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REVIEW ARTICLE

Monitoring *in vitro* thrombus formation with novel microfluidic devices

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Abstract

Cardiovascular disease is a major cause of mortality globally and is subject to ongoing research to improve clinical treatment. It is established that activation of platelets and coagulation are central to thrombosis, yet at different extents in the arterial and venous system. *In vitro* perfusion chamber technology has contributed significant knowledge on the function of platelets in the thrombotic process under shear conditions. Recent efforts to downscale this technique with a variety of microfluidic devices has opened new possibilities to study this process under precisely controlled flow conditions. Such microfluidic devices possess the capability to execute platelet function tests more quickly than current assays, while using small blood samples. Gradually becoming available to the clinic now, they may provide a new means to manage the treatment of cardiovascular diseases, although accurate validation studies still are missing. This review highlights the progress that has been made in monitoring aspects of thrombus formation using microfluidic devices.

Keywords: *Microfluidics*, *platelet function tests*, *real time thrombus formation*

First generation fluidic devices to assess platelet function under flow

Given the established role of platelets in normal hemostasis and in pathological thrombosis, many assay systems have been developed to determine mechanisms underlying platelet (dys)function. The origin of platelet testing can be traced back to the early 1900s, when Duke established the *in vivo* bleeding time as a measure of platelet function [1]. Nowadays, less invasive platelet function tests are routinely used for the diagnosis and management of patients with platelet dysfunctions as well as for monitoring the efficacy of antiplatelet therapies, such as with aspirin and purinergic receptor antagonists. Of more recent interest is the use of platelet function testing as a tool to aid the prediction of bleeding complications during surgery and to guide postsurgical hemostatic therapies.

A general limitation of the most platelet function assays is that they do not well correlate with the clinical outcome in terms of bleeding or thrombosis. As described in recent overviews, the use of flow chamber technology has promising potential to overcome this limitation [2, 3]. Conventional platelet function assays mostly exclude coagulation and only few assays introduce shear stress as an additional parameter (e.g. PFA-100). On the other hand, flow perfusion chambers allow the incorporation of platelet function, coagulation, and shear stress at the same time. In addition, they allow the use of various platelet-adhesive substrates as a surface, most typically collagen type I/III, von Willebrand factor (vWF), fibrinogen, tissue factor, fibronectin, extracellular matrix from endothelium, and human atherosclerotic plaque [4–6]. Baumgartner pioneered the first successful *in vitro* flow device, the annular perfusion chamber in which denuded and inverted arterial segments were used as an adhesive surface [7]. Later, Sakariassen et al. developed a parallel-plate perfusion chamber model in which a glass coverslip is coated with an adhesive substrate or endothelial cells and thrombus formation can be evaluated at shear rates of 50–10 500 s^{-1} [8]. This model has also been used in the study of interactions of leukocytes with endothelium. To date, the majority of perfusion chambers still use the same parallel-plate principle to study mechanisms of platelet adhesion and aggregation over a large range of shear rates [9]. These systems have uncovered a multitude of pathways leading to Ca^{2+} signaling, integrin activation and secretion which contribute to thrombus formation and are tightly regulated by the hemodynamic environment [10–12].

Although flow chamber technology with parallel-plate flow chambers is a proven tool to test platelet function and antithrombotic therapies, it is yet to be introduced into mainstream clinical diagnostic practice, mainly due to a lack of standardization in chamber construction and the use of this method [13]. Further limitations are that these assays mostly rely on complex microscopic imaging analysis and require highly trained personnel. In addition, the volume of blood needed to perform multiple perfusions can be quite large; typically 5 ml or more, depending on the dimensions of the perfusion chamber and the required wall shear rate [14]. Nowadays, these limitations of

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in vitro perfusion chambers are being addressed with the use of microfluidic technology.

New types of microfluidic devices for blood flow perfusion

As indicated below, the use of microfluidic technology provides new in-depth knowledge of specific processes that regulate platelet activation under flow because of the unique and flexible design of such devices and their capability to high-throughput and small-dimension investigations into thrombus formation.

