Platelet Function Tests

Marie Lordkipanidzé and Paul Harrison

Abstract

Many platelet function assays have been developed over the last century in an attempt to capture the natural ability of platelets to form aggregates in response to vascular injury. From physiological assays, such as the bleeding time, to diagnostic assays used within specialized hematology departments, to point-of-care assays that are intended as clinical decision aids on wards and in operating rooms, and finally to high-throughput deep phenotyping assays intended for precision medicine, platelet function assays have become increasingly commonplace in many settings. This chapter presents an overview of the most commonly used platelet functions assays and discusses important variables to take into account when performing platelet function testing, ranging from pre-analytical issues to the clinical utility of individual tests.

Introduction

Many platelet function assays have been developed over the last century in an attempt to mimic the natural ability of platelets to form aggregates in response to vascular injury. From physiological assays, such as the bleeding time, to diagnostic assays, such as those used to this day in specialized hematology departments, to point-of-care assays that are intended as clinical decision aids on wards and in operating rooms, and finally to high-throughput deep phenotyping assays intended for precision medicine, platelet function assays have become increasingly commonplace in many settings. The continued interest in platelets and their contribution to health and disease continuously drives innovation in this field, tackling the challenges of working with live, dynamic, and sensitive cells.

In order to accomplish their many roles, not only in maintaining hemostasis, but also in inflammation, host defense and immunity, wound healing, fetal vascular remodeling, tumor growth and metastasis, liver disease, and angiogenesis, to name a few, platelets are a treasure trove of membrane receptors and anchoring proteins, diverse granular contents, and *de novo*-generated mediators (Nurden 2011; McFadyen and Kaplan 2015). These need to intervene in a coordinated fashion to ensure appropriate platelet activation, degranulation and aggregation, as any imbalance may lead to either bleeding or thrombotic events. Because of this biological complexity, with many redundant activation pathways working in concert, studying platelet function can be difficult and requires specialized methods to capture this diversity of platelet responses.

In this chapter, we discuss general principles in platelet function testing, with special attention to pre-analytical variables, and highlight areas of standardization where guidelines are available. We also present an overview of currently available platelet function assays and their clinical usefulness. Specific techniques of platelet flow cytometry (Carubbi et al. 2017) and platelet aggregometry (Hayward

M. Lordkipanidzé (✉)
Faculté de pharmacie, Université de Montréal, C.P. 6128, succursale Centre-ville, Montréal, QC, CanadaH3C 3J7,

Research Center, Montreal Heart Institute, 5000 rue Bélanger, Montréal, QC, CanadaH1T 1C8,
e-mail: marie.lordkipanidze@umontreal.ca

P. Harrison
Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

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and Moffat 2017) are detailed further in the following sections, along with applications including monitoring of antiplatelet therapy (Tantry et al. 2017) and diagnosis of platelet defects (Mezzano and Pereira 2017).

Bleeding Time: A Physiological Approach to Platelet Function Testing

The first assessment of platelet function to be successfully used was initially described as a coagulation test by M.G. Milian in 1901 (Milian 1901). Shown to be correlated with platelet dysfunction by W.W. Duke in 1910 (Duke 1910), the test underwent further refinement at the hands of A. C. Ivy in 1941 and has become known as the Duke-Ivy Bleeding Time (Ivy et al. 1941). In its most accepted form, the assay is carried out on the ventral surface of the forearm where skin thickness is uniform, with a blood pressure cuff applied to the upper arm and inflated to 40 mmHg. The technique consists of recording the time required for a blood clot to form at the site of a 5 mm long by 1 mm deep longitudinal incision, and the test ends when the flow of blood is stopped. Commercial spring-loaded devices containing sterile blades (e.g., Simplate II® by the Organon Teknika Corporation and later Surgicutt® by the International Technidyne Corporation) have further standardized the procedure, which has remained the most useful screening test of platelet function until the early 1990s (The British Society for Haematology BCSH Haemostasis and Thrombosis Task Force 1988). This assay has brought forward a new understanding of platelet function in vivo, including the effect of platelets on hemostasis, the interaction of platelets with vascular endothelial cells and other blood elements, co-activation of platelets and the coagulation system, platelet release reactions in vivo, and various platelet diatheses (Gresele et al. 1987, 2003; Kyrle et al. 1987; Michelson et al. 1991; Weiss and Lages 1993; Weltermann et al. 1999; Ciferri et al. 2000; Undas et al. 2001; Falcinelli et al. 2007; Lubsczyk et al. 2010; Giannini et al. 2011; Traby et al. 2016).

