Platelet function tests

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ABSTRACT

Traditionally developed for diagnosis of bleeding disorders, platelet function assays have become increasingly used in basic research on platelet physiology, in phenotype-genotype associations in bleeding disorders, in drug development as surrogate endpoints of efficacy for new antiplatelet therapy, and to an extent, in monitoring of antiplatelet therapy in clinical practice to predict thrombotic and bleeding risk. A multiplicity of platelet function assays is available to measure the level of platelet activity in various settings. These include assays that are restricted to a specialized laboratory as well as point-of-care instruments meant to investigate platelet function at patient bedside. Unlike tests that determine a defined quantity or measurement of a clinical biomarker (e.g. cholesterol or blood pressure), platelet function testing assesses the dynamics of living cells, which immediately presents a series of unique problems to any laboratory or clinic. This article presents currently used platelet function assays and discusses important variables to take into account when performing these assays, including pre-analytical issues and difficulties in interpreting platelet function test results.

KEYWORDS: Antiplatelet therapy; Bleeding disorders; Platelet function testing.

INTRODUCTION

New roles for platelets are constantly being discovered, with platelets contributing to modulating inflammation, host defense and immunity, wound healing, fetal vascular remodeling, tumor growth and metastasis, in addition to their well-established role in maintaining hemostasis.[1,2] For this multitalented and multifaceted purpose, platelets are a treasure trove of membrane receptors and anchoring proteins, diverse granular contents and various *de novo*-generated mediators, which intervene in a coordinated fashion to ensure appropriate platelet activation, degranulation and aggregation.[3] With numerous redundant platelet activation pathways working in concert, platelets are a complex cell to study and require specialized methods to capture this diversity of platelet responses. A large array of platelet function assays have been developed in response to this challenge, mostly for the purpose of diagnosing and managing patients presenting with bleeding problems.[4] However, as platelets are key contributors to atherothrombosis,[5,6] platelet function tests are increasingly being used to monitor the efficacy of antiplatelet drugs to inhibit pathological thrombus formation, and to try to identify patients at risk of future ischemic or bleeding events.[7] Widespread use of antiplatelet therapy can not be dissociated from its attendant bleeding risk, which can be life-threatening in during trauma and surgical procedures. Platelet function assays are therefore also being increasingly used as periprocedural tools to aid in the prediction and management of periprocedural bleeding.[8] The need for on-the-spot results along with the development of new, simpler tests and point-of-care instruments, has resulted in the increasing tendency of platelet function testing to be performed away from specialized clinical or research hemostasis laboratories, where the more traditional and complex tests are still performed.

Specific applications of platelet function assays for the diagnosis of bleeding disorders,[9] granule secretion disorders,[10] and monitoring of antiplatelet therapy[11] are discussed in more detail elsewhere in this issue of the journal. This article presents currently used platelet function assays and discusses important variables to take into account when performing these assays, including pre-analytical considerations and difficulties in interpreting platelet function assay results.

DUKE-IVY BLEEDING TIME, A PRELUDE TO PLATELET FUNCTION TESTING

Initially described as a coagulation test by M.G. Milian in 1901, the test subsequently known as the Duke-Ivy Bleeding Time was first successfully demonstrated to correlate with platelet dysfunction by W.W. Duke in 1910.[12,13] The bleeding time was further refined by A. C. Ivy in 1941, who standardized the assay by applying a blood pressure cuff to the upper arm inflated to 40 mm Hg and making a 5 mm long by 1 mm deep longitudinal incision on the ventral surface of the forearm where skin thickness is uniform.[14] The technique consists of recording the time required for a blood clot to form at the site of the incision and stop blood flow. Following the 1961 report by C.H. Mielke of a template to further standardize bleeding time,[15] the 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 made the bleeding time the most useful screening test of platelet function until the early 1990's.[16]

The bleeding time is simple and does not require expensive equipment or a specialized laboratory. It measures physiological hemostasis driven by platelets and plasma

adhesive proteins such as von Willebrand factor (VWF), and integrates the interplay of the blood components with the vessel wall. However, despite the use of standardized devices, the bleeding time is poorly reproducible, invasive, insensitive to many mild platelet defects and time-consuming. In addition, an accurate bleeding history is a more valuable screening test, as it captures mild bleeding tendency more robustly than the bleeding time.[17-19] 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.[20]