Manufacturing of polydimethylsiloxane microfluidic devices

Microfluidic technology comprises the design and manufacturing of devices for manipulation of fluids on a micrometer scale. Typically, this technique uses flow channel dimensions of 2–100 μm with fluid volumes inside these devices being in the lower microliter range. Although microfluidic devices can be fabricated with metal, glass or plastics, most devices that are currently used in cell biological research are made from the polymer, polydimethylsiloxane (PDMS). This silicone rubber is easy-to-use, inexpensive, biocompatible, and transparent, which make PDMS chambers a cost effective and disposable device to investigate thrombus formation with high optical resolution. As a direct result of this, many different types of PDMS devices are reported, often with increased throughput over single channel devices. An overview of the current use of PMDS and other microfluidics is given in Table I.

In general, for the construction of PDMS chambers, a photoresist layer, consisting of the epoxy-based, photo-cross-linkable polymer SU-8 is deposited on a silicon substrate and patterned as desired by using conventional lithography [15]. This process involves the placement of an optically dense mask with a pattern of channels, followed by exposure to ultraviolet radiation to harden the SU-8 polymer. After extensive washing, the silicon master plate with a negative of the patterned channels serves as a stiff, reusable mold. A mixture of PDMS oligomers is then poured on this mold, allowed to solidify by cross-linking and then peeled off from the mold. Holes are punctured in the PDMS block to reach the closed channel structure, and tubing is connected to the channels for fluid inlet. The PDMS cast is usually used in combination with a glass coverslip, which can be coated with platelet adhesive ligands as desired. The PDMS cast is bonded to the glass either by hydrophobic interaction or by plasma bonding. The result is a flow device with single or multiple channels, as desired (Table I).

Precisely manipulating the flow conditions

The ability to computationally predict and accurately control the fluid dynamics in microfluidic devices makes these ideal tools to mimic and study vascular processes that occur at predicted hemodynamic shear conditions [9]. It has been demonstrated that a channel ratio of width to height larger than 10 is desirable for uniform shear stress distribution across the microchannel, thereby yielding similar degrees of platelet adhesion to collagen type I compared to conventional parallel-plate devices [16]. Nevertheless, microfluidic chips are reported with a much larger range of channel dimension ratios. A key characteristic for channels with a low width-height ratio is the appearance of considerable hydrodynamic effects stemming from the sidewalls, which typically lead to increased platelet adhesion in areas near the sidewalls [16]. The impact of sidewall hemodynamics was exploited by Neeves and colleagues, who monitored platelet adhesion in various areas of the flow channel and found a proportional increase in platelet deposition near the sidewalls relative to the centre of the channel that was dependent on the applied input shear rate [17].

An innovative microfluidic design was recently reported that completely eliminated effects of the channel sidewalls [18]. In this method, the blood sample is hydrodynamically focused between two layers of buffer, such that it is transported as a cylindrically sheathed stream, which does not interact with the channel side walls (Figure 1A). Due to the presence of laminar flow conditions, dilutional effects are eliminated in this setup, thereby preventing the blood to mix with the adjacent buffer streams. The design is such that the sample volume could be reduced to less than 50 μL. Proof of concept of this system was demonstrated in that it showed a 50% inhibitory effect on platelet surface adhesion to vWF with the integrin $\alpha_{IIb}\beta_3$ inhibitor, abciximab.

Microfluidics have also been used to study effects of vessel stenosis on the thrombotic process. Nesbitt and Westein employed a microfluidics system with protruding sidewall features to induce platelet aggregation at the apex of the stenosis [19]. It was demonstrated that platelet aggregation under conditions of micro-shear gradients could occur independently of autocrine platelet stimulation by ADP or thromboxane A_2. The same group characterized the exact fluid dynamics patterns in microfluidic stenosis channels and showed that the magnitude of micro-shear gradients correlated with the platelet aggregation response [20].

Microfluidic devices have furthermore been fabricated with circular cross-sections by introducing a coaxial stream of a gas and a solution of silicone oligomer in an organic solvent [21]. The oligomer polymerizes outside the gas stream and leaves a circular cross section after removal of the solvent. This method is designed to mimic vascular conditions, and uses confluently grown endothelial cells, thus replicating a round blood vessel with relevant circular blood flow hemodynamics. Blood perfusion studies using this device have not been reported yet, but will be of significant interest.

