NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Microcirculation. Author manuscript; available in PMC 2011 March 15.

Published in final edited form as:
Microcirculation. 2009 January ; 16(1): 58–83. doi:10.1080/10739680802651477.

Platelet Adhesion under Flow

Zaverio M. Ruggeri
Roon Research Center for Arteriosclerosis and Thrombosis, Division of Blood Cell and Vascular Biology, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA

Abstract

Platelet adhesive mechanisms play a well-defined role in hemostasis and thrombosis, but evidence continues to emerge for a relevant contribution to other pathophysiological processes including inflammation, immune-mediated responses to microbial and viral pathogens, and cancer metastasis. Hemostasis and thrombosis are related aspects of the response to vascular injury, but the former protects from bleeding after trauma while the latter is a disease mechanism. In either situation, adhesive interactions mediated by specific membrane receptors support the initial attachment of single platelets to cellular and extracellular matrix constituents of the vessel wall and tissues. In the subsequent steps of thrombus growth and stabilization, adhesive interactions mediate platelet to platelet cohesion (aggregation) and anchoring to the fibrin clot. A key functional aspect of platelets is their ability to circulate in a quiescent state surveying the integrity of the inner vascular surface, coupled to a prompt reaction wherever alterations are detected. In many respects, therefore, platelet adhesion to vascular wall structures, to one another or to other blood cells are facets of the same fundamental biological process. The adaptation of platelet adhesive functions to the effects of blood flow is the main focus of this review.

Keywords

Hemostasis; Thrombosis; Vascular Biology; Extracellular Matrix; Collagen

Physiopathological significance of platelet adhesion

Mammalian platelets are fragments of cytoplasm released into circulating blood by megakaryocytes, a precursor hematopoietic cell residing in the bone marrow [1,2]. Without a nucleus, platelets have no control on gene expression but possess a translational machinery that can direct protein synthesis [3]. The physiological role of platelets consists in a fundamental contribution to hemostasis, the defense mechanism that prevents blood loss when the continuity of the vascular tree is interrupted by traumatic injury to tissues [4,5]. To exert their function, platelets survey the vascular system and adhere where alterations of the endothelial cell lining, accompanied or not by exposure of subendothelial matrix components, are detected [6]. More recent studies have highlighted a different and equally important host defense role of platelets, namely the ability to maintain normal vascular permeability and direct other effector cells to sites where microbial and viral pathogens induce inflammatory responses [7]. As evidenced in a mouse model of infection by lymphocytic choriomeningitis virus, a reduced platelet count of 10–20% of normal is sufficient to maintain a normal hematocrit and permit viral clearance dependent on antigen-specific cytotoxic T-lymphocytes. Spontaneous hemorrhage, mostly mucocutaneous in

Correspondence to: Zaverio M. Ruggeri, MD The Scripps Research Institute Mail drop: MEM 175 10550 North Torrey Pines Road, La Jolla, California 92037 Telephone: 858-784 8950 Facsimile: 858-784 2026 ruggeri@scripps.edu.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

nature, and death develop when platelets are further reduced in number to ~1% of normal or their function is pharmacologically inhibited. These findings suggest that platelets interacting with the inner vessel lining provide an essential control over vascular permeability. Because this activity is maintained with a low number of platelets, if they can be normally activated, and is independent of coagulation function [7], it is likely not mediated by the formation of thrombi, i.e. the platelet aggregates with clotted fibrin that seal broken vessel. Indeed, histological evaluation of infected mice that developed acute anemia demonstrated diffuse red cell extravasation around healthy looking skin capillaries and microvessels, suggesting that normal permeability may be maintained by transient interactions of single layers of platelets with the endothelial cell lining. Local platelet adhesion to virus-induced vascular alterations may also be the cue for the recruitment of cytotoxic T-lymphocytes required for clearing the infection. In essence, the adhesive properties of platelets contribute to maintaining vascular integrity and defending from external pathogens, thus playing a role that goes beyond the sealing of ruptured vessels and provides a link between the fundamental processes of hemostasis and inflammation [8,9]. In this regard, mammalian platelets function like the nucleated thrombocytes found in all other vertebrates [10–12].

Adherent platelets can rapidly recruit to the site of injury additional platelets, necessary to achieve hemostasis, or different types of leukocytes, which set off host defense responses. Such selective recruitment is orchestrated by activation pathways [13] stimulated by the initial adhesive interactions and by soluble agonists released or generated locally, which lead to the appearance on the platelet membrane of different molecules capable of attracting distinct circulating cells. All these functions depend on regulated adhesive properties and, as they develop in flowing blood, are influenced by fluid dynamic parameters. Platelets and the hemostatic system, however, cannot discriminate between alterations of the vessel wall caused by traumatic wounds and those resulting from pathologic processes. As a consequence, chronic vascular diseases can acutely induce massive platelet responses, usually initiated by the occurrence of destabilizing events such as the sudden rupture of an atherosclerotic plaque, which lead to the formation of intravascular occluding aggregates and fibrin clots (thrombi) [14]. Under such circumstances, the same processes that underlie the beneficial hemostatic function become a life-threatening disease mechanism that, by curtailing the supply of blood to vital organs, may cause ischemic syndromes of the heart and brain leading to death. In a similar fashion, inflammatory signal originating from pathological processes within organs may induce local platelet reactivity and thus attract cytotoxic T-lymphocytes capable of causing cell death and tissue damage. Such a platelet-mediated immunopathogenetic mechanism has been documented in a mouse model of viral hepatitis, in which platelet depletion prevents the destruction of hepatocytes expressing a specific hepatitis B virus antigen [15,16]. It is apparent, therefore, that the adhesive properties of platelets are of key relevance for essential host defense mechanisms but may also turn into mediators of serious illness, suggesting that anti-platelet therapy, now well established in the treatment of ischemic coronary artery disease, will likely be considered in the future for novel applications in acute and chronic inflammatory and infectious diseases [17].

Blood flow as a modulator of platelet adhesive functions

Fluid dynamic parameters influence platelet adhesion and aggregation

The adhesive properties of platelets support attachment to extracellular matrix (ECM) components or to the membrane of other cells, as well as homotypic intercellular contacts that link platelets to one another during thrombus growth. The latter process is designated as platelet aggregation and is often treated as separate from adhesion. The main reason for this dichotomy can be traced to the different experimental conditions typically used to study the

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

two phenomena *ex vivo*. Historically, aggregation has been evaluated with platelets in suspension in the absence of interactions with immobilized substrates other than on the platelet membrane, thus with continuous motion in a stirred fluid and poorly defined fluid dynamic parameters. Moreover, under such conditions aggregation can only follow platelet activation by an appropriate agonist. With respect to function *in vivo*, however, both adhesion and aggregation involve the transition from free flow to arrest onto a surface, a step that likely precedes activation under most situations, and may even depend on the same adhesive ligand and receptor pairs. In the case of adhesion, the surface onto which flowing platelets are captured is the vessel wall and surrounding tissues, and the adhesive substrates are endogenous matrix or cell membrane proteins and proteoglycans along with locally bound selected plasma components. In the case of aggregation, the surface is the membrane of other platelets that have already become immobilized at sites of thrombus formation and present membrane-bound adhesive substrates, either following activation-induced translocation from internal storage compartments or bound from plasma. An important consideration is that, by virtue of the mechanistic similarities, the same fluid dynamic constraints influence both platelet adhesion and aggregation.

Rheological properties of blood

Platelets are components of blood and perform their functions in an environment characterized by constant fluid motion. The function of blood is transport - of cells, chemicals, heat - to all areas of an organism, and motion (circulation) is the very essence of such a function. In the specific case of platelets, circulation is fundamental for surveying the integrity of all the conduits within the vascular tree. The effects of different flow regimens on thrombus formation are well recognized, for example by the fact that thrombi developing in the arterial side of the circulation – where flow velocity is higher - tend to be rich in platelets (white thrombi), while those developing in the venous side - characterized by a lower flow velocity - contain more red cells within a fibrin clot with relatively few platelets (red thrombi) [18]. Blood is a viscous fluid suspension constituted by a continuous part - plasma - and a suspended component - cells - that may become separated by the action of forces such as gravity or shear stress [19,20]. In flowing blood, gravity has a negligible effect and cells tend to move towards areas of higher fluid velocity. In a normal vessel containing blood with normal cell counts, erythrocytes represent most of the circulating cellular mass. As a consequence of lateral migration and erratic motion, they occupy the greatest part of the lumen [21,22], pushing white blood cells and platelets to the sides and creating a near wall platelet excess [23–25]. Interactions between erythrocytes mediated by fibrinogen tend to increase viscosity and are the main reason for the deviation of blood from the ideal behavior of a Newtonian fluid at low shear rates [26]. By definition, a Newtonian fluid is one that has constant viscosity at all shear rates, i.e. irrespective of velocity fields. Higher shear forces tend to break these interactions, thus at shear rates above 200 s^{-1} the behavior of blood can be considered Newtonian [21,27].

Blood flow is caused by the pump action of the heart and is driven by pressure differences and counteracting boundary movements due to the elastic properties of the vessel wall. The velocity profile of an incompressible Newtonian fluid moving steadily in a long cylindrical tube of constant cross-sectional area is parabolic (Poiseuille flow), meaning that at a given flow rate the fluid velocity is maximal in the center of the tube lumen and decreases towards the wall as a parabolic function of the radial distance from the central axis (Fig. 1). Consequently, the fluid can be imagined as divided in a series of adjacent layers (laminae or planes) moving at different velocity relative to one another (laminar flow). The velocity differential dependent on the distance from the center of the lumen creates the shearing flow, namely the sliding motion between two adjacent planes moving at different speed. Shear rate (γ) is the difference in flow velocity as a function of distance from the wall and is

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

expressed in cm/s per cm or the equivalent inverse second (s^{-1}). Shear stress (τ) is the resulting tangential force per unit area of contact between laminae resulting primarily from pressure differences within the vascular system, and represents the determinant cause of the shearing motion. The unit of shear stress in the International System of Units (SI) is the pascal (Pa), equivalent to one newton/m^2 (N/m^2), and in the CGS (centimeter/gram/second) system is the dyn/cm^2 (1 Pa = 10 dyne/cm^2). In ideal Newtonian fluids, the relationship between shear stress and shear rate is linear and the corresponding proportionality constant (τ/γ) defines the viscosity of the fluid (μ), which is expressed in Pa⊕s or dyne/cm^2⊕s, the latter equivalent to poise (P); thus, 1 P = 0.1 Pa⊕s. In blood, with a viscosity of ~3–4 millipascal (mPa)⊕s or ~3–4 centipoise (cP), a shear rate of 25 s^{-1} causes a shear stress of ~1 dyne/cm^2 (0.1 Pa). The highest wall shear rate in the normal human circulation, estimated to vary between 500 and 5,000 s^{-1} (corresponding to a shear stress of ~20–200 dyne/cm^2), has been estimated to occur in arterioles of ~50 μm diameter [28].

Although blood exhibits a pulsatile (not-steady) flow in arteries and a non-Newtonian behavior at lower (venous) shear rates, the principles of Poiseuille flow outlined above can be reasonably applied to events in the circulation. As mentioned above, in a flow field with high shear rate erythrocytes tend to move towards the center of the vessel creating a cell-depleted plasma sleeve near the wall [29,30] that can be considered as an ideal Newtonian fluid [31]. This layer of blood flows more rapidly than it would in a homogeneous liquid, such that the resulting velocity profile is not parabolic but more blunted (Fig. 1). The near-wall platelet excess facilitates adhesive interactions with the vascular surface. In fact, blood perfusion over immobilized von Willebrand factor (VWF) at relatively high shear rate results in efficient surface coverage by adherent platelets even when the blood platelet count is decreased by up to 90%, and only a more drastic reduction limits the extent of adhesion (Fig. 2). Such experimental findings confirm with functional evidence the existence of a near-wall platelet excess and explain the clinical observation - as well as the results in a mouse model of viral infection [7] that bleeding, particularly spontaneous, is usually not a serious problem until the platelet count drops below ~10,000/μl [32]. In fact, while formation of the large platelet aggregates required to seal post-traumatic wounds in vessels may be affected by even a relatively small reduction in platelet number, the platelet monolayer required to ensure control of vascular permeability (thus prevent spontaneous hemorrhage) is largely undepleted until the platelet counts drops significantly (Fig. 2). By the same token, since the near-wall platelet excess results from the presence of the erythrocyte mass, it is clear why anemia (low erythrocyte number) may predispose to excessive bleeding [33].