Among the advantages of this assay are its simplicity and its accessibility, as no expensive equipment or specialized laboratory is required. This ensured the test's widespread use in and outside of specialized centers for decades. The Duke-Ivy Bleeding Time measures physiological hemostasis driven by platelets and plasma adhesive proteins such as von Willebrand factor (VWF), as well as the interplay of blood components with the vessel wall. For this reason, it cannot pinpoint the origin of the defect, but an abnormally prolonged Bleeding Time is indicative of a hemostatic deficiency.

Several factors have led to the downfall of the Bleeding Time as a screening assay, in favor of novel platelet function tests. Chiefly, they included the poor reproducibility despite the use of standardized devices, the invasive nature of the test which could lead to scarring, and the relative insensitivity of the Bleeding Time to many mild platelet defects. In the early 1990s, several investigators concluded that an accurate bleeding history was a more valuable screening test, as it captured mild bleeding tendency more robustly than the Bleeding Time (Rodgers and Levin 1990; Lind 1991; Peterson et al. 1998). As a result, widespread use of the bleeding time has rapidly declined over the last 20 years, to be replaced by other less invasive platelet function assays carried out ex vivo on freshly collected blood samples (Harrison et al. 2011).

Important Considerations in Platelet Function Testing

Most platelet function assays have strict requirements in terms of blood drawing and sample preparations. Recent surveys have highlighted that platelet function testing practices can vary significantly between laboratories (Moffat et al. 2005; Jennings et al. 2008; Cattaneo et al. 2009; Gresele et al. 2014). These differences may explain some of the discrepancies reported between laboratories and emphasize the importance of standardized laboratory approaches. Because platelets can be artifactually activated by inappropriate sample handling, important pre-analytical variables must be taken into consideration before platelet function testing can be performed. Failure to apply some of these key considerations may lead to spurious results (Harrison et al. 2011). This is particularly important, as there are no widely available internal or external quality control materials for platelet function testing (Favaloro 2009), with the exception of the PFA-100/200® device where quality controls have been proposed (Favaloro 2013; Favaloro and Bonar 2014). For this reason, most assays are performed side-by-side with a fresh blood sample from a drug-naïve healthy volunteer, which ensures the viability of reagents and appropriate assay settings (Harrison and Lordkipanidzé 2013). Alternatively, some laboratories derive local reference ranges from a cohort of healthy volunteers, and carry out their platelet function testing according to rigorous standard operating procedures, to limit operator-induced variability. Finally, cartridge-based point-of-care assays have built-in electronic control measures that limit inappropriate test procedures, but pre-analytical conditions may still lead to aberrant test results. The main key pre-analytical considerations are detailed below.

Physiological and Medical Conditions

A number of physiological conditions may influence platelet function results. These include circadian rhythms (Hartley 2012), strenuous exercise (Davis et al. 1990), fasting (Ahuja

