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Flow chamber and microfluidic approaches for measuring thrombus formation in genetic bleeding disorders

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Abstract

Platelet adhesion and aggregation, coagulation, fibrin formation, and fibrinolysis are regulated by the forces and flows imposed by blood at the site of a vascular injury. Flow chambers designed to observe these events are an indispensable part of doing hemostasis and thrombosis research, especially with human blood. Microfluidic methods have provided the flexibility to design flow chambers with complex geometries and features that more closely mimic the anatomy and physiology of blood vessels. Additionally, microfluidic systems with integrated optics and/or pressure sensors and on-board signal processing could transform what have been primarily research tools into clinical assays. In this review, we describe a historical review of how flow-based approaches have informed mechanisms in genetic bleeding disorders, challenges and potential solutions for developing models of bleeding in vitro, and outstanding issues that need to be addressed prior to their use in clinical settings.

Keywords

microfluidics; platelets; coagulation; biorheology

Introduction

The relationship between blood flow and thrombus formation dates back at least to 19th century when Bizzozero developed the first flow chamber.[1] Since then, numerous mechanisms involved in hemostasis and thrombosis have been shown to be regulated by the forces imposed by blood flow on vascular and blood cells and the transport of plasma proteins to, into, and away from growing thrombus.[2] As such, it has been proposed that laboratory and diagnostic devices that incorporate forces and flows that mimic in vivo hemodynamics could be useful in identifying bleeding and thrombotic disorders, assigning bleeding and thrombotic risk, and dosing therapeutic agents.[3–6] The advent of microfluidic technologies make this a more feasible prospect compared to earlier flow chamber designs because of their low blood volume requirements and potential integration into automated laboratory or point-of-care systems.[7,8]

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Declaration of Interests

A brief historical aside provides some context for the reader with respect to the origin of flow-based approaches to measuring thrombus formation. The use of what are commonly called ‘flow assays’ was pioneered in the seminal work of Baumgartner and Turitto.[9–16] They popularized annular flow chambers that use everted and endothelial cell denuded blood vessels to present the subendothelium matrix to flowing blood. These annular chambers were replaced with parallel plate flow chambers that provide a more controlled presentation of prothrombotic substrates and are compatible with optical microscopy.[17–19] Over the last fifteen years, microfluidic versions of the parallel plate flow chamber have become more popular as several commercially systems are now available.[20–24] Beyond miniaturizing the parallel plate flow chamber, microfluidic systems have been created in a variety of geometries to mimic the anatomy of the microvasculature, leaky or injured vessels, and vessels with stenoses.[25–30] The design, use, and limitations of microfluidic flow chambers have been reviewed elsewhere.[7,20]

Genetic bleeding disorders such as hemophilia and von Willebrand disease (VWD) provide a test case for the utility of microfluidics in hemostasis and thrombosis because current clinical assays are poor predictors of bleeding risk. For example, factor VIII levels do not correlate with bleeding risk in hemophilia A.[31] Recent genome wide association studies have found modifier loci of von Willebrand factor (VWF) levels, yet 60–70% of the heritability is still unknown.[32,33] In this review, we focus on studies using microfluidic and other types of flow chambers using whole blood from individuals with genetic bleeding disorders and opportunities and challenges for their translation into the clinical environment. Each section is organized by the evolution of flow-based approaches starting the 1970s through the present day.

Flow assays in von Willebrand disease

VWD is an ideal pathology to test a flow-based assay because VWF structure, size, and function as well as platelet adhesion are force-dependent phenomena that are difficult to replicate in conventional platelet function and coagulation assays.[34–38] Early annular flow chambers were essential in quantifying the relationship between platelet adhesion and VWF deficiencies.[10,13,14,39] For example, the discovery that VWF adsorption to the subendothelium was a necessary step that precedes platelet adhesion at arterial shear rates was first demonstrated in a flow chamber.[40]

Table 1 summarizes flow chamber studies using whole blood from individuals with VWD. Fressinaud and colleagues showed differences in platelet adhesion and thrombus volume for types 1, 2A, 2B, and 3 VWD in a parallel plate flow chamber.[41] Type 1 VWD is quantitative defect in VWF levels. Type 2A VWD is characterized by loss of high molecular weight multimers and type 2B VWD is characterized by spontaneous binding of VWF to GP1bα. Type 3 VWD is the almost complete absence of VWF. There are no differences between controls and any VWD subtype at a shear rate of 100 s^{-1}, whereas at a shear rate of 2600 s^{-1} significant reductions were observed in thrombus volume. These data support the necessary role of VWF for platelet adhesion and aggregation at arterial shear rates. Importantly, thrombus volumes differ between VWD subtypes; type 1 > type 2A > type 2B, type 3. Sugimoto and colleagues further demonstrated differences in the evolution of thrombi

