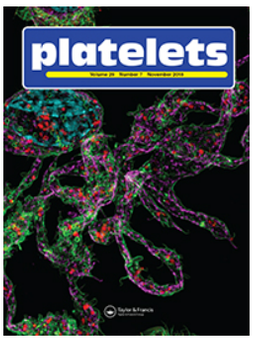

Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: https://www.tandfonline.com/loi/iplt20

High-throughput measurement of human platelet aggregation under flow: application in hemostasis and beyond

Sanne L. N. Brouns, Johanna P. van Geffen & Johan W. M. Heemskerk

To cite this article: Sanne L. N. Brouns, Johanna P. van Geffen & Johan W. M. Heemskerk (2018) High-throughput measurement of human platelet aggregation under flow: application in hemostasis and beyond, Platelets, 29:7, 662-669, DOI: 10.1080/09537104.2018.1447660

To link to this article: https://doi.org/10.1080/09537104.2018.1447660

© 2018 The Author(s). Published by Taylor & Francis

Published online: 14 Mar 2018.

Submit your article to this journal

Article views: 1987

View related articles

View Crossmark data

Citing articles: 15 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=iplt20

http://www.tandfonline.com/iplt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

platelets

Platelets, 2018; 29(7): 662–669
© 2018 The Author(s). Published by Taylor & Francis. DOI: https://doi.org/10.1080/09537104.2018.1447660

Taylor & Francis
Taylor & Francis Group

SPECIAL REVIEW: PLATELETS ON AGGREGOMETRY

Check for updates

High-throughput measurement of human platelet aggregation under flow: application in hemostasis and beyond

Sanne L. N. Brouns, Johanna P. van Geffen, & Johan W. M. Heemskerk

Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands

Abstract

In recent years, considerable progress has been made in understanding the mechanisms involved in platelet activation during hemostasis and thrombosis. Parallel-plate flow chambers and other microfluidic devices have markedly contributed to this insight. Conversely, such flow devices are now increasingly used to monitor the combined processes of platelet aggregation, thrombus formation, and coagulation in human blood. Currently, by combining microspotting and multi-color fluorescence microscopy, this technology offers the capability of high-throughput measurement of platelet activation processes, even in small blood samples. Here we review the potential of flow chamber devices for complex (multiparameter) platelet and coagulation phenotyping, focusing on patients with (genetic) platelet- or coagulation-based bleeding disorders as well as monitoring of antithrombotic medication. Animal studies are not discussed.

Keywords

Adhesion, fibrin procoagulant, thrombus

History

Received 10 December 2017
Revised 26 February 2018
Accepted 27 February 2018
Published online 14 March 2018

Introduction

Since the early flow chamber studies, where blood was used directly taken from the arm of a subject and platelet surface-area coverage was analyzed offline, the microfluidics technology is developing into a high-throughput test, capable of detecting aberrations in multiple platelet responses.

Development of flow devices to measure hemostatic and thrombotic processes

Baumgartner, Badimon, Sixma, and colleagues have pioneered in the use of flow chamber devices to study platelet adhesion and aggregation on extracellular matrix components of the arterial wall (1–3). Initial investigations revealed the importance of a well-controlled blood flow and indicated that platelet adhesion markedly increases at higher with blood flow and wall-shear rates. One approach was to perfuse blood directly originating from the arm of a volunteer through the flow chamber *ex vivo*, thus allowing formation of platelet thrombi without anticoagulant intervention (4,5). Yet, the first flow chamber experiments were long-lasting, required large blood volumes, used roller pumps influencing platelet activity, and required offline analysis of thrombus formation (6). In most cases, the chambers contained deliberately damaged endothelium, endothelial matrix, or blood vessels, all of heterogeneous composition (7,8). Yet, it was immediately recognized that the devices could mimic the *in vivo* situation of hemostasis and thrombosis, where platelets rapidly interact with a damaged or diseased vessel wall to form clots of aggregated platelets and fibrin.

Already in early parallel-plate flow studies, Sakariassen and McIntyre (9,10) used purified collagen (applied with a paint brush!) as an effective platelet-adhesive surface. Immobilized fibrillar collagen type I (pipette coating) now appears to be the standard surface for measurements of platelet adhesion and thrombus formation under flow conditions (11). For clinical application, the use of more widely applied commercial flow chambers can be considered. However, with such commercial systems (which are lower in costs and have a great ability for integration platforms) the possibility of applying more than one coating in the chamber is limited. For a more in-depth overview of strengths and weaknesses of different (commercial) microfluidic systems, we refer to elsewhere (12).

The widespread use of collagen-I is promoted by three sets of findings. First, thrombus formation on collagen-I relies on the synergy between three important receptor complexes on platelets, namely glycoprotein (GP)VI, integrin $\alpha_2\beta_1$ and GPIb-V-IX; the latter is required for shear-dependent trapping of platelets to von Willebrand factor (VWF) that is bound to collagen (13,14). Second, for many genetic mouse models, it has been noticed that abnormal collagen-induced thrombus formation *in vitro* corresponds well with aberrant arterial thrombus formation *in vivo* (especially for $FeCl_3$- and ligation-induced injury models) (15–17). Third, in atherothrombosis models of experimental plaque rupture, thrombus formation relies on collagen–GPVI interactions next to thrombin generation (18). Similarly in flow devices, collagen-I (allowing GPVI activation) as well as tissue factor (producing thrombin) are key components of coated plaques to stimulate platelet thrombus and fibrin

Color versions of one or more of the tables in the article can be found online at www.tandfonline.com/iplt.

Correspondence: Johan Heemskerk, Department of Biochemistry (CARIM), Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands. E-mail: jwm.heemskerk@maastrichtuniversity.nl

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

formation (19–21). In addition to the primary collagen–GPVI interaction, secondary roles in thrombus buildup have been identified for autocrine-released ADP acting through the platelet $P2Y_{1/12}$ receptors (22,23), and of factor XIIa stimulating the intrinsic coagulation pathway via collagen (24). In multiple laboratories, hence, collagen-I with or without tissue factor is the preferred surface for flow-based assays of thrombus formation using human or animal blood, aiming to obtain overall information on platelet activity in hemostatic and thrombotic processes.

In parallel-plate chambers with constant blood flow, a thrombus will grow but complete occlusion cannot be reached due to continuous pressure provided by the pump. In the pressure drop model established by Colace and colleagues, the blood can bypass occluding channels by moving toward an open channel. This mimics the physiological situation, where a thrombus grows until it reaches full occlusion (25). Another remark is that, when a thrombus is obstructing a channel, the flow pattern will be disturbed, resulting in very high shear rates (26,27).

