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ORIGINAL RESEARCH

A Microflow Chip Technique for Monitoring Platelets in Late Pregnancy: A Possible Risk Factor for Thrombosis

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Purpose: To study the platelet adhesion and aggregation behaviour of late pregnancy women under arterial shear rate using microfluidic chip technology and evaluate the risk of thrombosis in late pregnancy.

Methods: We included pregnant women who were registered in the obstetrics department of our hospital between January 2021 and October 2022 and underwent regular prenatal examinations. Blood samples were collected at 32–35 weeks of gestation for routine blood tests and progesterone, oestradiol, and platelet aggregation function. A microfluidic chip was used to construct an in vitro stenosis vascular model to explore the platelet reactivity at shear rates of 1000s-1, 1500s-1 and 4000s-1. Flow cytometry was used to analyse the effect of shear rate induction on the expression of platelet membrane surface fibrin receptor (PAC-1) and P-selectin (CD62P) in pregnant women.

Results: Compared to the non-pregnant healthy control group, the white blood cell count increased and platelet count decreased significantly in late pregnant women ($P < 0.05$), and platelet reactivity to agonists increased under non-flow conditions (adhesion and aggregation rates, $P < 0.05$). Microfluidic chip technology showed that platelet aggregation in late pregnant women increased significantly ($P < 0.05$) in the shear-rate environment and was positively correlated with the shear rate. The degree of aggregation at $4000s^{-1}$ was more evident, but the stability of platelet aggregates was low. Shear rate increased PAC-1 and CD62P expression.

Conclusion: Microfluidic chip technology was used to analyse the platelet aggregation function under arterial shear rate combined with flow cytometry to detect platelet activation, which was consistent with the traditional non-flow conditions used to evaluate platelet function. However, microfluidic technology can simulate a more realistic in vivo shear rate environment, providing more effective clinical application data and a theoretical basis for the diagnosis and prevention of platelet dysfunction and thrombotic diseases during pregnancy.

Keywords: microfluidic chip, late pregnancy, platelets, adhesion aggregation, thrombosis

Introduction

Pregnancy is a special physiological period for women, and pregnant women are hypercoagulable due to hormonal leaves and elevated fibrinogen levels during pregnancy, which can not only provide prophylactic haemostasis protection for bleeding from the stripped surface of the placenta and laceration after delivery but also facilitate the formation of arteriovenous thrombosis.[1–4] The changes in hormone levels and coagulation status during pregnancy vary with increasing gestational age with further increases in the levels of factors II, V, VII, X, and other coagulation factors in late pregnancy. They result in further decreases in antithrombin activity and fibrous activity, and aggravation of hypercoagulability, which is beneficial for placental separation during delivery and reduces postpartum haemorrhage, but increases the risk of thrombosis in pregnant women.[5] With the increasing attention being paid to postpartum haemorrhage, the disease mortality rate decreased, but venous thromboembolism is still the leading cause of maternal death in some developed countries; therefore, it is of great significance for the early diagnosis of thrombotic diseases

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during pregnancy.[6–8] Additionally, 20% of thrombi in pregnancy are arterial, and the remaining 80% are venous,[9,10] and venous thrombosis is common in lower limbs and pelvic thrombosis. Currently, the diagnosis of the disease is still mainly based on imaging examinations, such as colour Doppler ultrasound; however, imaging examinations have a certain lag, which is not conducive to early diagnosis and treatment of the disease.[11,12] Prevention of arteriovenous thromboembolism is essential, often occurring after surgery and delivery, and in severe cases can lead to death. Platelet aggregation is markedly enhanced in women in early, middle and late pregnancy, with activation of platelets or platelet activation, which may be associated with the synthesis and release of large amounts of platelet-activating factor (PAF)[13] and vascular endothelial cell damage.[14] Moreover, Edwards et al[15,16] found that the PAF, CD62p, and CD63 were significantly increased in the third trimester and postpartum, with expression levels positively associated with platelet aggregation rate and increased platelet aggregation activity in patients with venous thrombosis. Moreover, platelet cell levels of Ca2+ and plasma β-thromboglobulin (bTG) are significantly increased.[17,18] Thus, platelet adhesion, aggregation, and activation in vivo are important parts of thrombosis.[19]