PRE-ANALYTICAL VARIABLES

Recent surveys have highlighted important differences between laboratories in platelet function testing practices.[21-24] These differences may explain some of the variability reported between laboratories, but also emphasize the importance of controlling for pre-analytical variables when performing platelet function assays. Some of the key considerations are detailed below, as failure to apply these precautions may lead to spurious results.[20] With the exception of PFA-100/200® where quality controls have been proposed,[25,26] there are no widely available internal or external quality control materials available for platelet function testing.[27] Most assays are performed on fresh blood and so many laboratories either establish normal ranges using control blood obtained from healthy volunteers and/or assay samples known to be normal in parallel to ensure that each test/reagent is viable.[28]

Blood sampling conditions

Several conditions have been shown to influence platelet function, including circadian rhythms,[29] exercise,[30] fasting,[31], coffee and caffeine-containing beverage consumption,[32,33]

and smoking.[34] Although ideally, samples for platelet function studies should only be collected from fasting and resting subjects who have refrained from smoking, caffeine ingestion and rigorous exercise on the day of testing, as well as 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, pragmatically many of these conditions are hard to control.[20,35,36] An unexpected finding in platelet function results should, however, prompt investigations into these potential confounders, and repeat testing on a fresh blood sample under more suitable conditions may be warranted.

Venipuncture

Blood should be collected from the antecubital vena fossa by an experienced phlebotomist applying a standardized, atraumatic protocol of a clean venipuncture using minimum tourniquet pressure.[20] To avoid sheer-induced platelet activation, 19-21 gauge needles or butterfly cannulae should be used, in conjunction with either an evacuated tube system or plastic syringe. Traditionally, the first 5-ml / tube collected should be discarded to avoid tissue factor-induced aggregation from the venipuncture, but this practice is rarely applied in clinical centers and evidence of nefarious effects is lacking.[37] Underfilling or overfilling of collection tubes is an important problem, and efforts should be made to filling the tube to 90% capacity or to the manufacturer-specified mark to avoid incorrect sample dilution / anticoagulation.[37] Samples should be handled gently and inverted three to six times to provide adequate mixing of test sample with anticoagulant.

Anticoagulants

For clinical platelet function testing, the most commonly employed anticoagulant is trisodium citrate (105-109 mM final concentration).[20] Other commonly used anticoagulants include hirudin, D -phenylalanyl- L -prolyl- L -arginine chloromethyl ketone (PPACK) a potent thrombin inhibitor, heparin, the dual thrombin/factor Xa inhibitor benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA), and acid-citrate-dextrose (ACD). Although there is a healthy debate about the most suitable anticoagulant for platelet function testing,[38] most assays can be performed in samples anticoagulated with any of these agents. However, if EDTA-containing tubes are required (e.g. for complete blood counts), these should be collected last to avoid potential carryover effects.[37]

Specimen processing

All blood samples should be maintained at ambient temperature (20-25 °C) and should not be placed in a refrigerator/ice pack or in a warm water bath prior to platelet function testing.[20] Transport of samples should avoid vibration, shaking, vortexing or agitation; as such, pneumatic tube systems are considered inappropriate for transporting platelet function testing specimens.[39] The time delay between collection, transport and analysis should ideally be between 30 min and 2h, but no more than 4h.[20]

If preparation of platelet-rich plasma (PRP) is required, centrifugation of whole blood samples should be at 200g for 10 min, at ambient temperature without using a brake.[36] This maximizes the quality of PRP by reducing contamination with other blood cells and optimizing platelet reactivity.[40] Platelet-poor plasma (PPP) should be prepared by centrifuging whole blood, or the tubes of blood from which PRP was removed, at ambient temperature at 1500g for 15 min.[36] The platelet count of PRP samples should not be adjusted

to a standardized value with autologous PPP, as this practice alters platelet reactivity.[41,42]

PLATELET FUNCTION ASSAYS

Increasingly, platelet function instruments are easy to use and have made their way outside of specialized laboratories. However, the more involved and complex assays remain restricted to specialist centers, as they require not only expensive machinery, but perhaps more importantly, expertise and a relatively high volume of testing to maintain test quality and facilitate interpretation of results. Some tests are meant as global screening tests of platelet hemostatic function, but most tests are specific assays that assess platelet responses, either in whole blood or in PRP. Table 1 provides a summary of currently available platelet function tests, their major advantages and disadvantages as well as frequency of use. The more commonly used assays are described in more detail below.