Microspotting and high-throughput testing

No coagulation (high Ca^{2+}/Mg^{2+} with thrombin inhibitors)

Regardless of the widespread use of collagen-I, platelets contain several other non-collagen receptors that can play a role in interaction with the activated or injured vessel wall (28,29). In a recent paper, our group compared 52 adhesive proteins and peptides (with single or multiple components), encompassing all major adhesive receptors, for the capability to support platelet adhesion, activation and full-size thrombus formation (15). It appeared that, next to the collagen/VWF receptors (GPVI, $\alpha_2\beta_1$, GPIb-V-IX), also CLEC-2 (podoplanin, rhodocytin receptor), integrin $\alpha_{IIb}\beta_3$ (fibrinogen/WVF receptor), and $\alpha_6\beta_1$ (laminin receptor) supported adhesion in perfused whole blood. Furthermore, at low shear rate, we noticed a role of CD36 (thrombospondin receptor).

As early described, rapid screening of the 52 surfaces could only be performed by microspotting (1–2 mm Ø) and application of 2–3 microspots in the same flow chamber (15). To further increase the throughput, it is necessary to capture both enhanced-contrast brightfield and fluorescence microscopic images. Aiming to perform full platelet function analysis, brightfield images provide information on platelet deposition (surface-area coverage) and aggregation (aggregate/feature size). Fluorescence images in different colors inform on stable platelet adhesion (membrane probe), secretion (CD62P or CD63 expression), integrin activation (probing for activated $\alpha_{IIb}\beta_3$ or fibrinogen), and procoagulant activity (phosphatidylserine exposure). The combination of multiple microspotted surfaces with different fluorescent stains thus results in a high-throughput test, e.g., allowing measurement of 3×8 end-stage parameters of thrombus formation in a single flow run with less than 0.5 mL of blood (15,30). See also Box 1.

Controlled coagulation

In the majority of *in vivo* hemostasis and thrombosis models, platelet activation/aggregation is known to be accompanied by coagulation (thrombin and fibrin formation), in particularly triggered by tissue factor (31–34). A common procedure to achieve controlled coagulation *in vitro* is to co-perfuse citrate-anticoagulated blood with a $CaCl_2$/$MgCl_2$ mixture over microspots that contain collagen-I whether or not co-spotted with tissue factor (see Box 1). Tissue factor can be applied in a range of concentrations to fine-tune the extent of coagulation triggering (25). Such coagulation experiments require the collection of kinetic information, e.g., by recording of time series of brightfield and fluorescence images, since the buildup of platelet aggregates with fibrin is more time-dependent.

Box 1. Useful protocols.

A. Construction of flow chambers for blood perfusion. Both commercial and home-made parallel-plate flow devices can be used for whole blood perfusion studies to measure platelet deposition, activation, and aggregation by the use of brightfield and fluorescence microscopy (73). Soft-material polydimethylsiloxane (PDMS) chambers though may contain irregularities in the flow channels, so that hard-plastic chambers with high-precision dimensions are preferred. The Maastricht flow chamber is made of polymethyl methacrylate with channel dimensions of 50 μm height, 3 mm width, and 20 mm length. At one side it is covered with a rectangular glass coverslip. The small channel proportions reduce the amount of blood needed per flow run to about 0.5 mL (27). Flow disturbances are prevented by tubular inlets entering the channel at low angle of 11 . Coverslips are usually coated with microspots of 1–2 mm Ø (0.5–2 μL applied per spot) for obtaining a consistent pattern of thrombi (15). A variety of platelet-adhesive ligands can be used, but most common are collagen type-I which binds VWF (73). Protocol details are given elsewhere (74).

B. Whole-blood thrombus formation without coagulation. Preferably, blood is collected on thrombin inhibitors. Alternatively citrate-anticoagulated blood is supplemented, prior to the experiment, with a $CaCl_2$/$MgCl_2$ mixture in the presence of thrombin inhibitors, in order to achieve physiological (millimolar) concentrations of free Ca^{2+} and Mg^{2+} (74). In our experience, dual thrombin inhibition is required to prevent "background" clotting (e.g. PPACK/fragmin or hirudin/fragmin), certainly when using hyper-coagulant or mouse blood. We prefer to perfuse whole blood samples in a plastic syringe in push mode, thus limiting leakage artifacts. A large set of fluorescent labels is available for quantifying purposes (27). For most surfaces, thrombus formation tends to maximize in a time span of 5–8 min, meaning that end-stage images can be recorded only. Labeling for platelet activation then is done *post hoc*, i.e., while recording (enhanced-contrast) brightfield images. Additional recording of fluorescence images (e.g. using spectrally non-overlapping green fluorescent protein (GFP), red fluorescent protein (RFP), and Cy5 filter sets) ultimate provides multiparameter insight into the content, structure, and platelet-activation properties of a thrombus (15).

C. Whole-blood thrombus formation with coagulation. Samples of citrate-anticoagulated blood may be supplemented with $CaCl_2$/$MgCl_2$ mixture, and then perfused over a collagen/tissue factor surface. However, a disadvantage of this procedure is ongoing contact activation in the blood before reaching the tissue factor (this is partly prevented by addition of corn trypsin inhibitor). Our preferred way of operation is to continuously co-infuse citrated-anticoagulated whole blood with a $CaCl_2$/$MgCl_2$ mixture (two plastic syringes in push mode), with tissue factor either added to the mix (56) or present with collagen in microspots (57). On microspots with tissue factor alone, no fibrin is formed due to the absence of platelets (57). Since the formation of thrombin and fibrin is an ongoing process, preferably kinetic information is collected, i.e., by recording time series of microscopic images. Fluorescent probes can be added to the blood samples, e.g., detecting platelets, procoagulant activity and fibrin (using GFP, RFP, and Cy5 filter sets).

D. Microscopic image analysis. High-resolution digital microscopic (≥8-bit, brightfield and fluorescence) images can be scored for thrombus morphology, and analyzed for coverage of platelets and labeled activation markers. Image analysis using FIJI software (75) is convenient since the program allows to write scripts for different optics and image types. Scripts may include a correction for background illumination (fast Fourier transform bandpass filter), followed by (manual) adjustment of a threshold setting, and a measurement of the surface area coverage of supra-threshold pixels. For brightfield images, it is useful to include Gray morphology conversions (large-/medium-sized close, followed by a small dilate) in order to reduce striping and improve the detection accuracy.

Simultaneous assaying of different platelet functions

In the last decade, microfluidic devices are increasingly applied for characterization of the platelets from patients with several genetically linked diseases. In the majority of the work, collagen-I was used as platelet adhesive surface; with a perfusion protocol consisting of perfusion of anticoagulated/recalcified blood at arterial wall-shear rates of 800–1600 s^{-1}. Thrombi formed on collagen-I were usually observed by brightfield microscopy, sometimes in combination with fluorescence microscopy. In some cases, flow pressure changes, accompanying platelet aggregation, were recorded. In only few papers, information was obtained on thrombus formation at other adhesive surfaces than collagen-I. Table I provides an overview of the published studies so far.