In addition to the well-known agonist-induced platelet activation, the other way is to interact with the cells and extracellular matrix components of the blood vessel wall at different shear rates, resulting in mechanical forces that cause platelet aggregation and thrombosis. Such shear-induced platelet aggregation (SIPA) is a specialized platelet aggregation that is rare in normal haemostatic mechanisms. The first step in thrombosis management is the accumulation of platelets at sites of vascular injury. Shear-induced platelet aggregation is related to the interaction between plasma vWF and platelet membrane surface receptors GPIb and GPIIb/IIIa. In contrast, the mechanical stimulation of the protein targets associated with shear stress, platelet membrane surface mechanical ion channel piezo1, platelet endothelial adhesion molecule 1 (PECAM-1), and the directed receptor plexin D1, stimulate shear stress and convey downstream protein activation that in turn affects platelet activation. The binding of platelets to adhesion proteins and subendothelial stroma such as von Willebrand factor (vWF), fibrinogen, or collagen involves specific receptors expressed on platelet membranes (GPIb/IX/V, GPIIb/IIIa, GPVI, and GPIa/IIa, respectively). Once triggered, these receptors cause platelet adhesion, activation, and spread, and granular secretion amplifies recruitment of additional platelets to form platelet thrombus.[20,21] And these can be detected by Ca release and expression of the platelet surface marker CD62, CD63. Researchers have been devoted to the study of the formation, prevention, and treatment of pathological thrombosis in pregnancy, with fewer studies. Platelet adhesion aggregation function and thrombotic risk in most normal pregnant women and the detection of platelet aggregation function under static conditions cannot comprehensively assess the transient interactions between platelets and increased shear forces. Limited ability to test the efficacy of platelet adhesion aggregation in the hemodynamic environment experienced by platelets in vivo. Therefore, in this study, the platelet function was studied using microfluidic chips by simulating micro vessel size and structural characteristics in vivo to precisely regulate microfluidic dynamics.

The microfluidic microarray used in this study simulates microchannel structures similar in size and structure to microvessels in vivo and accurately regulates the flow dynamics, thus serving as an ideal in vitro microvessel model for exploring relevant physiologic pathological mechanisms such as coagulation and platelet formation under the control of shear forces. We used microfluidic chip technology to simulate the shear rate of venous vessel walls at ~500s^{-1}, small arterial duct wall shear at 1500s^{-1}, and arterial wall moderate stenosis shear at 4000s^{-1} in vitro to investigate platelet adhesion aggregation behaviour and thrombotic risk. In this study, we found that platelet adhesion aggregation function was generally enhanced in women in the third trimester. Platelet adhesion increased at a low shear rate with slow flow, and thrombosis formed larger aggregates that were easily washed away, whereas at a higher shear rate, thrombosis was less and all aggregates were small and were less easily washed away. Therefore, we believe that platelet aggregation function is enhanced in late pregnancy, but that thrombotic capacity is reduced under normal blood flow conditions, which increases the risk of thrombosis in patients with other pregnancy complications.

Materials and Methods

Ethical Approval

The study complies with the Declaration of Helsinki.

Collection and Processing of Blood Samples

Blood samples were collected from pregnant women who were registered in the Department of Obstetrics, Yongchuan Hospital of Chongqing Medical University from January 2021 to December 2022 and delivered naturally in the Department of Obstetrics, Yongchuan Hospital of Chongqing Medical University. Twenty healthy volunteers were recruited from the physical examination centre of Yongchuan Hospital of Chongqing Medical University. Enrolment criteria: pregnant women: first-trimester singletons aged 20–35 years with a prepregnancy body mass index (BMI) of 18.5–23.9, no history of thrombosis, normal platelet count, no medications related to platelet count and function within 2 weeks, no pregnancy complications. Eligible nonpregnant volunteers: aged 20–35 years with a BMI of 18.5–23.9, no history of cardiovascular disease, and no recent medications: clopidogrel, aspirin, and other antiplatelet and statin lipid-lowering drugs, normal platelet count. This study was approved by the Ethics Committee of Chongqing Medical University Affiliated Yongchuan Hospital (approved number 2021086), all of the participants were informed about the purpose of the study and signed a consent form. Vessel samples were collected by vacuum, using 1:9 (v/v) 3.2% sodium citrate (fibrinogen, D-dimer, platelet function assay), EDTA-K2 anticoagulant (white blood cell count, haemoglobin level, and platelet count), without anticoagulant blood vessel collection (progesterone, estradiol assay) within 2 h. Blood samples for microflow control were fluorescently labelled with Calcein AM for platelets in blood samples. Calcein AM fluorescent dye at a concentration of 1 mmol/L was added 1 ： 500 (v/v) to the blood samples during the experiment, which were gently shaken and incubated in a photophobic chamber at 37°C for 10 min.

Microchannel Chip Design and Processing

The microchannel chip design and processing were based on a previous report by the research group.[22] Straight microchannels with width and depth of 1000 μm and 70 μm were selected. Adding narrow microchannels on the basis of the previous study, simulated the pathological high shear rate in small artery stenosis. The narrow microchannels used in this study had a width of 200 μm (80% narrowness) at the narrow point and a length of 0.5 mm (Figure 1a), with other components being homostenic channels.

Microfluidic Chip Technology Analysis of Platelet Aggregation Function

The microchannel platelet aggregation experiment was based on a previous report.[22] In this study, the shear rate of straight microchannel wall was set at 300/s and 1500/s, which represented the shear rate of blood flow wall in human veins and arteries under physiological conditions, and 4000/s represented the shear rate of blood flow wall in narrow