et al. 2009), coffee and caffeine-containing beverage consumption (Varani et al. 2000; Natella et al. 2008), and smoking (Rival et al. 1987). Although guidelines in the past have suggested that platelet function studies should only be performed on samples obtained from individuals who were fasting and resting, and who refrained from smoking, caffeine ingestion, and rigorous exercise on the day of testing, pragmatically many of these conditions are hard to control and only have a minor influence on platelet function results (Kottke-Marchant and Corcoran 2002; Harrison et al. 2011; Cattaneo et al. 2013). However, it is necessary to ensure that subjects have refrained from medication or substances known to affect platelet function (which include non-steroidal anti-inflammatory drugs, antiplatelet agents, phosphodiesterase inhibitors, certain psychotropics, and herbal remedies) for 10–14 days, to account for normal platelet turnover (Kottke-Marchant and Corcoran 2002; Harrison et al. 2011; Lordkipanidzé 2012; Cattaneo et al. 2013). This washout period can be longer in case of drugs with a long effective half-life, such as the antiplatelet drug vorapaxar which may take weeks or even months to wear off (Gurbel et al. 2011). An unexpected finding in platelet function results should prompt investigations into these potential confounders, and repeat testing on a fresh blood sample under more suitable conditions may be warranted.

In some instances, deferring platelet function testing or stopping antiplatelet medications cannot be considered. For example, in the context of acute coronary syndromes or in patients requiring chronic antiplatelet therapy as secondary prevention of acute thrombotic events, platelet function testing must be carried out promptly and on-treatment. A number of expert opinion documents have been published with recommendations in terms of interpretation of platelet function results in this context (Michelson et al. 2005; Pulcinelli and Riondino 2006; Kuliczkowski et al. 2009; Tantry et al. 2013; Aradi et al. 2014), and these are treated in more detail in a dedicated section in this book (Tantry et al. 2017).

Venipuncture

Blood should be collected by an experienced phlebotomist, from the antecubital vena fossa, applying a standardized, atraumatic protocol of a clean venipuncture using minimum tourniquet pressure (Harrison et al. 2011). As shear is an important inducer of platelet activation, needles or butterfly cannulae of 19–21 gauge should be preferred, in conjunction with either an evacuated tube system or plastic syringe. A discard tube of 5 ml should be drawn first to avoid tissue factor-induced aggregation from the venipuncture, but this practice is rarely applied in clinical centers with little to no impact on the quality of the blood sample (Favaloro et al. 2008). Underfilling or overfilling of collection tubes is a frequently encountered problem. Collection tubes should be filled to 90 % capacity or to the manufacturer-specified mark, to avoid incorrect sample dilution/anticoagulation which can negatively impact platelet function tests (Favaloro et al. 2008). Samples should be handled gently and inverted three to six times to provide adequate mixing of test sample with the anticoagulant.

Anticoagulants

The most commonly employed anticoagulant for platelet function testing is sodium citrate (Harrison et al. 2011). Other commonly used anticoagulants include acid-citrate-dextrose (ACD), the potent thrombin inhibitor D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK), hirudin, heparin, and the dual thrombin/factor Xa inhibitor benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA). There is no consensus on the best anticoagulant for platelet function testing, and most assays can be performed in samples anticoagulated with any of these agents (Kaiser et al. 2012). Particular precautions must be taken to avoid EDTA-containing tubes, and whenever these are required (e.g., for full blood counts), these should be collected last to avoid potential contamination by carryover (Favaloro et al. 2008).

Specimen Processing

Blood samples collected for platelet function testing should ideally be left to rest for 30 min at room temperature (20–25 °C). Placing samples in a refrigerator/ice pack or in a warm water bath prior to platelet function testing negatively impacts platelet responses (Harrison et al. 2011). Ideally, samples should be collected close to the laboratory, but if transport is necessary, it is important to avoid vibration, shaking, vortexing, or agitation. For this reason, pneumatic tube systems are considered inappropriate for transporting platelet function testing specimens (Thalen et al. 2013). The time delay between collection, transport and analysis should ideally be between 30 min and 2 h, but should not exceed 4 h (Harrison et al. 2011).

For platelet function assays that require the preparation of platelet-rich plasma (PRP), whole blood samples should be centrifuged without using a brake at 200 g for 10 min and at room temperature (Cattaneo et al. 2013). These conditions were shown to result in high quality PRP, by reducing contamination with other blood cells and optimizing platelet reactivity (Femia et al. 2012). Once the PRP has been removed by gentle pipetting, the remaining portion of the blood sample can be centrifuged at room temperature at 1500 g for 15 min to prepare platelet-poor plasma (PPP) (Cattaneo et al. 2013). There is no need to adjust platelet

count in PRP with autologous PPP to a standardized value, as this practice induces extra variability to platelet function results (Cattaneo et al. 2007; Linnemann et al. 2008).