Platelet procoagulant defects

Patients with Scott syndrome suffer from a mild bleeding disorder (35), which is linked to the inability of platelets to expose phosphatidylserine in a Ca^{2+}-dependent way (36,37). The syndrome is associated with defective expression of the *ANO6* gene (alternatively termed *TMEM16F*), which encodes the Ca^{2+}-activated ion channel, anoctamin-6 (37,38). High-throughput whole-blood flow measurements indicated that platelet adhesion, aggregation, and secretion were normal on collagen-I, whereas phosphatidylserine exposure of the Scott platelets was severely decreased (37,39).

Other essential players in platelet Ca^{2+} homeostasis are the store-operated Ca^{2+} channel, ORAI1, and its Ca^{2+}-sensing binding partner in the endoplasmic reticulum, STIM1 (36). Hetero- or homozygous mutations in either gene can lead to severe combined immunodeficiency, but are mostly not accompanied with an overt bleeding phenotype. An impairment in store-regulated Ca^{2+} entry is frequently observed in the platelets from patients with loss-of-function mutations in either *ORAI1* or *STIM1*. For the few patients studied so far in multispot flow chamber studies (Table II), it appeared that the *ORAI1* mutation was accompanied by a stronger defect in platelet adhesion and aggregation than the *STIM1* mutation (30). On the other hand, a gain-of-function mutation in *ORAI1* (high store-regulated Ca^{2+} entry) is associated with relatively high platelet adhesion, secretion, and integrin activation under flow (15,30). Markedly, for collagen-I and also other surfaces, platelet procoagulant activity correlated well with the loss- or gain-of-function mutation in *ORAI1*. Interestingly, the collagen-I surface appeared to be most sensitive for a moderate lowering in platelet count often seen in such patients when compared to VWF/rhodocytin or VWF/fibrinogen (30).

Platelet signaling receptors and cytoskeletal defects

Several bleeding disorders are linked to changes in the platelet cytoskeleton (40). So far, only patients with May–Hegglin syndrome have been examined for changes in thrombus formation. This syndrome is linked to a mutation in the gene for non-muscle myosin heavy chain 9 and is characterized by macrothrombocytopenia. On collagen-I, and to a lesser extent on other microspots (15), platelet aggregates from a patient with May–Hegglin anomaly were low in most activation parameters, except for phosphatidylserine exposure, which was comparable to the control level (Table I).

Platelet secretion defects

In several groups of patients, the risk of bleeding is linked to a defect in platelet α-granule secretion (no P-selectin expression) and/or δ-granule secretion (no CD63 expression in Hermansky–Pudlak patients). In both cases, mutations have been detected in multiple genes (40). The Gray platelet syndrome is an example of α-granule deficiency, often in combination with mild thrombocytopenia. In

Table I. Changed parameters of platelet thrombus formation on a collagen-I surface (no coagulation) observed for patients with genetically linked disorders.

Disorder	OMIM ID	Collagen	Shear rate (s^{-1})	Adhesion	Aggregation	Secretion	Integrin+	Procoagulant	Platelet phenotype	Ref.
Scott (*ANO6*)	262890		1k	=	=	=		↓	Impaired PS exposure	*a*
SCID (*ORAI1* GOF*)	610277		1.6k	↓/↑	↓	=/↑	=/↑	=	High calcium entry	*b*
SCID (*ORAI1* LOF*)	610277		1.6k	↓	↓	=/↓	↓/↑	↓	Impaired calcium entry	*b*
SCID (*STIM1* LOF*)	605921		1.6k	=	=	=	=	=	Impaired calcium entry	*c*
May-Hegglin (*MYH9*)	155100		1.6k	↓	↓	↓	↓	=	Altered cytoskeleton, macrothrombocytopenia	*d*
Gray platelet (multiple genes)	139090		1.6k	↓	↓	↓	↓	=	Absence of alpha-granules, platelet dysfunction	*d*
Hermansky Pudlak (*HPS3*)	606118		1.6k	=	↓	=	=	=	Absence of dense-granules, aggregation defect	*d*
δ-Storage pool deficiency (multiple genes)	185050		1.0-2.0k	↓					Absence of dense-granules, aggregation defect	*e*
Glanzmann (*ITGA2B, ITGB3*)	273800		50, 1.6k	=/↓	↓	=	↓	=	Defective platelet adhesion, aggregation	*f*
LAD-III (*FERMT3*)	607901		1.0k	↓	↓	↓	↓	↓	Defective platelet adhesion, aggregation	*b*
Bernard-Soulier (multiple genes, GPIb-V-IX)	231200		1.5k	↓					Large platelets, defective adhesion, aggregation	*g*
Bernard-Soulier (multiple genes, GPIb-V-IX)	231200		50	=					Large platelets, defective adhesion,aggregation	*g*
GPVI (*GP6*, deficient platelets)	614201		0.5, 1.6, 3.0k	↓	↓				Defective platelet activation, aggregation	*h*
GPVI (*GP6*, compound heterozygous)	614201		0.5k	↓/↑	↓				Defective platelet activation, aggregation	*i*
GPVI (*GP6*, SNP)	rs1613662		1.0k	↓					Differential GPVI expression	*j*
Von Willebrand 2B (*GPIBA*, GOF*)	613554		1.5-2.5k	=/↓	↓		↓		Increased aggregation, spontaneous VWF binding	*k*
Von Willebrand 2B (*GPIBA*, GOF*)	613554		50, 0.7k	=	=				Increased aggregation, spontaneous VWF binding	*k*
Von Willebrand 1,2A,3 (*VWF*)	193400, 277480		1.0-1.5k	↓	↓				Low or defective function of VWF	*l*
Von Willebrand 1,2A,3 (*VWF*)	193400, 277480		50, 0.3-0.7k	=	=				Low or defective function of VWF	*l*
Afibrinogenemia (multiple genes)	202400		50, 1.5k	=	↓				Low fibrinogen	*m*

Shear rates are indicated in 1000× (k). Color intensity reflects reported frequency (of effect). References: a (37,39); b (30); c (15,30); d (15); e (41); f (15,42); b (30); g (42); h (43,44); i (45,46); j (47); k (48,50); l (48–52); and m (49).

Table II. Changed parameters of platelet-fibrin thrombus formation on a collagen-I surface with(out) tissue factor observed for patients with coagulation deficiencies.