Light transmission aggregometry

Considered the historical gold standard, light transmission aggregometry (LTA) measures platelet aggregation as a function of light transmission through PRP, a turbid suspension of cells relatively opaque to light transmission.[43] As platelets start to aggregate in response to a platelet agonist, turbidity of the suspension is reduced and translates into in increased light transmission (PPP being used to define 100% light transmission). The dynamics of platelet aggregation (expressed as %) is therefore measured in real time as platelets aggregate. An adaptation of this technology allows for luminescence to be read concomitantly to light transmission, thus measuring secretion of nucleotides from dense granules during aggregation by using a luciferin-luciferase substrate added to PRP. [44]

Among the advantages of this assay is its flexibility, as platelet aggregation can be

induced by numerous agonists in varying concentrations.[20,45] Kinetic information of platelet-platelet interactions that is gleaned from this assay corresponds with specific phases of platelet activation, from shape change to degranulation and unstable vs. firm platelet aggregation as confirmed by electronic microscopy (**Figure 1**).[46] As a result, LTA offers the possibility of studying platelet activation pathways in detail, and has been the preferred platelet function assay in most specialized laboratories over the last 50 years.

On the other hand, LTA is relatively non-physiological, as platelets are isolated from other whole blood components, stirred under low shear conditions and only form aggregates after addition of agonists. These conditions do not entirely mimic platelet adhesion, activation and aggregation as they would occur in response to damage to the vessel wall.[28] Conventional LTA using a full panel of agonists requires large blood volumes and significant expertise to perform the tests and to interpret the tracings,[47] which has triggered attempts at automation of LTA,[48] as well as development of new, easier-to-use platelet function assays.

High throughput adaption of LTA using 96-well plates

In an attempt to palliate some of the difficulties in performing LTA, including the requirement for a large blood volume, for dedicated machinery and for experienced personnel, several laboratories have developed a modified technique based on light transmission principles but applied to standard 96-well plates.[49-51] Platelet agonists either in solution or pre-coated onto wells are used to trigger platelet aggregation upon addition of PRP. When the plate is stirred, the formation of platelet aggregates changes the absorbance of light passing through the 96-well plate, which can be measured either kinetically or as an endpoint (e.g. after 5 minutes) in a conventional plate reader. Absorbance is then converted into percentage of aggregation in a similar fashion to LTA based on measuring both control

PRP and PPP samples.[51,52] Example results from a healthy volunteer obtained using the *Optimul* assay are shown in **Figure 2**.[52]

This technique can provide a rapid assessment of most platelet activation pathways within minutes, including detailed dose-response curves which are rarely obtainable from LTA due to blood volume restrictions, the number of channels available and time.[53] The assay is a promising tool for screening of bleeding disorders and monitoring of antiplatelet therapy, as well as understanding basic pathophysiology of platelets.[53-55]

Whole blood aggregometry

Whole blood aggregometry (WBA) is based on a change in electrical impedance resulting from platelet aggregation, in response to classical agonists, onto two electrodes immersed directly in saline-diluted whole blood.[56] The Multiplate® instrument is the most widely used apparatus in clinical laboratories, as it is semi-automated and uses disposable cuvettes/electrodes with preselected agonists for different applications, including diagnosis of bleeding and monitoring of antiplatelet therapy.[57-59]