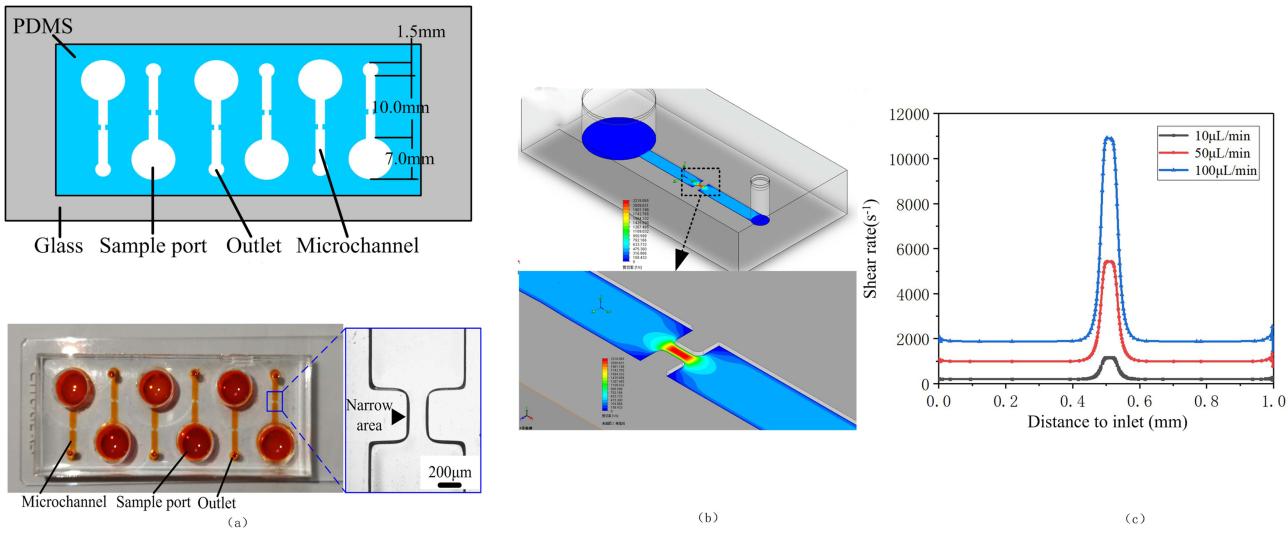

Figure 1 (**a**) Physical map of the microfluidic chip. (**b**) Chromatic scale of the shear rate of a narrow microchannel and shear-rate distribution at the stenosis. (**c**) Shear-rate distribution in the narrow microchannel.

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arteries. The whole blood samples were perfused into the microchannel for 200 s, and fluorescent images were taken for analysis.

Fluid Dynamics Analysis of Narrow Microchannel in Microfluidic Chip

The finite element analysis module of the Solidwork3D modelling software was used to analyse the fluid dynamics of the narrow microchannels (Figure 1b and c). The flow rate of narrow microchannels was set to 50ul/min, and the cut rate of the walls through finite element analysis could reach 5000/s. The splicing rate distribution was uniform across most of the splicing area of the narrow microchannel, with a narrow microchannel length of 0.50 mm and a region of upstream and downstream splicing rate gradient change at 0.75 mm before and after the narrow microchannel, with a gradient decreasing from the narrow microchannel to both ends. Anticoagulant whole blood is perfused through narrow microchannels in which platelets enter upstream of the high shear area and subsequently out into the lower shear area where they aggregate to form a steady platelet aggregate. Therefore, the platelet aggregation image of this study selected the aggregation area downstream of the stenosis at 0.75 mm.

Intravenous whole blood samples were collected in the pregnant women group and non-pregnant women healthy control group at the same time. Progesterone and estradiol were detected by Mindray 6000, fibrinogen and D-dimer by the automated coagulation analyser, and white blood cell count, haemoglobin level and platelet count by automatic blood cell analyser. All reagents are instrument specific accessory reagents.

Detection of Platelet Adhesion Aggregation Function by Continuous Multiparameter Method

Platelet aggregation induced by AA, ADP, and COL was analysed using a continuous multiparameter method based on Aggrestra (PL-12). The collected anticoagulated whole blood samples were maintained at equilibrium at room temperature for 10 min. The detection program was Aggrestra (PL-12). The inducers AA, ADP and COL corresponding to the program setting were added to each 30 ul at the specified position of the reagent. Next, 300 ul of sodium citrate anticoagulant was added to the whole blood at the sample detection position. At the end of the assay, the maximum adhesion rate and maximum aggregation rate of platelets induced by different inducing agents were recorded.

Analysis of Platelet Activation by Flow Cytometry

Anticoagulant whole blood, normal control group and whole blood of women in late pregnancy were incubated at 37 °C for 30 min. After that, the blood was perfused into the bovine serum albumin-closed straight-through and 80% narrow microchannels. Blood samples were collected at a volume flow rate of 52 μL / min and 100 μL / min.5 μL of each sample was incubated with anti-human CD61, CD62 P, PAC-1 at room temperature for 20 min in the dark. Before perfusion, blood samples from Group 1 were used as negative controls. Labelled samples were added to 1% paraformaldehyde 1 mL, fully mixed, and fixed for 10 min. The expression of CD62 P and PAC-1 was analysed by flow cytometry using three-colour fluorescence, and the data were analysed using Flowjo 10.5.3.

Statistical Analysis

SPSS 26.0 statistical software was used for data analysis. Measurement data were expressed as mean ± standard deviation. Multiple-group comparisons of mean values were designed with randomized blocks for analysis of variance, multiple comparisons were performed with the LSD test, paired t tests were used for comparisons of mean values between two groups, and the Shapiro–Wilk experiment was used for normality testing. $P < 0.05$ was statistically significant as a difference.

