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Platelet biomechanics, platelet bioenergetics, and applications to clinical practice and translational research

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Abstract

The purpose of this review is to explore the relationship between platelet bioenergetics and biomechanics and how this relationship affects the clinical interpretation of platelet function devices. Recent experimental and technological advances highlight platelet bioenergetics and biomechanics as alternative avenues for collecting clinically relevant data. Platelet bioenergetics drive energy production for key biomechanical processes like adhesion, spreading, aggregation, and contraction. Platelet function devices like thromboelastography, thromboelastometry, and aggregometry measure these biomechanical processes. Platelet storage, stroke, sepsis, trauma, or the activity of antiplatelet drugs alters measures of platelet function. However, the specific mechanisms governing these alterations in platelet function and how they relate to platelet bioenergetics are still under investigation.

Keywords

Platelet bioenergetics; biomechanics

Introduction

The study of platelet bioenergetics has attracted renewed attention with the emergence of its relationship with platelet biomechanics and its potential to be a biomarker of platelet function (1). Platelet function testing platforms like aggregometry and viscoelastometry continue to gain acceptance as methods that demonstrate clinical utility (2). There is increasing clinical potential in merging the current understanding of platelet bioenergetics and mechanics with the data derived from platelet function devices.

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Declaration of interest

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Platelets are released into the circulation as anucleate cells from megakaryocytes. Their primary function is to initiate hemostasis by adhering to damaged tissues, aggregation and catalysis of thrombin generation to ultimately build a fibrin network and stable clot. Platelets normally exist in a quiescent state unless activated by sub-endothelial collagen exposed by vascular injury, soluble agonists like epinephrine, ADP, thromboxane A_2, or the final common platelet activator – thrombin. When activated, platelets initiate a complex sequence of bioenergetic and biomechanical events. Platelet biomechanical processes like adhesion, aggregation, spreading, and contraction are governed by and reflect distinct metabolic avenues including glycolysis and oxidative phosphorylation. These biomechanical signals have a wide dynamic range allowing correlation with clinical conditions like sepsis or coagulopathy. Platelet function devices capture and isolate these signals. For example, optical aggregometry measures platelet aggregation (3). Viscoelastometry measures clot stiffening through platelet aggregation, fibrin polymerization, and platelet contraction (4).

Abnormal platelet function measured by these devices has a pathophysiological cause leading to a clinical consequence. Causes of clinical platelet dysfunction include mediation of inflammation in sepsis, platelet exhaustion in stroke, coagulopathy in trauma, or diseases causing mechanical dysfunction in adhesion. The results for platelet function devices in these clinical situations potentially reflect underlying platelet bioenergetics.

In this review, we examine the relationship between platelet bioenergetics and platelet biomechanics. We discuss how clinical platelet function devices reflect platelet bioenergetic processes and also how deranged platelet bioenergetics are associated with clinically abnormal platelet function.

Definition of platelet bioenergetics

Platelet bioenergetics refers to the production and consumption of energetic substrates. The primary avenues of energy production in platelets are glycolysis and oxidative phosphorylation (5). However, platelet bioenergetics is not a simple dichotomy of these two avenues. Platelets demonstrate an integrated energetic response to stimulation allowing metabolic plasticity between these two energetic avenues and with use of energetic substrates like glucose, fatty acids, or glutamine. A recent paper by Ravi et al. demonstrated that either primary metabolic avenue in dysfunctional platelets allows compensation by the other to respond to increased energy demands (6). The pentose phosphate pathway plays a minor role in platelet metabolism and is not significant in the overall energy production.

In quiescence, platelets require energy in the form of ATP to function at a basal level. In an early study, Akkerman et al. measured glycolysis reflected by the proton production rate and oxidative phosphorylation reflected by the oxygen consumption rate to estimate metabolic contribution from either pathway in resting platelets. The authors reported that glycolysis provided up to 65% of required ATP in inactivated platelets and platelet mitochondria supply the rest (7). A more recent study by Chacko et al., performed in a similar manner, demonstrated glycolysis providing 45% of the required ATP during quiescence. A key difference in this more recent study was the addition of prostaglandin I_2 during the platelet

preparation process to inhibit inadvertent platelet activation, potentially providing a more accurate assessment of platelet quiescence.

When stimulated, platelets increase the rates of glycolysis, fatty acid oxidation, and glutaminolysis (8,9). Depending on environmental conditions like oxygen tension or substrate availability, platelets are able to modulate energy production to provide ATP necessary for hemostatic processes like adhesion, aggregation and contraction. Relative increases and distributions of this increase between glycolysis and oxidative phosphorylation are unknown (10). From the total pool of available platelet adenine nucleotides, one-third reside in the platelet cytoplasm and the remaining two-thirds reside in dense granules (11).