between types 2A and 2B VWD.[42] Both types 2A and 2B showed similar platelet surface coverage at shear rates of less than 340 s^{-1}, suggesting that initial platelet adhesion to collagen is not impaired. At higher shear rates, there is a significant reduction in both surface coverage and thrombus volume for type 2A, indicating the importance of high molecular weight multimers in platelet aggregation at arterial shear stresses. Notably, unlike in ristocetin-induced platelet aggregation (RIPA), there is a difference between type 2A and type 3 in flow chamber experiments because RIPA does not include platelet adhesion to collagen at high shear stresses. Results with type 2B VWD are more difficult to generalize as some samples showed similar surface coverage as healthy controls, while others showed significant reductions. However, at shear rates of 2040 s^{-1} and greater, both type 2B samples show reduced thrombi volumes and lower integration of VWF into the thrombus. Consequently, although type 2B VWD shows a gain-of-function in RIPA, under physiologic flow conditions platelet aggregation is reduced. This result is consistent with bleeding risk in these patients.

The aforementioned studies, along with the inability of standard assays to predict clinical bleeding,[43–45] provide the rationale for the development of a flow-based assay for VWD. The Platelet Function Analyzer (PFA-100) was the first widely used commercial system that integrated flow into a whole blood assay.[46] The PFA-100 consists of a capillary in series with a small aperture coated with collagen and either ADP or epinephrine through which whole blood is aspirated until the aperture is occluded to give a ‘closure time’. The simplicity and ease-of-use make the PFA-100 an attractive alternative to laborious custom flow chambers. PFA-100 closure time is sensitive to VWF deficiencies and dysfunction in all types of VWD, while being less invasive than bleeding times.[47,48] In a study of type I VWD (n = 107), the PFA-100 was found to be no better than the VWF ristocetin cofactor assay (VWF:RCo) or VWF antigen levels in predicting bleeding scores.[49] However, in a sixteen year retrospective study of over 4000 patients referred to bleeding disorder clinic, 213 of which confirmed to have VWD, the PFA-100 was a more efficient screening tool than VWF:RCo. Note that the PFA-100 is sensitive to many other factors that affect platelet function (platelet count, hematocrit, aspirin) and therefore is cannot provide a specific diagnosis for VWD. Additionally, the system uses citrated blood and is therefore not sensitive to most coagulopathies including FVIII and FIX deficiencies.[50] It can however still be a valuable screening tool in the absence of time or access to VWF functional assays.[50]

The third evolution in flow chambers, following annular and parallel plate flow chambers, occurred in the mid-2000’s as part of the rapidly growing field of microfluidics enabled by a prototyping method called soft lithography.[51] In the context of blood flow and platelet adhesion assays, microfluidics provided a lower volume requirements compared to larger parallel plate flow chamber and, when combined with micropatterning, the ability to control the spatial presentation of prothrombotic proteins.[52–54] Yet, perhaps a more important criterion for clinical translation is the need for a simple, yet quantitative output to characterize thrombus growth, something akin to the closure time in the PFA-100. One approach is to perfuse blood at a constant flow rate and measure pressure changes as a platelet aggregate or thrombus grows into the lumen of a microfluidic channel coated with prothrombotic proteins.[55] This approach has been integrated into the Total Thrombus-formation Analysis System (T-TAS®).[56] Here, hirudin treated whole blood is perfused

through a collagen coated channel, or citrated and corn trypsin inhibitor (CTI) treated whole blood is recalcified and perfused through a collagen-tissue factor (TF) coated channel. Metrics of thrombus formation include time for the pressure to increase 10 or 30 kPa (partial occlusion), an area under the pressure curve (AUC) for the first 10 or 30 min, and occlusion time. In a study of five patients with types 1, 2A, 2N, and 3 VWD, the T-TAS® system showed reduced AUC in both collagen and collagen-TF coated channels and prolonged closure times in collagen-TF channels from samples from symptomatic VWD patients, while an asymptomatic type 1 VWD patient showed normal values (Fig. 1A).[57] Patients treated with desmopressin (DDAVP) or plasma-derived VWF showed marked changes in thrombus formation metrics. A larger study of 50 individuals with type I VWD using T-TAS® correlated partial closure times with a bleeding score (BS) questionnaire and VWF ristocetin cofactor activity (VWF:RCo).[58] Partial closure time on collagen coated chips could differentiate BS for samples from individuals with severe reduction in VWF function, defined as VWF:RCo less than 10 IU dL^{-1}.