Result

Basic Data

Hormone levels in late pregnancy and postpartum women increased, the number of red blood cells and platelets decreased, the number of white blood cells increased slightly, and protein C, protein S activity, and plasma antithrombin III and D-dimer levels increased significantly (Table 1).

Table 1 Basic Data of Research Objects (n=52)

Items	Control	Pregnant	*P*
Estradiol (ng/mL)		25.14 ± 3.19	
Progesterone (ng/mL)		215.12 ± 30.58	
Red blood cells (*10^{12}/L)	3.77 ± 0.35	3.26 ± 0.29	0.516
White blood cells (*10^{9}/L)	6.41 ± 1.95	9.53 ± 3.31	0.006
Platelets (*10^{9}/L)	239.4 ± 50.05	196.4 ± 48.22	0.015
D-dimer (ug/mL)	1.58 ± 0.21	2.74 ± 1.51	0.021
FiB (g/L)	2.76 ± 0.35	5.95 ± 0.72	0.008

Note: P < 0.05 indicates a statistically significant difference.

Effect of Platelet Adhesion and Aggregation Induced by AA, ADP, and COL

In this study, the platelet adhesion rates induced by AA, ADP and COL in the control group were 25.15 ± 2.18%, 29.48 ± 2.48% and 23.18 ± 4.25%, respectively. The maximum aggregation rates induced by AA, ADP and COL were 54.8 ± 5.43%, 61.16 ± 4.09%, and 46.46 ± 6.32%, respectively. The adhesion and aggregation rates of pregnant women in the third trimester were 32.58 ± 4.62%, 40.16 ± 5.96%, 36.87 ± 6.21%, 65.97 ± 4.13%, 74.25 ± 3.54%, and 72.39 ± 5.83%, respectively. The reactivity of platelets to inducers in pregnant women in late pregnancy was significantly increased, and the aggregation function was significantly enhanced (P < 0.05). (Table 2 and Figure 2)

Platelet Adhesion Aggregation Effect and Activation Under Shear Rate Condition

After the blood of women in late pregnancy was allowed to flow for 200 s at 300/s, 1500/s, and 4000/s, the platelet adhesion and aggregation images showed that there were fewer platelet aggregates at 300/s, and the distribution was punctate and nondirectional (Figure 3). At 1500/s, the platelet adhesion aggregates increased, and the brightness increased significantly and extended linearly along the flow direction. When the shear rate of blood in the simulated stenotic artery was 4000/s, the platelet aggregation image showed obvious platelet aggregation downstream of the pathologically high shear rate, which was highlighted at the stenosis outlet. With a gradual increase in the wall shear rate, the total area and coverage of platelet aggregation increased significantly (P < 0.05, Figures 3 and 4). This is consistent with our previous results.[23]

In this study, fluorescein-labelled antiplatelet monoclonal antibody immunofluorescence-stained specimens were flowed through enclosed microchannels, and the expression of platelet surface activation markers (PAC-1 and CD62P-positive platelet percentages) was evaluated by flow cytometry, which showed that the blood of pregnant women increased the expression of P-selectin under either wall shear ratio condition and showed an upward trend with increasing shear ratio (Figure 5).

Discussion

Pregnancy represents a period of accelerated cardiovascular adaptation, where the body undergoes profound changes to accommodate foetal growth and development. To maintain adequate perfusion of both maternal and foetal vascular systems, hemodynamic alterations commence within the blood vessels. Additionally, maternal hypercoagulability emerges as a physiological response facilitating childbirth, yet this heightened coagulation state, coupled with the foetus'

Table 2 Platelet Adhesion and Aggregation Rate in the Third Trimester (n=52)

Ttem	Control		Pregnant		*P*
	Adhesion Rate	Aggregation Rate	Adhesion Rate	Aggregation Rate	
AA	25.15 ± 2.18	54.8 ± 5.43	32.58 ± 4.62	65.97 ± 4.13	<0.01
ADP	29.48 ± 2.48	61.16 ± 4.09	40.16 ± 5.96	74.25 ± 3.54	<0.01
COL	23.18 ± 4.25	46.46 ± 6.32	36.87 ± 6.21	72.39 ± 5.83	<0.01

Note: P < 0.05 indicates a statistically significant difference.