Disorder	Collagen	TF	Shear rate (s^{-1})	Adhesion	Aggregation	Fibrin	Time to fibrin	Coagulation phenotype	Ref.
Hemophilia A, mild (*F8*)			0.1-0.7k, 1.5k	=	=	=/↓	↑	FVIII 6-30%	*a*
Hemophilia A, mild (*F8*)			0.1k	=		=		FVIII 6-30%	*b*
Hemophilia A, moderate (*F8*)			0.1k, 1.0k	=/↓		↓	↑	FVIII 1-5%	*c*
Hemophilia A, severe (*F8*)			0.1-0.7k, 1.5k	=/↓	↓	↓	↑	FVIII <1%	*d*
Hemophilia A, severe (*F8*)			0.1k	↓		↓		FVIII <1%	*e*
Hemophilia B, moderate (*F9*)			1.0k	=		↓	↑	FIX 5%	*f*
Hemophilia B, severe (*F9*)			0.1k	↓		↓	↑	FIX <1%	*g*
Hemophilia C, moderate (*F11*)			0.1k	↓		↓	↑	FXI 2%	*g*
Dilution coagulopathy			1.0k	↓		↓		Plasma dilution	*f*

Shear rates are indicated in 1000× (k). Color intensity reflects reported frequency (of effect).
References: a (48,54,55); b (53); c (53,54,56); d (48,54,55); e (53); f (56,57); and g (54).

high-throughput flow chamber studies, platelets from a patient with this syndrome showed decreased adhesion, secretion, and aggregation (collagen-I and other GPVI- and CLEC-2-activating surfaces), whereas phosphatidylserine exposure remained normal (15). In case of absent δ-granules, i.e., patients with δ-storage pool disease or Hermansky–Pudlak syndrome (*HPS3* gene), platelet adhesion and aggregation on collagen-I were found to be impaired (15,41). These changes are explained by the combination of a lower platelet count and platelet dysfunction.

Platelet adhesion and receptor defects

Platelets from patients with bleeding and Glanzmann's thrombasthenia, carrying loss-of-function mutations in the *ITGA2B* or *ITGB3* genes, are characterized by absence of integrin $\alpha_{IIb}\beta_3$ or a qualitative defect of integrin activation and, hence, inability to aggregate. Affected aggregate formation and integrin activation in Glanzmann patients has also been observed using flow assays assessing platelet adhesion and thrombus formation on collagen and other surfaces, independent of shear rate (15,42). Similarly, patients with a combined immune disease and bleeding disorder, i.e., leukocyte adhesion deficiency-III, due to homozygous dysfunctional mutations in *FERMT3* (a gene coding for the integrin-regulating protein kindlin-3), have platelets that are unable of $\alpha_{IIb}\beta_3$ activation and aggregation. High-throughput flow assays with blood from such a patient or the heterozygous parents showed a marked reduction for all parameters of thrombus formation on collagen-I and other surfaces (30).

A clear shear-dependent difference in thrombus formation under flow is observed for patients with the Bernard–Soulier syndrome. This is a bleeding disorder characterized by macrothrombocytopenia due to mutations in genes encoding for components of the GPIb-V-IX complex (40). In flow chamber assays, the patients' platelets displayed decreased adhesion on collagen at high shear rate (1500 s^{-1}), but normal adhesion at low shear rate (50 s^{-1}) (42). Thrombus formation is also assessed in blood from patients with (an unknown cause of) GPVI deficiency or with a genetic compound heterozygous deficiency in GPVI. For these patients, a decreased platelet adhesion and aggregation on collagen was seen (43–45); in one case, the overall adhesion of single platelets was increased, whereas aggregates were not formed at all (46). In healthy subjects, a common genetic variant that associates with low GPVI expression also reduces parameters of thrombus formation on collagen-I (47).

Von Willebrand disease and afibrinogenemia

Subtypes of von Willebrand disease (VWD) are defined according to the altered way of VWF-GPIb-V-IX interaction and the severity of the bleeding disorder. In VWD type I, 2A or 3, categorized by a low or defective plasma VWF function, published reports pointed to a (severely) reduced platelet adhesion and aggregation at high shear (1000–1500 s^{-1}) flow conditions (42,48–52). However, platelet adhesion was in the normal range at lower shear rates (50–670 s^{-1}) (42,48–52). In type 2B vWD, characterized by a gain-of-function of GPIbα, enlarged platelets are present showing spontaneous binding to VWF (40). Especially at high-shear flow conditions, platelets from these patients showed a deficiency in aggregation and integrin activation on collagen surfaces (48,50). As expected, also low plasma fibrinogen (afibrinogenemia) resulted in low platelet aggregation under flow (49). Taken together, although for only some of these patient groups specific platelet responses (integrin activation, secretion, procoagulant activity) have been measured, the obtained flow results are in general agreement with the known platelet phenotypes (Table I).

Simultaneous assaying of platelet activation and coagulation

Coagulation studies under flow, using surfaces consisting of collagen with(out) tissue factor, have been performed to characterize abnormalities in the clotting process of patients with genetically linked or acquired coagulation disorders (Table II). In the majority of studies performed so far, both platelet adhesion and fibrin formation have been measured.

Hemophilia A

Several authors (48,53,54) reported a normal formation of a fibrin-containing thrombus on collagen with(out) tissue factor with blood from patients with mild hemophilia A (6–30% factor VIII activity) at low or high shear rates (Table II). Only one paper describes a decreased fibrin deposition at a shear rate of 1500 s^{-1} (55). For patients with moderate hemophilia A (1–5% factor VIII activity), platelet adhesion was normal to decreased and accompanied by a marked delayed and reduced formation of fibrin fibers (53,54,56). In severe hemophilia A (<1% activity), a severe bleeding phenotype, all parameters of thrombus formation were reported to be abrogated (with the exception of one paper showing

only decreased aggregation and fibrin deposition) without tissue factor (48,54,55).

Other coagulant disorders

By multiparameter testing, our group (56,57) has reported normal platelet adhesion on collagen/tissue factor surfaces with blood from patients with moderate hemophilia B (factor IX deficiency), whereas fibrin formation is retarded (Table II). A more striking dysfunction is described with blood from (bleeding) patients with severe hemophilia B, that is, low platelet adhesion as well as fibrin formation (54). The same is true in blood from a patient with mild hemophilia C (factor XI deficiency) (54). Acquired dilution coagulopathy is a clinical condition with high bleeding risk, caused by massive blood dilution due to infusion of colloids and crystalloids in major surgery (58). Blood samples from patients with dilution coagulopathy demonstrated low platelet adhesion and fibrin formation on a collagen/tissue factor surface (57). In most papers monitoring the effects of coagulation disorders, no information is provided on specific platelet responses (integrin activation, secretion, procoagulant activity).

Clinical use: thrombosis and effects of antithrombotic medication

Flow devices, mostly single-spot, have also been used to assess altered platelet functions in whole blood as a consequence of common antithrombotic medication, either in healthy subjects or in patients with increased risk of cardiovascular disease. An overview is given in Table III.