Up-scaling the throughput of blood samples

Perhaps the most promising aspect of microfluidic technology to investigate thrombus formation is the ease and flexibility with which special PDMS microfluidic channel configurations can be designed. This makes the microfluidics technology ideally suited to up-scaling of the throughput by incorporating multiple flow channels into a single perfusion round, while keeping the required blood volume to a minimum. This aspect is a clear advantage of microfluidics over standard parallel-plate flow devices. Hosokawa et al. studied collagen-dependent thrombus formation in 25 parallel capillary channels, which were fed from a single blood sample reservoir (Figure 1B). Using this approach, the efficacy of

Table I. Microfluidic devices to study aspects of thrombus formation.

Reference	Type of chamber	Key feature	Platelet detection	Application
Single flow channels				
[16]	PDMS microfluidics	Microchannel with discontinuous collagen coating	Mepacrine	Measurement of collagen-induced thrombus formation under flow
[18]	PSA microchannel	Hydrodynamic focusing of blood between fluid streams	DiOC6	Platelet adhesion under flow (μl volume)
[19]	PDMS microfluidics	Microchannel with stenotic sidewall features	DiOC6	Testing of anti-platelet drugs under shear gradient conditions
[20]	Laser-cut PSA	Microchannel with stenotic sidewall features	DiOC6	Platelet aggregation under flow at stenotic geometries
[36]	PDMS microfluidics	Microchannel with membrane for agonist influx	Labeled anti-CD41 mAb	Thrombus formation under flow in the presence of agonist influx
[37]	microcapillary	Microchannel allowing passage of single platelets	Calcium dye	Single platelet calcium signaling in response to soluble agent
[38]	PDMS microfluidics	Size-based lateral displacement of blood cells	Particle counting	Measurement of platelet size on chip
[46, 52]	PDMS microfluidics	Microchannel connected to wells, various inlet designs	Calcein	High-throughput analysis of platelet adhesion
[48]	Laser-cut PSA	Microchannel	DiOC6	Tracking of platelet translocation under flow
[53]	Plexiglass microchannel	Circular fluid flow system, induced by surface acoustics	Labeled vWF	Unfolding and adhesion of vWF under flow
Multiple flow channels				
[22]	Microfluidics	25 microchannels with 1 inlet	Flow pressure drop	Anti-platelet drug testing under shear conditions
[24]	PMDS microfluidics	Eight microchannels with varying outlet lengths and 1 inlet	Mepacrine	Platelet adhesion and signaling under flow
[45]	PMDS microfluidics	Six separate microchannels coated with endothelial cells	CMFDA	Platelet adhesion to endothelium under flow
[47]	PMDS microfluidics	32 microchannels coated with endothelial cells, 1 inlet	Rhodamine 6 G	Blood cell adhesion under flow
[49]	PDMS microfluidics	Four parallel microchannels with stenotic region, 1 inlet	Transmitted light absorption	Measurement of label-free thrombus formation under shear gradient conditions
Multiple (discontinuous) ligands				
[17]	PDMS microfluidics	Seven separate microchannels with collagen microspots	Labeled anti-CD41 mAb	Measurement of collagen-induced thrombus formation under flow
[23]	PDMS microfluidics	Eight microchannels with shared outlet	Labeled anti-CD41 mAb	Testing of anti-platelet drugs under flow
[31]	Capillary glass tube	Patterned substrate (tissue factor) generated by laser ablation	Brightfield microscopy	Coagulation under shear at varying concentrations of substrate tissue factor
[33, 34]	Parallel plate chamber	Chamber with microarray of printed substrates	Labeled anti-CD42b mAb	High-throughput monitoring of thrombus formation on multiple substrates
[35]	Parallel plate chamber	Patterned substrates in separate microfluidic channels	Brightfield microscopy	Rolling of blood cells on discrete patches of substrates

Notes: Abbreviations: PSA, pressure-sensitive adhesive (ArCARE 8890) made from polyester film coated with medical grade acryl. DiOC6, 3,3′-Dihexyloxacarbocyanine iodide. CMFDA, 5-chloromethylfluorescein diacetate.