Flow assays in hemophilia

Measuring coagulopathies in microfluidic assays has additional technical challenges to measuring platelet function alone.[59] Blood needs to be collected into an anticoagulant that is either reversible, most commonly sodium citrate, or alternatively inhibits only parts of the coagulation pathway, for example blocking the contact pathway with CTI. Initiating coagulation via the extrinsic pathway with tissue factor (TF) requires quantification of its surface concentration because of the shear rate dependent threshold response to TF.[60] Developing a quantifiable and repeatable surface with collagen and TF often requires more advanced conjugation techniques beyond physical adsorption.[61] Despite these challenges, the motivation for measuring thrombus formation in hemophilia blood under flow stems from the well-documented regulation of coagulation by blood flow and the role of fibrin in establishing the mechanical properties of thrombi.[62–64]

Table 2 summarizes some of the key studies using hemophilia blood in flow chambers. Like VWD, initial studies of hemophilia were conducted in annular flow chambers using animal derived subendothelium.[16] Fibrin deposition, fibrinopeptide A (FPA) generation, and thrombus volume are significantly diminished in FVIII and FIX deficiencies.[16] A more modest reduction in fibrin deposition and FPA generation in FXI and FXII deficiencies is observed with no difference in thrombus volume.[16] These results reflect clinical bleeding; severe FVIII and FIX deficiencies can result in major bleeding, severe FXI deficiency leads to relatively mild bleeding, and FXII deficiency is often asymptomatic.[65,66] FVIII and FIX deficiencies results in bleeding primarily in the muscles and joints, while bleeding in FXI deficiencies is associated with injury to tissues with activators of fibrinolysis including the oral cavity, nose, tonsil and urinary tract.[65] A study of hemophilia A (mild and severe) patients using a parallel plate flow chamber and reconstituted type III collagen shows the shear rate sensitivity of FVIII deficiencies on thrombus formation.[41] Differences in fibrin surface coverage and thrombus size are most pronounced at venous and moderate arterial shear rates because there is less dilution of coagulation products by blood flow.[62] Similar observations of diminished thrombus volume were reported on type I collagen using thrombin inhibitors and hemophilia A samples.[67] The T-TAS® system was used to evaluate

thrombus formation on collagen-TF surfaces in blood from a FVIII$^{-/-}$ mouse and with human blood treated with an anti-FIXa aptamer.[68] The onset of thrombus formation is unchanged from normal controls; however the partial closure time (time to 40 kPa) is prolonged at low (110 s^{-1}), but not high (1100 s^{-1}) shear rates.

Onasoga and colleagues measured the dynamics of thrombus formation in a cohort of individuals with severe, moderate, and mild FVIII deficiencies with a custom microfluidic chamber using a collagen-TF surface and recalcified citrated whole blood (Fig. 1B).[69] Dynamics are characterized by three parameters—lag time to 10% surface coverage, velocity of fibrin accumulation, and maximum fibrin density—using deposition of fluorescently labeled fibrin(ogen) as a marker for coagulation. While velocity and maximum fibrin density were strongly correlated to FVIII antigen levels and were different between mild and moderate deficiencies, neither metric could distinguish moderate from severe deficiencies because of minimal fibrin deposition in both cases. Interestingly, the amount of platelet accumulation was different between moderate and severe deficiencies.

Individuals with inhibitors to FVIII were treated with recombinant FVIIa (rFVIIa) and in the microfluidic device show a reduced lag time and velocity and maximum fibrin that exceeds even normal controls one hour after treatment.[69] A mathematical model of the experiment suggests differences in the local thrombin concentration between a moderate and severe deficiency could reflect reduced activation of PAR1 on platelets. The model predicts that the low thrombin concentration is a direct consequence to reduced FXa generation via the intrinsic tenase complex on platelets. This was corroborated in experiments with FVIII and FIX deficient mice where FXa generation via intrinsic tenase is attenuated leading to reduced phosphatidylserine exposure on platelets and fibrin deposition.[70]

Colace and colleagues conduced a similar study of hemophilia blood in a microfluidic system, except here coagulation is initiated through the intrinsic pathway via type I collagen and lightly anticoagulated CTI treated whole blood (Fig. 1C).[71] On average, fibrin deposition in patient samples was similar to healthy controls for residual FVIII activity greater than 13% and severely diminished at levels less than 13%. Similar to results by Onasoga and colleagues when using TF as an extrinsic pathway initiator, there is a significant reduction in platelet accumulation between severe (<1% residual activity) and moderate (1–6% residual activity) deficiencies when the intrinsic pathway is initiated by type I collagen. Platelet accumulation could be rescued with rFVIIa for severe deficiencies, but initiation via the contact pathway is necessary to reestablish normal fibrin deposition.[72] These studies together suggest that reduced platelet activation, likely through PAR1/PAR4 signaling,[52] results in small and less stable thrombi for severe FVIII deficiencies in both TF-rich and TF-poor environments.