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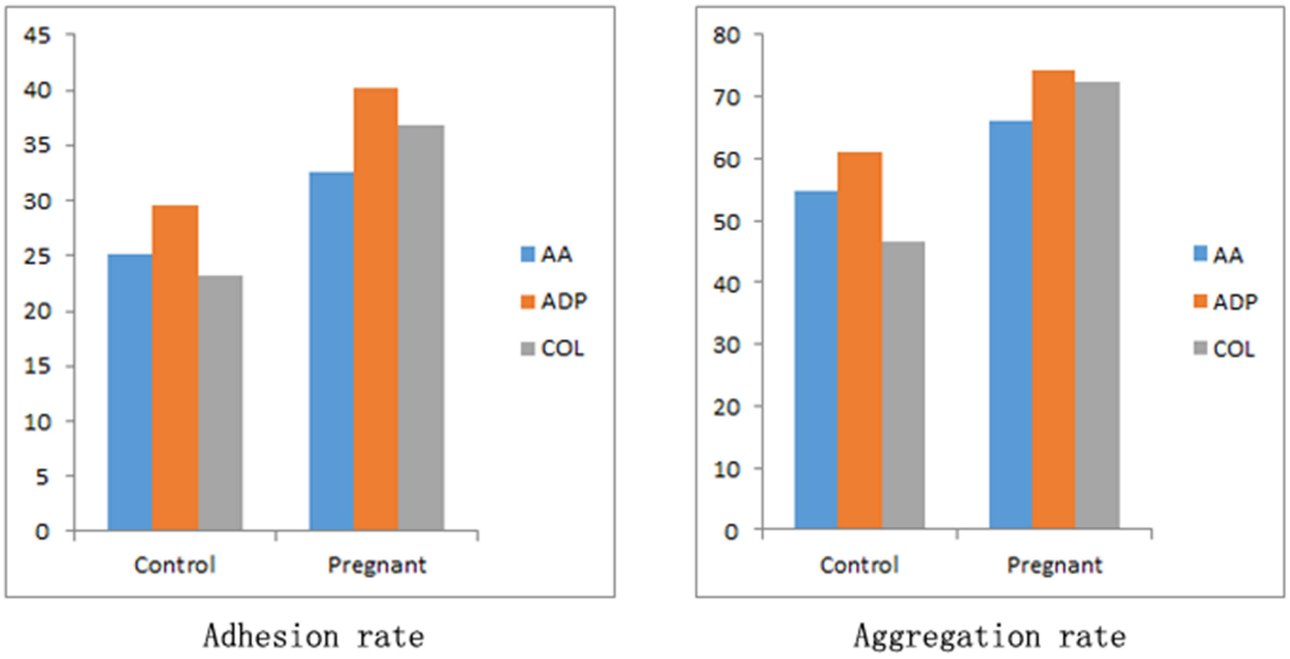

Figure 2 Platelet adhesion and aggregation rate in the presence of inducer.