Definition of platelet biomechanics

Platelet biomechanics refers to platelet mechanotransduction to support efficient platelet adhesion, spreading, aggregation, and contraction. These energy intensive processes require extensive platelet cytoskeletal rearrangements and platelets derive this energy through glycolysis, oxidative phosphorylation and stored ATP/ADP in dense granules. Interestingly, platelets are also mechanosensitive and are capable of responding to a wide dynamic range of shear stresses in flow as well as varying substrate stiffness (12).

Platelet adhesion begins with platelet glycoprotein receptors adhering to exposed vascular subendothelium. The glycoprotein (GP) Ib-IX complex attaches to the subendothelium via the ligand von Willebrand's Factor (VWF). These initial attachments are relatively weak, in the range of hundreds of pico-Newtons, and function to overcome shear forces imparted onto attaching platelets by circulating blood (13,14). These initial attachments also serve to expose the A1 domain of VWF which binds to the GP Ibα subunit of the GP Ib-IX complex (15). A recently identified mechanosensory domain (MSD) within the GP Ib-IX subunit is responsive to tensile stress with an unfolding force of 10–20 pN (16). Unfolding of the MSD triggers intracellular signaling via GP Ib-IX. This marks the initial molecular event in platelet activation which leads to downstream signals including release of intracellular calcium and filopdia formation (17). Mechanically induced platelet signaling also occurs extracellularly. Shear force imparted onto platelets has been linked to the expression of TGF-β1, possibly due to a thiol-disulfide exchange (18).

Platelet spreading strengthens adhesion by increasing the number of GP IIb-IIIa attachments to the subendothelium and decreasing platelet surface area normal to blood flow, thus decreasing imparted shear force (19). As platelet activation proceeds, GP IIb-IIIa is exposed externally and facilitates stronger adhesive forces (20,21). Adhesion forces between spread platelets and collagen are much stronger than the initial attachments via GP Ib-IX-V and are as high as 34 nN (22). Aggregation occurs as GP IIb-IIIa binds soluble plasma components like fibrinogen and VWF to form platelet–platelet crosslinks. These platelet–platelet adhesions are weaker than platelet–collagen adhesions, requiring 3 nN to cause disaggregation (23).

Myosin IIa and actin in the platelet cytoplasm form the contractile unit responsible for platelet contraction. Myosin IIa ATPase facilitates actin filament assembly, and forces

created inside the platelet are transmitted via actin polymers to the external fibrin network through GP IIb-IIIa (24). Actin binding with GP IIb-IIIa promotes further exposure of the receptor and stiffening of the platelet aggregate (25). Single platelets have been shown to generate up to 70 nN of force through the fibrin network and an average maximum contractile force of 29 nN (26).

Platelet biomechanics play a crucial role in all stages of clot formation, clot structural organization and clot stabilization. Platelet biomechanics could be a potential indicator for understanding the interaction of platelets with various biomaterials or even devices such as extra-corporeal membrane oxygenators (ECMO) or left ventricular assist devices (LVAD) (27). Furthermore, gaining better understanding of platelet biomechanics through clinical assays may help the clinicians to delineate hemostasis and thrombosis, and make important treatment decisions.

The relationship between platelet bioenergetics and platelet biomechanics

A clear shift in energy demand occurs when quiescent platelets are activated by agonists like thrombin or subendothelial collagen. Biomechanical processes like platelet adhesion, spreading, aggregation, and contraction require increased levels of ATP. However, different metabolic avenues at different times are utilized to fuel each process. A number of studies have demonstrated correlation between platelet bioenergetics and biomechanics.

Platelet adhesion is one of the few platelet biomechanical processes that is energy independent. In an early study investigating platelet interaction with collagen, Misselwitz et al. demonstrated adhesion of platelets to collagen despite the presence of the glycolytic inhibitor 2-deoxy-d-glucose (2DG) and the oxidative phosphorylation inhibitor antimycin A. In the same study, platelet spreading was shown to be sensitive to only 2DG and not antimycin A, suggesting that platelet spreading is dependent on energy supplied by glycolysis (28). A later study by Smith et al. agreed that platelet spreading was energy dependent, however their method of metabolic inhibition was not specific – both glycolysis and oxidative phosphorylation were inhibited without isolating either pathway (29). The cumulative findings in these studies suggest that adhesion is energy independent and platelet spreading is dependent on glycolysis.