Aspirin and cardiovascular disease

High-throughput assessment of thrombus formation indicated that, for patients with stabilized peripheral arterial disease and taking aspirin, platelet adhesion was within the normal range, whereas aggregate formation and procoagulant activity were reduced (59). This partial loss of activity was ascribed to the use of aspirin. Several studies have examined the effect of aspirin *per se*, either *in vivo* or *in vitro*, on thrombus formation on collagen-I. Depending on the particular microfluidics conditions (Table III), reduced platelet adhesion and fibrin formation by aspirin was described by some authors (60–63), whereas no effect was reported by others (64,65). In terms of kinetics, one group described that aspirin *in vitro* mainly affected secondary aggregation (66), while another group demonstrated reduced aggregate stability (63). Taken together, this points to a relatively late effect of aspirin in the thrombus buildup on collagen-I.

Other antithrombotic drugs

Attention has been paid to the effect of dual antiplatelet agents, i.e., aspirin in combination with clopidogrel, the latter producing an active metabolite that causes irreversible $P2Y_{12}$ receptor inhibition (Table III). Several groups showed that this combination of agents reduced platelet adhesion, aggregate formation, and fibrin deposition on a collagen surface (60,63,65,67). Similarly, *in vitro* addition of aspirin and the active metabolite of clopidogrel decreased platelet adhesion and aggregate formation (68). On the other hand, an early paper reported no effects of $P2Y_{12}$ or $P2Y_1$ receptor antagonists on platelet deposition on collagen-I in an open flow system (69). Very little information is still available on other platelet responses (integrin activation, secretion, procoagulant activity).

Under coagulating conditions, both dabigatran (thrombin inhibitor) and rivaroxaban (factor Xa inhibitor) were found to reduce platelet aggregate formation and fibrin deposition (by microscopy or deduced from pressure changes in a flow chip) on collagen/tissue factor surfaces (56,70,71). However, one study demonstrated an increase of platelet adhesion and thrombus formation on VWF, collagen, and human atherosclerotic plaque tissue after dabigatran treatment when compared to vitamin K antagonists (72). Apixaban (another factor Xa inhibitor) gave similar results as rivaroxaban. Under conditions of limited coagulation (no tissue factor), rivaroxaban did not affect platelet aggregation (71).

Conclusions

For the limited number of clinical blood samples examined, mostly from patients with genetically linked bleeding disorders (Table I), high-throughput analysis of thrombus formation on collagen-I has provided relevant, all-in-one information about the platelet phenotype. Additional surfaces (e.g., triggering platelet adhesion via CLEC-2, integrin $\alpha_{IIb}\beta_3$, $\alpha_6\beta_1$, or CD36) give more detailed insight into the altered platelet reactivity, if only

Table III. Changed parameters of platelet-fibrin thrombus formation on collagen surface with(out) tissue factor observed for antithrombotic drugs.

Intervention	Subjects	Collagen TF	Shear rate (s^{-1})	Adhesion	Aggregation	Secretion	Procoagulant	Fibrin	Time to fibrin	Ref.
In vivo										
Aspirin (*COX1*)	Control		0.2k, 2.6k	=/↓				↓		*a*
Aspirin (*COX1*)	peripheral arterial disease		1.6k	=	↓		↓			*b*
Aspirin + clopidogrel (*COX1, P2RY12*)	Control		0.3k, 1.5k, 2,6k	↓	↓			↓		*c*
Aspirin + clopidogrel (*COX1, P2RY12*)	Acute coronary syndrome		0.3k, 1.5k, 2,6k	↓	↓			↓		*c*
In vitro										
Aspirin (*COX1*)	Control		0.2k, 0.5k, 1.6k	=/↓	=/↓	↓	↓			*d*
Aspirin + clopidogrel* (*COX1, P2RY12*)	Control		1.6k	↓	↓					*e*
Rivaroxaban (*F10*)	Control		0.2k, 0.6k, 1.0k	=	↓			↓	↑	*f*
Dabigatran (*F2*)	Control		0.2k, 0.6k	=	↓			↓	↑	*g*

* active metabolite, coinfusion with ADP

Shear rates are indicated in 1000× (k). Color intensity reflects reported frequency (of effect).

References: a (60,64,65,76); b (59); c (60,63,65,67); d (59,61–63,66,76); e (66,68); f (56,70,71); and g (71).

because these are less sensitive to changes in platelet count. Flow perfusion measurements under coagulating conditions, e.g., with collagen/tissue factor surfaces, can distinguish between conditions of mild, moderate, and severe hemophilia, which appears to be in accordance with the bleeding risk of the patients. Given this, we consider that further fine-tuning of the technology for high-throughput microfluidic assays is needed for optimal assessment of platelet function and effects of antithrombotic medication.

Acknowledgments

We thank T. Vu for expert assistance.

Declaration of Interest

The authors declare that no relevant conflict of interest exist.

Funding

This study was supported by the Interreg Euregio Meuse Rhine programme, PolyValve.