The flow chambers described above are models of intravascular thrombus formation whereby a thrombus forms on immobilized prothrombotic proteins adsorbed on a solid wall and grows into the lumen of a channel. This type of model corresponds with our view of thrombosis, for example following the rupture of an atherosclerotic plaque, but is perhaps not consistent with bleeding in hemophilia where blood escapes from the vessel and coagulation can be initiated by TF in the extravascular space. Our group recently developed

a microfluidic model of hemostasis that consists of an intravascular, vascular injury, and extravascular channels (Fig. 2).[73] The 'injury' channel is coated with collagen and TF and connects the intravascular and extravascular channels, mimicking the physical separation between TF and the intravascular compartment.[74] Rather than setting a wall shear rate, as is typical for flow assays, we set a pressure difference across the injury channel and measure the time to closure as well as the kinetics of platelet and fibrin accumulation. This provides both a measure of the spatiotemporal dynamics of a hemostatic formed under physiologic blood pressures and a simple time to closure metric. The time to closure of the injury channel is approximately five minutes using healthy whole blood. No closure is observed using an anti-FVIII antibody, rather platelets build-up and embolize repeatedly over 45 minutes, consistent with reduced platelet activation and a mechanically unstable thrombus. Treatment with the P2Y12 antagonist 2-MeSAMP yield prolonged closure of approximately 15 minutes as measured by the transport of erythrocytes across the injury channel, however these thrombi are still permeable to plasma, suggesting a low quality thrombus that could be prone to rebleeding as in animal models of hemostasis.[75] The ability to differentially define the vascular, injury, and extravascular compartments with different pro- and antithrombotic proteins could be exploited to model different tissues since VWD, hemophilia, and platelet disorders have distinct bleeding patterns in certain tissues.

Flow assays in congenital platelet disorders

Congenital platelet disorders such as Bernard-Soulier syndrome and Glanzmann thrombasthemia were used to identify the relative roles of different platelet receptors on thrombus formation under flow in early studies.[14,76,77] Flow chamber studies have been used to test therapeutic strategies in these rare disorders. In the case of platelet dense granule defects, a novel therapy was proposed whereby elevated hematocrit could rescue platelet accumulation in a ADP dependent manner.[78] For Glanzmann thrombasthemia, studies on endothelial cells and collagen showed that recombinant FVIIa could enhance platelet deposition in the TF-independent manner.[79] The PFA-100 is sensitive to platelet disorders with significantly a significantly prolonged closure time for Bernard-Soulier syndrome and Glanzmann thrombasthemia, while milder platelet defects like Hermansky-Pudlak syndrome and storage pool and secretion defects are subject to slightly higher false negative.[80]

In an elegant demonstration of the potential of microfluidic flow chambers, de Witt and colleagues measured thrombus formation on 52 different adhesive surfaces characterized by eight outputs.[81] These surfaces are combinations one, two, or three adhesive proteins known to interact with platelet receptors. Using this approach they were able to identify unique signatures of severe immune deficiency syndrome, May-Hegglin anomaly, gray platelet syndrome, Glanzmann thrombasthemia, and Hermansky Pudlak syndrome (Fig. 3).

Current limitations and future work

The diversity of microfluidic flow chamber designs and operating conditions have made them valuable research tools for exploring platelet function, coagulation, and thrombus formation. Whether these devices will be useful as a clinical assay remains to be seen; as of this writing it is unclear whether data from these devices provides additional information to the already large number of assays used to characterize platelet disorders and

coagulopathies. Whole blood flow assays may be poorly suited for diagnosing specific diseases because they are influenced by many factors including hematocrit, platelet count, and antiplatelet and anticoagulant drugs. A more promising direction for these assays may be in emergent and point-of-care environments as a rapid measure of hemostatic potential and response to therapy, much like thromboelastography is being used in trauma.[82,83] Alternatively, flow assays that use plasma rather than whole blood could be used to eliminate some of the variability inherent in whole blood.[84]

Tables 1 and 2 show that even the largest flow chamber studies are quite modest in size for clinical studies and do not have the statistical power to draw strong correlations with bleeding scores. Certainly, larger cohorts and multi-center studies are needed to prove the utility of microfluidic flow chambers for screening or predicting bleeding. Furthermore, while the diversity of designs has been a desirable feature for basic research, it has also hampered comparison across studies. Small changes in geometry and dimensions can have significant effects on platelet and fibrin accumulation even for studies performed at identical shear rates.[85] The dynamics of thrombus formation are also dependent on the choice of anticoagulant. Studies focused on platelet function often use thrombin inhibitors like hirudin, heparin, and PPACK which results in larger, less compact thrombi compared to those formed in the presence of coagulation.[86] Studies that incorporate coagulation typically use either sodium citrate followed by recalcification of blood prior to entry into the flow chamber, or the FXIIa inhibitor CTI. A challenge with sodium citrate is the method for recalcification where a continuous or semi-batch, rather than batch, mixing is preferable for longer assays (> 5 min).[87,88]

A clear limitation of the devices reviewed here is the lack of a functional endothelium, which regulates platelet function, coagulation, and fibrinolysis. Several innovative methods for culturing vascular cells in microfluidic formats have been reported, but the technical challenges and high cost associated with these methods may make them challenging in a clinical laboratory.[89–92] Some features of the endothelium such as nitric oxide release or presentation of adhesive proteins are achievable without using live cells.[93–95] Even given the ability to introduce vascular cells into these devices, the phenotype of endothelial cells varies considerably between organs.[96] Individuals with VWD, hemophilia, and platelet disorders bleed at specific sites that may influenced by the endothelial cell phenotype, for example high fibrinolytic activity in mucocutaneous tissues.