Differential effects of shear rate and shear stress on platelet adhesive interactions

Circulating platelets and the vessel wall can be viewed as two juxtaposed surfaces one of which the platelet membrane - is moving while the other is stationary. Molecules on the two surfaces are separated by a layer of viscous fluid, the plasma, and cannot interact as long as the separation distance is greater than 100 nm. As this decreases, long-range forces including electrostatic interactions become stronger, and at 10 nm or less proper intermolecular bonds can form. The minimum distance compatible with an adhesive receptor-ligand interaction depends also on the length of the molecules involved and on the position of the reacting sites. The question of what forces can bring two surfaces in close juxtaposition, and how, has not yet been fully answered with definitive quantitative and theoretical approaches, but deviations from perfect planarity appear to be important for enhancing bond formation across surfaces. Once a flowing platelet is at a “latching distance” from reactive sites on the vessel wall, membrane receptor binding to adhesive ligands occurs if the corresponding intrinsic association rate is faster than the relative velocity at which the molecules move with respect to each other. In other words, binding must be established

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

while the two molecules are sufficiently close to interact, a time that shortens as the velocity of platelets, i.e. the shear rate, increases. As a consequence, cell recruitment onto the surface decreases with increasing shear rate. Shear stress, in contrast, influences the lifetime of an adhesive bond once formed, thus the off rate of the interaction, resulting in detachment of adherent cells with increasing shear stress [34]. Different pathways of platelet adhesion are variably affected by shear force depending on the biomechanical properties of each receptor-ligand pair. Above a threshold shear rate of 500–800 s^{-1} in human blood [35,36] and 2,000–5,000 s^{-1} in mouse blood [37], under controlled experimental conditions, only the interaction between immobilized VWF A1 domain (VWF-A1) and membrane glycoprotein (GP) Ibα has a sufficiently fast on-rate to initiate platelet adhesion [38]. The higher threshold in the mouse is likely the consequence of a smaller platelet size, which reduces the drag on the particle for a given shear stress. Thrombus development alters the hemodynamic conditions by restricting the lumen through which blood flows. To maintain the same volumetric flow rate (i.e., the volume of blood transported per unit time) in spite of the restriction, the velocity of flow must increase resulting in higher shear rate and stress. This explains why shear rates in excess of 20,000–40,000 s^{-1} develop at or just upstream of a severe stenosis in a human atherosclerotic coronary artery [39–41]. For the same reason, the shear rate on the membrane of immobilized platelets exposed to flowing blood at the surface of an arterial thrombus is progressively higher as the protrusion into the vessel lumen increases. Thus, the fluid dynamic constraints influencing the adhesion of single platelets to the vessel wall affect also the recruitment of circulating platelets into a growing thrombus, as demonstrated by the required function of GP Ibα and VWF-A1 to support platelet aggregation above a threshold shear rate (Fig. 3). It is important to note that the threshold discussed here is not a minimum shear rate value to engage the function of immobilized VWF-A1, which can mediate platelet tethering even under venous slow flow conditions [38]; rather, it is an upper limit for the function of most other platelet adhesive bonds in the absence of VWF. variably affected by shear force depending on the biomechanical properties of each receptor-ligand pair. Above a threshold shear rate of 500–800 s^{-1} in human blood [35,36] and 2,000–5,000 s^{-1} in mouse blood [37], under controlled experimental conditions, only the interaction between immobilized VWF A1 domain (VWF-A1) and membrane glycoprotein (GP) Ibα has a sufficiently fast on-rate to initiate platelet adhesion [38]. The higher threshold in the mouse is likely the consequence of a smaller platelet size, which reduces the drag on the particle for a given shear stress. Thrombus development alters the hemodynamic conditions by restricting the lumen through which blood flows. To maintain the same volumetric flow rate (i.e., the volume of blood transported per unit time) in spite of the restriction, the velocity of flow must increase resulting in higher shear rate and stress. This explains why shear rates in excess of 20,000–40,000 s^{-1} develop at or just upstream of a severe stenosis in a human atherosclerotic coronary artery [39–41]. For the same reason, the shear rate on the membrane of immobilized platelets exposed to flowing blood at the surface of an arterial thrombus is progressively higher as the protrusion into the vessel lumen increases. Thus, the fluid dynamic constraints influencing the adhesion of single platelets to the vessel wall affect also the recruitment of circulating platelets into a growing thrombus, as demonstrated by the required function of GP Ibα and VWF-A1 to support platelet aggregation above a threshold shear rate (Fig. 3). It is important to note that the threshold discussed here is not a minimum shear rate value to engage the function of immobilized VWF-A1, which can mediate platelet tethering even under venous slow flow conditions [38]; rather, it is an upper limit for the function of most other platelet adhesive bonds in the absence of VWF.

Substrates and receptors for platelet adhesion to the vessel wall

Platelets circulate close to the endothelial cell lining of the vascular wall without displaying any stable interaction, mainly because endothelial cells actively maintain an anti-adhesive

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

phenotype [42,43]. Perturbation of this normal equilibrium, caused either by mechanical disruption of vascular integrity (wound) or pathogenic stimuli, promptly leads to platelet adhesion onto the injured site. The interaction with the vessel wall, including endothelial cells, smooth muscle cells, fibroblasts and ECM components, is the key initiating event in all host defense activities of platelets as well as in the pathological processes in which they are involved. The response to vascular injury is contingent on the nature of the lesion. Depending on the matrix proteins exposed to blood and the hemodynamic conditions, platelet adhesion requires the synergistic function of different receptors ultimately leading to activation and aggregation. A brief description of the main interactions that support the adhesive properties of platelets in flowing blood is presented below, followed by a more extensive analysis of the platelet-VWF interaction as the uniquely relevant pathway for adhesion under elevated shear stress.

Platelet adhesion to collagen

Different collagens are present in the vessel wall. Of these, types I, III, and VI are known to support platelet adhesion and aggregation [44]. Several putative collagen-binding proteins have been identified on platelets, including the integrin α2β1 (GP Ia–IIa in the pre-integrin era) [45–47], GP VI [48,49], GP IV (CD-36) [50], and the 65 kDa protein (p65) reportedly specific for type I collagen [51]. Of the latter two, however, GP IV can be absent from platelets [52], as in 5% of Japanese, without affecting platelet function [53] and hemostasis [54], and no confirmed information has followed the original report on p65 existence. Thus, only α2β1 and GP VI have an undisputed potential role in collagen-mediated platelet adhesion and aggregation, but their relative importance remains debated [6,55–57]. Conflicting opinions supported by results obtained with genetically manipulated or pharmacologically treated mice may be explained by factors that are only beginning to emerge, such as the role of modifier genes controlling the expression of proteins with still ill-defined effects on thrombus formation. One cogent example of this is represented by he identification of the Modifier of Hemostasis (MH) locus on mouse chromosome 4, which influences the prothrombotic and hemostatic phenotype in GP VI-deficient mice [58]. Moreover, native collagen is an insoluble protein whose thrombogenic activity is likely influenced by multiple interactions with other matrix components in a complex supramolecular assembly. Collagen preparations used in *ex vivo* experiments undergo manipulations that alter the structurally integrity present in the matrix, with poorly understood and, possibly, underestimated consequences on the interaction with platelet receptors. For example, acid-insoluble fibers display a characteristic banded pattern due to the regular staggering of collagen monomers [44,59], while pepsin-solubilized microfibrils lack this pattern and form spiral structures [60]. Spiraled collagen formed by fibrils in helical assembly has been found in both normal and pathological vascular tissues, and may result from the degradation of fibers by matrix metalloproteases (MMP), particularly MMP-1 [61]. When immobilized, all these substrates are thrombogenic but elicit platelet adhesion and aggregation through different mechanisms. This is best illustrated by the distinct consequences of blocking one of the platelet collagen receptors, the integrin α2β1, which impairs thrombus formation on spiral microfibrils but not on banded collagen fibers [60]. In contrast, blocking the other receptor, GP VI, impairs thrombus formation on all types of collagen fibers [62,63]. At the present state of knowledge, it seems reasonable to assume that both α2β1 and GP VI play a physiopathological role in mediating platelet interactions with collagens. In the future, it should be possible to define clearly how the relative function of these two receptors may vary as a result of differences in the composition of the thrombogenic surface exposed to flowing blood at a site of vascular injury, in turn dependent on the causing trauma or underlying disease process.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Platelet adhesion to fibrinogen/fibrin

Fibrinogen is present in blood as a soluble protein and a releasable pool is also contained within the α-granules of platelets, but it is not a normal constituent of ECM. As such, fibrinogen is required for normal platelet aggregation (see below) but may not contribute to the first localization of platelets onto an injured vascular surface. Nonetheless, in experimental conditions, immobilized fibrinogen is a substrate for platelet arrest under flow, selectively mediated by αIIbβ3 in the conformation present on nonactivated platelets [38]. Fibrin, the final product of the coagulation process, is a cross-linked insoluble polymer of fibrinogen that is essential for the definitive consolidation of platelet aggregates in thrombi but also retains the ability to support platelet adhesion. Thus, fibrin deposited onto injured vascular surfaces may become a substrate for initial platelet adhesion, and in so doing may synergize with immobilized VWF bound to its GP Ib receptor [64]. Because fibrin is rapidly generated in vessels with low shear rate, its contribution to initial platelet adhesion may be more relevant in the venous than arterial circulation. In perfusion systems, platelets adhere to purified fibrinogen and fibrin in a shear rate-limited fashion, with decreasing efficiency up to a limit of 1,000–2,000 s^{-1} [38]. Of note, adherent platelets activate and spread on surfaces coated with immobilized fibrinogen/fibrin, but only small thrombi form in the absence of other adhesive substrates.

Platelet adhesion to fibronectin

Fibronectin is an essential adhesive substrate in many fundamental biological processes [65]. Platelets possess two main receptors for this protein, α5β1 and αIIbβ3 [66], of which the latter requires activation in order to function [67]. It has been known for some time that α5β1 supports platelet adhesion to endothelial ECM [36], and more recently direct evidence has been presented that fibronectin may contribute to thrombus formation. A key observation has been that platelets from mice deficient in both VWF and fibrinogen can form thrombi at sites of vascular damage [68]. Typically, in these animals there is no occlusion at the site of lesion, which can be explained by the required role of VWF in an area of increasing shear rate, but platelet aggregates are unstable and embolize causing downstream blockage of flow. In contrast, mice that lack αIIbβ3 are unable to form thrombi under the same conditions [69], suggesting that a β3 ligand different from VWF and fibrinogen can contribute to platelet cohesion. A candidate for this function has been identified by demonstrating that mice with a conditional depletion of plasma fibronectin exhibit a delayed thrombus growth and decreased stability of platelet aggregates [70], suggesting that fibronectin can synergize with VWF and fibrinogen in supporting inter-platelet cohesion through activated αIIbβ3. This apparently satisfactory explanation is challenged by a recent report that highlights the complexity of the issue. In fact, mice with a triple deficiency of VWF, fibrinogen and plasma fibronectin, which would be expected to have a very marked diminution of thrombus formation, display a less severe abnormality than mice with the double deficiency of VWF and fibrinogen [71]. The conclusion supported by these new findings is that plasma fibronectin is not essential for VWF/fibrinogen-independent thrombus formation; rather, soluble plasma fibronectin appears to be an inhibitory factor for platelet aggregation. To address the contradiction at hand - plasma fibronectin deficiency causes unstable thrombi when isolated [70], but ameliorates the defect of VWF/fibrinogen-deficient mice [71] – the suggestion has been made that plasma (soluble) fibronectin may inhibit the function of pro-adhesive αIIbβ3 ligands, but after transitioning to insoluble ECM-like fibrils might support thrombus formation [71].

The above-mentioned hypothesis is not yet proven and still cannot explain while the switching of plasma fibronectin from inhibitory to supportive of thrombus formation would occur differently in wild type as opposed to VWF/fibrinogen-deficient mice. It is known, however, that soluble plasma fibronectin can assemble into fibrillar networks on the surface

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

of fibroblasts, platelets [72] and, possibly, other cells, thus providing a substrate that can contribute to the stable attachment of platelet aggregates [73]. Fibronectin assembled into fibrillar structures may also support initial platelet adhesion, either directly or indirectly by association with collagen and/or VWF. In experimental conditions *ex vivo*, purified fibronectin is a rather inefficient substrate for platelet adhesion [74], and this may reflect the importance of supramolecular assembly with other ligands for the synergistic expression of adhesive function. Of note, a fibronectin isoform containing Extra Domain A (EDA), which is absent from normal plasma but elevated in several pathological conditions, has been found to accelerate thrombus formation both in perfusion experiments *ex vivo* and in mouse models of vascular injury [75]. Its significance as a risk factor for thrombotic disease in humans remains to be established.

Platelet adhesion to thrombospondin

Thrombospondins are a family of adhesive proteins [76], of which thrombospondin-1 is contained in platelet α-granules. After activation-induced secretion, thrombospondin-1 binds to the platelet membrane and mediates adhesion [77]. The significance of thrombospondin-1 in platelet thrombus formation is still unclear, although it is intriguing to note that this glycoprotein is abundant in atherosclerotic plaques. In experimental models, immobilized thrombospondin-1 has been shown to support stable platelet attachment up to a shear rate of 4,000 s^{-1}, a value typically associated with the participation of VWF in the process. Adhesion to thrombospondin-1, however, was proven to be independent of VWF albeit mediated by GP Ib with a minor contribution by GP IV (CD36) only when platelets were activated [78]. The suggestion that thrombospondin-1 may mediate platelet adhesion under arterial flow conditions in lieu of VWF remains to be verified.

Thrombospondin-2 is a relevant constituent of ECM but is not present in platelets; nonetheless, its absence has been associated with a congenital hemostatic defect in mice [79]. The extent to which this abnormality is directly associated with impaired adhesion is not clear, and alternative explanations are possible as megakaryocytes from thrombospondin-2 deficient mice produce platelets that are not fully activated by agonist stimulation [80]. This may exemplify a mechanism for an indirect effect on platelet thrombus formation caused by an adhesive molecule required during megakaryocytic maturation and/or thrombocytopoiesis.

Platelet adhesion to laminin

Different forms of laminin are highly expressed in the subendothelial ECM, and when exposed to blood are potential substrates for platelet adhesion. The subendothelial forms are laminins 8 (α4β1γ1) and 10 (α5β1γ1). Platelets also contain and secrete upon activation laminins 8 and 10, as well as laminin 11 (α5β2γ1) [81]. It has been reported that platelets can adhere to laminin [82] but are not directly activated following the interaction [81]. Other investigators, however, found that human laminin stimulates formation of filopodia and lamellipodia in human and mouse platelets through an adhesion/activation pathway that involves the integrin α6β1, a well-known laminin receptor, and GP VI. Adhesion to laminin appears to depend on α6β1 with no required contribution by GP VI, while the latter is necessary for the formation of lamellipodia but not filopodia [83]. These findings delineate a synergistic role for α6β1 and GP VI as laminin receptors, coupled to distinct activation pathways convergent on the function of the Syk tyrosine kinase [83]. The relative contribution of adhesion to laminin in platelet thrombus formation remains to be delineated.