As its name states, WBA is performed in whole blood, which is among its main advantages as opposed to LTA. However, a number of factors are known to influence WBA, which include hematocrit and platelet count, the anticoagulant used, and the delay between blood sample collection and platelet function testing.[38,60-62] The use of smaller sample volumes, no requirement of prior sample manipulation by centrifugation, which reduces the risk of platelet activation or potential loss of platelet subpopulations, as well as gains in time make it an interesting clinical assay, especially in the monitoring of antiplatelet therapy and as a perioperative assay.[59,63] The paucity of published data on platelet dysfunctions with these methods is, however, a limitation for laboratories investigating inherited platelet disorders.[64]

VerifyNow®

Specifically developed to monitor antiplatelet therapy, the VerifyNow® system is fully automated point-of-care test where fibrinogen-coated polystyrene beads agglutinate in whole blood in response to either arachidonic acid (Aspirin cartridge), ADP and prostaglandin E_1 (PGE_1; P2Y12 cartridge) or thrombin receptor activating peptide (TRAP; GPIIbIIIa cartridge). Although it is robust and highly standardized, a number of factors influence the performance of the assay, which include fibrinogen levels, hematocrit and platelet count, blood triglyceride levels and time from blood sampling to testing.[65,66] Lately, there has been some question as to the clinical usefulness of the assay, because large clinical trials using VerifyNow® P2Y12 to guide antiplatelet therapy have failed to show benefit.[7] Nonetheless, VerifyNow® P2Y12 remains recommended in consensus documents as a predictor of future cardiovascular and bleeding events.[59]

Platelet Function Analyzer (PFA-100/200®)

The PFA-100® instrument has been recently updated to PFA-200®.[26] It is a cartridge-based assay, in which a small volume of blood is aspirated through an aperture in a membrane coated with platelet agonists until the aperture is completely occluded by a platelet plug, which is reported by the system as "closure time". 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).[55,56] The INNOVANCE P2Y cartridge was developed in view of relative insensitivity of previous cartridges to $P2Y_{12}$ receptor inhibitors, and appears promising in evaluating congenital P2Y12 defects as well as in monitoring P2Y12 receptor inhibitors.[67-70]

This global test of platelet function is easy to use, rapid, requires a small volume of blood (0.8 ml) and does not require substantial specialist training. Platelet count and hematocrit influence closure time, and results need to be carefully interpreted in patients with a platelet count below 50 x 10^9/L or hematocrit below 25%.[71] Blood group O was associated with longer closure times than non-O groups, which should be taken into account when deriving normal ranges.[72] The closure time is also influenced by the concentration of sodium citrate used as anticoagulant, with 3.8% giving greater stability of results.[73] The test is highly dependent on VWF levels because of the high-shear conditions within the cartridge capillary and aperture, which makes it suitable for screening of von Willebrand disease, but makes it unsuitable for platelet function testing in this cohort.[74,75] In view of the limited sensitivity of the PFA-100® in the detection of mild platelet function defects including secretion and release defects, a normal PFA-100® result is useful in ruling 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, where specific assays of platelet function are preferable.[20,76]

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. [77,78] The assays use whole blood incubated in a heated cup in which a pin, connected to a computer, is suspended. In the TEG, the cup oscillates whereas in the ROTEG or ROTEM, the pin oscillates. In normal anticoagulated blood, the pin is unaffected, but as a blood clot develops, the motion of the cup/pin is impeached, and this is translated as a curve

depicting clot strength. Whole blood or re-calcified plasma can be used, with or without activators of the tissue factor or contact factor pathways.[77] Arachidonic acid and ADP can be used as agonists to pre-activate platelets within the TEG system (PlateletMapping™ technology). As such, the assay is theoretically suitable to study platelet function and monitor antiplatelet drugs, but the test lacks sensitivity to detect moderate changes in platelet function.[77,79,80]

TEG®/ROTEM® tests are relatively rapid to perform (<30 minutes) and have traditionally been used as point-of-care tests during surgery. Their main advantage is to provide a complete profile of clot formation, including the kinetics of clotting, clot strength and fibrinolysis. Both tests thus provide a global portrait of clot formation within whole blood and allows for interactions between whole blood elements including platelets and the coagulation system. A group of investigators from different countries have formed the TEG-ROTEM Working Group, with the intention to standardize assay performance, and increase reproducibility and consistency using TEG®/ROTEM.[81] Their initial observations suggest that there is significant inter-laboratory variation. Also, both tests exhibit relative insensitivity to various aspects of platelet function and they are therefore not routinely recommended for platelet function testing.