Finally, specific to bleeding disorders, most flow chamber do not recreate the essential anatomy and physiology of a compromised microvasculature, specifically those of the joints and muscles where individuals with hemophilia A and B bleed, and mucocutaneous tissues where individuals with VWD, hemophilia, and platelet disorders bleed. This is partially due to the paucity of data on the events that trigger initial bleeding in these disorders and a lack of understanding of coagulation in the extravascular space. Microfluidic devices have proven useful in elucidating biophysical mechanisms that regulate thrombosis in stenotic arteries,[29,97,98] and perhaps similar insight will be garnered from devices that recapitulate bleeding in the microvasculature.

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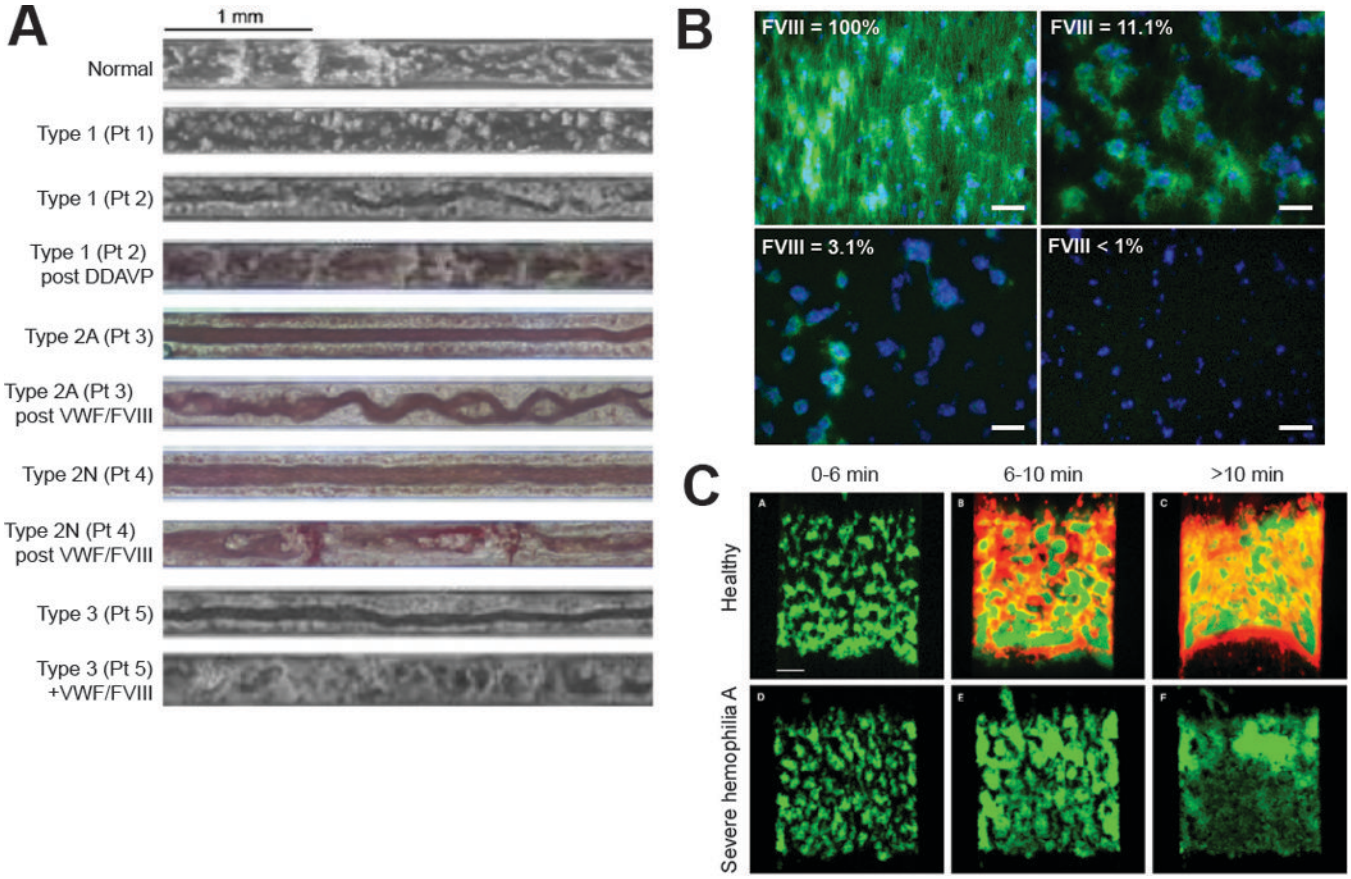

Figure 1.