Platelet adhesion to other ECM components

Other ECM components have shown the potential of interacting with platelets, but their contribution to the mechanisms of adhesion and thrombus formation is still uncertain.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Among these are fibulin [84] and vitronectin [85]. After the initial interest [86], limited confirmatory work has been published on fibulin and the uncertainty about its role in relation to platelet function persists. The contribution of vitronectin to thrombus formation is also controversial, since both inhibitory and supportive roles have been reported, but recent evidence obtained in vitronectin-deficient mice supports a clearer definition of the issue [87]. In *ex vivo* studies, the thrombin-induced aggregation of plasma-free platelets obtained from vitronectin-deficient animals was impaired, but aggregation induced by ADP in vitronectin-deficient platelet-rich plasma was enhanced. This enhancement was reduced by plasma-derived vitronectin, suggesting that vitronectin released from platelets may contribute to a more stable platelet aggregation, while plasma vitronectin may be inhibitory. While this conclusion may still be debatable, the evidence from *in vivo* studies using vascular injury models indicates more unequivocally that thrombi formed in the absence of vitronectin are unstable, resulting in delayed vessel occlusion and frequent reopening [88]. Vitronectin binds to and stabilizes plasminogen activator inhibitor 1 (PAI-1) and thus could protect fibrin from premature lysis acting as a thrombus stabilizer. Indeed, the thrombotic phenotype of mice with a combined deficiency in PAI-1 and vitronectin does not differ significantly from that of mice with the corresponding single deficiencies, suggesting that PAI-1 and vitronectin may influence thrombus stability by regulating a common pathway [89].

A recent addition to the list of matrix components with a potential role in supporting platelet adhesive functions is microfibril-associated glycoprotein-1 (MAGP1), a component of microfibrils and vascular elastic fibers that is not found in plasma or platelets. Mice lacking this protein exhibit delayed thrombus formation following arterial injury and a prolonged tail-bleeding time, suggesting that MAGP1 may play a role in hemostasis and thrombosis [90]. Of note, infusion of recombinant MAGP1 before injury appeared to restore function in deficient animals, a finding not necessarily expected with an integral matrix protein. The mechanism of action of MAGP1 during platelet thrombus formation remains to be elucidated, and a complicating factor is that MAGP1-deficient mice have a lower than normal platelet count. The protein appears to have the potential to interact with fibronectin, fibrinogen, and VWF, but has no effect on blood coagulation or platelet aggregation tested *ex vivo* [90].

In general, one can assume that all tissue components capable of interacting with platelets can contribute to the initiation of thrombus formation when exposed to blood, even though only a few may be essential. Of note, experiments with purified proteins are useful to establish specific mechanisms of action but may not reflect functions within the complex supramolecular assembly of ECM. As a consequence, it remains to be elucidated in detail the relative role that several adhesive interactions may play in supporting platelet adhesion to vascular surfaces. This is true in particular for insoluble proteins that exist only in the ECM, such as collagen, or for proteins that may exist in a soluble form in plasma, such as fibronectin, but differ in their structure and conformation when they assemble into complex matrices [65,72].

The unique role of von Willebrand factor in platelet adhesion under flow

Subendothelial matrix VWF and immobilized plasma-derived VWF

Among the substrates required for thrombus formation, VWF is thought to be unique for its role in initiating platelet adhesion and sustaining platelet aggregation under conditions of elevated shear stress [36,38]. These functions are carried out primarily trough the tethering of GP Ibα in the platelet membrane GP Ib-IX-V receptor complex [91] to the A1 domain of immobilized VWF exposed to flowing blood. As a constitutive component of the ECM of endothelial cells [92], in which it is associated with collagen type VI filaments [93,94],

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

subendothelial VWF can directly support platelet adhesion [95–98]. Nonetheless, hemostasis can be normal in the absence of endogenous endothelial VWF if plasma VWF is present. The relative contribution of these two forms of VWF to platelet adhesion was elucidated by studies on the transplant of normal pig bone marrow into pigs affected by severe von Willebrand disease, characterized by a complete deficiency of VWF [99]. As expected, the procedure could not correct the endothelial cell defect, thus there continued to be no VWF secretion into blood or subendothelial matrix while the VWF content in platelet α-granules was normalized. This was not sufficient to restore a normal hemostasis, although some transplanted animals showed a partial amelioration of the prolonged bleeding time. The latter finding is in agreement with the concept that α-granule-derived VWF, released after activation, can contribute to thrombus growth but not to the initial platelet adhesion occurring before activation [100]. In contrast, hemostasis was completely normal in the transplanted pigs after infusion of normal plasma VWF. The prolonged bleeding time of pigs with severe von Willebrand disease was also normalized by transfusion of VWF concentrates alone, even though their platelet VWF remained absent, but a considerably higher dose was required than in transplanted pigs with normal platelet VWF [101]. These results prove that plasma VWF can initiate platelet adhesion after binding to the vessel wall in the absence of matrix VWF and support subsequent thrombus growth with the contribution of platelet-released VWF, even though the latter is not strictly essential. Consequently, the interaction of circulating VWF with exposed vascular and perivascular tissues is a key early event in thrombus formation.

The transition from soluble to immobilized VWF

Plasma VWF can become immobilized onto subendothelial surfaces through the binding to ECM components and through self-association with other VWF multimers. The main substrate capable of binding VWF is collagen, particularly types I and III present in deeper layers of the vessel wall and microfibrillar collagen type VI in the subendothelial matrix. VWF-A1, comprising residues 497–716 of the mature subunit (add 763 to obtain the corresponding residue number in pre-pro-VWF) [102], was initially shown to interact with collagen types I and III [103], but its main role may be binding to collagen type VI [104,105]. The latter contains VWF type A domains in its non-collagenous regions [106] that may become engaged in homotypic interactions with VWF-A1. The VWF A3 domain (VWF-A3), comprising residues 910–1111, also binds to collagen types I and III, and is apparently necessary and sufficient to mediate the interaction with fibrillar collagens [36,107]. The VWF-A3 residues involved in collagen binding have been mapped [108,109], and a high affinity binding site for VWF has been identified in collagen type III [110]. Fluid dynamic conditions and mechanical forces may modulate the VWF-collagen interaction, and the interplay of domains A1 and A3 may be necessary to support VWF immobilization onto extracellular matrices containing various collagen types [105,111]. Of note, VWF multimer size directly correlates with the affinity for collagen binding [112].

Contrasting the information on the role played by VWF-A3 in the interaction with fibrillar collagens *in vitro*, supported by the demonstrated anti-thrombotic activity of a function-blocking anti-VWF A3 antibody [113], stands the evidence that mutations preventing collagen binding (such as Ser968Thr) are compatible with normal hemostasis *in vivo* [114,115]. It appears, therefore, that different VWF domains can ensure the interaction with collagen in ECM, possibly depending on the nature of a lesion, and/or extracellular substrates other than collagen can support VWF in initiating platelet adhesion. In favor of the first hypothesis, evidence has been obtained that collagen type VI may play an important role in VWF binding by interacting with the A1 domain [94]. Moreover, particularly under flow conditions, VWF-A1 can substitute for VWF-A3 in supporting binding to collagen, in which the sites interacting with the two domains appear to be overlapping [111]. Concerning

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

the second hypothesis, it is known that VWF can interact with ECM components independently of collagen. The A1 domain contains a heparin-binding site [116,117] that has been localized to the sequence Tyr565-Ala587 [118]. A second, lower affinity heparin-binding sequence exists within the first 272 residues of the mature VWF subunit [119].[28] These heparin-binding sites may reflect the ability to interact with matrix proteoglycans that contain sulfated carbohydrates. For example, the small proteoglycan decorin, which associates with several matrix components and contributes to matrix assembly, has been reported to bind VWF in an interaction mediated by the glycosaminoglycan chain and regulated by the degree of sulfation [120]. In addition, VWF binds to sulfated glycosphingolipids (sulfatides) [121] that are present on cellular membranes and may serve an accessory role in promoting localization on wounded tissues. The binding site for sulfatides has been localized within A1 domain residues 512–673, possibly with a more direct involvement of residues 569–584 [122] and/or 626–646 [123]. Sulfatides can inhibit platelet adhesion to VWF mediated by GP Ibα, suggesting an overlap of interacting sites [124]. Because VWF is multimeric, sulfatides may mediate binding to surfaces and still allow platelet adhesion to different A1 domains in the same immobilized polymer, and the same may be true for heparin bound to the A1 domain [125]. Another pathway to VWF immobilization may be through interaction with a forming clot. Thus, the cross-linking of VWF to the α-chain of fibrin [126,127] can contribute to platelet deposition onto altered vascular surfaces, and this may become a relevant adhesion mechanism in areas where acute or chronic inflammation causes fibrin formation.

The ability to self-associate on a surface represents an additional mechanism supporting the transition from soluble to immobilized VWF, in which case circulating multimers interact with matrix-bound and endogenous subendothelial VWF [128]. This mechanism was demonstrated by immobilizing a mutant VWF devoid of domain A1 (ΔA1-VWF), thus unable to promote platelet adhesion, onto collagen and showing that GP Ibα-mediated tethering was restored by the presence of soluble VWF in plasma (Fig. 4). As reported, this phenomenon is fully reversible. Very large VWF multimers locally released by stimulated endothelial cells [129][42]may enhance the efficiency of the process, as these molecules form high strength bonds with GP Ibα [130]. Self-association of VWF multimers can occur onto the platelet surface [131] under conditions of hydrodynamic shear that favor the binding of soluble VWF [132]. The self-association of VWF apparently involves multiple domains [133] and none has been identified as essential, including A1 and A3 [128].

A distinct form of self-association may involve thiol-disulfide exchange. In this mechanism, as recently reported, surface-exposed free thiols present in at least some circulating VWF multimers can form new disulfide bonds under the effect of shear stress >50–100 dynes/cm^2, and the resulting increase in VWF multimer size may support enhanced binding to platelets [134]. This process of self-association apparently applies to VWF multimers in solution, i.e. precedes immobilization onto a surface, but a similar mechanism involving free thiol groups has been shown to support the elongation of ultralarge VWF strings released from endothelial cells. In this case, VWF strings on the endothelial surface, i.e. immobilized, increase in size through the covalent association of plasma VWF multimers that are recruited from solution [135]. Of note, inter-subunit disulfide bonds formed during biosynthesis inside the cell are the foundation of the polymeric VWF structure. The reported findings, which need to be confirmed, indicate that disulfide exchange involving VWF multimers may continue after secretion of the molecule into blood and potentially represent a relevant mechanism regulating adhesive functions dependent on platelet binding. One obvious question posed by these experiments concerns the possibility that shear-induced enhancement of VWF adhesive functions could be irreversible, a conclusion that apparently conflicts with the evidence that shear-induced VWF-mediated platelet aggregation is largely reversible if shear stress decreases before platelet activation takes place.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

In summary, the available evidence suggests that different types of injury may elicit distinct pathways for the local immobilization of soluble VWF. As a consequence, for example, VWF binding to collagen may not be essential to ensure normal hemostasis, but may be a primary determinant of the pathological thrombogenic response caused by the rupture of collagen-rich atherosclerotic plaques. From an experimental point of view, it is difficult to recreate such a functional diversity using purified molecules, which can explain some of the inconsistencies found in the literature with respect to the mechanisms of VWF binding to vascular surfaces.

The distinctive functional properties of immobilized and soluble VWF

Platelets have no measurable interaction with circulating VWF in solution, but adhere to immobilized VWF. Such a regulation prevents intravascular platelet aggregation, and has led to the concept that the interaction with GP Ibα depends on a conformational change in surface-bound VWF. Indeed, VWF molecules may change shape depending on hemodynamic conditions, so that upon binding to the vessel wall under high shear stress they appear as elongated filaments rather than the loosely coiled structures seen under static or low shear stress conditions [136]. Uncoiling may expose the repeating functional sites present in multimeric VWF, allowing a more efficient multivalent binding to platelets. Three-dimensional structural studies [137] have shown that more subtle conformational changes can occur in the GP Ibα-binding VWF A1 domain as a result of amino acid substitutions, such as those causing type 2B von Willebrand disease [138], which overcome the affinity barrier for soluble VWF binding to platelets. These findings indicate that conformation can influence the interaction between VWF-A1 and GP Ibα, but they provide no evidence that conformational changes occur as a result of the transition from soluble to surface-immobilized VWF. Studies with a specific “nanobody” [139] support the concept of a common “active” conformation in the VWF A1 domain of surface-bound multimers, soluble ultralarge multimers released by endothelial cells and mutant type 2B plasma VWF, in contrast to the “inactive” conformation of normal plasma VWF. In fact, the “nanobody” binds preferentially to the A1 domain of VWF species with enhanced affinity for GP Ibα, indicating that they may share the same conformation. It remains to be determined whether such a conformation is dynamically transient or reflects one of the known crystallized structures seen in the VWF-A1 complex with GP Ibα [140,141]. A particularly relevant “active” form of soluble VWF is represented by the ultralarge multimers released from the storage granules of stimulated endothelial cells and platelets [142,143]. Ultralarge VWF multimers function locally, but under normal conditions they do not accumulate in circulating blood [144] because they are processed by a specific protease, ADAMTS-13 (ADAMTS: A Disintegrin And Metalloproteinase with a TromboSpondin type 1 motif) [145].