Similar to platelet spreading, platelet aggregation is fueled primarily by glycolysis. Holmsen et al. demonstrated a correlation between platelet energy demand and aggregation. In their study, the mitochondrial inhibitor antimycin and glycolytic inhibitor 2DG decreased platelet aggregation with an associated decrease in ATP. However, this study did not isolate the effects of inhibiting only glycolysis or oxidative phosphorylation (30). In a later study, Ravi et al. isolated the glycolytic pathway and demonstrated that platelet aggregation progresses normally despite inhibition of mitochondrial metabolism. In his study, platelet aggregation was measured in a 96-well plate as changes in light transmittance through platelet suspensions activated by thrombin. In the presence of the mitochondrial inhibitor antimycin A, aggregation was not significantly different from controls. However, in the presence of glycolytic inhibitors like 2-deoxy-glucose, aggregation was 50% less compared to controls. Combining glycolytic inhibitors with mitochondrial inhibitors decreased aggregation by a

further 10%, suggesting that platelet glycolysis drives aggregation with oxidative respiration playing a secondary role (6). A major advantage of the study by Ravi et al. compared to previous studies using 2DG is the inclusion of pyruvate in their platelet assay buffer. After incubation with 2DG, mitochondrial respiration can be affected due to depletion of pyruvate (31). This off-target effect of 2DG on oxidative phosphorylation could obscure conclusions regarding platelet metabolics. However, this effect takes up to an hour to occur while measuring platelet spreading and aggregation takes just minutes.

Platelet contraction, unlike platelet adhesion or aggregation, does not depend primarily on glycolysis. Rather, platelet contraction demonstrates a correlation with oxidative phosphorylation. Mürer et al. studied clot size in samples of platelet rich plasma inhibited with the glycolytic inhibitor 2DG and the mitochondrial inhibitors antimycin or oligomycin. Clot formation was initiated with thrombin and measured as serum expressed from clots in glass tubes. There was no significant change in clot size in samples treated with only antimycin, oligomycin, or 2DG, however antimycin or oligomycin in combination with 2DG caused a near complete absence of retraction. This early study suggests that either metabolic avenue can provide energy required for actin–myosin cycling that drives platelet contraction. However, there was no quantification of metabolic inhibition in this study (32). In a follow up study, Chao et al. described similar findings by inhibiting platelet contraction with antimycin and 2DG (33). As mentioned previously however, the off-target effect of 2DG blocking pyruvate supply was not accounted for in these studies.

In a more recent study, Misztal et al. expanded on work by Mürer and Chao by studying the effect of peroxynitrite on platelet bioenergetics and biomechanics. Peroxynitrite inactivates mitochondrial enzymes responsible for oxidative phosphorylation and decreases levels of available ATP (34). Misztal found that peroxynitrite inhibits energy production via oxidative phosphorylation which is associated with decreased platelet contraction. After adding concentrations from 10–100 μM of peroxynitrite to samples of platelet rich plasma, glycolytic activity reflected by lactate production showed a dose-dependent increase while mitochondrial activity reflected by consumption of oxygen demonstrated a dose dependent decrease. Despite the increase in glycolytic activity in response to decreased mitochondrial function due to peroxynitrite, platelet contraction was decreased. Platelet contraction is therefore potentially dependent primarily on oxidative phosphorylation, with glycolysis holding a secondary role (35). Although this study avoids the issues with 2DG inhibition, peroxynitrite also leads to off-target effects. Peroxynitrite concentrations of 50 μM induce increased platelet expression of the cell adhesion molecule P-selectin (36) which can stabilize platelet aggregates (37). This off-target effect could potentially lead to unwanted stabilization of a contraction clot.

Pharmacological inhibition with small molecules still exists as the mainstay for investigation into platelet bioenergetics. Many of the previously mentioned studies utilize the SeaHorse analyzer, a state-of-the-art assay that requires sequential inhibition with different metabolic inhibitors to delineate mitochondrial oxygen consumption. However, more recent studies have expanded small molecule inhibition to include correlation with platelet metabolomics. Slatter et al. compared the lipidome of resting and activated platelets with and without the presence of Aspirin. Cytosolic phospholipase A2 was identified as a key regulator in

provision of lipid substrates to support energy demands during platelet activation. Lipids were structurally identified with only 50% of them being present in established databases. This approach offers a unique opportunity into the study of platelet bioenergetics and how it relates to disease (38).