References

1. Baumgartner HR, Muggli R, Tschopp TB, Turitto VT. Platelet adhesion, release and aggregation in flowing blood: effects of surface properties and platelet function. Thromb Haemost. 1976; 35: 124–138.
2. Bolhuis PA, Sakariassen KS, Sixma JJ. Adhesion of blood platelets to human arterial subendothelium: role of factor VIII-Von Willebrand factor. Haemostasis. 1979; 8: 312–323.
3. Badimon L, Badimon JJ, Galvez A, Chesebro JH, Fuster V. Influence of arterial damage and wall shear rate on platelet deposition. Ex Vivo Study in a Swine Model. Arteriosclerosis. 1986; 6: 312–320.
4. Orvim U, Roald HE, Stephens RW, Roos N, Sakariassen KS. Tissue factor-induced coagulation triggers platelet thrombus formation as efficiently as fibrillar collagen at arterial blood flow conditions. Arterioscler Thromb. 1994; 14: 1976–1983. doi:10.1161/01.ATV.14.12.1976.
5. Sakariassen KS, Joss R, Muggli R, Kuhn H, Tschopp TB, Sage H, Baumgartner HR. Collagen type III induced ex vivo thrombogenesis in humans. Role of Platelets and Leukocytes in Deposition of Fibrin. Arteriosclerosis. 1990; 10: 276–284.
6. De Groot PG, Sixma JJ. Perfusion chambers. In: Michelson AD. ed. Platelets. New York: Acad. Press; 2002. Chapter 24: p. 347–350.
7. Hanson SR, Reidy MA, Hattori A, Harker LA. Pharmacologic modification of acute vascular graft thrombosis. Scan Electron Microsc. 1982; 2: 773–779.
8. Weiss HJ, Turitto VT, Baumgartner HR. Role of shear rate and platelets in promoting fibrin formation on rabbit subendothelium. Studies Utilizing Patients with Quantitative and Qualitative Platelet Defects. J Clin Invest. 1986; 78: 1072–1082.
9. Sakariassen KS, Aarts PA, de Groot PG, Houdijk WP, Sixma JJ. A perfusion chamber developed to investigate platelet interaction in flowing blood with human vessel wall cells, their extracellular matrix, and purified components. J Lab Clin Med. 1983; 102: 522–535.
10. Hubbell JA, McIntire LV. Technique for visualization and analysis of mural thrombogenesis. Rev Sci Instrum. 1986; 57: 892–897. doi:10.1063/1.1138830.
11. Heemskerk JW, Sakariassen KS, Zwaginga JJ, Brass LF, Jackson SP, Farndale RW. Collagen surfaces to measure thrombus formation under flow: possibilities for standardization. J Thromb Haemost. 2011; 9: 856–858. doi:10.1111/j.1538-7836.2011.04230.x.
12. Roest M, Reininger A, Jj Z, Mr K, Jw H. Flow chamber-based assays to measure thrombus formation in vitro: requirements for standardization. J Thromb Haemost. 2011; 9: 2322–2324. doi:10.1111/j.1538-7836.2011.04492.x.
13. Savage B, Almus-Jacobs F, Ruggeri ZM. Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. Cell. 1998; 94: 657–666. doi:10.1016/S0092-8674(00)81607-4.
14. Siljander PR, Munnix IC, Smethurst PA, Deckmyn H, Lindhout T, Ouwehand WH, Farndale RW, Heemskerk JW. Platelet receptor interplay regulates collagen-induced thrombus formation in flowing human blood. Blood. 2004; 103: 1333–1341. doi:10.1182/blood-2003-03-0889.
15. De Witt SM, Swieringa F, Cavill R, Lamers MM, van Kruchten R, Mastenbroek T, Baaten C, Coort S, Pugh N, Schulz A and others. Identification of platelet function defects by multi-parameter assessment of thrombus formation. Nat Commun. 2014; 5: 4257. doi:10.1038/ncomms5257.
16. Konopatskaya O, Matthews SA, Harper MT, Gilio K, Cosemans JM, Williams CM, Navarro MN, Carter DA, Heemskerk JW, Leitges M and others. Protein kinase C mediates platelet secretion and thrombus formation through protein kinase D2. Blood. 2011; 118: 416–424. doi:10.1182/blood-2010-10-312199.
17. Stegner D, Nieswandt B. Platelet receptor signaling in thrombus formation. J Mol Med (Berlin). 2011; 89: 109–121. doi:10.1007/s00109-010-0691-5.
18. Kuijpers MJ, Gilio K, Reitsma S, Nergiz-Unal R, Prinzen L, Heeneman S, Lutgens E, van Zandvoort MA, Nieswandt B, Egbrink MG and others. Complementary roles of platelets and coagulation in thrombus formation on plaques acutely ruptured by targeted ultrasound treatment: a novel intravital model. J Thromb Haemost. 2009; 7: 152–161. doi:10.1111/j.1538-7836.2008.03186.x.
19. Penz S, Reininger AJ, Brandl R, Goyal P, Rabie T, Bernlochner I, Rother E, Goetz C, Engelmann B, Smethurst PA and others. Human atheromatous plaques stimulate thrombus formation by activating platelet glycoprotein VI. Faseb J. 2005; 19: 898–909. doi:10.1096/fj.04-2748com.
20. Cosemans JM, Kuijpers MJ, Lecut C, Loubele ST, Heeneman S, Jandrot-Perrus M, Heemskerk JW. Contribution of platelet glycoprotein VI to the thrombogenic effect of collagens in fibrous atherosclerotic lesions. Atherosclerosis. 2005; 181: 19–27. doi:10.1016/j.atherosclerosis.2004.12.037.
21. Reininger AJ, Bernlochner I, Penz SM, Ravanat C, Smethurst P, Farndale RW, Gachet C, Brandl R, Siess W. A 2-step mechanism of arterial thrombus formation induced by human atherosclerotic plaques. J Am Coll Cardiol. 2010; 55: 1147–1158. doi:10.1016/j.jacc.2009.11.051.
22. Penz SM, Reininger AJ, Toth O, Deckmyn H, Brandl R, Siess W. Glycoprotein Ibalpha inhibition and ADP receptor antagonists, but not aspirin, reduce platelet thrombus formation in flowing blood exposed to atherosclerotic plaques. Thromb Haemost. 2007; 97: 435–443.
23. Nergiz-Unal R, Cosemans JM, Feijge MA, van der Meijden PE, Storey RF, van Giezen JJ, Oude Egbrink MG, Heemskerk JW, Kuijpers MJ. Stabilizing role of platelet P2Y12 receptors in shear-dependent thrombus formation on ruptured plaques. PLoS One. 2010; 5: e10130. doi:10.1371/journal.pone.0010130.
24. Van der Meijden PE, Munnix IC, Auger JM, Govers-Riemslag JW, Cosemans JM, Kuijpers MJ, Spronk HM, Watson SP, Renne T, Heemskerk JW. Dual role of collagen in factor XII-dependent thrombus formation. Blood. 2009; 114: 881–890. doi:10.1182/blood-2008-07-171066.
25. Colace TV, Muthard RW, Diamond SL. Thrombus growth and embolism on tissue factor-bearing collagen surfaces under flow: role of thrombin with and without fibrin. Arterioscler Thromb Vasc Biol. 2012; 32: 1466–1476. doi:10.1161/ATVBAHA.112.249789.
26. Colace TV, Tormoen GW, McCarty OJ, Diamond SL. Microfluidics and coagulation biology. Annu Rev Biomed Eng. 2013; 15: 283–303. doi:10.1146/annurev-bioeng-071812-152406.
27. Van Kruchten R, Cosemans JM, Heemskerk JW. Measurement of whole blood thrombus formation using parallel-plate flow chambers - a practical guide. Platelets. 2012; 23: 229–242. doi:10.3109/09537104.2011.630848.
28. Ruggeri ZM. Platelets in atherothrombosis. Nat Med. 2002; 8: 1227–1234. doi:10.1038/nm1102-1227.
29. Versteeg HH, Heemskerk JW, Levi M, Reitsma PH. New fundamentals in hemostasis. Physiol Rev. 2013; 93: 327–358. doi:10.1152/physrev.00016.2011.
30. Nagy M, Mastenbroek TG, Mattheij NJA, de Witt S, Clemetson KJ, Kirschner J, Schulz AS, Vraetz T, Speckmann C, Braun A. et.al. Variable impairment of platelet functions in patients with severe, genetically linked immune deficiencies. Haematologica. 2018; 103: 540–549.