ADAMTS-13 and the regulation of VWF-mediated platelet adhesion and aggregation

VWF released by endothelial cells and/or activated platelets at sites of vascular injury promotes both platelet adhesion and aggregation, particularly under high shear stress conditions, thus exerting a potent prothrombotic effect. The largest, most active VWF multimers are present inside cellular storage granules but are not normally found in the circulation. The reason for this is the efficient processing of all secreted VWF by the metalloprotease, ADAMTS-13, which cleaves one single peptide bond in the VWF subunit [146] and in so doing reduces multimer size [147]. Absence of ADAMTS-13 results in a thrombotic microangiopathy [148], suggesting that the physiologic function of the protease is to limit the activity of the most active VWF multimers to the sites where they are released from cells. Recently, the results of *ex vivo* perfusion experiments have added to this concept by showing that ADAMTS-13 can further cleave circulating VWF multimers while they mediate activation-independent inter-platelet cohesion induced by elevated shear stress,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

resulting in a time-dependent dispersion of the aggregates [149]. In contrast, the protease appeared to have no effect, at least under the *ex vivo* conditions studied, when thrombus formation was induced by blood exposure to a collagen surface.

The latter finding stands in apparent contradiction with the results of *in vivo* studies in mouse thrombosis models, which have shown the ability of recombinant ADAMTS-13 to dissolve experimentally-induced thrombi in the arteriolar circulation leading to the conclusion that the protease could be used as an antithrombotic agent [150]. While the effect of ADAMTS-13 on microarteriolar thrombi is in agreement with the phenotype caused by its deficiency, i.e. microarteriolar thrombosis, the situation may be different in larger arteries. In this case, the antithrombotic activity of ADAMTS-13 may depend on the extent to which adhesive molecules such as fibrinogen and fibronectin, rather than VWF, contribute to platelet aggregation. Thus, the antithrombotic activity of ADAMTS-13 may be selective for platelet aggregation under high shear stress conditions in which VWF is important for platelet cohesion [151]. During hemostasis, ADAMTS-13 activity may be needed to avoid the propagation of platelet aggregates beyond the limits of a vascular wound, which typically involves the microarteriolar circulation with rapidly flowing blood. It remains to be demonstrated whether ADAMTS-13 may limit the potential role of VWF in mediating the occlusion of stenotic arteries where pathologically elevated shear rates develop. In this regard, it is intriguing to observe that a recent study found a positive correlation between ADAMTS-13 levels and the risk of myocardial infarction in men [152], a finding that is in apparent contrast with the suggestion that ADAMTS-13 may act as an anti-thrombotic agent. The mechanism through which increased ADAMTS-13 levels and/or activity might constitute a risk for arterial thrombosis remains to be understood.

Membrane receptors and the mechanism of platelet tethering to VWF

Platelets have two main binding sites for VWF [153,154], GP Ibα in the GP Ib-IX-V complex [91] and the integrin αIIbβ3 [155], which recognizes the ligand Arg-Gly-Asp (RGD) sequence. A second β3 integrin, αvβ3, albeit present at much lower density than αIIbβ3 [156], may contribute to VWF binding, a function shown on endothelial cells [157]. Both platelet receptors for VWF are promiscuous and bind several ligands that may mediate adhesion to other platelets and cells. In particular, the GP Ib-IX-V complex is a counter-receptor for P-selectin [158] and for the leukocyte integrin Mac-1 (α2βM) [159], supporting two interactions that may contribute more to inflammatory responses than to platelet thrombus formation. The integrin αIIbβ3, on the other hand, binds several ligands, in addition to VWF, that are key to the process of platelet adhesion and aggregation, primarily fibrinogen [160], fibronectin [67] and CD40 ligand [161].

The distinguishing feature of the interaction between GP Ibα and VWF-A1 is the ability to support activation-independent platelet tethering to thrombogenic surfaces even when the velocity of blood is elevated. The bond has a fast dissociation rate and, for this property, it is considered selectin-like [162]. Also akin to selectin-mediated bonds [163], recent studies have shown that increasing tensile stress prolongs the lifetime of the interaction between VWF-A1 and GP Ibα when force is relatively small but shortens it above a certain threshold, indicating a shear dependent transition from the properties of a “catch” bond to those of a “slip” bond [164]. A catch bond requires a high shear stress to achieve functionally efficient lifetimes, and such a property could prevent VWF-A1 from interacting with platelets in the circulation. Intuitively, when a molecule in solution interacts with a membrane receptor on a flowing cell, the resulting bond is unlikely to be subjected to significant tensile stress as long as the bound ligand flows with the cell, thus a catch bond would not achieve a significant lifetime. Of interest, VWF-A1 containing type 2B mutations does not appear to form catch bonds with GP Ib, rather it forms typical slip bonds with relatively long bond lifetimes even when exposed to low shear stress [164]. Such a functional property is consistent with the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

significantly slower rolling velocity of platelets interacting with immobilized type 2B VWF-A1 [137], and can explain the ability of soluble type 2B VWF to induce platelet agglutination in circulating blood [165]. In fact, the lifetime of the bond between type 2B VWF-A1 and GP Ib on circulating platelets may be sufficient to mediate efficient adhesion even without exposure to a minimum level of tensile stress [164]. Recent results of molecular dynamics simulation have suggested a structural basis to explain how flow-induced conformational changes in GP Ibα, in addition to the intrinsic properties of VWF-A1 discussed above, can contribute to the functional modulation as well as enhanced adhesive properties of the GP Ib/VWF-A1 adhesive bond under increasing shear stress [166].

Under most circumstances, platelets tethered to the vessel wall solely through VWF-GP Ibα move slowly in the direction of flow and, until recently, it was thought that this interaction by itself cannot support irreversible adhesion [38]. Initial transient interactions between platelets and reactive surfaces my be essential for allowing a modulated response to injury, as commitment to irreversible adhesion after each initial contact could have adverse consequences, including tissue damage. In inflamed tissues, this function may mediate platelet contact with stimulated endothelial cells [167], a surface onto which the only adhesive substrates may be membrane-bound VWF and P-selectin, which also mediates cell adhesion and rolling [168]. The presence of additional structures signifying a serious lesion may be the required trigger for subsequent steps such as irreversible platelet adhesion and accumulation. The GP Ibα-mediated translocation velocity onto immobilized VWF is typically less than 2% of the free flow velocity of non-interacting platelets at the same distance from the luminal surface. This slow motion allows the establishment of additional bonds through receptors that belong mostly, but not necessarily, to the integrin superfamily. Such receptors, many of which depend on platelet activation to express function, typically have an intrinsically slower rate of bond formation but are capable of mediating stable interactions that lead to the definitive arrest of individual platelets and subsequent thrombus development. Notable in this regard is the role of the activated integrin αIIbβ3 and of collagen receptors. When VWF is bound to collagen, the transition from rolling to stable adhesion occurs more rapidly than on immobilized VWF alone and thrombus development occurs at higher shear rates than on collagen without VWF [36]. Such considerations highlight the true synergistic function of the VWF-collagen complex.

An integrated view of VWF-mediated platelet adhesion and aggregation

The concept that the VWF-GP Ibα interaction cannot support long-lasting adhesion must be modified in view of the recently demonstrated ability of non-activated platelets to form aggregates that attach firmly to immobilized VWF under extremely high shear stress conditions (Fig. 5) [169]. Several unique features characterize this mechanism of platelet adhesion to extracellular surfaces and to one another, marking substantial differences with the process of single platelet rolling. Perhaps the most relevant distinction is that GP Ibα-mediated long-lasting adhesion and aggregation only occur above a threshold shear rate of ~10,000 s^{-1}, a feature that highlights its potential importance for pathological arterial thrombosis. Platelets that become firmly adherent to the surface are stretched considerably in the direction of flow, but whether this morphological change is the cause or consequence of adhesion under elevated shear stress remains to be established [169]. Of note, a 3-D multi-scale computational model has been used to calculate the shear rate threshold required for measurable VWF-mediated platelet aggregation [170,171], and the zero efficiency of binding predicted for shear rates <8000 s^{-1} is in remarkable agreement with experimental findings obtained independently [169]. It is also noteworthy that, at the levels of shear stress required for VWF-mediated and activation-independent platelet aggregation (>400 dyne/ cm^2 wall shear stress), the bond between VWF-A1 and GP Ibα exhibits the properties of a

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

slip bond (the transition from catch to slip bond occurring at 10–20 dyne/cm^2 wall shear stress; see reference [164]), thus should become less efficient with increasing shear stress. Experimental evidence is in contrast with such a conclusion [169], indicating that mechanisms not directly dependent on the intrinsic biomechanical properties of single bonds (for example, regulation of the number of bonds formed) may play a key role in VWF and GP Ib-mediated platelet adhesion/aggregation. A second key distinction is that platelet adhesion and aggregation at pathologically elevated shear rates depends on soluble as well as surface-bound VWF, while single platelet adhesion and rolling require only immobilized VWF [169]. A third and equally relevant feature is that GP Ibα-mediated and VWF-dependent firm platelet adhesion and aggregation occur without any requirement for platelet activation and integrin function. Such a statement should not be taken to indicate that activation has no influence on platelet thrombus formation at pathologically elevated shear rates, as it remains essential for the stability of aggregates [13]. It is intuitive, however, that the ability of platelets to aggregate onto surfaces even before activation greatly favors the establishment of growing thrombi in a high shear rate environment, in which elevated tensile stress limits the efficiency of adhesive bonds and rapid flow reduces the concentration of agonists required for activation. Under challenging hydrodynamic conditions, therefore, platelet interactions with adhesive surfaces and with one another appear to be synergistic. Of note, ADAMTS-13 can cleave circulating VWF multimers while they mediate activation-independent inter-platelet cohesion under high shear stress, thus dispersing the aggregates [149].

Substrates and receptors for platelet aggregation

Following the initial events that lead to adhesion and activation at sites of vascular injury, platelets bind soluble adhesive proteins and form a reactive surface for continuing platelet deposition. Subsequent thrombus growth is therefore strictly dependent on the formation of inter-platelet bonds. Aggregation has typically been studied with agonist-stimulated platelets in suspension, without surface interactions, and under stirring conditions that create a turbulent flow with low shear rates [172]. Studies of this kind have led to the assumption that fibrinogen binding to αIIbβ3 is the only interaction relevant for platelet aggregation [173]. The view has changed with the use of the cone-and-plate viscometer, where platelets in suspension can be exposed to defined levels of shear stress in a laminar flow field. High shear stress by itself, without addition of exogenous agonists, can lead to platelet aggregation that is mediated by VWF and its two membrane receptors [174,175]. Indeed, at elevated shear rates, typically in excess of 5000 s^{-1}, VWF binds specifically to platelets in a process that involves sequentially GP Ibα and αIIbβ3 [176]. Thus, fibrinogen and VWF, as well as GP Ibα and αIIbβ3, have distinct but complementary roles in platelet aggregation depending on fluid dynamic conditions.

The study of platelet aggregation in suspension fails to reproduce the correct spatial and temporal sequence of events that occur during thrombus growth *in vivo*, but alternative experimental approaches can measure in real time the three-dimensional growth of platelet aggregates under defined hemodynamic conditions [151]. *Ex vivo* perfusion experiments indicate a synergistic role for fibrinogen and VWF in supporting platelet aggregation onto collagen fibrils. Without fibrinogen, thrombi mediated by VWF grow rapidly at high shear rate but are unstable; with both VWF and fibrinogen, thrombi grow more slowly but are stable [151]. In mice selectively deficient in VWF, platelet adhesion at sites of experimental vascular lesion is delayed, but stable platelet aggregates eventually develop even though arterial occlusion is often impaired [68]. Because these studies have been performed in the microcirculation, their significance with respect to the role of VWF in the thrombotic occlusion of larger arteries remains to be defined. In mice selectively deficient in fibrinogen, in contrast, platelet thrombi develop rapidly but detach from the surface and embolize

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

causing vascular occlusion downstream of the lesion [68]. Thus, in agreement with the *ex vivo* perfusion studies outline above, both VWF and fibrinogen are required to ensure stable aggregates. These results provide a plausible explanation for the altered hemostatic properties of platelets from patients with isolated congenital deficiency of either fibrinogen [177] or VWF [178]. Because neither protein by itself can sustain the development of stable thrombi under relevant flow conditions, hemostasis cannot be normal unless both are present and functional.

Platelet adhesion and thrombus stabilization

Platelet aggregation in itself is not an irreversible process, which is an important characteristic allowing for the intervention of regulatory mechanisms capable of preventing an excessive, and potentially dangerous, propagation of thrombus growth. After activation and aggregation have occurred in response to a vascular lesion, distinct adhesive mechanisms become operative that consolidate the stability of the forming thrombus. The identification and characterization of the molecules involved in these processes have become the focus of increasing attention in recent years because of their seemingly important influence on hemostatic efficiency and, in pathological conditions, arterial thrombotic complications. It is apparent that newly recruited platelets at the growing edge of a thrombus transmit feedback signals to aggregated platelets in deeper layers that are necessary to prevent disaggregation. The propagation of these signals throughout the thrombus is visible as recurrent cycles of intracytoplasmic Ca^{2+} elevations (Fig. 6) [179]. Both ADP receptors, $P2Y_1$ and $P2Y_{12}$, are involved in the process, as demonstrated experimentally by two-stage perfusion experiments in which normal blood is first exposed to a thrombogenic surface, such as collagen type I fibers, followed by blood containing specific inhibitors. In such a manner, it has been shown that a thrombus can begin to disperse even after several minutes of growth if ADP activation pathways are blocked, intracytoplasmic Ca^{2+} elevations are prevented or inhibitors of αIIbβ3 function are added [180]. These findings indicate that platelet activation must be sustained in order to keep adhesive ligands bound to αIIbβ3 in a stable manner. It should be considered, however, that *ex vivo* experiments might overemphasize platelet-dependent mechanisms of this kind because anticoagulants used to handle blood outside of vessels markedly suppress α-thrombin generation and/or activity and reduce the deposition of fibrin that normally contributes to thrombus stability. Nevertheless, sustained platelet activation may be critical for maintaining the integrity of platelet aggregates, particularly in rapid arterial flow conditions and in the early stages of thrombus development.