Platelet function devices

Platelet aggregometry is a well-defined and clinically relevant platelet function test. Born et al. originally described optical aggregometry in 1962 (3). It is considered the gold standard test to assess platelet function. Platelet rich plasma in a cuvette with a stir bar is heated and agonists induce aggregation of platelets. Light transmittance through the sample increases as platelets aggregate. The device signal depends on platelet-to-platelet aggregation via GP IIb/IIIa. Impedance aggregometry is a newer approach which measures changes in electrical impedance across wires in a whole blood sample. This method is advantageous because it requires less sample preparation and more closely mimics physiological conditions by using whole blood. Both of these versions of aggregometry record measurements such as the lag phase, rate, and maximal aggregation. Aggregometry is useful in detecting the effect of anti-platelet drugs and has recently demonstrated potential in trauma as a measure of platelet dysfunction (39).

The PFA-100 (Siemens Corp., Washington D.C.) simulates in vitro shear stress and measures platelet adhesion and aggregation. Blood is injected through a collagen coated capillary tube until occlusion which signifies the primary metric, closure time (CT). The device accepts whole blood and does not require elaborate sample preparation like optical aggregometry. The test is valuable clinically to aid in diagnosis of clotting disorders like von Willebrands disease, however due to its lack of specificity it is limited to screening purposes only. In addition, the assay provides limited information in that it only reports one metric of blood coagulation (40).

Thromboelastography (TEG) and thromboelastometry (ROTEM) are similar viscoelastic tests measuring torque on a pin induced by a clotting blood sample. These tests provide metrics such as time to initiate clotting, maximum clot strength, and rates of fibrinolysis. They identify coagulopathy in settings such as trauma or acute care surgery and can guide blood product administration (41). Viscoelastometry is not focused on the direct measurement of platelet function. However, specialty assays like TEG Platelet Mapping (TEG-PM) or the ROTEM EXTEM/FIBTEM paired assays extrapolate the platelet contribution to clot strength – calculated by the difference in clot shear modulus with and without platelet activation (42,43). TEG-PM measures platelet function using the agonists adenosine diphosphate (ADP) or arachidonic acid (AA) in a thrombin free environment and compares these to maximal activation with thrombin in a kaolin activated sample. Maximal amplitude (MA) is one metric from the TEG-PM assay that has proven correlation to platelet contraction (44).

While the focus of this review is on clinical platelet function devices, platelet function devices used in translational research deserve brief mention. These devices measure an array of platelet bioenergetic or platelet biomechanical function. For example, devices to measure

platelet bioenergetics include the Seahorse XF24 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA) or the Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) (10,45–47). These devices are useful in determining platelet mitochondrial function and accommodate the use of metabolic inhibitors. Measurements are possible using intact cells rather than mitochondrial isolates. Because they are a plate based assay, they allow higher throughput than classical oxygen electrode assays. However, one limitation of these devices is the requirement for high levels of ADP which precludes calculation of ATP produced per oxygen atom reduced by the respiratory chain, or the P/O ratio (48). Another device specific limitation of the Oxygraph-2k is that it requires stirring of platelets which likely leads to unwanted activation.

Measuring dynamic activity of single platelets is possible using the device recently reported by Myers et al. (49). A micro-fabricated chip hosts an array of paired fibrinogen microdots with varying stiffness inside microfluidic channels to control shear by controlling flow rates. Platelets span the paired fibrinogen microdots and displacement of the dots is quantified as the platelet contraction force. The assay allows biochemical control by addition of different agonists or inhibitors and mechanical control by defining flow rates that determine local shear force. Another device that measures platelet contraction forces on a macro scale is the Hemodyne described by Carr et al. (50). This device uses whole blood or platelet rich plasma to detect clot contraction with time and offers insight into the kinetics of platelet driven clot contraction. Experimental devices like these are extremely useful in characterizing platelet function in a controlled manner however they are not clinically translatable due to high cost and complexity.

Clinical platelet function devices reflect platelet bioenergetic processes

Clinical platelet function devices are engineered to capture a specific platelet biomechanical signal (Figure 1). Each of these biomechanical signals is associated with an adaptive bioenergetic balance between glycolysis and oxidative phosphorylation. Thus, platelet biomechanics and the devices that measure them can potentially be considered as a surrogate for platelet bioenergetics. The following studies demonstrate that platelet function devices correlate with platelet bioenergetics. These studies commonly use inhibitors of platelet bioenergetics to demonstrate the loss of energy production capability and corresponding changes in biomechanics (Figure 2).