Platelet Function Assays

An impressive array of platelet function assays are available to platelet researchers (Table 1). Many require dedicated skill, expertise, and machinery to run and to interpret, but increasingly, point-of-care instruments are being developed with the intent of applying this technology outside of specialized laboratories. The more commonly used assays are described in more detail below.

Light Transmission Aggregometry

First described by Gustav Born in 1962 and considered by many as the historical gold standard in platelet function testing (Born 1962), light transmission aggregometry (also known as optical aggregometry) measures the increase in light transmission through a PRP sample as platelets aggregate in response to an external stimulus. Addition of a platelet agonist induces the platelets to activate and clump together, which decreases the turbidity of the sample and results in greater light transmission (autologous PPP being used to define 100 % light transmission). The aggregometer captures this dynamic interaction of platelets with each other in real time, resulting in a time-dependent platelet aggregation curve (described in further detail in Section 2.4). An adaptation of this technology allows for luminescence to be read concomitantly to light transmission. By using a luciferin-luciferase substrate added to PRP, a measurement of dense granule secretion can then be performed in parallel with platelet aggregation (Pai et al. 2011) (described in further detail in Wersäll et al. 2017).

This assay offers several advantages. It is very flexible, as there is no restriction to the platelet agonists or agonist concentrations that can be used to stimulate platelets (Hayward et al. 2010; Harrison et al. 2011). The real-time nature of the assay also provides with kinetic information of platelet–platelet interactions; this includes initial platelet shape change, degranulation, and reversible or irreversible aggregate formation (Cazenave et al. 2004). These factors have contributed to making the assay the preferred platelet function test in most specialized laboratories over the world for the better part of the last century (Cattaneo et al. 2013).

Among the disadvantages of the assay, it is important to note that LTA is carried out in PRP, making it relatively non-physiological, stirred at low shear conditions not representative of arterial shear rates where platelet activation normally occurs, and platelets in this assay only form aggregates after a platelet agonist has been added. These conditions do not entirely mimic platelet adhesion, activation and aggregation as they would occur in response to damage to the vessel wall (Harrison and Lordkipanidzé 2013). It is also a time-, labor-, and blood-volume intensive technique. To run a full panel of agonists, conventional LTA requires large blood volumes and a dedicated and experienced operator (Dawood et al. 2012). Interpretation of tracings can also be challenging, which often limits its use to specialist tertiary centers that can maintain their expertise through a high patient volume. This has triggered several attempts at automation of LTA (Lawrie et al. 2014), as well as development of new, and faster approaches to LTA (Sun et al. 2001; Moran et al. 2006; Chan et al. 2011) that are described below.

96-Well Plate-Based Light Transmission Aggregometry

The requirement for a dedicated aggregometer has hampered the availability of LTA in many laboratories. However, as modern 96-well plate readers capable of recording light transmission through multiple samples within a few minutes have become widely available in standard laboratories, several groups have attempted to adapt LTA principles to a high-throughput 96-well plate design (Bednar et al. 1995; Sun et al. 2001; Moran et al. 2006; Chan et al. 2011). This requires the coating of wells with platelet agonists, either in solution or lyophilized, to trigger platelet aggregation after PRP is added to each well with a multichannel pipette. The plate is quickly transferred to a heater-shaker that can maintain the samples at 37 °C and stir the samples to facilitate platelet–platelet interactions. Depending on the plate reader setting, light transmission can be read either at set intervals (e.g., 15 s) or at the end of a fixed period (e.g., 5 min), thus providing either kinetic curves similar to LTA, or an endpoint assessment of platelet aggregation (Chan et al. 2011; Chan and Warner 2012). Similarly to LTA, light transmission is converted into % aggregation by measuring light transmission in PRP and autologous PPP samples.