Adhesive molecules bound to or expressed onto the membrane of activated platelet are crucial for the rapid growth of stable thrombi. One example of this function is the role that fibrinogen (likely after conversion to fibrin) plays in anchoring aggregated platelets to the site of vascular injury, thus preventing downstream embolization under the effects of flow [68]. Several specific interactions have been identified that directly enhance the cohesion between platelets initially established through well-known adhesive ligands (VWF, fibrinogen, fibronectin) and receptors (GP Ibα and αIIbβ3). Two main mechanisms can be envisioned to operate in this regard, one resulting in the generation of signals that enhance or sustain platelet activation, and the other based on homophilic or heterophilic interactions that add adhesive strength to platelet aggregates. The hormone leptin is an example of the first type of action, as it may reinforce the response of platelets to weak agonist stimulation thus contributing to the stability of platelet aggregation [181]. The effect of leptin on thrombus growth has been confirmed with *in vivo* experiments in obese mice, who lack leptin and exhibit delayed thrombotic occlusion with frequent embolization in injured arteries [182]. Moreover, inhibition of endogenous leptin has been found to protect mice from arterial and venous thrombosis [183]. In human obesity, therefore, in which leptin

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

resistance leads to high circulating levels of the hormone, the effect of leptin on platelet activation may represent an additional prothrombotic factor. It should be noted, however, that the reported effect of leptin on platelet aggregation has not yet been confirmed and, for reasons still unknown, may be detectable only in a subpopulation of individuals.

Cellular pathways involved in inflammatory and immune responses also appear to have prothrombotic effects. CD40 ligand (CD40L), a member of the tumor necrosis factor family of ligands, has been detected on the surface of activated platelets where it interacts with αIIbβ3 through an integrin recognition sequence (KGD). In the absence of CD40L, platelet aggregates formed in experimental *in vivo* models of thrombosis are unstable, confirming the ability of this protein to support platelet-platelet cohesion under high shear stress conditions via an αIIbβ3-dependent mechanism [161].

Growth arrest-specific gene 6 product (Gas6) is a vitamin K-dependent protein homologous to the anticoagulant cofactor, protein S, whose absence or inactivation was reported to protect mice from fatal thromboembolism [184]. Gas6 is present in plasma and in the α-granules of mouse platelets, from which it is secreted upon activation, but may be absent in human platelets [185]. Because of the latter observation, the relevance of Gas6 in human platelet physiology is being reevaluated as it may depend on the function of the plasma-derived protein only. On the other hand, all three known Gas6 receptors (Axl, Sky, and Mer) are present on mouse and human platelets, and their inactivation or stimulation results in the expected inhibition or enhancement, respectively, of agonist-induced platelet activation responses [186]. Thus, Gas6-dependent pathways may represent an amplification mechanism for platelet aggregate stability.

Eph kinases and ephrins are families of membrane bound molecules that interact with each other, exhibiting a major role in neuronal organization and as early markers for vascular commitment to arterial or venous development. Platelet-expressed Eph/ephrins include EphA4 and ephrinB1, which appear to contribute to "outside-in" signals originating from ligand-occupied αIIbβ3. Inhibition of Eph/ephrin interactions results in a diminution of the volume of thrombi formed on a collagen type I surface, presumably as a consequence of decreased aggregate cohesion [187].

A strategy has been proposed to identify molecules that are phosphorylated following the induction of platelet aggregation, as this could indicate a specific role in controlling thrombus stability [188]. Two studies have recently verified the validity of this hypothesis. In one, a novel membrane protein has been identified, platelet endothelial aggregation receptor 1 (PEAR1), that appears to signal secondary to αIIbβ3-mediated platelet-platelet contacts [188]. Future studies will indicate the pathophysiological significance of this finding. In another study, evidence has been presented for the prothrombotic function of CD84 and SLAM (signaling lymphocyte activation molecule), both members of the same family of homophilic adhesion receptors. Immobilized CD84 can promote platelet microaggregation, and $SLAM^{-/-}$ mice exhibit decreased agonist-induced platelet aggregation, a defect confirmed by intravital studies of thrombus formation in injured vessels [189]. Because numerous cell signaling and adhesive pathways potentially involved in controlling platelet thrombus integrity have already been identified, an obvious challenge for the future will be the integration of this analytical knowledge into a comprehensive representation of the mechanisms of platelet response to vascular injury.

Conclusions

The last few years have witnessed major advances in our understanding of the mechanisms that support platelet thrombus formation in flowing blood. The results of *ex vivo* flow

NIH-PA Author Manuscript

experiments and intravital microscopy studies have shed new light on the processes underlying hemostasis and thrombosis. Animal models with targeted gene deletions or mutations have greatly contributed to these advances and will certainly provide more insights in the future. Significant progress in genomics and proteomics has generated information relevant to elucidating the integrated processes that link platelet-substrate interactions and signaling pathways to thrombus growth and stability. Finally, the advent of improved drug development technologies is likely to permit the translation of this fundamental knowledge into more efficient therapeutic approaches to prevent excessive bleeding and thrombosis.

Acknowledgments

We gratefully acknowledge the many contributions that all the colleagues in the Ruggeri laboratory have made to the original work discussed here. The original work by the author referred to in this review was supported by grants from the National Heart Lung and Blood Institute of the National Institutes of Health (HL-78784, HL-31950, HL-42846 and HL-80718).

Abbreviations

ECM	extracellular matrix
VWF	von Willebrand factor
VWF-A1	A1 domain of VWF
GP	glycoprotein
MMP	matrix metalloproteinases
MAGP1	microfibril-associated glycoprotein-1
VWF-A3	A3 domain of VWF
ADAMTS	A Disintegrin And Metalloproteinase with a TromboSpondin type 1 motif
CD40L	CD40 ligand
Gas6	Growth arrest-specific gene 6 product
PEAR1	platelet endothelial aggregation receptor 1
SLAM	signaling lymphocyte activation molecule
PPACK	D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone dihydrochloride

References

1. Schulze H, Shivdasani RA. Mechanisms of thrombopoiesis. J Thromb Haemost 2005;3:1717–1724. [PubMed: 16102038]
2. Junt T, Schulze H, Chen Z, Massberg S, Goerge T, Krueger A, Wagner DD, Graf T, Italiano JE Jr. Shivdasani RA, von Andrian UH. Dynamic visualization of thrombopoiesis within bone marrow. Science 2007;317:1767–1770. [PubMed: 17885137]
3. Weyrich AS, Lindemann S, Tolley ND, Kraiss LW, Dixon DA, Mahoney TM, Prescott SP, McIntyre TM, Zimmerman GA. Change in protein phenotype without a nucleus: Translational control in platelets. Semin Thromb Hemost 2004;30:491–498. [PubMed: 15354270]
4. Weiss HJ. Platelet physiology and abnormalities of platelet function (first of two parts). New Engl J Med 1975;293:531–541. [PubMed: 168489]
5. Weiss HJ. Platelet physiology and abnormalities of platelet function (second of two parts). New Engl J Med 1975;293:580–588. [PubMed: 1097915]
6. Ruggeri ZM, Mendolicchio GL. Adhesion mechanisms in platelet function. Circul Res 2007;100:1673–1685.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

7. Iannacone M, Sitia G, Isogawa M, Whitmire JK, Marchese P, Chisari FV, Ruggeri ZM, Guidotti LG. Platelets prevent IFN-α/β-induced lethal hemorrhage promoting CTL-dependent clearance of lymphocytic choriomeningitis virus. Proc Natl Acad Sci U S A 2008;105:629–634. [PubMed: 18184798]
8. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest 2005;115:3378–3384. [PubMed: 16322783]
9. Wagner DD, Burger PC. Platelets in inflammation and thrombosis. Atertio Thromb Vasc Biol 2003;23:2131–2137.
10. Soslau G, Prest PJ, Class R, George R, Paladino F, Violetta G. Comparison of sea turtle thrombocyte aggregation to human platelet aggregation in whole blood. Comparative Biochemistry and PhysiologyBBiochemistry and Molecular Biology 2005;142:353–360.
11. Jagadeeswaran P, Gregory M, Day K, Cykowski M, Thattaliyath B. Zebrafish: A genetic model for hemostasis and thrombosis. J Thromb Haemost 2005;3:46–53. [PubMed: 15634265]
12. Meseguer J, Esteban MA, Rodriguez A. Are thrombocytes and platelets true phagocytes? Microsc Res Tech 2002;57:491–497. [PubMed: 12112431]
13. Petrich BG, Marchese P, Ruggeri ZM, Spiess S, Weichert RA, Ye F, Tiedt R, Skoda RC, Monkley SJ, Critchley DR, Ginsberg MH. Talin is required for integrin-mediated platelet function in hemostasis and thrombosis. J Exp Med 2007;204:3103–3111. [PubMed: 18086863]
14. Ruggeri ZM. Platelets in atherothrombosis. Nat Med 2002;8:1227–1234. [PubMed: 12411949]
15. Iannacone M, Sitia G, Isogawa M, Marchese P, Castro MG, Lowenstein PR, Chisari FV, Ruggeri ZM, Guidotti LG. Platelets mediate cytotoxic T lymphocyte-induced liver damage. Nat Med 2005;11:1167–1169. [PubMed: 16258538]
16. Iannacone M, Sitia G, Ruggeri ZM, Guidotti LG. HBV pathogenesis in animal models: Recent advances on the role of platelets. J Hepatol 2007;46:719–726. [PubMed: 17316876]
17. Iannacone M, Sitia G, Narvaiza I, Ruggeri ZM, Guidotti LG. Antiplatelet drug therapy moderates immune-mediated liver disease and inhibits viral clearance in mice infected with a replication-deficient adenovirus. Clin Vaccine Immunol 2007;14:1532–1535. [PubMed: 17881509]
18. Baumgartner HR. The role of blood flow in platelet adhesion, fibrin deposition and formation of mural thrombi. Microvascular Research 1973;5:167–179. [PubMed: 4694283]
19. Turitto VT, Baumgartner HR. Effect of physical factors on platelet adherence to subendothelium. ThrombDiathHaemorrh 1974;60:17–24.
20. van Breugel HHFI, de Groot PG, Heethaar RM, Sixma JJ. Role of plasma viscosity in platelet adhesion. Blood 1992;80:953–959. [PubMed: 1498335]
21. Goldsmith HL, Turitto VT. Rheological aspects of thrombosis and haemostasis: Basic principles and applications. Thromb Haemost 1986;55:415–435. [PubMed: 3750272]
22. Basmadjian D. The effect of flow and mass transport in thrombogenesis. Ann Biomed Eng 1990;18:685–709. [PubMed: 2281887]
23. Aarts PA, van den Broek SA, Prins GW, Kuiken GDC, Sixma JJ, Heethaar RM. Blood platelets are concentrated near the wall and red blood cells, in the center in flowing blood. Arteriosclerosis 1988;8:819–824. [PubMed: 3196226]
24. Eckstein EC, Tilles AW, Millero FJ Iii. Conditions for the occurrence of large near-wall excesses of small particles during blood flow. Microvascular Research 1988;36:31–39. [PubMed: 3185301]
25. Wang SK, Hwang NHC. On transport of suspended particulates in tube flow. Biorheology 1992;29:353–377. [PubMed: 1298451]
26. Turitto VT, Baumgartner HR. Platelet interaction with subendothelium in a perfusion system: Physical role of red blood cells. Microvascular Research 1975;9:335–344. [PubMed: 1128284]
27. Schmid-Schonbein H, Born GVR, Richardson PD, Cusack N, Rieger H, Forst R, Rohling-Winkel I, Blasberg P, Wehmeyer A. Rheology of thrombotic processes in flow: The interaction of erythrocytes and thrombocytes subjected to high flow forces. Biorheology 1981;18:415–444. [PubMed: 7326385]
28. Tangelder GJ, Slaaf DW, Arts T, Reneman RS. Wall shear rate in arterioles in vivo: Least estimates from platelet velocity profiles. American Journal of Physiology 1988;254:H1059–H1064. [PubMed: 3381893]