31. Stalker TJ, Traxler EA, Wu J, Wannemacher KM, Cermignano SL, Voronov R, Diamond SL, Brass LF. Hierarchical organization in the hemostatic response and its relationship to the platelet-signaling network. Blood. 2013; 121: 1875–1885. doi:10.1182/blood-2012-09-457739.
32. Heemskerk JW, Mattheij NJ, Cosemans JM. Platelet-based coagulation: different populations, different functions. J Thromb Haemost. 2013; 11: 2–16. doi:10.1111/jth.12045.
33. Van Geffen JP, Swieringa F, Heemskerk JW. Platelets and coagulation in thrombus formation: aberrations in the Scott syndrome. Thromb Res. 2016; 141: S12–6. doi:10.1016/S0049-3848(16)30355-3.
34. Kuijpers MJ, Munnix IC, Cosemans JM, Vlijmen BV, Reutelingsperger CP, Egbrink MO, Heemskerk JW. Key role of platelet procoagulant activity in tissue factor-and collagen-dependent thrombus formation in arterioles and venules in vivo differential sensitivity to thrombin inhibition. Microcirculation. 2008; 15: 269–282. doi:10.1080/10739680701653517.
35. Satta N, Toti F, Fressinaud E, Meyer D, Freyssinet JM. Scott syndrome: an inherited defect of the procoagulant activity of platelets. Platelets. 1997; 8: 117–124. doi:10.1080/09537109709169326.
36. Nurden AT, Nurden P. Inherited disorders of platelet function: selected updates. J Thromb Haemost. 2015; 13(Suppl 1): S2–9. doi:10.1111/jth.12898.
37. Mattheij NJ, Braun A, van Kruchten R, Castoldi E, Pircher J, Baaten CC, Wulling M, Kuijpers MJ, Kohler R, Poole AW and others. Survival protein anoctamin-6 controls multiple platelet responses including phospholipid scrambling, swelling, and protein cleavage. Faseb J. 2016; 30: 727–737. doi:10.1096/fj.15-280446.
38. Nagata S, Suzuki J, Segawa K, Fujii T. Exposure of phosphatidylserine on the cell surface. Cell Death Differ. 2016; 23: 952–961. doi:10.1038/cdd.2016.7.
39. Solari FA, Mattheij NJ, Burkhart JM, Swieringa F, Collins PW, Cosemans JM, Sickmann A, Heemskerk JW, Zahedi RP. Combined quantification of the global proteome, phosphoproteome, and proteolytic cleavage to characterize altered platelet functions in the human scott syndrome. Mol Cell Proteomics. 2016; 15: 3154–3169. doi:10.1074/mcp.M116.060368.
40. Nurden P, Nurden AT. Congenital disorders associated with platelet dysfunctions. Thromb Haemost. 2008; 99: 253–263.
41. Minami H, Nogami K, Ogiwara K, Furukawa S, Hosokawa K, Shima M. Use of a microchip flow-chamber system as a screening test for platelet storage pool disease. Int J Hematol. 2015; 102: 157–162. doi:10.1007/s12185-015-1819-8.
42. Tsuji S, Sugimoto M, Miyata S, Kuwahara M, Kinoshita S, Yoshioka A. Real-time analysis of mural thrombus formation in various platelet aggregation disorders: distinct shear-dependent roles of platelet receptors and adhesive proteins under flow. Blood. 1999; 94: 968–975.
43. Moroi M, Jung SM, Shinmyozu K, Tomiyama Y, Ordinas A, Diaz-Ricart M. Analysis of platelet adhesion to a collagen-coated surface under flow conditions: the involvement of glycoprotein VI in the platelet adhesion. Blood. 1996; 88: 2081–2092.
44. Kojima H, Moroi M, Jung SM, Goto S, Tamura N, Kozuma Y, Suzukawa K, Nagasawa T. Characterization of a patient with glycoprotein (GP) VI deficiency possessing neither anti-GPVI autoantibody nor genetic aberration. J Thromb Haemost. 2006; 4: 2433–2442. doi:10.1111/j.1538-7836.2006.02173.x.
45. Dumont B, Lasne D, Rothschild C, Bouabdelli M, Ollivier V, Oudin C, Ajzenberg N, Grandchamp B, Jandrot-Perrus M. Absence of collagen-induced platelet activation caused by compound heterozygous GPVI mutations. Blood. 2009; 114: 1900–1903. doi:10.1182/blood-2009-03-213504.
46. Hermans C, Wittevrongel C, Thys C, Smethurst PA, Van Geet C, Freson K. A compound heterozygous mutation in glycoprotein VI in a patient with a bleeding disorder. J Thromb Haemost. 2009; 7: 1356–1363. doi:10.1111/j.1538-7836.2009.03520.x.
47. Petersen R, Lambourne JJ, Javierre BM, Grassi L, Kreuzhuber R, Ruklisa D, Rosa IM, Tome RA, Elding H, van Geffen JP and others. Platelet function is modified by common sequence variation in megakaryocyte super enhancer. Nat Commun. 2017; 8: 16058. doi:10.1038/ncomms16058.
48. Fressinaud E, Sakariassen KS, Rothschild C, Baumgartner HR, Meyer D. Shear rate-dependent impairment of thrombus growth on collagen in nonanticoagulated blood from patients with von Willebrand disease and hemophilia A. Blood. 1992; 80: 988–994.
49. Matsui H, Sugimoto M, Mizuno T, Tsuji S, Miyata S, Matsuda M, Yoshioka A. Distinct and concerted functions of von Willebrand factor and fibrinogen in mural thrombus growth under high shear flow. Blood. 2002; 100: 3604–3610. doi:10.1182/blood-2002-02-0508.
50. Sugimoto M, Matsui H, Mizuno T, Tsuji S, Miyata S, Matsumoto M, Matsuda M, Fujimura Y, Yoshioka A. Mural thrombus generation in type 2A and 2B von Willebrand disease under flow conditions. Blood. 2003; 101: 915–920. doi:10.1182/blood-2002-03-0944.
51. Hansen RR, Tipnis AA, White-Adams TC, Di Paola JA, Neeves KB. Characterization of collagen thin films for von Willebrand factor binding and platelet adhesion. Langmuir. 2011; 27: 13648–13658. doi:10.1021/la2023727.
52. Grabowski EF, Curran MA, Van Cott EM. Assessment of a cohort of primarily pediatric patients with a presumptive diagnosis of type 1 von Willebrand disease with a novel high shear rate, non-citrated blood flow device. Thromb Res. 2012; 129: e18–24. doi:10.1016/j.thromres.2011.12.016.
53. Onasoga-Jarvis AA, Leiderman K, Fogelson AL, Wang M, Manco-Johnson MJ, Di Paola JA, Neeves KB. The effect of factor VIII deficiencies and replacement and bypass therapies on thrombus formation under venous flow conditions in microfluidic and computational models. PLoS One. 2013; 8: e78732. doi:10.1371/journal.pone.0078732.
54. Colace TV, Fogarty PF, Panckeri KA, Li R, Diamond SL. Microfluidic assay of hemophilic blood clotting: distinct deficits in platelet and fibrin deposition at low factor levels. J Thromb Haemost. 2014; 12: 147–158. doi:10.1111/jth.12457.
55. Mizuno T, Sugimoto M, Matsui H, Hamada M, Shida Y, Yoshioka A. Visual evaluation of blood coagulation during mural thrombogenesis under high shear blood flow. Thromb Res. 2008; 121: 855–864. doi:10.1016/j.thromres.2007.07.019.
56. Swieringa F, Kuijpers MJ, Lamers MM, van der Meijden PE, Heemskerk JW. Rate-limiting roles of the tenase complex of factors VIII and IX in platelet procoagulant activity and formation of platelet-fibrin thrombi under flow. Haematologica. 2015; 100: 748–756. doi:10.3324/haematol.2014.116863.
57. Swieringa F, Baaten CC, Verdoold R, Mastenbroek TG, Rijnveld N, van der Laan KO, Breel EJ, Collins PW, Lance MD, Henskens YM and others. Platelet control of fibrin distribution and microelasticity in thrombus formation under flow. Arterioscler Thromb Vasc Biol. 2016; 36: 692–699. doi:10.1161/ATVBAHA.115.306537.
58. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, Mackway-Jones K, Parr MJ, Rizoli SB. Yukioka T and others. The coagulopathy of trauma: a review of mechanisms. J Trauma. 2008; 65: 748–754. doi:10.1097/TA.0b013e3181877a9c.
59. Van GJP, Kleinegris MC, Verdoold R, Baaten CC, Cosemans JM, Clemetson KJ, Ten Cate H, Roest M, de Laat B, Heemskerk JW. Normal platelet activation profile in patients with peripheral arterial disease on aspirin. Thromb Res. 2015; 135: 513–520. doi:10.1016/j.thromres.2014.12.029.
60. Cadroy Y, Bossavy JP, Thalamas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. Circulation. 2000; 101: 2823–2828. doi:10.1161/01.CIR.101.24.2823.
61. Hernandez MR, Tonda R, Pedreno J, Salas E, Arderiu G, Pino M, Serradell M, Escolar G. Effects on primary haemostasis of an anti-inflammatory agent with 5-lipoxygenase and cyclooxygenase inhibitory activity. J Cardiovasc Med. 2006; 7: 859–865. doi:10.2459/01.JCM.0000253829.17700.4f.
62. Mendolicchio GL, Zavalloni D, Bacci M, Corrada E, Marconi M, Lodigiani C, Presbitero P, Rota L, Ruggeri ZM. Variable effect of P2Y12 inhibition on platelet thrombus volume in flowing blood. J Thromb Haemost. 2011; 9: 373–382. doi:10.1111/j.1538-7836.2010.04144.x.
63. Lucitt MB, O'Brien S, Cowman J, Meade G, Basabe-Desmonts L, Somers M, Kent N, Ricco AJ, Kenny D. Assaying the efficacy of dual-antiplatelet therapy: use of a controlled-shear-rate microfluidic device with a well-defined collagen surface to track dynamic platelet adhesion. Anal Bioanal Chem. 2013; 405: 4823–4834. doi:10.1007/s00216-013-6897-y.