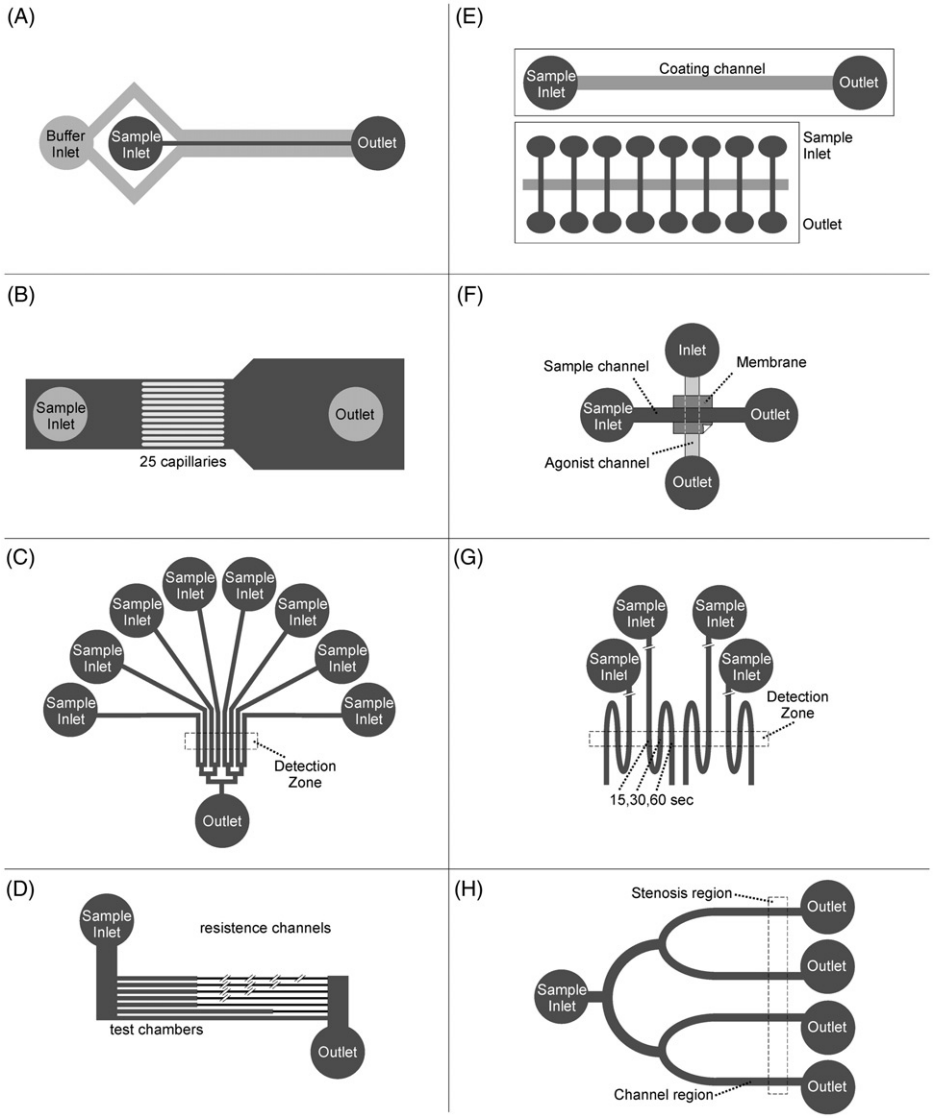

Figure 1. Microfluidic flow devices with special design. (A) Microfluidic device from polymethyl methacrylate, consisting of a top plate, a channel of two laser-patterned PSA gaskets for buffer and sample and a coated coverslip. The buffer gasket allows two streams of buffer flowing parallel with the blood sample, resulting in three parallel streams in the main channel [Adapted from 18]. (B) Microchip device (width 40 μm, height 40 μm) containing 25 micro-capillaries, which is placed on a surface with immobilized collagen [Adapted from 22]. (C) Multi-inlet single outlet flow device with eight parallel perfusion channels in combination with a common collagen coating. [Adapted from 23]. (D) Microfluidic networks containing 24 μm deep flow channels. Additional resistance channels (in gray) vary in length to create different flow rates per flow channel. Channels are connected to a common sample inlet at controlled pressure [Adapted from 24]. (E) Device of parallel microfluidic flow channels (100 μm width) connected to a functionalized coverslip, coated with a perpendicular strip of collagen. [Adapted from 17]. (F) Microfluidic device consisting of a top channel with flowing blood, a track-etched polycarbonate membrane and a bottom channel that contains agonists, mounted to a vacuum holder. The cross-section area is 100×100 μm, where the flux of the agonist molecules is controlled by the membrane [Adapted from 36]. (G) Caliper Technologies FS-417 four-sipper cell chip designed and used to detect agonist-induced calcium flux. Samples are drawn from the four ''cell wells'' and streamed through the chip. Agonists mixing is performed by diffusion while sample is continuously flown through the chip past the fluorescence detection zone. Flow rate is dependent on the combination of applied vacuum and the viscosity of the sample [Adapted from 37]. (H) Microfluidic system designed to induce platelet aggregation at four distinct shear rates in whole blood within a high shear stenotic region (single inlet, multi-outlet). An optical system measures light transmission in the stenotic region [Adapted from 23].