29. Karnis A, Mason SG. Particle motions in sheared suspensions xix. Viscoelastic media. Transactions of the Society of Rheology 1966;10:571–592.
30. Eckstein EC, Belgacem F. Model of platelet transport in flowing blood with drift and diffusion terms. Biophys J 1991;60:53–69. [PubMed: 1883945]
31. Fung, YC. Biodynamics circulation. Springer-Verlag; New York: 1984.
32. Slichter SJ, Harker LS. Thrombocytopenia: Mechanisms and management of defects in platelet production. Clinics in Hematology 1978;7:523–538.
33. Livio M, Gotti E, Marchesi D, Mecca G, Remuzzi G, De Gaetano G. Uraemic bleeding: Role of anaemia and beneficial effect of red cell. Lancet 1982;2:1013–1015. [PubMed: 6127502]
34. Chen S, Springer TA. Selectin receptor-ligand bonds: Formation limited by shear rate and dissociation governed by the bell model. Proc Natl Acad Sci USA 2001;98:950–955. [PubMed: 11158576]
35. Weiss HJ, Turitto VT, Baumgartner HR. Effect of shear rate on platelet interaction with subendothelium in citrated and native blood. I. Shear rate-dependent decrease of adhesion in von Willebrand's disease and the Bernard-Soulier syndrome. The Journal of Laboratory and Clinical Medicine 1978;92:750–764. [PubMed: 309498]
36. Savage B, Almus-Jacobs F, Ruggeri ZM. Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. Cell 1998;94:657–666. [PubMed: 9741630]
37. Konstantinides S, Ware J, Marchese P, Almus-Jacobs F, Loskutoff D, Ruggeri ZM. Distinct antithrombotic consequences of platelet glycoprotein Ibα and VI deficiency in a mouse model of arterial thrombosis. J Thromb Haemost 2006;4:2014–2021. [PubMed: 16961609]
38. Savage B, Saldivar E, Ruggeri ZM. Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. Cell 1996;84:289–297. [PubMed: 8565074]
39. Mailhac A, Badimon JJ, Fallon JT, Fernandez-Ortiz A, Meyer B, Chesebro JH, Fuster V, Badimon L. Effect of an eccentric severe stenosis on fibrin(ogen) deposition on severely damaged vessel wall in arterial thrombosis. Relative contribution of fibrin(ogen) and platelets. Circulation 1994;90:988–996. [PubMed: 8044972]
40. Siegel JM, Markou CP, Ku DN, Hanson SR. A scaling law for wall shear rate through an arterial stenosis. Journal of Biomechanical Engineering 1994;116:446–451. [PubMed: 7869720]
41. Bluestein D, Niu L, Schoephoerster RT, Dewanjee MK. Fluid mechanics of arterial stenosis: Relationship to the development of mural thrombus. Ann Biomed Eng 1997;25:344–356. [PubMed: 9084839]
42. Ware JA, Heistad DD. Platelet-endothelium interactions. New Engl J Med 1993;328:628–635. [PubMed: 8429855]
43. Marcus AJ, Broekman MJ, Drosopoulos JHF, Islam N, Alyonycheva TN, Safier LB, Hajjar KA, Posnett DN, Schoenborn MA, Schooley KA, Gayle RB. The endothelial cell ecto-adpase responsible for inhibition of platelet function is cd39. J Clin Invest 1997;99:1351–1360. [PubMed: 9077545]
44. Farndale RW, Sixma JJ, Barnes MJ, de Groot PG. The role of collagen in thrombosis and hemostasis. J Thromb Haemost 2004;2:561–573. [PubMed: 15102010]
45. Nieuwenhuis HK, Akkerman JWN, Houdijk WPM, Sixma JJ. Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia. Nature 1985;318:470–472. [PubMed: 2933589]
46. Santoro SA. Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. Cell 1986;46:913–920. [PubMed: 3757029]
47. Kunicki TJ, Nugent DJ, Staats SJ, Orchekowski RP, Wayner EA, Carter WG. The human fibroblast class II extracellular matrix receptor mediates platelet adhesion to collagen and is identical to the platelet glycoprotein Ia-IIa complex. J Biol Chem 1988;263:4516–4519. [PubMed: 2832397]
48. Moroi M, Jung SM, Okuma M, Shinmyozu K. A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. J Clin Invest 1989;84:1440–1445. [PubMed: 2808700]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

49. Moroi M, Jung SM, Shinmyozu K, Tomiyama Y, Ordinas A, Diaz-Ricart M. Analysis of platelet adhesion to a collagen-coated surface under flow conditions: Involvement of glycoprotein VI in the platelet adhesion. Blood 1996;88:2081–2092. [PubMed: 8822927]
50. Diaz-Ricart M, Tandon NN, Carretero M, Ordinas A, Bastida E, Jamieson GA. Platelets lacking functional CD36 (glycoprotein IV) show reduced adhesion to collagen in flowing whole blood. Blood 1993;82:491–496. [PubMed: 7687161]
51. Chiang TM, Rinaldy A, Kang AH. Cloning, characterization, and functional studies of a nonintegrin platelet receptor for type I collagen. J Biol Chem 1997;100:514–529.
52. Yanai H, Chiba H, Fujiwara H, Morimoto M, Abe K, Yoshida S, Takahashi Y, Fuda H, Hui SP, Akita H, Kobayashi K, Matsuno K. Phenotype-genotype correlation in CD36 deficiency types I and II. Thromb Haemost 2000;84:436–441. [PubMed: 11019968]
53. McKeown L, Vail M, Williams S, Kramer W, Hansmann K, Gralnick H. Platelet adhesion to collagen in individuals lacking glycoprotein IV. Blood 1994;83:2866–2871. [PubMed: 7514049]
54. Yamamoto N, Akamatsu N, Yamazaki H, Tanoue K. Normal aggregations of glycoprotein IV (CD36)-deficient platelets from seven healthy japanese donors. Br J Haematol 1992;81:86–92. [PubMed: 1381610]
55. Nieswandt B, Watson SP. Platelet-collagen interaction: Is GPVI the central receptor? Blood 2003;102:449–461. [PubMed: 12649139]
56. He L, Pappan LK, Grenache DG, Li Z, Tollefsen DM, Santoro SA, Zutter MM. The contributions of the α2β1 integrin to vascular thrombosis in vivo. Blood 2003;102:3652–3657. [PubMed: 12893751]
57. Sarratt KL, Chen H, Zutter MM, Santoro SA, Hammer DA, Kahn ML. GPVI and α2β1 play independent critical roles during platelet adhesion and aggregate formation to collagen under flow. Blood 2005;106:1268–1277. [PubMed: 15886326]
58. Cheli Y, Jensen P, Marchese P, Habart D, Wiltshire T, Cooke M, Fernandez JA, Ware J, Ruggeri ZM, Kunicki TJ. The modifier of hemostasis (mh) locus on mouse chromosome 4 controls in vivo hemostasis of gp6 −/− mice. Blood 2008;111:1266–1273. [PubMed: 17991808]
59. Prockop DJ, Kivirikko KI. Collagens: Molecular biology, diseases and potentials for therapy. AnnRevBiochem 1995;64:403–434.
60. Savage B, Ginsberg MH, Ruggeri ZM. Influence of fibrillar collagen structure on the mechanisms of platelet thrombus formation under flow. Blood 1999;94:2704–2715. [PubMed: 10515874]
61. Ishii T, Asuwa N. Spiraled collagen in the major blood vessels. Modern Pathology 1996;9:843–848. [PubMed: 8871926]
62. Nieswandt B, Brakebusch C, Bergmeier W, Schulte V, Bouvard D, Mokhtari-Nejad R, Lindhout T, Heemskerk JW, Zirngibl H, FÑssler R. Glycoprotein VI but not α2β1 integrin is essential for platelet interaction with collagen. EMBO J 2001;20:2120–2130. [PubMed: 11331578]
63. Kato K, Kanaji T, Russell S, Kunicki TJ, Furihata K, Kanaji S, Marchese P, Reininger A, Ruggeri ZM, Ware J. The contribution of glycoprotein VI to stable platelet adhesion and thrombus formation illustrated by targeted gene deletion. Blood 2003;102:1701–1707. [PubMed: 12738669]
64. Hantgan RR, Hindriks G, Taylor RG, Sixma JJ, de Groot PG. Glycoprotein Ib, von Willebrand factor, and glycoprotein IIb:IIIa are all involved in platelet adhesion to fibrin in flowing whole blood. Blood 1990;76:345–353. [PubMed: 2369638]
65. Hynes, RO. Fibronectins. Springer-Verlag; New York: 1989.
66. Ginsberg MH, Xiaoping D, O'Toole TE, Loftus JC, Plow EF. Platelet integrins. Thromb Haemost 1993;70:87–93. [PubMed: 8236121]
67. Ginsberg MH, Forsyth J, Lightsey A, Chediak J, Plow EF. Reduced surface expression and binding of fibronectin by thrombin-stimulated thrombasthenic platelets. J Clin Invest 1983;71:619–624. [PubMed: 6826726]
68. Ni H, Denis CV, Subbarao S, Degen JL, Sato TN, Hynes RO, Wagner DD. Persistence of platelet thrombus formation in arterioles of mice lacking both von Willebrand factor and fibrinogen. J Clin Invest 2000;106:385–392. [PubMed: 10930441]
69. Smyth SS, Reis ED, Vaananen H, Zhang W, Coller BS. Variable protection of β3-integrin-deficient mice from thrombosis initiated by different mechanisms. Blood 2001;98:1055–1062. [PubMed: 11493451]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

70. Ni H, Yuen PS, Papalia JM, Trevithick JE, Sakai T, Fassler R, Hynes RO, Wagner DD. Plasma fibronectin promotes thrombus growth and stability in injured arterioles. Proc Natl Acad Sci USA 2003;100:2415–2419. [PubMed: 12606706]
71. Reheman A, Yang H, Zhu G, Jin W, He F, Spring CM, Bai X, Gross PL, Freedman J, Ni H. Plasma fibronectin depletion enhances platelet aggregation and thrombus formation in mice lacking fibrinogen and von Willebrand factor. Blood First Edition Paper. 2008 prepublished online November 25, 2008; DOI 2010.1182/blood-2008-2004-148361.
72. Cho J, Mosher DF. Impact of fibronectin assembly on platelet thrombus formation in response to type I collagen and von Willebrand factor. Blood 2006;108:2229–2236. [PubMed: 16735600]
73. Cho J, Mosher DF. Role of fibronectin assembly in platelet thrombus formation. J Thromb Haemost 2006;4:1461–1469. [PubMed: 16839338]
74. Beumer S, Heijnen HF, Ijsseldijk MJ, Orlando E, de Groot PG, Sixma JJ. Platelet adhesion to fibronectin in flow: The importance of von Willebrand factor and glycoprotein Ib. Blood 1995;86:3452–3460. [PubMed: 7579450]
75. Chauhan AK, Kisucka J, Cozzi MR, Walsh MT, Moretti FA, Battiston M, Mazzucato M, De Marco L, Baralle FE, Wagner DD, Muro AF. Prothrombotic effects of fibronectin isoforms containing the eda domain. Arterioscler Thromb Vasc Biol 2008;28:296–301. [PubMed: 17991876]
76. Adams J, Lawler J. The thrombospondin family. Curr Biol 1993;3:188–190. [PubMed: 15335796]
77. Wencel-Drake JD, Painter RG, Zimmerman TS, Ginsberg MH. Ultrastructural localization of human platelet thrombospondin, fibrinogen, fibronectin, and von Willebrand factor in frozen thin section. Blood 1985;65:929–938. [PubMed: 2579691]
78. Jurk K, Clemetson KJ, de Groot PG, Brodde MF, Steiner M, Savion N, Varon D, Sixma JJ, Van Aken H, Kehrel BE. Thrombospondin-1 mediates platelet adhesion at high shear via glycoprotein Ib (GPIb): An alternative/backup mechanism to von Willebrand factor. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2003;17:1490–1492. [PubMed: 12824298]
79. Kyriakides TR, Zhu Y, Smith LT, Bain SD, Yang Z, Lin MT, Danielson KG, Iozzo RV, LaMarca M, McKinney CE, Ginns EI, Bornstein P. Mice that lack thrombospondin 2 display connective tissue abnormalities that are associated with disordered collagen fibrillogenesis, an increased vascular density, and a bleeding diathesis. J Cell Biol 1998;140:419–430. [PubMed: 9442117]
80. Kyriakides TR, Rojnuckarin P, Reidy MA, Hankenson KD, Papayannopoulou T, Kaushansky K, Bornstein P. Megakaryocytes require thrombospondin-2 for normal platelet formation and function. Blood 2003;101:3915–3923. [PubMed: 12732502]
81. Nigatu A, Sime W, Gorfu G, Geberhiwot T, Anduren I, Ingerpuu S, Doi M, Tryggvason K, Hjemdhal P, Patarroyo M. Megakaryocytic cells synthesize and platelets secrete α5-laminins, and the endothelial laminin isoform laminin 10 (α5β1γ1) strongly promotes adhesion but not activation of platelets. Thromb Haemost 2007;95:85–93. [PubMed: 16543966]
82. Hindriks G, Ijsseldijk MJW, Sonnenberg A, Sixma JJ, deGroot PG. Platelet adhesion to laminin: Role of Ca 2+ and Mg 2+ ions, shear rate, and platelet membrane glycoproteins. Blood 1992;79:928–935. [PubMed: 1737101]
83. Inoue O, Suzuki-Inoue K, McCarty OJ, Moroi M, Ruggeri ZM, Kunicki TJ, Ozaki Y, Watson SP. Laminin stimulates spreading of platelets through integrin alpha6beta1- dependent activation of GPVI. Blood 2006;107:1405–1412. [PubMed: 16219796]
84. Godyna S, Diaz-Ricart M, Argraves WS. Fibulin-1 mediates platelet adhesion via a bridge of fibrinogen. Blood 1996;88:2569–2577. [PubMed: 8839849]
85. Asch E, Podack E. Vitronectin binds to activated human platelets and plays a role in platelet aggregation. J Clin Invest 1990;85:1372–1378. [PubMed: 1692034]
86. Tran H, Tanaka A, Litvinovich SV, Medved LV, Haudenschild CC, Argraves WS. The interaction of fibulin-1 with fibrinogen: A potential role in hemostasis and thrombosis. J Biol Chem 1995;270:19458–19464. [PubMed: 7642629]
87. Reheman A, Gross P, Yang H, Chen P, Allen D, Leytin V, Freedman J, Ni H. Vitronectin stabilizes thrombi and vessel occlusion but plays a dual role in platelet aggregation. J Thromb Haemost 2005;3:875–883. [PubMed: 15733060]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

