Platelet function under high shear conditions

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Summary
Blood platelets are the first line of defense against bleeding and as such involved in the haemostatic repair of damaged vasculature. Their true prowess seems to be displayed under high shear conditions where platelets interact with a variety of plasma proteins, all of which are tightly regulated to close the leak but at the same time prevent lumen occlusion and thromboembolism. The first task is to arrest fast flowing platelets on exposed collagen of the damaged sub-endothelial surface. Although platelets are endowed with several collagen receptors, most notably integrin α2β1 and the immunoglobulin superfamily member GPVI, they can not arrest platelets at high shear rates. The latter requires binding of the platelet receptor GPIbα to the A1-binding domain of von Willebrand factor (VWF), which first has to be immobilized from the flowing blood onto the site of injury. Under high shear conditions further accrual of newly arriving platelets again requires VWF, which has to bind to collagen to allow adhesive on the vessel wall is mainly plasma VWF, which has to bind to collagen to allow platelet arrest at high shear rates. This would make it more difficult for the platelets to finish the last percentages of occlusion, were they not staffed with an ability to adhere under extreme shear forces. The platelet membrane appears to attach via so called DAPs (discrete adhesion points), i.e. membrane areas of 0.05 to 0.1 μm² comprising approximately 75 to 100 GPIbα receptors that can oppose a force of 160 pN (1). Simultaneous engagement of the receptors would correspond to only 1.6 pN per single bond, and would diminish the risk of bond breakage or even extrusion of the receptor from the membrane. Such a distribution of high adhesive forces over multiple bonds may be a unique requirement for platelets, which must function at high shear stress. The DAPs may represent the structural unit for the coordination of multivalent GPIbα-VWF-A1 interactions. Platelet adhesion at high shear rates is supported by the properties of VWF molecules, which uncoil and stretch from their globular shape when exposed to shear rates of approximately 2000 s⁻¹, thereby presenting the A1-binding domains for the platelet GPIbα receptor (5, 6).

Platelet attachment under adverse shear conditions

Platelet attachment to thrombogenic surfaces above a shear rate of approximately 1500 s⁻¹ appears to be absolutely dependent on the platelet GPIbα receptor (1, 2). The necessary adhesive on the vessel wall is mainly plasma VWF, which has to bind to collagen to allow platelet arrest at high shear rates; for video footage of this phenomenon see also online supplemental material of (1–3). Another protein that could possibly substitute for VWF may be thrombospondin (4).

The unique task of the GPIbα-VWF couple is to enhance binding while the shear forces and thus the adverse conditions for adhesion increase. Elevated shear rates may not only be present under pathologic conditions such as severe stenosis in diseased arteries. When regular hemostasis closes a bleeding wound, the shear rate increases the more the deposited platelets narrow the orifice of the leak. This would make it more difficult for the platelets to finish the last percentages of occlusion, were they not staffed with an ability to adhere under extreme shear forces. The platelet membrane appears to attach via so called DAPs (discrete adhesion points), i.e. membrane areas of 0.05 to 0.1 μm² comprising approximately 75 to 100 GPIbα receptors that can oppose a force of 160 pN (1). Simultaneous engagement of the receptors would correspond to only 1.6 pN per single bond, and would diminish the risk of bond breakage or even extrusion of the receptor from the membrane. Such a distribution of high adhesive forces over multiple bonds may be a unique requirement for platelets, which must function at high shear stress. The DAPs may represent the structural unit for the coordination of multivalent GPIbα-VWF-A1 interactions. Platelet adhesion at high shear rates is supported by the properties of VWF molecules, which uncoil and stretch from their globular shape when exposed to shear rates of approximately 2000 s⁻¹, thereby presenting the A1-binding domains for the platelet GPIbα receptor (5, 6).

Platelet signaling and activation in flow

When perfused over immobilized VWF the binding of GPIbα to the VWF A1 domain elicits signals that may contribute to platelet activation (7). Once activated, platelets can bind permanently to VWF by engagement of their integrin αIIbβ3 receptors with the Arg-Gly-Asp sequence in the VWF C1 domain (8, 9). The signals, which include elevation of cytosolic Ca²⁺ and activation of protein kinase C and tyrosine kinases, can be detected under static conditions (10, 11), but may be particularly relevant for platelet activation under high shear stress conditions (12). In platelets, a first rise in Ca²⁺ released from intracellular stores is designated an α/β peak and occurs during the initial platelet contacts with immobilized VWF that are still potentially reversible and always precede stable platelet attachment. A second Ca²⁺ signal, designated a γ peak, occurs in platelets firmly attached via the integrin αIIbβ3 and appears to be required for subsequent aggregation with fibrinogen acting as ligand. Type γ peaks involve a trans-membrane Ca²⁺ flux. A sustained influx of extracellular Ca²⁺ occurs by a mechanism known as store-operated Ca²⁺ entry (SOCE) (13, 14).

Recently, stromal interaction molecule 1 (STIM1) was identified as a calcium sensor that mediates SOCE and is critical for platelet function (15), mainly by contributing to stable thrombus formation under flow conditions (16). The SOC channel moiety, Orai1, in platelet SOCE is strongly expressed in human and mouse platelets and is essential for thrombus formation in vitro and in vivo.(17) Orai1 appears to be crucial for the platelets pro-coagulant response rather than for other Ca²⁺-dependent cellular responses (18).