Despite the fact that various versions of this technique are in use in research laboratories, the experience in clinical settings remains limited. In a head-to-head comparison of the assay with LTA in a group of patients with idiopathic bleeding, the assay was shown to be a promising tool for the screening of bleeding disorders and monitoring of antiplatelet drugs (Lordkipanidzé et al. 2014). Its main use presently is as a tool for a better understanding of basic pathophysiology of platelets (Mylotte et al. 2012; Cooke et al. 2013; Lordkipanidzé et al. 2014).

Table 1 Non-exhaustive list of commonly used platelet function assays

Name of test	Principle	Advantages	Disadvantages	Frequency of use
Global assays of hemostasis				
Bleeding time	In vivo cessation of blood flow	In vivo test, physiological POC	Insensitive, invasive, scarring, high CV	Was widely used, now less popular
Thromboelastography (TEG® or ROTEM®)	Monitoring of rate and quality of clot formation	Global whole blood test	Measures clot properties only, largely platelet-independent unless platelet activators are used	Widely used in surgery and trauma
TEG-6S	Monitoring of rate and quality of clot formation	POC	POC equivalent to TEG or ROTEM	No clinical experience
Electron microscopy	Ultrastructural analysis of platelets	Diagnostic Whole mount technique useful for dense granular imaging	Expensive, specialized equipment	Only available in special units
Platelet activation-based assays				
Flow cytometry	Measurement of platelet glycoproteins and activation markers by fluorescence	Whole blood test, small blood volumes, wide variety of tests	Specialized operator, expensive, samples prone to artifact unless carefully prepared	Frequently used either in core laboratories or as benchtop instruments in research labs
VASP	Flow cytometric or ELISA measurement of phosphorylation of VASP	Measurement of $P2Y_{12}$ receptor signaling	Insensitive to intermediate inhibition of $P2Y_{12}$	Increasing use
Platelet aggregation-based assays				
Light transmission aggregometry (LTA)	Low shear platelet-to-platelet aggregation in response to classic agonists	Gold standard	Time-consuming, sample preparation, poorly standardized	Widely used in specialized labs
96-well-plate based assays in PRP	Based on LTA principles	Lower blood/PRP volumes than LTA. Many replicates and dose–response curves possible	Little widespread experience	Little widespread experience
Plateletworks®	Platelet counting pre- and post-activation	Rapid, simple, POC, small blood volume	Indirect test measuring count after aggregation	Used in surgery and cardiology
VerifyNow®	Fully automated platelet aggregometer to measure antiplatelet therapy	Simple, POC, 3 test cartridges (aspirin, $P2Y_{12}$, and $\alpha_{IIb}\beta_3$)	Inflexible, cartridges can only be used for single purpose	Widely used
WBA	Monitors changes in impedance in response to classic agonists	Whole blood test, multichannel version available	Dependent on platelet count, older instruments require electrodes to be cleaned and recycled	Widely used in specialized labs although less than LTA
Shear-based assays				
PFA-100/200®	High-shear platelet adhesion and aggregation during formation of a platelet plug	Whole blood test, high shear, small blood volumes, simple, rapid, POC, 3 test cartridges (CEPI, CADP and INNOVANCE P2Y)	Inflexible, VWF-dependent, Hct-and platelet count dependent	Widely used
Microfluidic devices	Miniaturized multichannel devices	Whole blood, real-time thrombus formation	Little widespread experience	Research only at present
Assays measuring platelet release reactions				
Lumi-aggregometry	Combined WBA or LTA and nucleotide release	Monitors release reaction with secondary aggregation	Semiquantitative	Widely used in specialized labs, although less than LTA
Adenine nucleotides	Measurement of total and released nucleotides by luminescence or HPLC	Sensitive	Sample preparation, assay calibration, extra equipment	Restricted to specialized labs

(continued)