Platelet aggregometry has been applied clinically to investigate the effect of storage on platelet concentrates meant for transfusion. Termed the “platelet storage lesion,” there is an established consensus that storage of platelets for transfusion has detrimental effects on platelet function (51). Ravi et al. investigated this principle by measuring platelet bioenergetics and aggregation in stored versus fresh platelets. They found that platelet concentrates stored at room temperature for 6–9 days compared to fresh platelets demonstrated decreased mitochondrial respiration measured by oxygen consumption and increased glycolytic function measured by extra-cellular acidification. Aggregation measured by optical aggregometry was significantly decreased in the stored platelet samples after hypotonic challenge (52). In a following study, Bynum et al. similarly showed that aggregation measured by impedance aggregometry was decreased in stored platelets which

correlated with decreases in mitochondrial respiration and oxidative burst. Additionally, this storage lesion was partially mitigated by storage at 4°C instead of room temperature (53). These cumulative findings demonstrate that the aggregation signal captured in optical aggregometry is linked to platelet glycolysis and oxidative phosphorylation.

While there are a number of experimental devices that directly measure platelet contraction, there is no device approved for clinical use. However, viscoelastometry partially reflects platelet contraction through measures like maximum amplitude in thromboelastography or maximum clot firmness (MCF) in thromboelastometry. One study measuring clot strength using thromboelastography in healthy individuals found that platelets contribute 80% to clot strength while fibrin contributes the remaining 20% (54). In the study mentioned previously by Misztal et al. (22), thromboelastometry was employed to study the effect of inhibition of glycolysis or oxidative phosphorylation on clot retraction. The mitochondrial inhibitor cyanide (CN) or the glycolytic inhibitor 2DG was added to samples of platelet rich plasma. ATP content of formed clots with and without addition of inhibitors was also performed. Inhibition with 2DG demonstrated no change in MCF while inhibition with cyanide demonstrated a 9% decrease in MCF compared to controls. ATP content of clots inhibited with 2DG was unchanged from controls while it was decreased by 16% when inhibited by CN. With no change in clot ATP content after 2DG inhibition, it is possible that glycolysis plays a secondary role in platelet energy production after activation has occurred compared to oxidative phosphorylation.

Deranged platelet biomechanics and bioenergetics are associated with clinically abnormal platelet function

As discussed previously, the biomechanical signal captured by platelet function devices reflects a balance of energy production in the platelet. Therefore, abnormal platelet function recorded by platelet function devices is potentially a result of deranged platelet biomechanics or bioenergetics. Clinical research supporting this relationship is limited; however it has been suggested with platelet function devices that measure aggregation and contraction in the following studies.

Protti et al. investigated the relationship between mitochondrial respiratory chain activity and platelet aggregation in patients with sepsis or cardiogenic shock. They found that in both patient groups the complexes I, III and IV of the platelet mitochondrial respiratory chain were depressed. In the septic patients, platelet aggregation was significantly lower compared to controls when stimulated with adenosine diphosphate (ADP). Platelet aggregation measurements in cardiogenic shock would have been confounded by all of these patients taking anti-platelet drugs, and were excluded. In the septic patient group, the degree of mitochondrial inhibition was found to correlate with the degree of inhibition of aggregation. Metrics of glycolytic metabolism however were not measured, thus glycolysis cannot be excluded as possibly correlating with inhibition of aggregation in this study. Regardless, this paper establishes the relationship between platelet mitochondrial dysfunction in sepsis with platelet hyporesponsiveness measured by optical aggregometry (55). Further studies using platelet function devices to correlate clinical platelet dysfunction to deranged platelet

bioenergetics could allow platelet function to serve as a metabolic biomarker of disease states.

Tutwiler et al. investigated the biomechanics of platelet driven clot contraction in stroke patients. A novel optical analyzer (56) tracked *in-vitro* contraction of whole blood clots of healthy and stroke patients. The primary finding of the study was that blood from patients with ischemic stroke demonstrated reduced clot contraction when compared to healthy controls. This finding is clinically meaningful as appropriate clot contraction would potentially mitigate cerebral ischemia as a result of vessel occlusion. A potential explanation for this difference is desensitization of stroke patient platelets to thrombin. Platelets from stroke patients demonstrated reduced surface expression of P-selectin and GP IIb-IIIa after stimulation with thrombin, leading to decreased platelet aggregation, decreased fibrin-platelet cross-linking, and ultimately diminished platelet contraction (57). Thrombin desensitization in platelets from stroke patients has been validated in other studies and is potentially due to internalization of protease-activated receptors or metabolic exhaustion (58). Other factors that could impede clot contraction in the study by Tutwiler et al. were higher levels of fibrinogen and lower platelet counts amongst stroke patients. Correlating the results of this study with measures of platelet mitochondrial dysfunction would be especially interesting and further delineate potential underlying derangements in platelet bioenergetics.