several antiplatelet drugs (aspirin, ADP receptor antagonists, abciximab) could be evaluated under physiological flow conditions by monitoring flow pressure and by microscopic imaging [22]. In this system, dual platelet inhibition with aspirin and $P2Y_{12}$ antagonist was more effective in thrombus inhibition than either drug alone. Although the method purposely averaged the flow pressure drop over all 25 channels, it must be noted that fluid flow in individual parallel channels fed from a common inlet reservoir is affected by the flow in other channels, when extensive thrombus formation occurs. This limitation is overcome by restraining the thrombus build-up. In this manner, Maloney et al. investigated the effects of $P2Y_1$ and $P2Y_{12}$ antagonists on collagen-induced platelet aggregation in a device with eight parallel channels

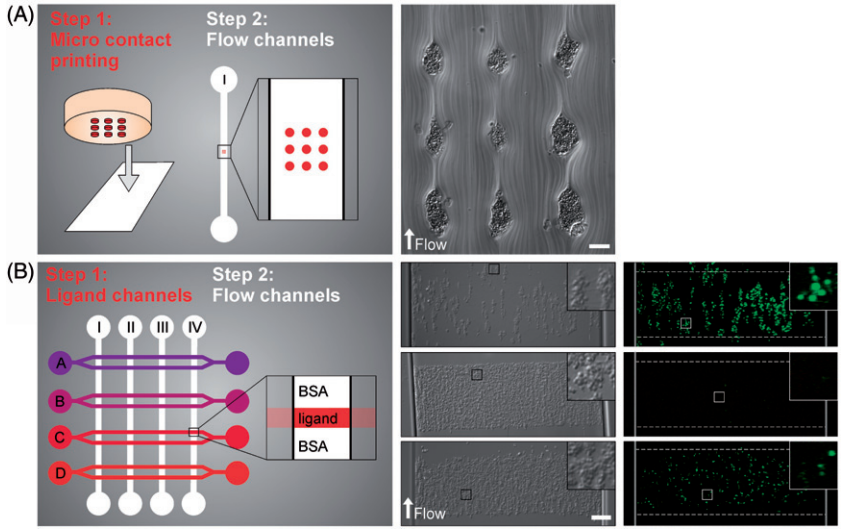

Figure 2. High-throughput flow microfluidics with discontinuously immobilized ligands. (A) Step 1: Micro-contact printing of collagen type III onto a glass coverslip, giving spot sizes of 20 µm. Step 2: A PDMS flow channel is placed over the microspot pattern and whole blood perfusion is done at 1600 s^{-1}. The result is platelet adhesion specifically on the collagen-coated microspots. Scalebar, 20 µm. (B) Step 1: Four PDMS-coating channels with dual configuration (175 µm width) are used to immobilize up to four ligands on the glass resulting in 4 × 2 coated strips. Step 2: A second PDMS device with four independent flow channels (I–IV) is placed perpendicular over the coated strips, allowing thrombus formation in a multi-ligand perfusion. Right panels: Platelet adhesion to collagen type I (top), fibrinogen (middle) and collagen type III (bottom). Platelets and phosphatidyl serine exposure are visualized with brightfield and fluorescence microscopy, respectively. Insets: zoomed area. Scale bar, 50 µm.