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Name of test	Principle	Advantages	Disadvantages	Frequency of use
Soluble platelet release markers and sheddome (e.g., serotonin, PF4, βTG, sCD40L, sCD62P, GPV, and GPVI)	Usually by ELISA, also available through bead-based flow cytometry	Relatively simple	Prone to artifact during blood collection and processing	Fairly widely used in research
Serum thromboxane B_2	Immunoassay	Dependent upon platelet COX-1 activity	Prone to artifact	Widespread use
AspirinWorks®	Immunoassay of urinary 11-dehydrothromboxane B_2	Measures stable thromboxane metabolite, dependent upon COX-1 activity	Indirect assay, not platelet-specific, renal function-dependent	Increasing use

COX-1 cyclooxygenase 1, *CV* coefficient of variation, *ELISA* enzyme-linked immunoassay, *GP* glycoprotein, *Hct* hematocrit, *HPLC* high-performance liquid chromatography, *LTA* light transmission aggregometry, *PFA-100/200®* platelet function analyzer 100/200, *PF4* platelet factor 4, *POC* point of care, *PPP* platelet-poor plasma, *PRP* platelet-rich plasma, *sCD40L* soluble CD40 ligand, *sCD62P* soluble CD62P (P-selectin), *βTG* β-thromboglobulin, *VASP* vasodilator-stimulated phosphoprotein, *WBA* whole blood aggregometry

Whole Blood Aggregometry

As its name suggests, whole blood aggregometry (WBA) measures platelet aggregation as it occurs ex vivo, in whole blood. The technique is based on electrical impedance resulting from aggregate formation onto two electrodes immersed in saline-diluted whole blood stimulated with platelet agonists (Cardinal and Flower 1980). There are currently two manufacturers of whole blood aggregometers. The Chronolog apparatus is a two- to four-channel computerized aggregometer, which is compatible with both a disposable single-use set of electrodes and reusable electrodes that require thorough and careful cleaning between uses. The more commonly used instrument in clinical laboratories is the Multiplate® analyzer, mainly because it is semi-automated, highly standardized and uses disposable cuvettes/electrodes with preselected agonists for different applications, including diagnosis of bleeding and monitoring of antiplatelet therapy (Solomon et al. 2011; Valarche et al. 2011; Aradi et al. 2014).

The assay's advantages include performance in whole blood, thus eliminating potential artefactual activation of platelets during the PRP preparation steps; relative ease of use and rapidity, as compared with LTA; the compatibility with a large array of platelet agonists at various concentrations, thus allowing for a better understanding of various activation pathways; and the smaller blood volume requirement as opposed to LTA (Aradi et al. 2014; Kong et al. 2015). However, the assay is influenced by a number of factors, which include platelet count and hematocrit, the anticoagulant used, and the delay between blood sample collection and platelet function testing (Stissing et al. 2011; Kaiser et al. 2012; Rubak et al. 2012; Wurtz et al. 2014). Its use in the clinic is mostly centered on monitoring of antiplatelet therapy (Tantry et al. 2013; Aradi et al. 2014), as there is little published data on the assay's usefulness in investigating inherited platelet disorders (Albanyan et al. 2015).

VerifyNow®

The VerifyNow® system is perhaps the most commonly used point-of-care assay. It is specifically developed to monitor antiplatelet therapy, with dedicated cartridges to assess the effect of aspirin, $P2Y_{12}$ ADP receptor inhibitors, and GPIIbIIIa antagonists. Its ease-of-use, speed, and requirement for little to no technical expertise have made this assay highly popular in settings where platelet function testing could be used to guide antiplatelet therapy, such as in cardiac catheterization laboratories. However, its clinical predictiveness has been questioned lately in view of large clinical trials using the VerifyNow® $P2Y_{12}$ assay to guide antiplatelet therapy having failed to provide clinical benefit (Siller-Matula et al. 2015). Notwithstanding, consensus documents still recommend the use of the VerifyNow® $P2Y_{12}$ assay, as a potential predictor of future cardiovascular and bleeding events (Aradi et al. 2014).