Examples of microfluidic devices for measuring thrombus formation in genetic bleeding disorders. A. Bright field microscopy images of thrombi formed after 12 min at 240 s^{-1} in a T-TAS® system with a collagen-TF surface using recalcified citrated and CTI treated whole blood from individuals with VWD before and after treatments.[57] B. Epifluorescence images of thrombi formed after 5 min at 100 s^{-1} in a microfluidic flow chamber on a collagen-TF surface (extrinsic pathway) with recalcified citrated whole blood from individuals with hemophilia A with varying plasma FVIII levels. Overlay of platelets (blue) and fibrin(ogen) (green). Scale bar = 25 μm.[69] C. Epifluorescence images of thrombi formed at 100 s^{-1} in a microfluidic flow chamber on a collagen surface (intrinsic pathway) using whole blood collected into a low level of CTI (4 μg/mL) from a healthy control and an individual with severe hemophilia A. Overlay of platelets (green) and fibrin (red). Scale bar = 50 μm.[71]

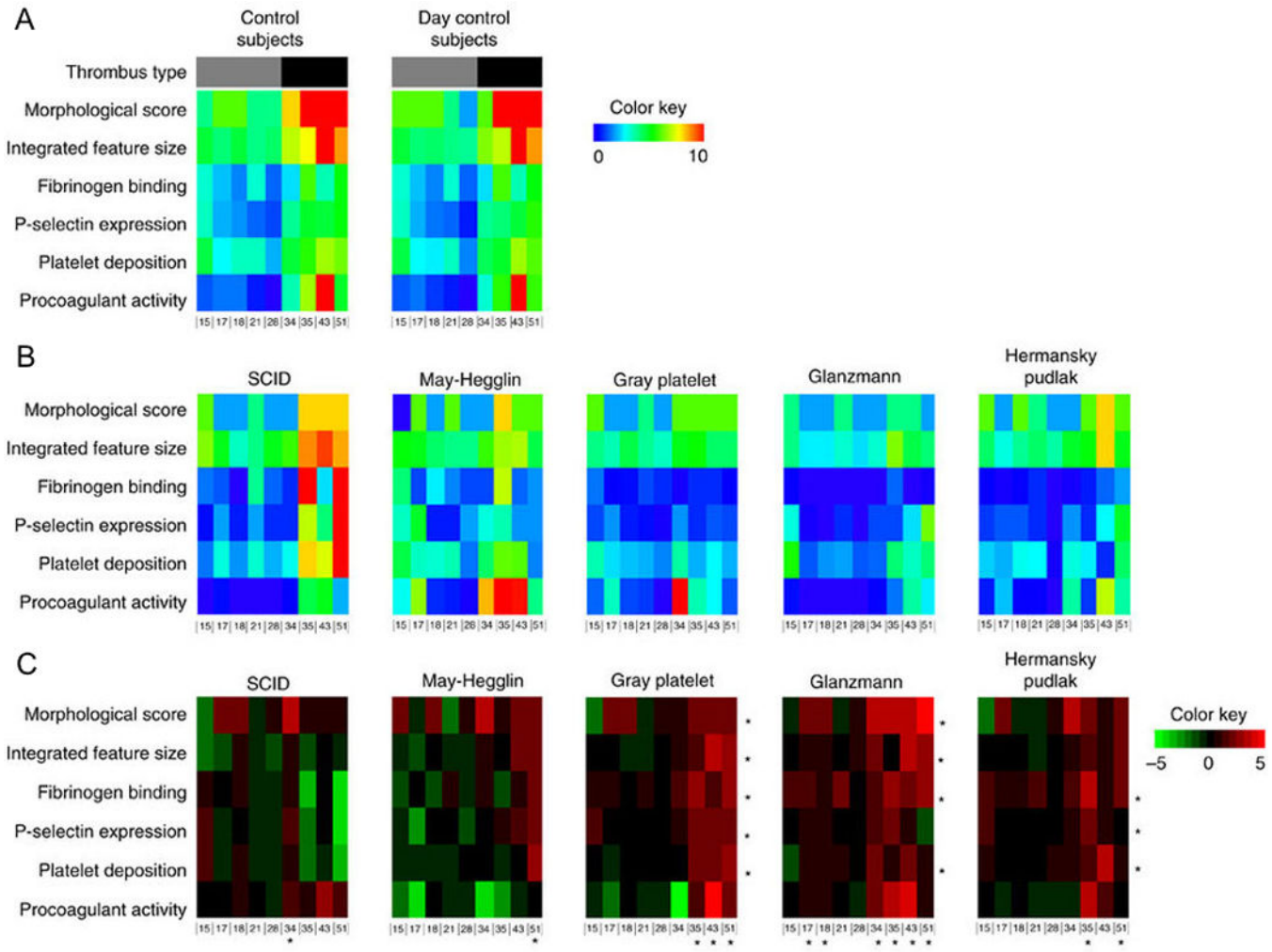

Figure 2.