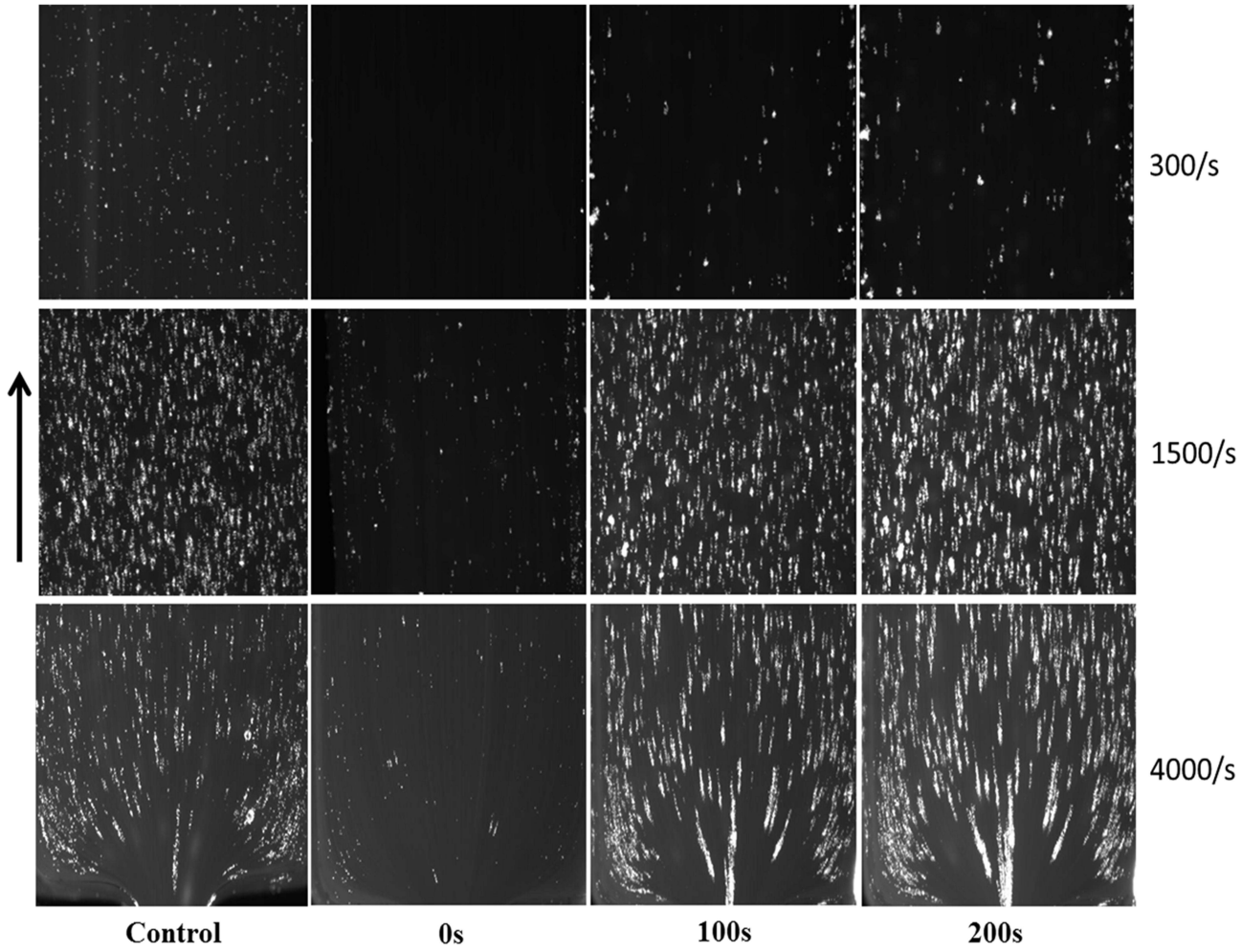

Figure 3 Arrow indicates flow direction; platelet adhesion and aggregation at shear rates of 300/s, 1500/s, and 4000/s.

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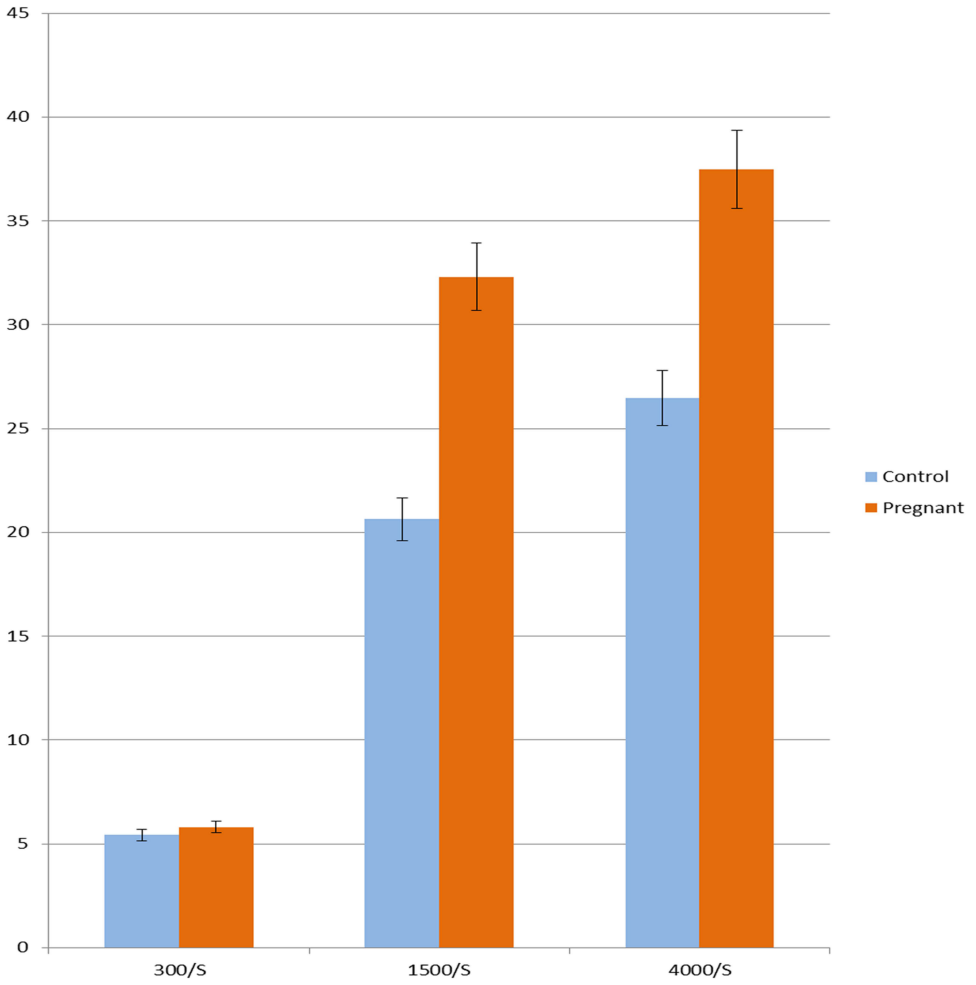

Figure 4 Platelet aggregation coverage under different shear rates.

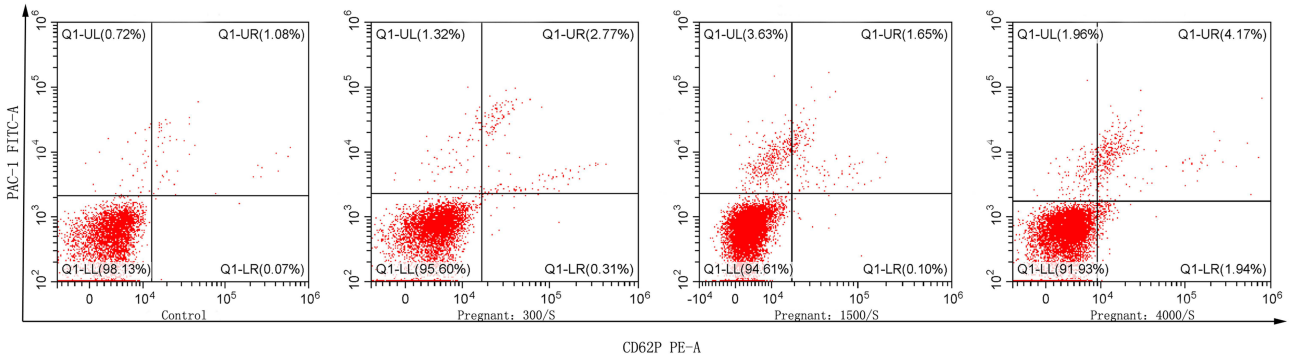

Figure 5 Flow cytometry of platelet activation marker.