Platelet Function Analyzer (PFA-100/200®)

The recently updated PFA-200® instrument is a cartridge-based assay. It was intended to provide an in vitro equivalent to the bleeding time, and its mode of action requires blood to be aspirated through an aperture in a membrane coated with platelet agonists, and to record the time for a platelet plug to occlude the aperture which is reported by the system as "closure time" (Favaloro and Bonar 2014). Three cartridges are currently available; the CADP cartridge contains collagen and ADP, the CEPI cartridge contains collagen and epinephrine and the INNOVANCE P2Y cartridge contains ADP and PGE_1 supplemented with calcium, with a smaller aperture (100 μm vs 150 μM) (Favaloro 2008; Koessler et al. 2011). The INNOVANCE P2Y cartridge is significantly more sensitive to P2Y12 receptor inhibition than the CADP cartridge and has shown promise in monitoring of antiplatelet drugs as well as in evaluation of congenital

$P2Y_{12}$ receptor defects (Koessler et al. 2011, 2012; Edwards et al. 2012; Scavone et al. 2014).

The assay has gained popularity as a general screening tool for hemostatic disorders due to its ease of use, speed, small blood volume requirement, and the need for little to no specialist training. It is also the only platelet function assay where an external quality control program is available (Favaloro 2009; Favaloro and Bonar 2014). However, a number of factors are known to affect the assay's performance, and these must be taken into account when interpreting the results. Hematocrit and platelet count influence closure time, which makes the assay not suitable for patients with a platelet count below 50×10^9/L or hematocrit below 25 % (Carcao et al. 2002). Patients with non-O blood groups have shorter closure times than blood group O patients, potentially requiring adapted reference ranges that take into account the patient's blood group (Cho et al. 2008). The concentration of sodium citrate used to anticoagulate the sample also influences the results, with 3.8 % giving greater stability of results (Jilma 2001). The high dependence of the assay on von Willebrand factor (due to the high-shear conditions when blood is aspirated through the aperture) makes it an interesting assay for screening of von Willebrand disease, but makes it unsuitable for platelet function testing in this cohort (Favaloro 2002, 2008).

Recent guidelines have suggested that the assay could be used as a screening test to rule out a significant platelet defect in a patient whose clinical history for bleeding is unlikely to point to an inherited platelet disorder, but should not be used as evidence of absence of a platelet defect in patients at high suspicion of inherited platelet dysfunction (Hayward et al. 2006; Harrison et al. 2011).

Thromboelastography® (TEG®)

Thromboelastography® (TEG) and Rotational TEG (ROTEG® or ROTEM®) are similar technologies that assess the hemostatic function as a whole, from thrombus formation to lysis (Luddington 2005; Chen and Teruya 2009). The instruments differ slightly, but the methodology is the same, as a whole blood sample is stirred in a cup with a suspended pin. In anticoagulated samples, the motion between the cup and the pin is unaffected. However, as clot formation is triggered by addition of coagulation or platelet activators, the motion of the cup/pin is hampered, and this translates into a curve depicting clot strength. Both re-calcified plasma and whole blood can be used in this assay, with activators of the tissue factor or contact factor pathways (Chen and Teruya 2009). Arachidonic acid and ADP can also be used as agonists to pre-activate platelets within the TEG system (PlateletMapping™ technology).

The TEG®/ROTEM® tests are well established, mostly in the context of assessing global hemostasis for the management of bleeding and thrombotic risk in surgical, cardiovascular, and trauma patients despite a relatively weak clinical evidence-base (Kozek-Langenecker et al. 2013; Hunt et al. 2015). They are, however, labor-intensive, which has prompted the development of a novel point-of-care microfluidic assay named TEG-6S (Gurbel et al. 2016). It provides all the same test results as traditional TEG, but is a cartridge-based point-of-care rapid analyzer. How this new assay compares with traditional TEG, and whether it will provide clinically meaningful results remains to be established.

Flow Cytometry

Important technological advances have made flow cytometers available not only in specialized core laboratories, but also as benchtop instruments in non-dedicated laboratories. A vast array of antibodies coupled with fluorochromes is commercially available, specifically targeted against individual platelet proteins, granules, and lipid membranes. Flow cytometry is therefore a powerful and popular tool to study many aspects of platelet biology and function, and is described in more detail in Carubbi et al. (2017).