Microfluidic model of bleeding. Whole blood is perfused through a vascular channel and flows through an ‘injury’ in the vessel wall out into an extravascular channel with a user-defined pressure gradient. A. Computational fluid dynamics simulation of velocity field in the microfluidic channels. Note the velocity is highest in the injury channel. B. Occlusive thrombus formed in a collagen-TF coated vascular injury channel for a 0.68 kPa pressure drop between the blood channel and extravascular channel.[73]

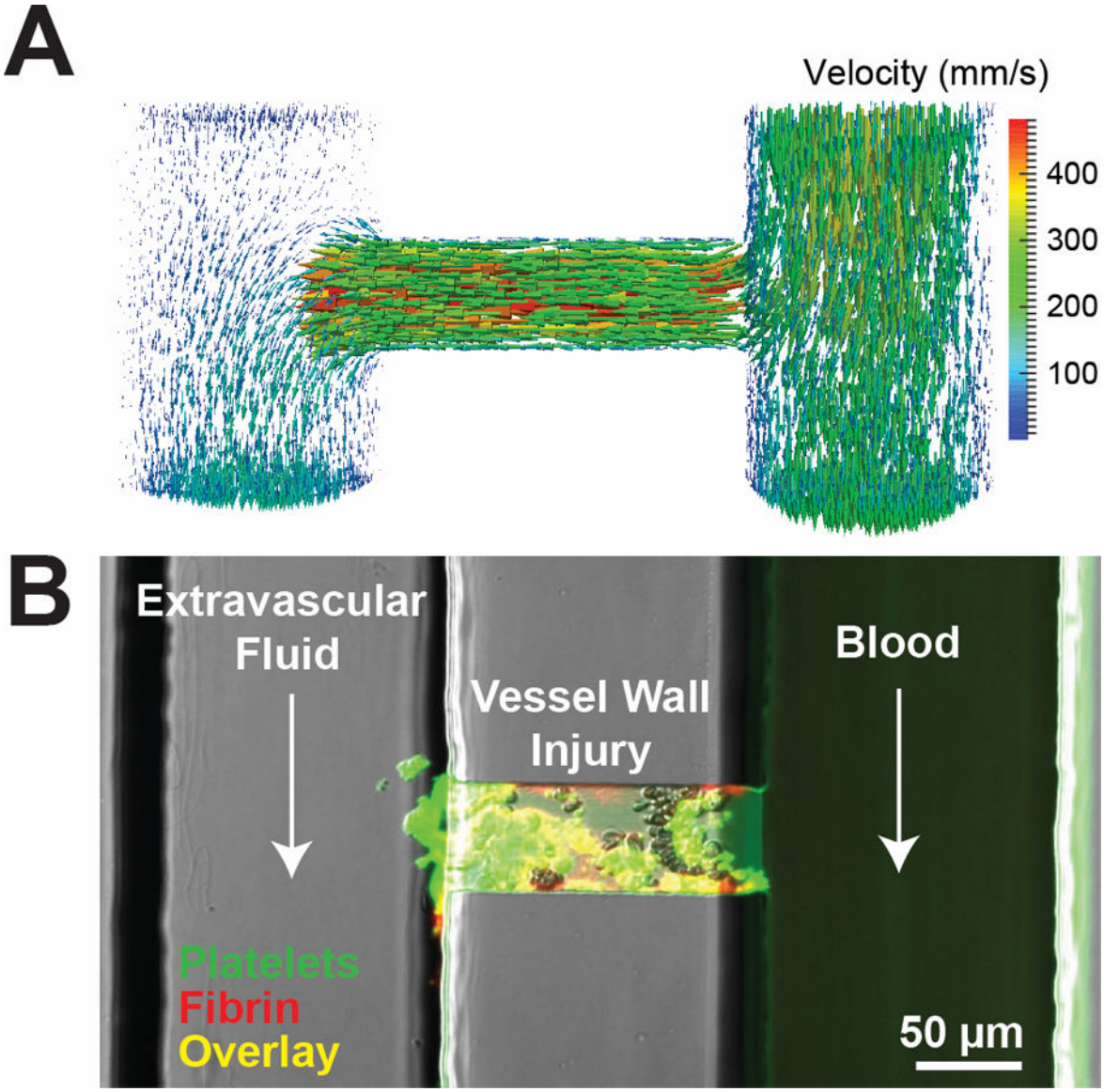

Figure 3.