Platelets as a bio-marker for disease

Platelets are a readily available cell type in blood with detectable glycolysis and oxidative phosphorylation. They reflect mitochondrial dysfunction during sepsis similar to other tissues like skeletal muscle (59). Platelet metabolic dysfunction is also a recognized downstream effect in the pathogenesis of common diseases like diabetes, stroke, or heart failure (60,61). While organ biopsy for metabolic studies is highly invasive, venous blood is easily accessible and isolation of platelet concentrates is easily performed. Clinical platelet function devices thus have the potential to assay individual components of platelet bioenergetics in a minimally-invasive manner.

Tyrrell et al. investigated platelets as a biomarker for systemic metabolic status. This study demonstrated that maximal respiration in platelet mitochondria is correlated with oxidative capacity of muscle and cardiac tissue (62). Platelet and tissue respirometry were recorded using an XF24 analyzer from a cohort of African green monkeys of varying metabolic status. Maximal platelet oxidative capacity correlated with cardiac and skeletal muscle. Thus platelets, and specifically their mitochondria, can potentially be used as a blood based bioenergetic marker to gain insight into systemic pathophysiology (63).

Cardenes et al. investigated platelets as a biomarker for platelet reactivity in sickle cell disease (SCD). This study offers a model for investigating platelets as biomarkers for a common disease and provides an example of causation between alterations in platelet bioenergetics and changes in platelet biomechanics. They demonstrated that free hemoglobin in SCD patients correlated with platelet bioenergetic dysfunction, specifically inhibition of platelet mitochondrial complex V (64). This bioenergetic inhibition correlated with an increase in platelet aggregation, an increase in glycolytic metabolism, and an

increase in oxidant production. Platelet metabolic function was measured with an XF24 analyzer and platelet aggregation was measured with a Chronolog aggregometer. The causality between this bioenergetics dysfunction and platelet aggregation was established by reproducing these results in platelets from healthy subjects treated with increasing levels of free hemoglobin. Similar to results from SCD patients, complex V activity in healthy subjects decreased and platelet aggregation increased in a dose dependent manner in response to free hemoglobin. Additional findings included consistent ATP production among the two groups, potentially due to a significant upregulation of glycolysis in the SCD patients. Glycolytic upregulation in the setting of mitochondrial dysfunction demonstrates again the platelet metabolic plasticity mentioned previously by Ravi et al. (6).

Layios et al. investigated platelets as a biomarker for the development of sepsis in an adult ICU. They found that levels of fibrinogen bound to circulating platelets correlate with patients developing sepsis. In patients with high sequential organ failure (SOFA) scores, 87% developed sepsis when greater than 50% of their circulating platelets expressed bound fibrinogen. This prediction only applied when completed within 24 hours of ICU admission. Other platelet activation markers like P-selectin expression or platelet–leukocyte aggregates were not predictive of sepsis (65). Sepsis is a leading cause of mortality in adult ICUs and this study offers clinicians a simple clinical tool to identify at risk patients.

Avila et al. investigated platelets as a biomarker in type 2 diabetics (47). Oxygen consumption and anti-oxidant enzymes were measured in platelets from healthy and diabetic subjects. Platelets from diabetics demonstrated decreased oxygen consumption and lower levels of oxygen-dependent ATP synthesis. Increased mitochondrial stress was suggested in diabetic platelets by increased levels of anti-oxidant mitochondrial enzymes. This study presents platelets as a potential target for measuring efficacy of anti-diabetic therapeutics.

Dysfunction of the platelet mitochondrial respiratory chain has also recently been implicated in multiple neurological diseases, such as deficiency of platelet cytochrome oxidase in Alzheimer’s disease (66,67). In addition, it is becoming clear that platelet mitochondrial function is an important metric in evaluating platelet storage methods in transfusion medicine (53,68).

Conclusions

Platelet biomechanics are governed by platelet bioenergetics. Platelet function devices offer a potential view of platelet metabolic status as they measure biomechanical signals such as adhesion, aggregation and contraction. A device capable of mimicking *in-vivo* conditions and measuring all platelet biomechanical signals in a single assay would prove extremely useful.

Further studies of the regulation of glycolysis versus oxidative phosphorylation as drivers of platelet biomechanics are required. Studies to investigate the role of platelet contraction in clinical platelet dysfunction and its relationship to platelet bioenergetics are also needed to better characterize this under-studied contribution of platelets to hemostasis.

