Procoagulant Platelets: Not Just Full of Hot Air

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Abnormalities in coagulation are a leading cause of disease and death worldwide due to thrombotic events such as myocardial infarction, stroke, etc. It has been estimated that the cost of treatment of these disorders will rise to over 820 billion by the year 2030.\(^1\) At the center of thrombus formation is the platelet, a cell that is seen as the cornerstone of hemostasis and thrombosis. Platelets mainly function to secure hemostasis by acting as the “band-aid of the blood”. They are the first responders to sites of vascular injury, bringing with them a membrane surface that provides the “glue” for clot formation as well as a number of proteins essential for coagulation. Platelet involvement ultimately leads to thrombin generation and clot stabilization through fibrin formation.

The process of thrombus formation is highly orchestrated, but although much is known about the steps needed to complete the task, holes in our understanding still exist. At sites of vessel injury where the arterial wall shear rate is high, platelets adhere to von Willebrand factor via the glycoprotein (GP) VI receptor and collagen. Subsequently, platelet activation occurs and platelets aggregate together via the fibrinogen (GPIIb/IIIa) receptor that creates platelet/fibrinogen bridges, leading to thrombus formation.\(^2,3\) A subgroup of platelets, however, respond to collagen exposure without GPIIb/IIIa activation, and instead undergo a direct transformation from resting surveillance cells to active procoagulant cells. This process is characterized by an initial shape change that results in membrane asymmetry and resultant exposure of surface anionic phospholipids including phosphatidylserine (PS). Next, the platelets disintegrate, leading to shedding of procoagulant microparticles that provide further hemostatic support. The mechanism underlying this PS exposure is dependent on cystolic Ca\(^{2+}\) elevation via phospholipase-c-gamma and phosphatidylinositol 3-kinase signaling. The exposure of PS results in increased surface area, on which essential coagulation factors bind to become activated. The
platelet’s PS surface provides an ideal area for assembly of coagulation complexes including tenase (factors IXa and VIIIa) and prothrominase (factors Xa and Va) leading to efficient thrombin generation and subsequent fibrin formation.

It is thought that platelets exist in two states: a resting, discoid shape or an activated shape with characteristic blebbing structures on the outer surface of the platelet. Now, however, the identification of a discrete subpopulation of platelets with enhanced procoagulant activity has made it evident that there may be an intermediary stage in platelet activation. These platelets have been referred to by a number of different names including procoagulant platelets, coated platelets, platelets with sustained calcium-induced morphology, and balloon platelets. Regardless of the name used, they all share the common feature of a blebbing or balloon shape with sustained calcium influx and high PS surface exposure. These platelets are distinct from activated platelets in that the glycoprotein IIb/IIIa integrin is not engaged. The procoagulant quality of these platelets is solely dependent on the fact that they are able to bind more coagulation factors on their surface due to their enhanced surface area.

The role of the ballooning platelet’s shape in hemostasis has been reported previously. Heemskerk and colleagues demonstrated by phase contrast live-cell microscopy that platelets adherent to collagen generate balloon-like structures when exposed to extracellular Ca$^{2+}$, and that this change correlates with the development of procoagulant activity, thrombin generation, and exposure of negatively charged phospholipids (annexin V binding). $^{4,5}$ This was further solidified by Hess and colleagues who characterized the ultra-structural features of these ballooning platelets by electron microscopy. $^{6}$ The mechanism driving this transformation into a procoagulant surface, however, has not been well-studied, mainly due to the limited methods of investigation and poor microscopy imaging resolution. $^{7}$ In the manuscript by Agbani and
colleagues in this issue of Circulation, this process is more clearly elucidated.

Disturbances in procoagulant platelet activity are directly linked to clinically relevant bleeding disorders due to deficiencies in membrane cytoskeletal changes. One such disorder is Scott Syndrome, a rare inherited bleeding disorder that is characterized by impaired surface exposure of PS. This results in impaired thrombin generation due to the diminished surface area for coagulation factor activation. The main defect in platelets of patients with Scott Syndrome is impaired translocation of PS from the inner to the outer leaflet of the membrane on both erythrocytes and platelets. Not only do these patients have decreased platelet procoagulant activity, but they also have decreased production of PS-containing microparticles, which further disrupts hemostasis. The main cause of the hemostatic abnormalities seen in Scott Syndrome is not platelet aggregation or activation responses, but instead lack of fibrin deposition at the site of vascular injury because of disruption in PS exposure and subsequent lack of surface area for thrombin generation. The molecular mechanism underlying this platelet defect has been determined to be defective expression of the anoctamin-6 gene (Ano-6), which is an essential component of calcium-activated chloride channels which couple with Na+ nonselective cation channels to promote fluid influx. PS exposure is dependent on calcium-dependent scramblase activity through the formation of Ca2+-activated Cl– channels.