weight gain during the third trimester intensifies the burden on lower limb circulation, leading to a significant reduction in venous flow rates. Furthermore, maternal activity levels often diminish significantly compared to daily norms, thereby escalating the risk of thrombotic diseases during this stage. The abrupt onset of thromboembolic disorders, which can be fatal if not promptly and effectively addressed, underscores the paramount importance of predicting such complications during pregnancy. While imaging modalities are the gold standard for diagnosing thrombotic diseases in pregnancy, they

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possess inherent delays and limited predictive capabilities. Thus, there is a pressing need for earlier and more accurate means of identifying those at risk.

Typically, platelets are in a relatively stable state, and thrombosis occurs once the platelets have been converted to a condition with activating functions. There is a mild inflammatory response during normal pregnancy, and various inflammatory factors can lead to the activation and injury of vascular endothelial cells, often manifesting themselves as a prethrombotic state and a platelet-activating state.[24] After endothelial cell injury in the affected patients, there is a certain exposure of the patient to collagen under the endothelial tissue, and the exposed tissue is involved in the process of platelet activation, further exacerbating platelet activation. While platelet activation can also further contribute to the patient's microcirculatory thrombotic disorders, both promoting and influencing each other.[25,26]

Platelet function testing is used in the adjunctive diagnosis of platelet disease, prethrombotic status, efficacy monitoring, and personalised treatment of antiplatelet therapy. Currently, a variety of platelet-based workups are mainly the following: optical turbidimetry (LTA), thromboelastography (TEG), MEA, VerifyNow, and VASP method, etc.[27–30] LTA method is the "gold standard" for determining platelet aggregation, and test results correlate well with clinical outcomes. Numerous studies are conducted both domestically and internationally to adopt this method. TEG method is widely used and has high clinical acceptance owing to advantages such as simple operation and good reproducibility. However, there are certain differences in the current multiple assays for assessing platelet reactivity; that is, the results derived from one assay (HPR or LPR) may differ from the results of other assays.[31] Furthermore, the critical values of different methods are inconsistent.[6] Overall, the methods for assessing platelet function have significant limitations, usually by detecting changes in absorbance caused by aggregation of platelets under stimulation with chemoactivators such as thrombin, collagen, and adenosine diphosphate (ADP), which do not evoke a full response to platelet aggregation in the flow state, and do not reflect the shear effect that platelet flow may experience when passing through stenotic vessels. Shear stress is a critical factor in achieving platelet activation by modulating mechanical action. Moreover, in arterial thrombotic disease, vascular stenosis can cause blood flow to be subjected to much higher than physiological levels of shear forces that can directly induce rapid platelet activation/aggregation in vitro.[6,19,32] The effects included membrane receptor aggregation, membrane tension, integrin and cytoskeleton modifications, and signal transduction.

With the widespread application of micron processing technology, studies have reported that microfluidic technology offers unique advantages in studying platelet adhesion aggregation, including precise control of the fluid shear stress environment, simulation of the structural features of blood and vascular wall interactions in vivo, integration of endothelial-mediated antithrombotic and prothrombotic mechanisms, and simulation of subendothelial substrate exposure such as collagen, fibronectin, and vWF in direct contact with platelets. Evaluation of platelet function under flow conditions is an effective complement to traditional assays. In the in vivo environment, platelets are always exposed to different flow shear stress conditions. The shear rate at venous vessels is 10–200s^{-1}; platelet adhesion and aggregation are more dependent on vWF when the shear rate of arterial vessels exceeds 600 s^{-1}, especially for arterioles or stenotic arteries, which can be sheared at a rate of 5000 s^{-1}. Under conventional shear flow conditions, platelets can form stable adhesive aggregates that participate in the thrombosis and haemostasis processes, which is also consistent with the results of this study. In a previous study by this group, we found that platelets from patients with other thrombotic conditions, such as atherosclerosis and acute myocardial infarction, aggregate to form thrombi at a pathologically high shear rate. However, these platelet aggregations are not inhibited by current commonly used drugs, such as aspirin, which can inhibit platelet aggregation under the action of shear rates in physiological flow environments.[33,34] Therefore, we considered aspirin as a prophylactic medication for the prevention of platelet thrombosis caused by blood flow changes in pregnant women rather than by vasculopathy, resulting in an abnormal shear rate.