Flow cytometric analysis of platelet function

Flow cytometry is a laser-based technology that provides a wealth of information on individual cells based on the scatter of light that they produce as they cross the light source. It is a powerful and popular tool to study many aspects of platelet biology and function, and its recent developments are described in a separate paper in this issue.[82] Investigations are performed using diluted anticoagulated whole blood incubated with a variety of reagents

including antibodies and dyes that bind specifically to individual platelet proteins, granules and lipid membranes.[83] Platelet function is usually studied as a change in expression of activation markers (most commonly P-selectin expression on the platelet surface as a marker of α-granule secretion and the conformational change in integrin αIIbβ3 into its active state with the PAC-1 antibody), in the appearance of heterocellular aggregates (most commonly platelet-leukocyte conjugates) and as change in signaling pathways (most commonly phosphorylation of vasodilator-stimulated phosphoprotein [VASP] as a marker of $P2Y_{12}$ receptor activation-dependent signaling).[84,85] Fixation of samples allows for use of a core facility for analysis, thus rendering the assay more accessible to non-specialist settings.[84] An important advantage of flow cytometry is that it can be performed independently of platelet count, making it potentially suitable for assessing platelet function in patients with thrombocytopenia.[86] The interpretation of results remains somewhat subjective, which makes the results of this assay difficult to compare from one laboratory to another; however, efforts have been made to standardize the use of flow cytometry for platelet function testing.[84,87]

CONCLUSION

The last 50 years have truly been a golden era for platelet function testing. The use of LTA and later flow cytometry have driven our understanding of platelet biology, physiology and pathology.[88] In parallel, the understanding of platelet targets for effective thrombotic inhibition has led to a wide arsenal of antiplatelet drugs being developed.[89] The availability of easy to use point-of-care assays have taken platelet function testing outside of specialized hematology laboratories and closer to the patient. 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.[7,90]

Platelet function testing is at the cusp of a new era, that of precision medicine and deep phenotyping. Comprehensive platelet function testing is increasingly being investigated in research settings, where platelet biology and physiology drive understanding of complex biological systems, including inflammation, immunity and cancer. New approaches to LTA, including automation and transfer to 96-well plate-based assays, as well as novel microfluidic assays appear particularly promising for deep phenotyping of platelet responses.[48,53,91] The future of platelet function testing 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, with ideally pharmacological targets to influence progression of disease. Platelet function testing is therefore at a particularly interesting stage, as the development of new technologies and new instruments may have future utility in a variety of different clinical and laboratory settings.

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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, POC	Measures clot properties only, largely platelet-independent unless platelet activators are used	Widely used in surgery and anesthesiology
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	Increasingly used in specialized labs

VASP	Flow cytometric or ELISA measurement of phosphorylation of VASP	Measurement of $P2Y_{12}$ receptor signalling	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 GPIIb-	Inflexible, cartridges can only be used for single purpose	Increasing use

		IIIa)		
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	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	Sensitive	Sample preparation, assay	Restricted to specialized

	nucleotides by luminescence or HPLC		calibration, extra equipment	labs
Soluble platelet release markers and sheddome (e.g. serotonin, PF4, βTG, sCD40L, sCD62P, GPV and GPVI)	Usually by ELISA	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.

Figure legends

Figure 1: Morphological changes of washed platelets during ADP-induced aggregation

An aggregation response was obtained by stimulating platelets with 5 μM ADP (arrow). The platelets were fixed at different time points and their surface features were visualized by scanning electron microscopy (SEM). (A) Discoid cells in the resting state. (B) Formation of early pseudopods (7 s). (C) Full shape change and first platelet-platelet interactions (20 s). (D) Large platelet aggregates (45 s). (E) Isolated platelets after disaggregation (3 min). Bars = 1 μm.