64. Bossavy JP, Thalamas C, Sagnard L, Barret A, Sakariassen K, Boneu B, Cadroy Y. A double-blind randomized comparison of combined aspirin and ticlopidine therapy versus aspirin or ticlopidine alone on experimental arterial thrombogenesis in humans. Blood. 1998; 92: 1518–1525.
65. Florian P, Wonerow P, Harder S, Kuczka K, Dubar M, Graff J. Anti-GPVI Fab SAR264565 effectively blocks GPVI function in ex vivo human platelets under arterial shear in a perfusion chamber. Eur J Clin Pharmacol. 2017; 73: 949–956. doi:10.1007/s00228-017-2264-9.
66. Li R, Diamond SL. Detection of platelet sensitivity to inhibitors of COX1, P2Y1, and P2Y12 using a whole blood microfluidic flow assay. Thromb Res. 2014; 133: 203–210. doi:10.1016/j.thromres.2013.10.043.
67. Jahn K, Suchodolski K, Schafer A, Sahlmann B, Kuster U, Echtermeyer F, Calmer S, Theilmeier G, Johanning K. Effect of clopidogrel on thrombus tormation in an ex vivo parallel plate flow chamber model cannot be reversed by addition of platelet concentrates or vWF concentrate. Anesth Analg. 2017; 124: 1091–1098. doi:10.1213/ANE.0000000000001903.
68. Baaten CC, Veenstra LF, Wetzels R, van Geffen JP, Swieringa F, de Witt SM, Henskens YM, Crijns H, Nylander S, van Giezen JJ and others. Gradual increase in thrombogenicity of juvenile platelets formed upon offset of prasugrel medication. Haematologica. 2015; 100: 1131–1138. doi:10.3324/haematol.2014.122457.
69. Maloney SF, Brass LF, Diamond SL. P2Y12 or P2Y1 inhibitors reduce platelet deposition in a microfluidic model of thrombosis while apyrase lacks efficacy under flow conditions. Integr Biol (Camb). 2010; 2: 183–192. doi:10.1039/b919728a.
70. Hosokawa K, Ohnishi T, Sameshima H, Miura N, Koide T, Maruyama I, Tanaka KA. Comparative evaluation of direct thrombin and factor Xa inhibitors with antiplatelet agents under flow and static conditions: an in vitro flow chamber model. PLoS One. 2014; 9: e86491. doi:10.1371/journal.pone.0086491.
71. Sugihara H, Idemoto Y, Kuwano T, Nagata Y, Morii J, Sugihara M, Ogawa M, Miura SI, Saku K. Evaluation of the antithrombotic effects of rivaroxaban and apixaban using the total thrombus-formation analysis system: in vitro and ex vivo studies. J Clin Med Res. 2016; 8: 899–907. doi:10.14740/jocmr2773w.
72. Petzold T, Thienel M, Konrad I, Schubert I, Regenauer R, Hoppe B, Lorenz M, Eckart A, Chandraratne S, Lennerz C, Kolb C, Braun D, Jamasbi J, Brandl R, Braun S, Siess W, Schulz C, Massberg S. et.al. Oral thrombin inhibitor aggravates platelet adhesion and aggregation during arterial thrombosis. Sci Transl Med. 2016; 8: 367ra168. doi:10.1126/scitranslmed.aad6712.
73. Nagy M, Heemskerk JWM, Swieringa F. Use of microfluidics to assess the platelet-based control of coagulation. Platelets. 2017; 28: 441–448. doi:10.1080/09537104.2017.1293809.
74. De Witt S, Swieringa F, Cosemans JM, Heemskerk JW. Multiparameter assessment of thrombus formation on microspotted arrays of thrombogenic surfaces. Nat Protocol Exchange. 2014; protocol number: 3382 doi:10.1038/protex.2014.026.
75. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A, Schmid B and others. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012; 9: 676–682. doi:10.1038/nmeth.2019.
76. Li R, Fries S, Li X, Grosser T, Diamond SL. Microfluidic assay of platelet deposition on collagen by perfusion of whole blood from healthy individuals taking aspirin. Clin Chem. 2013; 59: 1195–1204. doi:10.1373/clinchem.2012.198101.