(Figure 1C) [23]. In a single perfusion with less than 100 µl of blood, the IC_{50} values of both types of antagonists were determined.

Upscaling of sample throughput has also been done with particular emphasis on the shear dependency of thrombus formation. PDMS devices were developed, allowing blood flow at different shear rates with one sample, reducing inter-assay variability. Figure 1(D) shows an example of a parallel eight-channel design with different shear rate conditions by varying the lengths, and hence the fluid resistance, of the channel outlets. With this device the shear-dependent contributions of intracellular and extracellular domains of platelet $\alpha_{IIb}\beta_3$ were determined [24].

Down-scaling the size of platelet adhesive surfaces

Another aspect of thrombus formation that can be studied using microfluidics is the effect of changing the adhesive surface, for instance by discontinuous coating procedures of small patches of adhesive ligands. The rationale for this is that endothelial damage in thrombosis is often focused on in nature, a situation that can be mimicked by discontinuous ligand coating. A practical benefit of such coating is that excessive aggregate growth leading to channel occlusion can be prevented. Various techniques are reported to create local microspots of platelet-adhesive ligands. Photolithography technology is used to create arrays of adhesive microspots surrounded by non-adhesive proteins or a lipid bilayer [25–27]. The size and shape of the spots is controlled by photolithography etching after placing a photomask with desired features and by irradiation with deep ultraviolet light. This method has been used to create circular microspots of phospholipids containing tissue factor [28]. It was elegantly demonstrated that, in addition to the absolute protein density, the size and the spatial arrangement of the microspots are critical in the ability of tissue factor to support coagulation [29, 30]. Using a similar technique, a 200 µm patch of phospholipids containing tissue factor was immobilized in a circular glass capillary tube and characterized for coagulation under flow conditions [31]. Phospholipid containing TF has recently been used in combination with immobilized collagen type I to study the stabilizing effects of fibrin formation on platelet aggregates [32]. By monitoring embolization events in the microfluidic channels, it was found that fibrin increased clot strength by 12 - to 28-fold.

PDMS-based devices can also be used for micro-contact printing, which presents another method to deliver discrete patches of adhesive ligands to a glass surface [25]. This method makes use of the high protein adsorption properties of PDMS. Ligands like collagen are incubated on a PDMS stamp with desired feature and are then transferred to glass by briefly stamping the PDMS block onto the glass surface. After blood perfusion, this results in thrombus formation precisely at the stamped areas (Figure 2A).

Alternatively, immobilizing discrete ligand patches can be achieved with microarray printing. Diamond and co-workers describe that microarray printing of collagen and vWF, using 176 µm wide microspots in which multiple ligand combinations were studied in a single blood perfusion run [33]. This same technology was also employed to print microarray spots of collagen containing various amount of tissue factor to determine threshold concentrations of triggers for coagulation in whole blood [34]. Calculations learned that about 10 molecules tissue factor per μm^2 are sufficient to initiate fibrin formation at 1000 s^{-1} shear rate. Although microarray printing has clear advantages, the relatively large diameter of the microspots commonly used (100–1000 µm) limits its

application with small microfluidic systems. Hence, microspotting is usually performed in combination with conventional parallel plate flow devices.

Discrete ligand patches can also be immobilized in microfluidic devices by infusing the ligand solution through separate microfluidic ''coating'' channels with defined width and allow the ligand to adsorb to the glass surface. Following removal of these channels, a second set of microfluidic channels is placed perpendicular to the direction of the first channel (Figure 2B). After blood perfusion, this results in thrombus formation selectively at the discrete coating strips. Using a similar setup it was demonstrated that PAR4 agonists stimulate platelet aggregation at the collagen surface (Figure 1E) [17]. This approach has also been used by others, who made immobilized strips of P-selectin, E-selectin, and vWF, perpendicular or parallel to the direction of blood flow, to study the rolling velocity of neutrophils and platelets [35].