The involvement of platelets in the coagulation process consists of secretion, activation, adhesion, and aggregation. Hypercoagulability and elevated thrombin concentrations during pregnancy can induce platelet activation, resulting in changes in platelet shape, membranous vacuoles, increased pseudopodia formation, increased and enlarged open microtubule systems, which allow platelet surface area to be stretched, increasing by 4 times, supporting increased secretion of various particles and extra particulate matter such as thromboxane A2 to TXA2, further promoting platelet activation and aggregation, and reducing platelet consumption by large amounts. During pregnancy, activation and facilitation between the coagulation system and platelets are bidirectional. Activated platelets stimulate erythrocytes to

promote platelet activation by releasing the platelet aggregation activator ADP and enhance platelet reactivity by promoting platelet granule release and recruitment of more platelets. Owing to the presence of fibrinogen-specific binding sites on both erythrocyte and platelet cell membranes, it allows for large amounts of fibrinogen to be bound. This platelet-red blood cell interaction, which is affected by increased oestrogen levels during pregnancy, results in hypercoagulable state of the blood by initiating platelet activation and promoting platelet aggregation.[35] Especially for some pregnancy complications, such as systemic lupus erythematosus, antiphospholipid antibody syndrome, and other diseases, due to changes in hormone levels during pregnancy, these autoimmune diseases relapse or become aggravated, and the risk of arteriovenous thrombosis increases. Anti-β2-glycoprotein I (aβ2GPI) antibodies are closely related to APS-related thrombosis. Aβ2GPI antibodies are often detected in SLE patients, and aβ2GPI antibodies are closely related to the severity of thrombosis. Therefore, anti-β2GPI antibodies are more specific than lupus anticoagulants and anticardiolipin antibodies in APS patients. The mechanism by which antiphospholipid antibodies promote thrombosis is complex and of great significance in the pathogenesis of APS thrombosis. The binding of aPLs to the cell surface can activate vascular endothelial cells, platelets, neutrophils, and monocytes, and promote their release of tissue factors and pro-inflammatory cytokines. APLs induce vascular endothelial cells to express adhesion molecules, promote thrombus adhesion, and activate the complement pathway; thus, c5 binds to vascular endothelial cells and increases cell adhesion. Regarding molecular mechanism, the possible reason could be that anti-β2-GPI antibody triggers the signal transduction pathway of LRP8 through lipid rafts in endothelial cells to induce LRP8 phosphorylation under the action of shear rate. This signalling pathway will lead to eNOS phosphorylation and insufficient intracellular nitric oxide production, thereby increasing the risk of thrombosis, and pretreatment with RAP or MβCD will reverse this phenomenon.[36–38] This also brings new therapeutic targets and hope for pregnancies complicated by autoimmune diseases and thrombosis.

In this study, we used microfluidic microarray technology to monitor whole-blood platelet thrombosis, which is a method for simulating human haemostasis and thrombosis in vitro. The detection of platelet aggregation under physiological conditions is particularly important. During pregnancy, the uterine blood vessels undergo significant vascular remodelling due to mediation of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), leading to potential platelet collagen matrix interactions and hemorheological changes. In this study, late platelets of pregnant women had enhanced platelet adhesion aggregation function and markedly elevated expression of platelet activation markers under inducer conditions. Platelet reactivity increases, adhesion and aggregation are markedly enhanced in the arterial shear environment, and platelet aggregation stability in the microchannels is low and is prone to being washed away. This result may help to counteract the hypercoagulability state in late pregnancy, reduce the risk of arterial thrombosis, or may be associated with partial depolymerization of platelet aggregation induced by pathological shear rates in vitro over a defined period, and the specific mechanisms require further investigation. PAC-1 is the fibrinogen binding site exposed after glycoprotein IIb/IIIa activation, ie activated glycoprotein IIb/IIIa; CD62P is a protein component of the resting platelet a granule membrane, which undergoes a granulating reaction upon platelet activation, where a granule membrane rapidly fuses with platelets, and P-selectin either shifts to the platelet surface or degranulates becomes a soluble P-selectin, and according to our independent study, CD62P is a reliable marker of shear-dependent platelet activation.[39] This study examined the expression of activation markers after spleen platelets were subjected to shear using flow cytometry and showed that increased spleen platelet activation resulted in increased platelet activation, but no significant platelet aggregation, possibly related to changes in flow status and hormone levels in the third trimester or may be related to the limited number of samples in this study. Our results suggest that the measurement of platelet function under arterial blood flow conditions may provide valuable information for determining the potential risk of thrombosis or bleeding in complex pregnancies. However, the application of microfluidic chip technology to study the adhesion and aggregation of platelets in a shear-rate environment remains. However, the molecular mechanisms underlying platelet activation and thrombosis have not yet been elucidated. Therefore, our group will study the adhesion and aggregation of platelets at different stages of pregnancy and the postpartum shear rate in the future.

In summary, platelet aggregation effects in pregnant women in the third trimester were significantly enhanced and activation was increased, but platelet aggregation stability was not significantly increased. The specific mechanism remains to be investigated. The results of this study provide preliminary evidence that microfluidic chip systems can be used to assess shear-induced platelet aggregation and activation function in the context of significant changes in blood

flow during late pregnancy and high levels of hormones, analyse platelet reactivity differences under different shear rates, and combine flow cytometry to assess the risk of thrombosis in late pregnancy.

Data Sharing Statement

Data used to support the findings of this study are available from the corresponding author upon request.

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Disclosure

The authors declare that there are no conflicts of interest in this work.

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