was caused by the non-physiological local shear stress-induced receptor shedding or shear-induced GPIIb/IIIa internalization, the GPIIb/IIIa concentration in microparticle-free plasma was measured. Figure 5 shows the GPIIb/IIIa concentration in the microparticle-free plasma from the baseline and sheared blood samples under the three levels of shear stresses for 0.05, 0.5, and 1.5 s, respectively. The GPIIb/IIIa concentration in microparticle-free plasma increased with increasing the shear stress level and exposure time. Comparing Fig. 5 with 3b, it can be seen that the reduction in the GPIIb/IIIa receptors, shed from the platelet surface under the non-physiological high shear stress conditions, corresponded to the increase in the GPIIb/IIIa in the plasma, Therefore, the result confirmed that the shedding of the platelet surface GPIIb/IIIa receptors was induced by the non-physiological high shear stress.

Discussion

The non-physiologic high shear stress often exists in BCMDs, where shear the shear stress level is beyond 100 Pa and the exposure time is very short [7, 36]. Shear-induced platelet activation had been investigated by many studies, which was believed to be related with thrombosis in patients with BCMDs [8–13]. It has been reported that platelet receptors could be shed by the non-physiological shear stress, mostly focusing on GPIbα and GPVI receptors. The loss of functional receptors in the platelets might be related to hemostatic dysfunction and even bleeding [23–25]. The GPIIb/IIIa is a major membrane protein complex on the surface of platelets and plays a key role in platelet stable adhesion, aggregation and hemostasis. Very few studies have been performed to investigate the shedding of the GPIIb/IIIa induced by the non-physiological shear stress.

In the present study, we investigated the influence of the non-physiological high shear stress on the platelet GPIIb/IIIa. The blood from healthy donors was exposed to three levels of high shear stresses (25, 75, 125 Pa) from physiological to non-physiological conditions with short exposure times (0.05, 0.5, and 1.5 s). The activation and shedding of the GPIIb/IIIa receptors and the expression of CD62P in the platelet surface were examined. The results

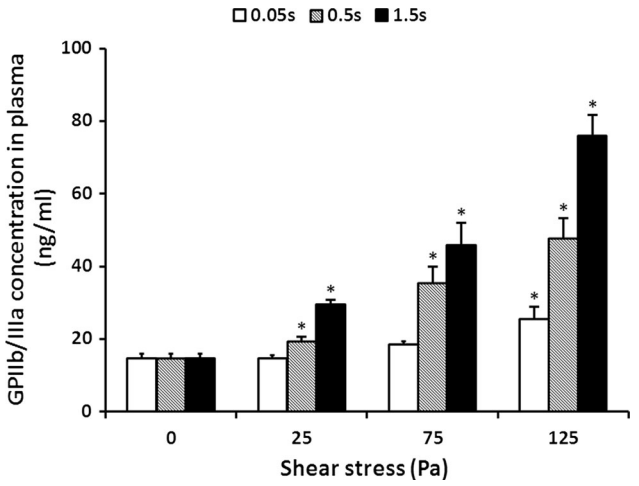


Fig. 5 The GPIIb/IIIa concentration in the microparticle-free plasma from the baseline and sheared blood samples exposed to the three levels of shear stresses (25, 75, 125 Pa) for 0.05, 0.5, and 1.5 s, respectively

clearly showed that the non-physiological shear stress caused the activation of the GPIIb/IIIa and CD62P expression in the platelets. The degree of activation of the GPIIb/IIIa and CD62P expression increased with increasing the shear stress level and exposure time. This observation is consistent with what have been reported in the literature. Interestingly, the copies of GPIIb/IIIa in platelet surface of the sheared blood samples decreased, indicating a reduction in the GPIIb/IIIa receptors (shedding). The reduction in the copies of the GPIIb/IIIa receptors was further confirmed by the reduced activation of the GPIIb/IIIa by ADP stimulation and the increase in the GPIIb/IIIa concentration in microparticle-free plasma.

Although we have not directly verified, the loss of the GPIIb/IIIa receptors on the platelet surface could lower the adhesion capacity of the platelets for the normal hemostasis. This is a paradoxical phenomenon in contrast to the common belief that the non-physiological shear stress induces platelet activation, thus enhances platelet aggregation and adhesion. In our study, we did observe that with increasing the shear stress level and exposure time, the number of platelets with the activated GPIIb/IIIa increased. However, the number of available copies of the GPIIb/IIIa on platelet surface reduced after being sheared by the non-physiological shear stress. Thus, under the non-physiological high shear stress conditions, shear-induced activation, and shedding of the GPIIb/IIIa receptors simultaneously occurred. The number of the GPIIb/IIIa on the platelet surface is 40,000–80,000 GPIIb–IIIa copies. While some of them could be shed from the surface, a portion of the remaining receptors could be activated at the same time by non-physiological high shear stress.

From the aforementioned results, we believe that the shear-induced platelet activation and shedding of the GPIIb/IIIa receptors could have two opposite effects occurring simultaneously in patients accompanied with both thrombosis and bleeding induced by BCMDs. Under the non-physiological high shear stress condition related to BCMDs, platelets were easily to be activated, leading to thrombosis within BCMDs. Thus, anticoagulation medication is prescribed to mitigate this potential problem. At the same time, the non-physiological shear-induced receptor shedding could impair the platelet function for hemostasis. It had been reported that the platelet receptor shedding, such as GPIbα and GPVI, could influence hemostasis and might be related with bleeding. Because of the importance of the GPIIb/IIIa in platelet adhesion and aggregation, the shedding of the GPIIb/IIIa from the platelet surface by the non-physiological shear stress could also influence hemostasis. Above all, more attention should be paid to avoid both shear-induced platelet activation and shear-induced receptor shedding during design and optimization of BCMDs. The activation and shedding of the GPIIb/IIIa induced by non-physiological high shear stress should be a key factor to be considered.

Conclusion

The study demonstrated that the non-physiological high shear stress could induce both activation and shedding of the GPIIb/IIIa receptors on the platelet surface. The activation and shedding increased with increasing the shear stress level and exposure time. While the activation of GPIIb/IIIa receptors would enhance the platelet aggregation and adhesion, leading to thrombotic potential, on the other hand the shedding of the GPIIb/IIIa receptors could reduce the platelet normal hemostatic function, leading to bleeding. This may be a paradoxical phenomenon in which both the activation and shedding occur simultaneously. This study may offer a new perspective to explain the reason of increased thrombosis and bleeding events in patient implanted with BCMDs.

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Compliance with Ethical Standards

Conflict of interest The authors declared that there are no conflicts of interests.

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