In this manuscript by Agbani and colleagues, the procoagulant activities of the platelet are characterized. They demonstrate that procoagulant platelets undergo distinctive cytoskeletal changes that define the balloon shape, enhance PS exposure, and ultimately lead to coagulation. Using 4D live cell microscopy imaging to define and visualize this dynamic process, they elucidate a process that was previously ill-defined. In addition, this manuscript defines platelet ballooning as distinct process apart from platelet blebbing; the balloon shape is the result of
disruption of the platelet microtubule cytoskeleton and an influx of fluid, and is dependent on Na\(^+\), Cl\(^-\) and water entry. They also demonstrate that the ballooning is linked to enhanced microparticle generation. To demonstrate the specificity of fluid entry in the process of platelet ballooning, they show that inhibition of Na\(^+\), Cl\(^-\), or water influx impairs ballooning, leading to disrupted procoagulant spreading and microparticle generation and ultimately leading to impaired thrombin generation. The authors demonstrate that there is an increase in internal hydrostatic pressure that results from a coordinated Na\(^+\), Cl\(^-\), and water entry, which leads to balloon inflation. This is distinct from other areas that are undergoing blebbing, which is not dependent on fluid entry. In addition, the authors show that the ballooned area is the essential area for microparticle release, another key component to maintaining hemostasis. They have identified and termed this particular population of platelets ballooned and procoagulant-spread, or “BAPS” platelets. BAPS platelets break up to form procoagulant microvesicles, and therefore increase the surface area of the PS-exposed membrane that supports procoagulant activity.

The authors delineate the mechanism for water influx by identifying the selective channel through which the water entry is regulated. Utilizing the defects identified in Scott syndrome, they linked this fluid entry to a mechanism of calcium entry resulting from defects in Ano-6. In Scott Syndrome, platelets lack Ano-6; the authors believe that this disrupts coagulation through functional changes in the platelet procoagulant response by decreased balloon formation, lack of PS exposure, and ultimately diminished microparticle release. Based on the authors’ findings, this lack of Ano-6 leads to inhibition of fluid entry due to disruption of Ca\(^{2+}\) entry and Na\(^+\), Cl\(^-\) shifts, leading to diminished platelet ballooning.

The notion of platelet ballooning, however, is not without controversy. One unique feature of these procoagulant platelets is that the processes of blebbing, PS exposure, and
microparticle generation resemble the process of cellular death. Others have hypothesized that platelets undergo the act of programmed cellular death to support the final stages of hemostasis. In addition, recent evidence suggests that platelet procoagulant activity occurs simultaneously with platelet death by necrosis; platelets with a morphology consistent with necrotic cell death (balloon-shaped) have been identified as undergoing necrotic cell death while simultaneously supporting coagulation and thrombin generation. Agbani and colleagues, however, have now provided microscopy-based visualization of platelets that demonstrates that the process of platelet ballooning is not merely a byproduct of programmed cell death, but instead an orchestrated process leading to enhanced procoagulant properties through dynamic changes in platelet water influx.

The implications of this enhanced understanding of procoagulant platelet activity are significant, and could lead to novel therapeutic manipulation of platelet shape change as a means of regulating hemostasis. As more patients are utilizing platelet-based therapies to decrease their risk of thrombotic events, it has become clear that regulating platelet activation is not enough to stop recurrent thrombotic events. Often patients are placed on dual platelet therapies with the hope that blockade of two pathways of platelet activation will yield better anti-thrombotic results. Yet, blocking platelet activation alone does not completely prevent platelet-dependent thrombosis. In addition, dual platelet blockade comes with increased risk of unwanted bleeding complications. Novel mechanisms that limit the role of platelets in coagulation by targeting distinct steps in thrombus formation beyond platelet activation may provide a more direct approach to anti-platelet therapy. Targeted inhibition of just the platelets that are active participants in thrombosis would spare nonparticipating platelets, allowing them to maintain their essential role in hemostasis without unwanted bleeding manifestations.
The mechanisms defining these procoagulant platelets suggest that regulation of salt and water influx could represent a novel target for drug therapies aimed at limiting thrombus formation and keeping platelets in a less activated state. This may represent a new area of investigation for anti-platelet therapies. Previous studies have documented that arterial thrombotic disease is associated with elevated levels of procoagulant platelets. Methods to identify patients with an increased level of these procoagulant platelets may represent a new biomarker for thrombotic risk. Also, targeting mechanisms that regulate Ca$^{2+}$ and water entry into platelets could directly prevent this platelet population from contributing to arterial thrombosis. Since these platelets are specifically found at sites of collagen exposure, this is also an exciting area of inquiry because it would enable us to directly target the platelets at the injury without impacting overall platelet function. Therefore, targeting procoagulant platelets could result in less bleeding complications and higher specificity in patients who require anti-platelet therapy.

Conversely, determination of the mechanism by which to increase procoagulant ballooning of platelets could also be utilized in patients with bleeding diathesis. Since these platelets are known to have high concentrations of clotting factors on their surface, determining the mechanisms by which resting platelets initiate ballooning may offer a therapeutic benefit to supplement hemostatic coagulation. If platelet surface area could be artificially “inflated” by triggering augmented ballooning, then better coagulation could be achieved in patients with bleeding diathesis.

Within the last decade, research has focused on the role of platelets in cardiovascular disease and thrombosis. Yet, as we have learned more about platelets, a better understanding of their role beyond hemostasis has emerged. Platelets have been shown to have roles in a myriad
of disease processes including inflammation, malignancy, and wound healing. As our understanding of the platelet’s role in disease has expanded, so too has our desire to find ways to manipulate platelet functionality. The work presented by Agbani and colleagues takes us a step further towards novel therapeutics aimed at regulating platelets not only by inhibiting activation but also by hindering their other procoagulant properties for anti-platelet drug development.

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**References:**


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