Activation-independent platelet aggregation at high shear rates

A new mechanism of platelet aggregation has been described that does not necessitate prior platelet activation (19). At shear rates exceeding 10 000 s⁻¹ non-activated platelets can interact with soluble VWF and thus form aggregates. At this shear rate threshold and above, active A1 domains in soluble VWF
multimers are exposed and allow the binding of platelet GPIbα and additional platelet recruitment without stimulation of platelets. This process appears to occur even in the vessels of live animals (19). Aggregates thus formed are unstable, whereas above shear rates of 20 000 s⁻¹ platelets adherent to immobilized VWF are stretched into elongated structures and become the core of aggregates that can persist on the surface for minutes. This synergy between platelets and VWF (soluble as well as immobilized) may be a key determinant of platelet accumulation in disease as well as normal hemostasis. Rapid initial deposition of a large number of unactivated platelets on lesion sites exposed to extremely high shear stress may occur at a bleeding wound and a ruptured atherosclerotic plaque alike. Stabilization of such deposited aggregates may occur after signaling dependent integrin activation, ligand binding, and fibrin formation.

**Platelet thrombus propagation under flow**

The time course of the platelet response to injury is influenced by the composition of the reactive surface exposed to flowing blood as well as soluble platelet agonists such as ADP, epinephrine, and α-thrombin. Synergy between agonists and collagen receptor activation may greatly enhance the efficiency of the haemostatic response. Indeed, irreversible platelet adhesion to collagen type I is essentially instantaneous even at the highest levels of shear stress, and aggregation occurs within seconds (20) (also own unpublished results). Successive layering of activated platelets to a vascular lesion is secured by immobilization of soluble adhesive proteins, mainly fibrinogen and VWF, onto the cell membranes of adherent platelets (19, 21). Activation of the newly recruited platelets continues the process until the thrombus mass grows sufficiently to arrest bleeding from an injured vessel or, in pathologic conditions, until the vascular lumen is occluded.

Thus, a thrombus is constituted essentially of platelets linked to one another with a firm basis of spread platelets, which has been reported for collagen substrates as well as for atherosclerotic plaque material and has been visualized under blood flow conditions (3, 22). Fibrinogen bound to activated integrin αIIbβ3 is generally considered the predominant adhesive bridge between aggregating platelets (23, 24), although a role for P-selectin and other ligands has been described (25, 26). At arterial shear rates above 1500 s⁻¹ a synergy may exist between fibrinogen and VWF in supporting platelet aggregation on collagen surfaces (26). Thrombus in the absence of fibrinogen have been seen in the mouse circulation (27). Apparently, neither protein – fibrinogen or VWF – by itself can sustain the full development of stable thrombi within the range of physiologically relevant flow conditions. Thrombus formation may persist even in the absence of both fibrinogen and VWF (27), and fibronectin and CD40L may support thrombus stability (28, 29).

An explanation for how shear stress can facilitate platelet thrombus formation while simultaneously limiting thrombus growth has been presented by two recent studies where the investigators examined the contribution of ADAMTS-13 activity to the growth of platelet thrombi at different shear stresses. Not only controlled ADAMTS-13 the VWF-reactivity with platelets by reducing the size and reactivity of ultra-large VWF forms, it also cleared VWF multimers forms normally found in plasma and participating in the buildup of thrombi. Their findings suggest a mechanism that allows repair of vessel wall defects without the repair process completely occluding the vessel (21, 30).

### Shear-induced platelet derived microparticles

Several mechanisms have been described for the formation of platelet derived microparticles (PMP), e. g. biochemical stimulation via collagen and thrombin (31) or biomechanical processes (32). Recently one of the possible biophysical mechanisms has been visualized under blood flow conditions in real-time (1). Under high shear conditions platelets attach to immobilized VWF via discrete adhesion points (DAPs). Although adherent platelets withstand the tensile force resulting from the hydrodynamic drag, this can, at the same time, create a pulling force on the platelet membrane. Thereby elongated membrane tubes, so-called tethers, as well as microparticles can form through cell-membrane disruption. Microparticles have been described to adhere at physiological shear rates (33) to

- collagen type I,
- fibrinogen,
von Willebrand factor and surface immobilized platelets.

On thrombogenic surfaces this biomechanical mechanism may generate and localize procoagulant and adhesive cell structures too small to be removed even under extreme flow conditions (32).

The same mechanism leading to platelet microparticle formation at an immobilized VWF surface may take place when VWF is bound to the membrane of platelets exposed to fluid shear stress in suspension (1). Such a conclusion was supported by the observation that the shear rate threshold for PMP formation, between 6000 s⁻¹ and 10000 s⁻¹, is essentially the same whether platelets adhere to immobilized VWF or to one another in the absence of aggregation. In either situation, the mechanical shearing of PMPs was passive, independent of calcium and platelet activation and aggregation, completely dependent on the interaction of GPIbα with VWF and not integrin αIIbβ3, and distinct from calcium dependent proteolytic shedding. Flow cytometry and electron microscopy defined the size of the microparticles generated and visualized under flow with a diameter of 0.05 to 0.1 µm (Fig. 1). The PMPs retained an intrinsic adhesive function and supported thrombin generation, either due to their expression of phosphatidylserine and/or tissue factor, which was detected at low levels on platelets and PMPs. Contrary to others, no evidence for a monocytic origin of the TF was found (31, 34).

References