Figure 2: Example results from the *Optimul* assay

Dose-response curves obtained with *Optimul* in a healthy volunteer, using 2.5 ml of platelet-rich-plasma.

Figure 1

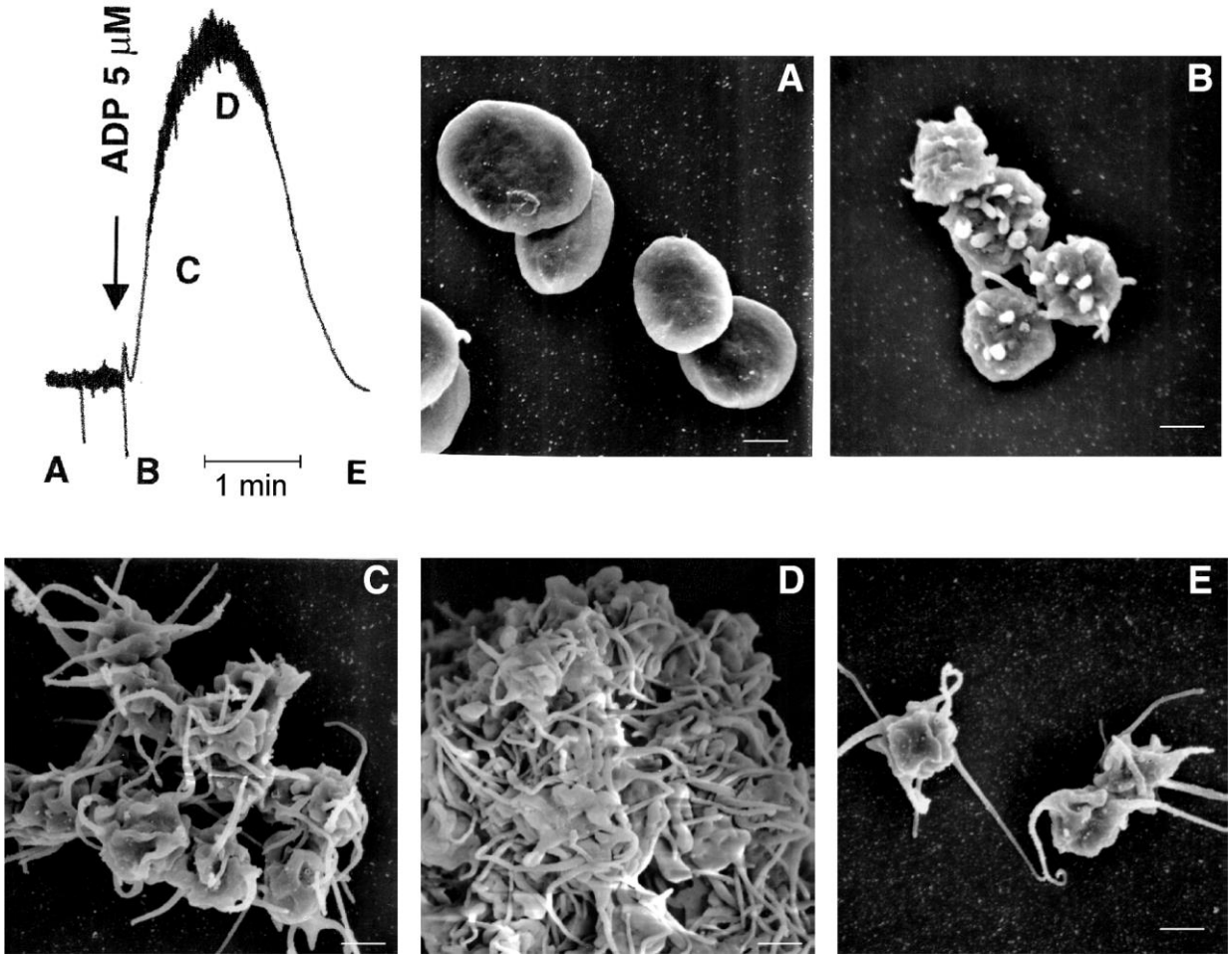