Multi-parameter assessment of thrombus formation in congenital platelet disorders. A. Heatmap of parameters for control subjects on nine difference surfaces (indicated by the number below the map). B-C. Heatmaps and subtraction heatmaps of parameters for individuals with SCID and several platelet disorders; Difference between disorder and controls indicated by *.[72] Heatmaps represent normalized values of a morphologic score that ranges 1–5; integrated feature size that accounts for the contribution of large and small thrombi; platelet deposition, anti-fibrinogen antibody (fibrinogen binding), anti-P-selectin antibody (P-selectin expression), and annexin A5 percent (procoagulant activity) are quantified by percent surface coverage. Subtraction heatmaps represent difference between patients and controls. Surfaces include 15, VWF and GFOGER-$(GPP)_n$; 17, VWF and vitronectin; 18, VWF and fibronectin; 21, VWF and laminin; 28, VWF; 34, VWF and fibrinogen; 35, VWF and rhodocytin; 43, VWF and GFOGER-$(GPO)_n$; 51, collagen I.

Table 1.

Summary of flow chamber studies using human whole blood samples from individuals with von Willebrand disease.

Subtype	Number of patients	Substrate	Chamber Dimensions	Shear rate(s)	Outcomes	Reference
Not reported	5	Rabbit subendothelium	Annular	830	Reduced thrombus surface coverage in VWD patients	[10]
Not reported	6	Rabbit subendothelium	Annular	1300, 2600, 3300	Reduced platelet adhesion and fibrin formation in VWD, but not correlated with VWF levels.	[13]
Not reported	1	Human renal arteries	Annular	805	Subendothelial bound VWF correlated to platelet adhesion. Bound VWF reduced in VWD plasma.	[40]
Not reported	5	Rabbit subendothelium	Annular	650, 1300, 3500	Reduced fibrin surface coverage and thrombus volume in VWD, but similar levels of platelet adhesion.	[39]
1, 2A, 2B	12	Human umbilical artery subendothelium	Annular	2500	DDAVP enhanced platelet adhesion in all type 1, half of type 2A, and decreased platelet adhesion in type 2B.	[99]
1, 2A, 2B	12	Human umbilical artery subendothelium	Annular	1000, 2500	Decreased adhesion in all subtypes compared to normal controls	[100]
1, 2A, 2B, 3	16	Type III collagen	Parallel plate	100, 650, 2600	VWD subtypes did not affect thrombus metrics at 100 s^{-1}; types 2A, 2B, and 3 show reduced volumes at 650 and 2600 s^{-1}, type 1 only reduced at 650 s^{-1}.	[41]
2A, 2B	7	Type I collagen	Parallel plate	50, 1500	No differences compared to controls at 50 s^{-1}. Type 2A had reduced thrombus size at 1500 s^{-1}, while type 2B varied between normal and defective.	[42]

Subtype	Number of patients	Substrate	Chamber Dimensions	Shear rate(s)	Outcomes	Reference
1, 2A, 2N, 3	5	Collagen; collagen-TF	Microfluidic	240, 1000, 2000	Symptomatic type 1 patients, but not asymptomatic, have delayed occlusion times in T-TAS. Types 2A, 2N, and 3 show delayed occlusion time.	[57]
1	50	Collagen; collagen-TF	Microfluidic	240, 1000, 2000	Time to 10 kPa metric in T-TAS correlates with bleeding score on collagen surface.	[58]

Table 2.

Summary of flow chamber studies using human whole blood samples from individuals with hemophilia.

Subtype	Number of patients	Substrate	Chamber Dimensions	Shear rate(s)	Outcomes	Reference
A, B, C	15	Rabbit subendothelium	Annular	650	Dramatic decreases in fibrin deposition, FPA release, thrombus volume and height in FVIII and FIX deficiencies. Modest reduction in fibrin deposition and FPA release, but no change in thrombus volume and height for FXI and FXII deficiencies	16
A	9	Type III collagen	Parallel plate	100, 650, 2600	Reduced fibrin deposition in mild and severe FVIII deficiencies at all shear rates, but thrombus volumes only decreased at 100 and 650 s^{-1} for severe deficiencies.	41
A	20	Type I collagen and TF	Microfluidic	100	Fibrin deposition decreased for severe and moderate, but not mild, FVIII deficiencies. Platelet aggregate area different between severe and moderate deficiencies.	69
A, B, C	21	Type I collagen	Microfluidic	100	Reduced fibrin deposition in samples with <13% FVIII activity, and reduced platelet deposition at <1% FVIII activity.	71
A, B, C	8	Type I collagen	Microfluidic	100	rFVIIa enhances platelet deposition on collagen in absence of contact pathway, and enhances both platelet and fibrin deposition in presence of contact pathway.	72
A, B	2	Type I collagen	Parallel plate	1000	Fibrin formation delayed in FVIII and FIX deficiencies with soluble TF concentration up to 100 pM	70