88. Konstantinides S, Schafer K, Thinnes T, Loskutoff DJ. Plasminogen activator inhibitor-1 and its cofactor vitronectin stabilize arterial thrombi after vascular injury in mice. Circulation 2001;103:576–583. [PubMed: 11157725]
89. Koschnick S, Konstantinides S, Schafer K, Crain K, Loskutoff DJ. Thrombotic phenotype of mice with a combined deficiency in plasminogen activator inhibitor 1 and vitronectin. J Thromb Haemost 2005;3:2290–2295. [PubMed: 16194205]
90. Werneck CC, Vicente CP, Weinberg JS, Shifren A, Pierce RA, Broekelmann TJ, Tollefsen DM, Mecham RP. Mice lacking the extracellular matrix protein MAGP1 display delayed thrombotic occlusion following vessel injury. Blood 2008;111:4137–4144. [PubMed: 18281502]
91. Andrews RK, Gardiner EE, Shen Y, Whisstock JC, Berndt MC. Glycoprotein Ib-IX-V. Int J Biochem Cell Biol 2003;35:1170–1174. [PubMed: 12757754]
92. Rand JH, Sussman II, Gordon RE, Chu SV, Solomon V. Localization of factor-VIII-related antigen in human vascular subendothelium. Blood 1980;55:752–756. [PubMed: 6244867]
93. Rand JH, Patel ND, Schwartz E, Zhou S, Potter BJ. 150-kd von Willebrand factor binding protein extracted from human vascular subendothelium is type VI collagen. J Clin Invest 1991;88:253–259. [PubMed: 2056120]
94. Denis C, Baruch D, Kielty CM, Ajzenberg N, Christophe O, Meyer D. Localization on von Willebrand factor binding domains to endothelial extracellular matrix and to type VI collagen. Atertio Thromb Vasc Biol 1993;13:398–406.
95. Sakariassen KS, Bolhuis PA, Sixma JJ. Human blood platelet adhesion to artery subendothelium is mediated by factor VIII/von Willebrand factor bound to the subendothelium. Nature 1979;279:636–638. [PubMed: 313016]
96. Stel HV, Sakariassen KS, de Groot PG, van Mourik JA, Sixma JJ. Von Willebrand factor in the vessel wall mediates platelet adherence. Blood 1985;65:85–90. [PubMed: 3917314]
97. Turitto VT, Weiss HJ, Zimmerman TS, Sussman II. Factor VIII/von Willebrand factor in subendothelium mediates platelet adhesion. Blood 1985;65:823–831. [PubMed: 3872140]
98. Baruch D, Denis C, Marteaux C, Schoevaert D, Coulombel L, Meyer D. Role of von Willebrand factor associated to extracellular matrices in platelet adhesion. Blood 1991;77:519–527. [PubMed: 1991166]
99. Bowie EJ, Solberg LA Jr. Fass DN, Johnson CM, Knutson GJ, Stewart ML, Zoecklein LJ. Transplantation of normal bone marrow into a pig with severe von Willebrand's disease. J Clin Invest 1986;78:26–30. [PubMed: 3088043]
100. Castillo R, Monteagudo J, Escolar G, Ordinas A, Magallon M, Villar JM. Hemostatic effect of normal platelet transfusion in severe von Willebrand disease patients. Blood 1991;77:1901–1905. [PubMed: 1902120]
101. Sawada Y, Fass DN, Katzmann JA, Bahn RC, Bowie EJ. Hemostatic plug formation in normal and von Willebrand pigs: The effect of the administration of cryoprecipitate and a monoclonal antibody to Willebrand factor. Blood 1986;67:1229–1239. [PubMed: 3486009]
102. Ruggeri ZM. Von Willebrand factor. Curr Opin Hematol 2003;10:142–149. [PubMed: 12579041]
103. Pareti FI, Niiya K, McPherson JM, Ruggeri ZM. Isolation and characterization of two domains of human von Willebrand factor that interact with fibrillar collagen types I and III. J Biol Chem 1987;262:13835–13841. [PubMed: 3498719]
104. Hoylaerts MF, Yamamoto H, Nuyts K, Vreys I, Deckmyn H, Vermylen J. von Willebrand factor binds to native collagen VI primarily via its A1 domain. Biochem J 1997;324:185–191. [PubMed: 9164855]
105. Mazzucato M, Spessotto P, Masotti A, De Appollonia L, Cozzi MR, Yoshioka A, Perris R, Colombatti A, De Marco L. Identification of domains responsible for von Willebrand factor type VI collagen interaction mediating platelet adhesion under high flow. J Biol Chem 1999;274:3033–3041. [PubMed: 9915842]
106. Colombatti A, Bonaldo P. The superfamily of proteins with von Willebrand factor type a-like domains: One theme common to components of extracellular matrix, hemostasis, cellular adhesion, and defense mechanisms. Blood 1991;77:2305–2315. [PubMed: 2039815]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

107. Lankhof H, van Hoeij M, Schiphorst ME, Bracke M, Wu YP, Ijsseldijk MJ, Vink T, de Groot PG, Sixma JJ. A3 domain is essential for interaction of von Willebrand factor with collagen type III. Thromb Haemost 1996;75:950–958. [PubMed: 8822592]
108. Romijn RA, Westein E, Bouma B, Schiphorst ME, Sixma JJ, Lenting PJ, Huizinga EG. Mapping the collagen-binding site in the von Willebrand factor-A3 domain. J Biol Chem 2003;278:15035–15039. [PubMed: 12582178]
109. Nishida N, Sumikawa H, Sakakura M, Shimba N, Takahashi H, Terasawa H, Suzuki EI, Shimada I. Collagen-binding mode of vWF-A3 domain determined by a transferred cross-saturation experiment. Nat Struct Biol 2003;10:53–58. [PubMed: 12447349]
110. Lisman T, Raynal N, Groeneveld D, Maddox B, Peachey AR, Huizinga EG, de Groot PG, Farndale RW. A single high-affinity binding site for von Willebrand factor in collagen III, identified using synthetic triple-helical peptides. Blood 2006;108:3753–3756. [PubMed: 16912226]
111. Bonnefoy A, Romijn RA, Vandervoort PA, Van Rompaey I, Vermylen J, Hoylaerts MF. von Willebrand factor A1 domain can adequately substitute for A3 domain in recruitment of flowing platelets to collagen. J Thromb Haemost 2006;4:2151–2161. [PubMed: 16805860]
112. Santoro SA. Preferential binding of high molecular weight forms of von Willebrand factor to fibrillar collagen. Biochimica et Biophysica Acta 1983;756:123–126. [PubMed: 6402037]
113. Wu D, Vanhoorelbeke K, Cauwenberghs N, Meiring M, Depraetere H, Kotze HF, Deckmyn H. Inhibition of the von Willebrand (VWF)-collagen interaction by an antihuman VWF monoclonal antibody results in abolition of in vivo arterial platelet thrombus formation in baboons. Blood 2002;99:3623–3628. [PubMed: 11986216]
114. Ribba AS, Loisel I, Lavergne JM, Juhan-Vague I, Obert B, Cherel G, Meyer D, Girma JP. Ser968thr mutation within the A3 domain of von Willebrand factor (VWF) in two related patients leads to a defective binding of VWF to collagen. Thromb Haemost 2001;86:848–854. [PubMed: 11583318]
115. Schneppenheim R, Budde U. Phenotypic and genotypic diagnosis of von Willebrand disease: A 2004 update. Semin Hematol 2005;42:15–28. [PubMed: 15662612]
116. Fujimura Y, Titani K, Holland LZ, Roberts JR, Kostel P, Ruggeri ZM, Zimmerman TS. A heparin-binding domain of human von Willebrand factor. Characterization and localization to a tryptic fragment extending from amino acid residue Val-449 to Lys-728. J Biol Chem 1987;262:1734–1739. [PubMed: 3100536]
117. Mohri H, Yoshioka A, Zimmerman TS, Ruggeri ZM. Isolation of the von Willebrand factor domain interacting with platelet glycoprotein Ib, heparin, and collagen, and characterization of its three distinct functional sites. J Biol Chem 1989;264:17361–17367. [PubMed: 2477370]
118. Sobel M, Soler DF, Kermode JC, Harris RB. Localization and characterization of a heparin binding domain peptide of human von Willebrand factor. J Biol Chem 1992;267:8857–8862. [PubMed: 1577724]
119. Fretto LJ, Fowler WE, McCaslin DR, Erickson HP, McKee PA. Substructure of human von Willebrand factor. Proteolysis by V8 and characterization of two functional domains. J Biol Chem 1986;261:15679–15689. [PubMed: 3536910]
120. Guidetti GF, Bartolini B, Bernardi B, Tira ME, Berndt MC, Balduini C, Torti M. Binding of von Willebrand factor to the small proteoglycan decorin. FEBS Lett 2004;574:95–100. [PubMed: 15358546]
121. Roberts DD, Williams SB, Gralnick HR, Ginsburg V. von Willebrand factor binds specifically to sulfated glycolipids. J Biol Chem 1986;261:3306–3309. [PubMed: 3485094]
122. Christophe O, Obert B, Meyer D, Girma JP. The binding domain of von Willebrand factor to sulfatides is distinct from those interacting with glycoprotein Ib, heparin, and collagen and resides between amino acid residues Leu 512 and Lys 673. Blood 1991;78:2310–2317. [PubMed: 1834252]
123. Andrews RJ, Booth WJ, Bendall LJ, Brendt MC. The amino acid sequence glutamine-628 to valine-646 within the A1 repeat domain mediates binding of von Willebrand factor to bovine brain sulfatides and equine tendon collagen. Platelets 1995;6:245–251. [PubMed: 21043708]

124. Borthakur G, Cruz MA, Dong JF, McIntire L, Li F, Lopez JA, Thiagarajan P. Sulfatides inhibit platelet adhesion to von Willebrand factor in flowing blood. J Thromb Haemost 2003;1:1288–1295. [PubMed: 12871332]
125. Sobel M, McNeill PM, Carlson PL, Kermode JC, Adelman B, Conroy R, Marques D. Heparin inhibition of von Willebrand factor-dependent platelet function in vitro and in vivo. J Clin Invest 1991;87:1787–1793. [PubMed: 2022745]
126. Hada M, Kaminski M, Bockenstedt P, McDonagh J. Covalent crosslinking of von Willebrand factor to fibrin. Blood 1986;68:95–101. [PubMed: 2872929]
127. Ribes JA, Francis CW. Multimer size dependence of von Willebrand factor binding to crosslinked or noncrosslinked fibrin. Blood 1990;75:1460–1465. [PubMed: 2107883]
128. Savage B, Sixma JJ, Ruggeri ZM. Functional self-association of von Willebrand factor during platelet adhesion under flow. Proc Natl Acad Sci USA 2002;99:425–430. [PubMed: 11756664]
129. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colannino NM, Azacar J, Seder RH, Hong SL, Deykin D. Unusually large plasma factor VIII:von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. New Engl J Med 1982;307:1432–1435. [PubMed: 6813740]
130. Arya M, Anvari B, Romo GM, Cruz MA, Dong JF, McIntire LV, Moake JL, Lopez JA. Ultralarge multimers of von Willebrand factor form spontaneous high-strength bonds with the platelet glycoprotein Ib-IX complex: Studies using optical tweezers. Blood 2002;99:3971–3977. [PubMed: 12010796]
131. Shankaran H, Alexandridis P, Neelamegham S. Aspects of hydrodynamic shear regulating shear-induced platelet activation and self-association of von Willebrand factor in suspension. Blood 2003;101:2637–2645. [PubMed: 12456504]
132. Goto S, Salomon DR, Ikeda Y, Ruggeri ZM. Characterization of the unique mechanism mediating the shear-dependent binding of soluble von Willebrand factor to platelets. J Biol Chem 1995;270:23352–23361. [PubMed: 7559492]
133. Ulrichts H, Vanhoorelbeke K, Girma JP, Lenting PJ, Vauterin S, Deckmyn H. The von Willebrand factor self-association is modulated by a multiple domain interaction. J Thromb Haemost 2005;3:552–561. [PubMed: 15748246]
134. Choi H, Aboulfatova K, Pownall HJ, Cook R, Dong JF. Shear-induced disulfide bond formation regulates adhesion activity of von Willebrand factor. J Biol Chem 2007;282:35604–35611. [PubMed: 17925407]
135. Li Y, Choi H, Zhou Z, Nolasco L, Pownall HJ, Voorberg J, Moake JL, Dong JF. Covalent regulation of ULVWF string formation and elongation on endothelial cells under flow conditions. J Thromb Haemost 2008;6:1135–1143. [PubMed: 18433456]
136. Siediecki CA, Lestini BJ, Kottke-Marchant K, Eppell SJ, Wilson DL, Marchant RE. Shear-dependent changes in the three-dimensional structure of human von Willebrand factor. Blood 1996;88:2939–2950. [PubMed: 8874190]
137. Celikel R, Ruggeri ZM, Varughese KI. von Willebrand factor conformation and adhesive function is modulated by an internalized water molecule. Nat Struct Biol 2000;7:881–884. [PubMed: 11017197]
138. Ruggeri ZM, Pareti FI, Mannucci PM, Ciavarella N, Zimmerman TS. Heightened interaction between platelets and factor VIII/von Willebrand factor in a new subtype of von Willebrand's disease. New Engl J Med 1980;302:1047–1051. [PubMed: 6767976]
139. Hulstein JJ, de Groot PG, Silence K, Veyradier A, Fijnheer R, Lenting PJ. A novel nanobody that detects the gain-of-function phenotype of von Willebrand factor in ADAMTS13 deficiency and von Willebrand disease type 2B. Blood 2005;106:3035–3042. [PubMed: 16014562]
140. Dumas JJ, Kumar R, McDonagh T, Sullivan F, Stahl ML, Somers WS, Mosyak L. Crystal structure of the wild-type von Willebrand factor A1-glycoprotein Ibα complex reveals conformation differences with a complex bearing von Willebrand disease mutations. J Biol Chem 2004;279:23327–22334. [PubMed: 15039442]
141. Huizinga EG, Tsuji S, Romijn RA, Schiphorst ME, de Groot PG, Sixma JJ, Gros P. Structures of glycoprotein Ibα and its complex with von Willebrand factor A1 domain. Science 2002;297:1176–1179. [PubMed: 12183630]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