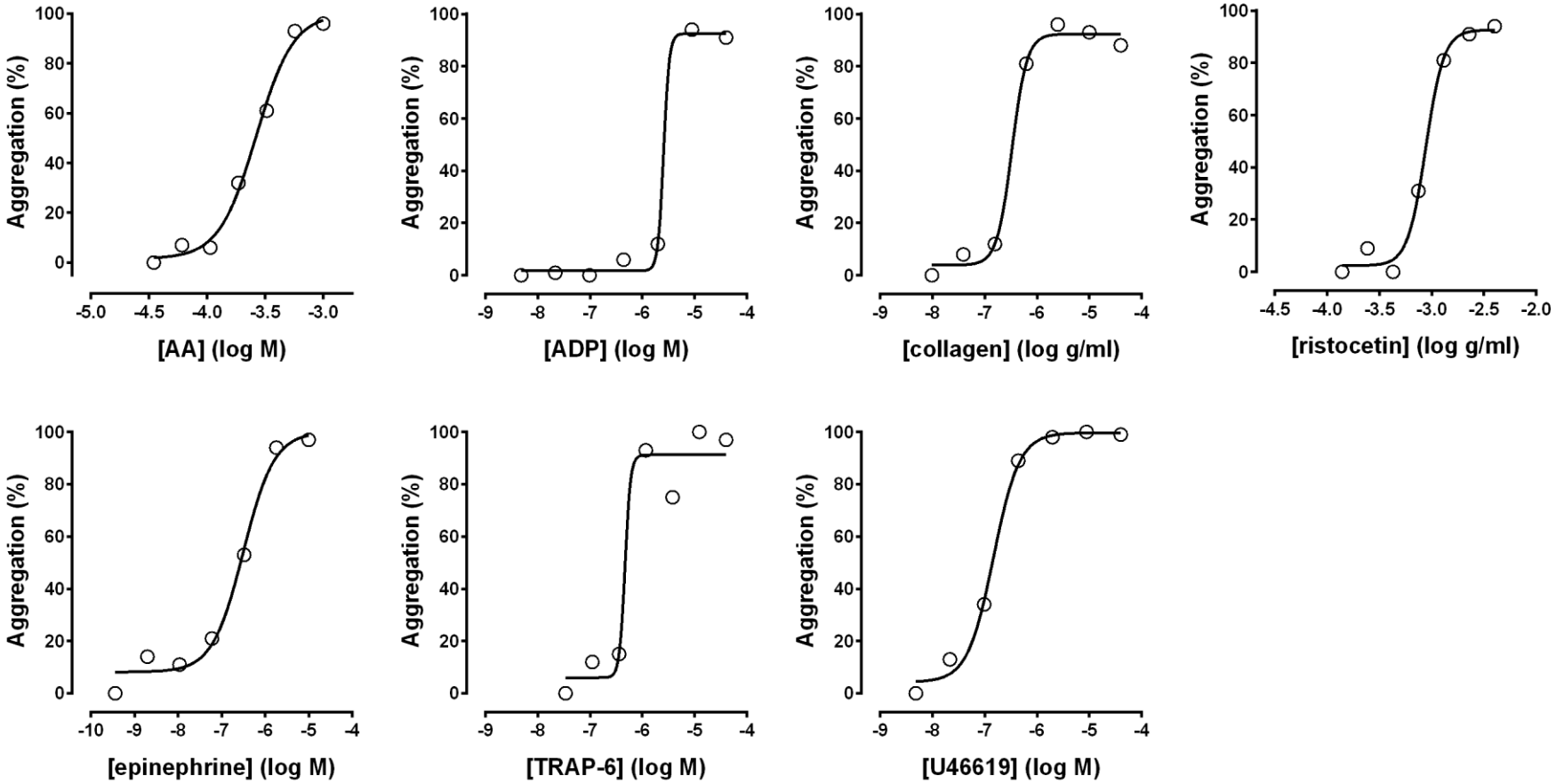


Figure 2


Aggregation (%)
[AA] (log M)
[ADP] (log M)
[collagen] (log g/ml)
[ristocetin] (log g/ml)
[epinephrine] (log M)
[TRAP-6] (log M)
[U46619] (log M)