Novel applications of microfluidic devices for platelet and vascular research

Novel microfluidic techniques have been used to elucidate platelet and endothelial cell functions under flow conditions.

Platelet activation under flow

Using sophisticated PDMS microfluidics, the spatio-temporal role of ADP activation during thrombus formation under flow was investigated [36]. Via two perpendicular channels, separated by a porous membrane, ADP was allowed to flux in a controlled way from the bottom channel into the upper perfusion channel (Figure 1F). It was demonstrated that the density of platelets within an aggregate increased with increasing ADP flux. In other work, a multi-channel system was used to simultaneously measure multiple concentrations of inhibitors of ADP-dependent platelet aggregation on a collagen surface, demonstrating that apyrase lacks inhibitory function under high shear conditions [23].

Microfluidics can also monitor the activation of single platelets in suspension, e.g. by detecting Ca^{2+} responses following on-chip mixing with agonists. By constructing a channel with a narrowing in the detection zone, individual responding platelets could be detected while passing through the channel (Figure 1G). This method demonstrated increases in intracellular Ca^{2+} levels following stimulation by the PAR-1 agonist SFLLRN, or ADP [37]. An innovative microfluidic method to measure label-free platelet activation is based on the principle of deterministic lateral displacement to separate platelets on-chip from other cell types based on their size [38]. This method exploits the fact that small particles have a stronger lateral displacement than larger particles in a fluid flow. The degree of displacement was measured separating the fluid flow into multiple parallel streams and counting platelet density in each parallel channel. This method proved to be accurate enough to detect the increased platelet size associated with thrombin activation.

Endothelial cell functions under flow

The endothelium has well-characterized inhibitory mechanisms to prevent platelet activation through the production of nitric oxide and prostacyclin. On the other hand, vWF and P-selectin, stored in and secreted from the endothelial Weibel-Palade bodies, are potent ligands to recruit platelets and leukocytes. This places endothelial cells in a central position to regulate platelet function and justifies their use in microfluidics to more closely mimic the physiological environment in which platelets can adhere.

Several flow assays focus on shear stress effects in relation to endothelial cell function, such as tests for cytoskeletal remodeling and protein secretion mechanisms [39–41]. Chau and colleagues demonstrated that shear stress triggers secretion of vWF from human umbilical vein endothelial cells cultured in a multichannel microfluidic device [42]. Similar systems have been used to study the endothelial migration response through a chemo-attractant gradient. Microfluidic devices have the exclusive possibility to create a fluid gradient due to their laminar flow characteristics, which prevent mixing of two adjacent fluid streams. This property has been exploited by some groups to study the migration effects of endothelial cells through an immobilized chemo-attractant gradient that was accomplished by introducing parallel streams of different chemotactic agents [43, 44].

The use of endothelial cells in microfluidic systems in combination with blood perfusion is only just emerging. A first paper, focusing on direct interactions of platelets with endothelial cells, demonstrated a role for ADP in platelet adhesion to endothelial cells, particularly after inhibition of endothelial nitric oxide production [45]. However, this study was performed under static conditions, and the translation to flow conditions has not been made. The feasibility of microfluidics with seeded endothelial cells to monitor results of blood flow was demonstrated by Conant et al. [46], who showed stable platelet adhesion and thrombus formation after endothelial stimulation with TNF-α. PDMS microfluidics using multiple parallel microchannels, lined with endothelial cells, could also model the pathophysiological processes of sickle cell disease and hemolytic uremic syndrome under blood flow conditions [47].

New detection technologies in combination with microfluidic devices

Detection methods in microfluidic devices are typically dedicated to monitor platelet adhesion and aggregation events, similarly to techniques used with parallel-plate flow chambers [14]. The most common applied technique is brightfield, contrast-enhanced microscopy, visualizing flow-adhered platelets in real time at high optical resolution. Microscopy readouts of microfluidic flow assays report on platelet density, ranging from individual platelet behaviour to large scale thrombus formation (Table I). Alternative approaches are the detection of platelets by labeling with DiOC6 or fluorescently labeled antibodies. Tracking software has been used to monitor the translocation of platelets on a vWF surface [48]. However, robust automated image analysis procedures for real-time quantification of thrombus formation still need to be developed.