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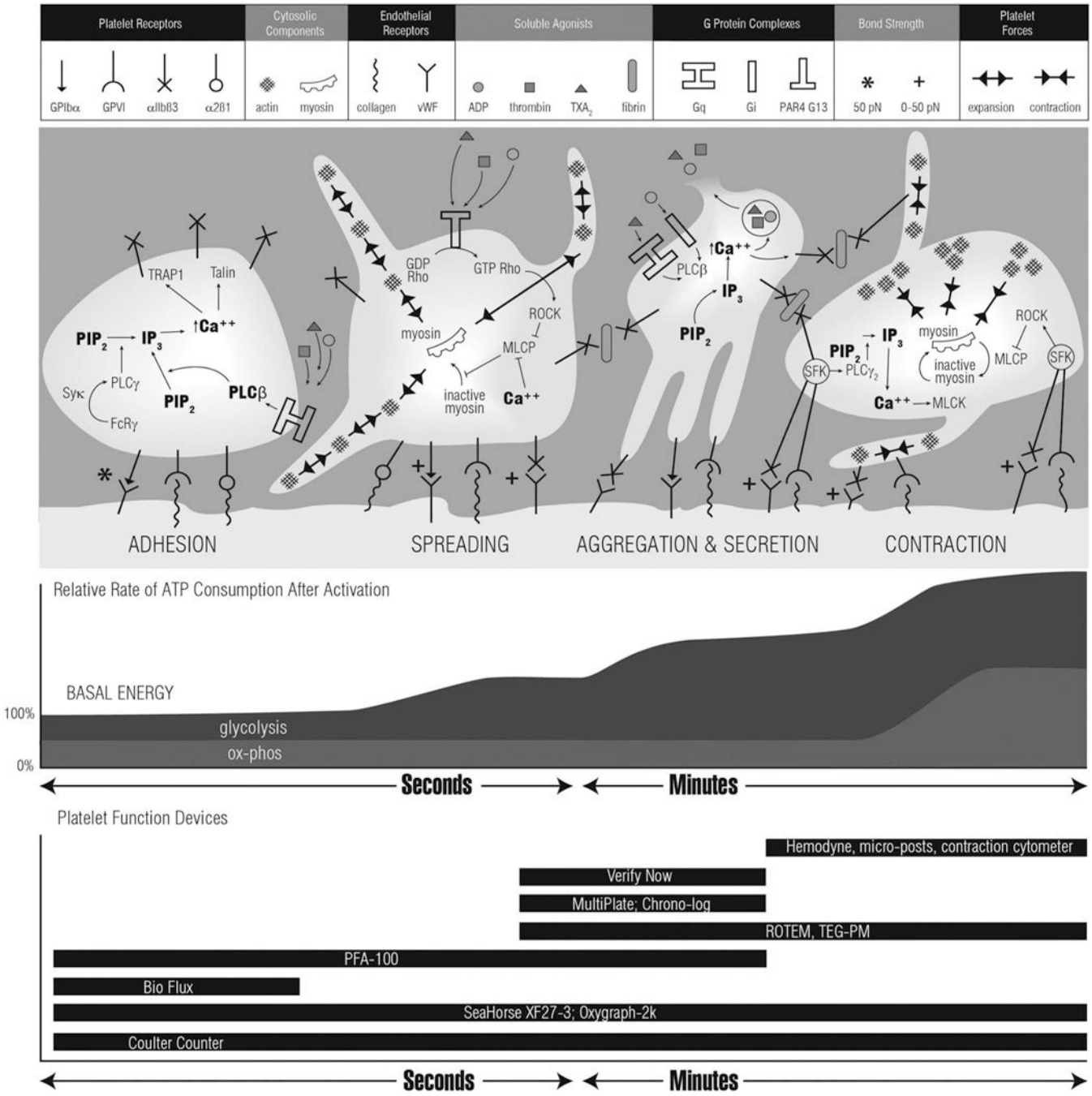

Figure 1.

Platelet activation through mechanosensation and relative rate of energy consumption. GPIb? initially tethers VWF attached to exposed collagen. GP VI binds exposed collagen leading to activation of Syk and PLC?, increasing levels of cytoplasmic calcium. Soluble agonists act via g-protein coupled receptors to increase cytosolic calcium – enhancing expression of GP IIb-IIIa through TRAP1 and talin dependent pathways. Platelet pseudopodia are driven by actin–myosin interactions and influenced by shear deceleration as platelet velocity decreases after adhesion. Pseudopodia restructuring during aggregation

stabilizes clot formation. Forces transmitted through GP IV and GP IIb-IIIa upregulates Src family kinases leading to myosin activation and platelet contraction via a ROCK and calcium dependent pathway. Energy consumption increases during aggregation and secretion. Devices exist to measure platelet function along the activation pathway. The XF 27–3 is able to continually measure oxygen consumption. FDA approved devices like the PFA-100, MultiPlate, Chrono-log and VerifyNow measure adhesion and aggregation. TEG-PM and ROTEM calculate platelet contribution to clot strength. Experimental devices measuring contraction include the Hemodyne, micro-posts and contraction cytometers.