Assessment of platelet function by flow cytometry can be performed on isolated platelets, but the use of diluted anticoagulated blood is preferred. The most common markers of platelet activation used in the literature are P-selectin expression on the platelet surface as a marker of α-granule secretion; the conformational change in integrin $\alpha_{IIb}\beta_3$ into its active state with the PAC-1 antibody; and phosphorylation of vasodilator-stimulated phosphoprotein [VASP] as a marker of $P2Y_{12}$ receptor activation-dependent signaling (Schmitz et al. 1998; Matzdorff 2005). However, the field is in constant progress, especially with novel multiplex technologies that allow for the concomitant measurement of multiple analytes in blood samples as small as a few microliters (Spurgeon et al. 2014). Moreover, the assay is being actively developed for assessing platelet function in patients with thrombocytopenia, as it is performed independently of platelet count (Frelinger et al. 2015). Efforts have been made to standardize the use of flow cytometry for platelet function testing (Schmitz et al. 1998; Lee et al. 2008), but it remains a challenging assay to homogenize, which makes comparisons between laboratories difficult. More recently, reagents have become available so that remote activation and fixing can be performed reliably before analysis within a central core laboratory (Dovlatova et al. 2015).

Conclusion

The last century has seen many developments in platelet function testing and has brought forward a better understanding of platelet biology, physiology, and pathology (Coller 2011). The time when functional assessment of platelets was confined to specialized laboratories and required dedicated equipment and personnel has given way to point-of-care, near-patient instruments of platelet function assessment and changed the way platelet studies are conducted (Harrison and Lordkipanidzé 2013). Today's challenges in platelet function testing include determining the true clinical usefulness of these assays for predicting thrombosis and bleeding, and the development of individualized approaches to mitigate risks in individual patients (Aradi et al. 2015; Siller-Matula et al. 2015).

Increasingly, a deeper phenotyping approach is required to capture the many functions platelets accomplish, through an intrinsically complex array of activation pathways. Comprehensive platelet function testing requires the development of new techniques that allow not only detailed characterization of platelet responses, but are also high-throughput to investigate a large number of individuals. Promising technologies include devices that measure global platelet reactivity in response to shear alone (Wurtz et al. 2012), microfluidic devices with pre-coated adhesion or activation molecules (Conant et al. 2011; Westein et al. 2012; Lucitt et al. 2013; de Witt et al. 2014), assays that measure calcium flux in platelets by fluorescent imaging (Liu and Abell 2006), ELISA-type assays that capture platelets on agonist-coated surfaces (Salles et al. 2010; Baker-Groberg et al. 2014), modified light transmission aggregometry techniques carried out on 96-well plates (Mylotte et al. 2011; Lordkipanidzé et al. 2014) and luminometric assays of platelet secretion in response to various platelet agonists (Sun et al. 2001). All of these techniques have significantly improved our ability to investigate platelet responses to multiple platelet agonists in a shorter time than traditional approaches. In the future, investigators will need to harvest the wealth of data generated by high throughput deep phenotyping approaches and translate these platelet function findings into actionable determinants of disease.

Take Home Messages

- An impressive array of platelet function assays are available to platelet researchers. Many require dedicated skill, expertise, and machinery to run and to interpret, but increasingly, point-of-care instruments are being developed with the intent of applying this technology outside of specialized laboratories.
- Most platelet function assays have strict requirements in terms of blood drawing and sample preparations, in order to help with the standardization and generalizability of results.
- Today's challenges in platelet function testing include determining the true clinical usefulness of these assays for predicting thrombosis and bleeding, and the development of individualized approaches to mitigate risks in individual patients.
- Increasingly, a deeper phenotyping approach is required to capture the many functions platelets accomplish, through an intrinsically complex array of activation pathways. In the future, investigators will need to harvest the wealth of data generated by high throughput deep phenotyping approaches and translate these platelet function findings into actionable determinants of disease.

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