Alternative detection techniques are being explored to characterize platelet adhesion and aggregation by measuring light absorbance. Transmitted light can quantify platelet-rich thrombi due to the displacement of red blood cells leading to

an increase in transmittance [49]. However, this detection method cannot inform on platelet activation levels or thrombus density. Another method to detect platelet deposition with high sensitivity uses a quartz crystal microbalance [50]. This method relies on the resonance frequency of a crystal in an alternating electric field, which is modulated by mass deposition at the detector surface. This detection modality could monitor effects of ADP stimulation and integrin $\alpha_{IIb}\beta_3$ inhibition of platelets on a fibrinogen surface. Also, this detection does not provide qualitative information on thrombus composition. Nevertheless, it is to be expected that microfluidic platelet function tests will benefit from current developments in sensitive detection methods.

Microfluidic device development towards preclinical testing

Microfluidic technology is regularly used in clinical routines, but this is limited to biochemical measurements of specific blood components. Platforms such as Spinit, Asklepios, Kumetrix, Fluidics-on-Flex and Piccolo Xpress have been developed for point-of-care diagnostics detecting a large range of proteins, lipids and electrolytes in the nano- to micromolar range. However, the use of microfluidic devices for monitoring platelet aggregation and thrombus formation is still limited to the academic setting, in the majority of cases for proof-of-principle experiments. An attempt to use PDMS microfluidics devices for whole-blood testing of thrombus formation in a point-of-care setting has been presented by Li et al. [49]. These authors used a chip with 4 parallel microchannels with stenotic region (shear rates 500–13 000 s^{-1}) to measure label-free thrombus formation by light absorption (Figure 1H). This method consumes blood without labeling, is fast and is relatively easy to perform. However, the assay is not yet validated for clinical applications, and its sensitivity to detect platelet hypo- or hyper-function is unknown. Limitations of this flow test are the relatively large blood sample volumes (2–8 ml), the inability to check for platelet activation markers and the use of only one adhesive substrate. The challenge will be to correctly integrate aspects from this and other microfluidics protocols to a test that is robust and sensitive enough to detect aberrant platelet function in a clinical setting.

An important reason for further development of microfluidic devices for pre-clinical research and patient diagnosis is that they can provide another approach in comparison to the current assays that target platelet activation in a point-of-care setting (Multiplate aggregometry, VerifyNow, Impact, PlateletWorks, PFA-100, Cone-and-Plate Analyzer). As reviewed elsewhere, the diagnostic and prognostic value of these assays is only moderate (*Review Harisson; this issue of Platelets*). This is in part due to the multiplicity of platelet activation and regulatory pathways, which are difficult to capture in a single function test. For example, light transmittance aggregometry, VerifyNow, PlateletWorks, and PFA provided only limited prognostic information to identify patients at higher risk of bleeding following stent implantation [51]. The clear advantage of PDMS microfluidic devices is that, using a small amount of blood, they can provide a means for high-throughput analysis of platelet function in the natural environment of flowing blood, at a range of shear rates and a variety of platelet-adhesive substrates. Especially in combination with multiple ligand micro-spotting, microfluidics have the potential to be developed as a clinical diagnostic tool for platelet function that may compete with other point-of-care tests.

Conclusions

Platelet activation in the *in vivo* environment is regulated by differentially exposed extracellular matrix components, complex rheological flow conditions and release or generation of soluble platelet agonists. Microfluidics have made it possible to investigate thrombus formation in a multi-variable manner with accurate control of wall shear rate conditions with small blood samples, while using multichannel perfusion and microspot ligand coating. Another important possibility is the use of multiple ligands in a single perfusion, allowing increased throughput and determination of ligand interactions. In comparison to the current point-of-care function tests, microfluidic assays may take the complex hemodynamic and biochemical environments present *in vivo* better into account. Integration of all these parameters into a user-friendly device will aid in the management of cardiovascular diseases by improving current platelet function tests.

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