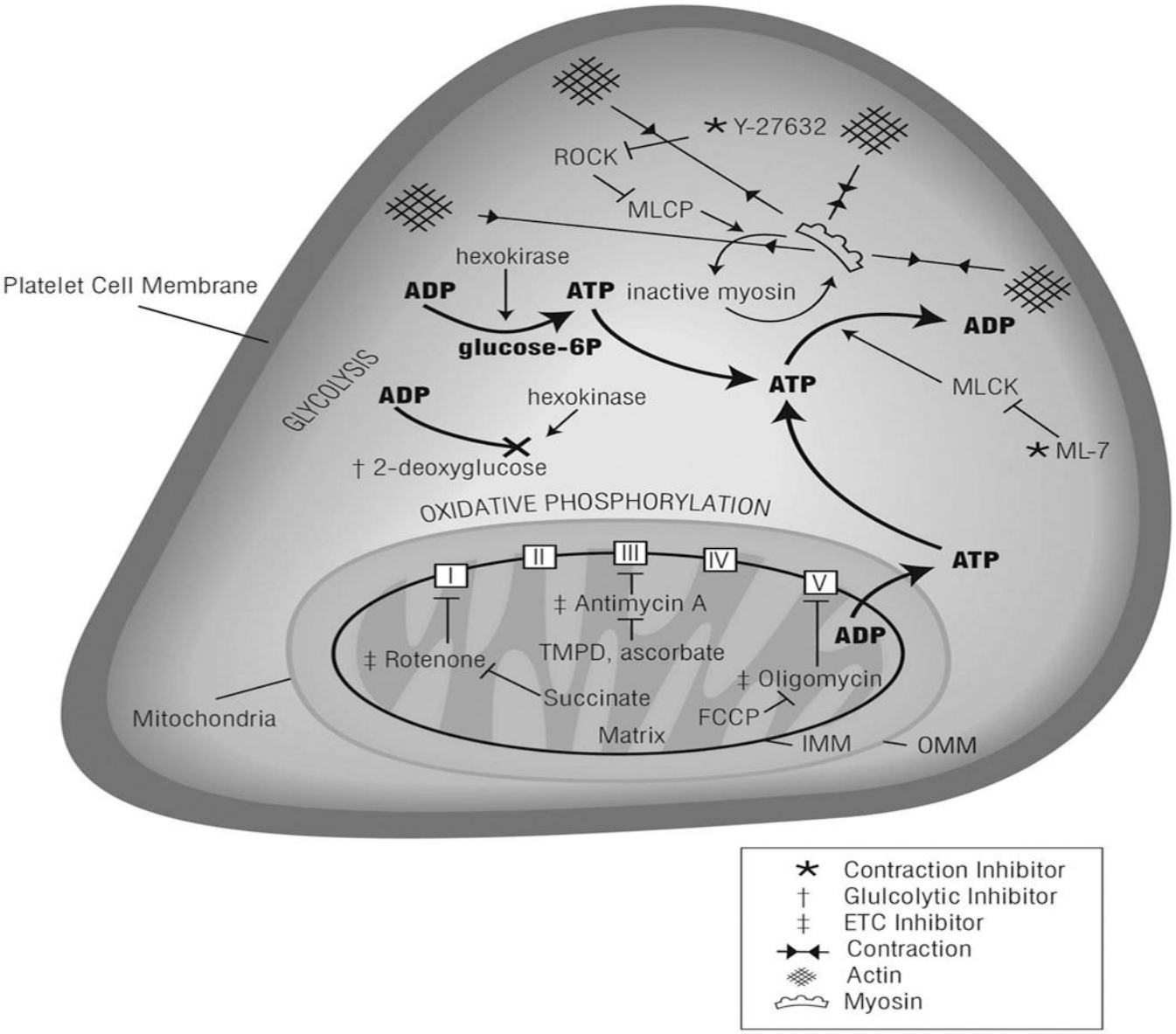

Figure 2.

Inhibitors to specific mechanical and metabolic pathways. Experimentally proven reversal agents exist for inhibitors of the electron transport chain like rotenone (succinate), antimycin A (TMPD, ascorbate), oligomycin (FCCP), and cyanide (glucose). 2-Deoxyglucose competitively inhibits the production of glucose via the glycolytic pathway. Y-27632 and ML-7 act to inhibit the actin–myosin contraction machinery.