142. Wagner DD. The Weibel-Palade body: The storage granule for von Willebrand factor and P-selectin. Thromb Haemost 1993;70:105–110. [PubMed: 7694385]
143. Lopez-Fernandez MF, Ginsberg MH, Ruggeri ZM, Batlle J, Zimmerman TS. Multimeric structure of platelet factor VIII/von Willebrand factor. The presence of larger multimers and their reassociation with thrombin-stimulated platelets. Blood 1982;60:1132–1138. [PubMed: 6982084]
144. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, Schade AJ, McIntire LV, Fujikawa K, Lopez JA. ADAMTS-13 rapidly cleaves newly secreted ultra-large von Willebrand factor multimers on the endothelial surface under flowing conditions. Blood 2002;100:4033–4039. [PubMed: 12393397]
145. Levy GG, Nichols WC, Lian EC, Foroud T, McClintock JN, McGee BM, Yang AY, Siemieniak DR, Stark KR, Gruppo R, Sarode R, Shurin SB, Chandrasekaran V, Stabler SP, Sabio H, Bouhassira EE, Upshaw JD Jr. Ginsburg D, Tsai HM. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature 2001;413:488–494. [PubMed: 11586351]
146. Dent JA, Berkowitz SD, Ware J, Kasper CK, Ruggeri ZM. Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type iia von Willebrand factor. Proc Natl Acad Sci USA 1990;87:6306–6310. [PubMed: 2385594]
147. Dent JA, Galbusera M, Ruggeri ZM. Heterogeneity of plasma von Willebrand factor multimers resulting from proteolysis of the constituent subunit. J Clin Invest 1991;88:774–782. [PubMed: 1909351]
148. Moake JL. Thrombotic microangiopathies. New Engl J Med 2002;347:589–600. [PubMed: 12192020]
149. Donadelli R, Orje JN, Capoferri C, Remuzzi G, Ruggeri ZM. Size regulation of von Willebrand factor-mediated platelet thrombi by ADAMTS-13 in flowing blood. Blood 2006;107:1943–1950. [PubMed: 16293606]
150. Chauhan AK, Motto DG, Lamb CB, Bergmeier W, Dockal M, Plaimauer B, Scheiflinger F, Ginsburg D, Wagner DD. Systemic antithrombotic effects of ADAMTS13. J Exp Med 2006;203:767–776. [PubMed: 16533881]
151. Ruggeri ZM, Dent JA, Saldivar E. Contribution of distinct adhesive interactions to platelet aggregation in flowing blood. Blood 1999;94:172–178. [PubMed: 10381510]
152. Chion CK, Doggen CJ, Crawley JT, Lane DA, Rosendaal FR. ADAMTS13 and von Willebrand factor and the risk of myocardial infarction in men. Blood 2007;109:1998–2000. [PubMed: 17053057]
153. Ruggeri ZM, Bader R, De Marco L. Glanzmann thrombasthenia: Deficient binding of von Willebrand factor to thrombin-stimulated platelets. ProcNatlAcadSci 1982;79:6038–6041.
154. Ruggeri ZM, De Marco L, Gatti L, Bader R, Montgomery RR. Platelets have more than one binding site for von Willebrand factor. J Clin Invest 1983;72:1–12. [PubMed: 6223940]
155. Ginsberg MH, Partridge A, Shattil SJ. Integrin regulation. Curr Opin Cell Biol 2005;17:509–516. [PubMed: 16099636]
156. Coller BS, Cheresh DA, Asch E, Seligsohn U. Platelet vitronectin receptor expression differentiates iraqi-Jewish from Arab patients with Glanzmann thrombasthenia in Israel. Blood 1991;77:75–83. [PubMed: 1702031]
157. Dejana E, Lampugnani MG, Giorgi M, Gaboli M, Federici AB, Ruggeri ZM. von Willebrand factor promotes endothelial cell adhesion via an arg-gly-asp-dependent mechanism. J Cell Biol 1989;109:367–375. [PubMed: 2545728]
158. Romo GM, Dong JF, Schade AJ, Gardiner EE, Kansas GS, Li CQ, McIntire LV, Berndt MC, Lopez JA. The glycoprotein Ib-IX-V complex is a platelet counterreceptor for P-selectin. J Exp Med 1999;190:803–814. [PubMed: 10499919]
159. Simon DI, Chen Z, Xu H, Li CQ, Dong JF, McIntire LV, Ballantyne CM, Zhang L, Furman MI, Berndt MC, Lopez JA. Platelet glycoprotein Ibα is a counterreceptor for the leukocyte integrin Mac-1 (cd11b/cd18). J Exp Med 2000;192:193–204. [PubMed: 10899906]
160. Coller BS, Peerschke EI, Scudder LE, Sullivan CA. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

normal platelets and binds to glycoproteins IIb and/or IIIa. J Clin Invest 1983;72:325–338. [PubMed: 6308050]

161. Andre P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR, Wagner DD. CD40L stabilizes arterial thrombi by a β3 integrin-dependent mechanism. Nat Med 2002;8:247–252. [PubMed: 11875495]
162. Doggett TA, Girdhar G, Lawshe A, Schmidtke DW, Laurenzi IJ, Diamond SL, Diacovo TG. Selectin-like kinetics and biomechanics promote rapid platelet adhesion in flow: The GPIbα-vWF tether bond. Biophys J 2002;83:194–205. [PubMed: 12080112]
163. Marshall BT, Long M, Piper JW, Yago T, McEver RP, Zhu C. Direct observation of catch bonds involving cell-adhesion molecules. Nature 2003;423:190–193. [PubMed: 12736689]
164. Yago T, Lou J, Wu T, Yang J, Miner JJ, Coburn L, Lopez JA, Cruz MA, Dong JF, McIntire LV, McEver RP, Zhu C. Platelet glycoprotein Ibα forms catch bonds with human WT vWF but not with type 2B von Willebrand disease vWF. J Clin Invest 2008;118:3195–3207. [PubMed: 18725999]
165. Ruggeri ZM. Type iib von Willebrand disease: A paradox explains how von Willebrand works. J Thromb Haemost 2004;2:2–6. [PubMed: 14717957]
166. Lou J, Zhu C. Flow induces loop-to-β-hairpin transition on the β-switch of platelet glycoprotein Ibα. Proc Natl Acad Sci U S A 2008;105:13847–13852. [PubMed: 18772372]
167. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM, Wagner DD. Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. Blood 2000;96:3322–3328. [PubMed: 11071623]
168. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: An interaction mediated by endothelial P-selectin. Proc Natl Acad Sci USA 1995;92:7450–7454. [PubMed: 7543682]
169. Ruggeri ZM, Orje JN, Habermann R, Federici AB, Reininger AJ. Activation-independent platelet adhesion and aggregation under elevated shear stress. Blood 2006;108:1903–1910. [PubMed: 16772609]
170. Mody NA, King MR. Platelet adhesive dynamics. Part I: Characterization of platelet hydrodynamic collisions and wall effects. Biophys J 2008;95:2539–2555. [PubMed: 18515387]
171. Mody NA, King MR. Platelet adhesive dynamics. Part II: High shear-induced transient aggregation via GPIbα-vWF-GPIbα bridging. Biophys J 2008;95:2556–2574. [PubMed: 18515386]
172. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962;194:927–929. [PubMed: 13871375]
173. Lefkovits J, Plow EF, Topol EJ. Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. New Engl J Med 1995;332:1553–1559. [PubMed: 7739710]
174. Peterson DM, Stathopoulos NA, Giorgio TD, Hellums JD, Moake JL. Shear-induced platelet aggregation requires von Willebrand factor and platelet membrane glycoproteins Ib and IIb-IIIa. Blood 1987;69:625–628. [PubMed: 3492225]
175. Ikeda Y, Handa M, Kawano K, Kamata T, Murata M, Araki Y, Anbo H, Kawai Y, Watanabe K, Itagaki I, Sakai K, Ruggeri ZM. The role of von Willebrand factor and fibrinogen in platelet aggregation under varying shear stress. J Clin Invest 1991;87:1234–1240. [PubMed: 2010539]
176. Goto S, Ikeda Y, Saldivar E, Ruggeri ZM. Distinct mechanisms of platelet aggregation as a consequence of different shearing flow conditions. J Clin Invest 1998;101:479–486. [PubMed: 9435321]
177. Weiss HJ, Rogers J. Fibrinogen and platelets in the primary arrest of bleeding-studies in two patients with congenital afibrinogenemia. New Engl J Med 1971;285:369–374. [PubMed: 5556576]
178. Sadler JE, Budde U, Eikenboom JC, Favaloro EJ, Hill FG, Holmberg L, Ingerslev J, Lee CA, Lillicrap D, Mannucci PM, Mazurier C, Meyer D, Nichols WL, Nishino M, Peake IR, Rodeghiero F, Schneppenheim R, Ruggeri ZM, Srivastava A, Montgomery RR, Federici AB. Update on the pathophysiology and classification of von Willebrand disease: A report of the subcommittee on von Willebrand factor. J Thromb Haemost 2006;4:2103–2114. [PubMed: 16889557]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

179. Nesbitt WS, Giuliano S, Kulkarni S, Dopheide SM, Harper IS, Jackson SP. Intercellular calcium communication regulates platelet aggregation and thrombus growth. J Cell Biol 2003;160:1151–1161. [PubMed: 12668663]
180. Goto S, Tamura N, Ishida H, Ruggeri ZM. Dependence of platelet thrombus stability on sustained glycoprotein IIb/IIIa activation through adenosine 5'-diphosphate receptor stimulation and cyclic calcium signaling. J Am Coll Cardiol 2005;47:155–162. [PubMed: 16386680]
181. Giandomenico G, Dellas C, Czekay RP, Koschnick S, Loskutoff DJ. The leptin receptor system of human platelets. J Thromb Haemost 2005;3:1042–1049. [PubMed: 15869602]
182. Konstantinides S, Schafer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. J Clin Invest 2001;108:1533–1540. [PubMed: 11714745]
183. Konstantinides S, Schafer K, Neels JG, Dellas C, Loskutoff DJ. Inhibition of endogenous leptin protects mice from arterial and venous thrombosis. Atertio Thromb Vasc Biol 2004;24:2196–2201.
184. Angelillo-Scherrer A, de Frutos P, Aparicio C, Melis E, Savi P, Lupu F, Arnout J, Dewerchin M, Hoylaerts M, Herbert J, Collen D, Dahlback B, Carmeliet P. Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. Nat Med 2001;7:215–221. [PubMed: 11175853]
185. Balogh I, Hafizi S, Stenhoff J, Hansson K, Dahlback B. Analysis of Gas6 in human platelets and plasma. Atertio Thromb Vasc Biol 2005;25:1280–1286.
186. Gould WR, Baxi SM, Schroeder R, Peng YW, Leadley RJ, Peterson JT, Perrin LA. Gas6 receptors Axl, Sky and Mer enhance platelet activation and regulate thrombotic responses. J Thromb Haemost 2005;3:733–741. [PubMed: 15733062]
187. Prevost N, Woulfe DS, Jiang H, Stalker TJ, Marchese P, Ruggeri ZM, Brass LF. Eph kinases and ephrins support thrombus growth and stability by regulating integrin outside-in signaling in platelets. ProcNatlAcadSciUSA 2005;102:9820–9825.
188. Nanda N, Bao M, Lin H, Clauser K, Komuves L, Quertermous T, Conley PB, Phillips DR, Hart MJ. Platelet endothelial aggregation receptor 1 (PEAR1), a novel epidermal growth factor repeat-containing transmembrane receptor, participates in platelet contact-induced activation. J Biol Chem 2005;280:24680–24689. [PubMed: 15851471]
189. Nanda N, Andre P, Bao M, Clauser K, DeGuzman F, Howle D, Conley PB, Terhorst C, Phillips DR. Platelet aggregation induces platelet aggregate stability via SLAM family receptor signaling. Blood 2005;106:3028–3034. [PubMed: 16037392]
190. Mazzucato M, Pradella P, Cozzi MR, De Marco L, Ruggeri ZM. Sequential cytoplasmic calcium signals in a two-stage platelet activation process induced by the glycoprotein Ibα mechanoreceptor. Blood 2002;100:2793–2800. [PubMed: 12351387]

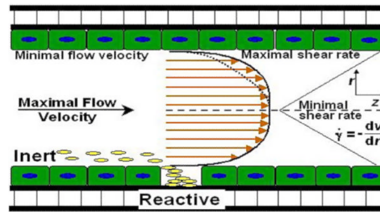

Figure 1. Schematic representation of blood flow in a vessel

Normal endothelial cells are non reactive for platelets, but exposed subendothelial structures induce rapid platelet adhesion and aggregation. Blood flow in a cylindrical vessel can be visualized as a series of fluid layers (laminae) moving at different velocity. The laminae near the center of the vessel have greater velocity than those near the wall (depicted by arrows of different length). The corresponding velocity profile (solid line) is more blunted than the parabolic profile expected with a homogeneous suspension (dotted line) because of cell depletion in the boundary layer near the wall. The shear rate is the rate of change of velocity with respect to distance measured perpendicularly to the direction of flow. The negative sign indicates that the gradient is defined from the center (where velocity is maximal) to the wall (where velocity is minimal).

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

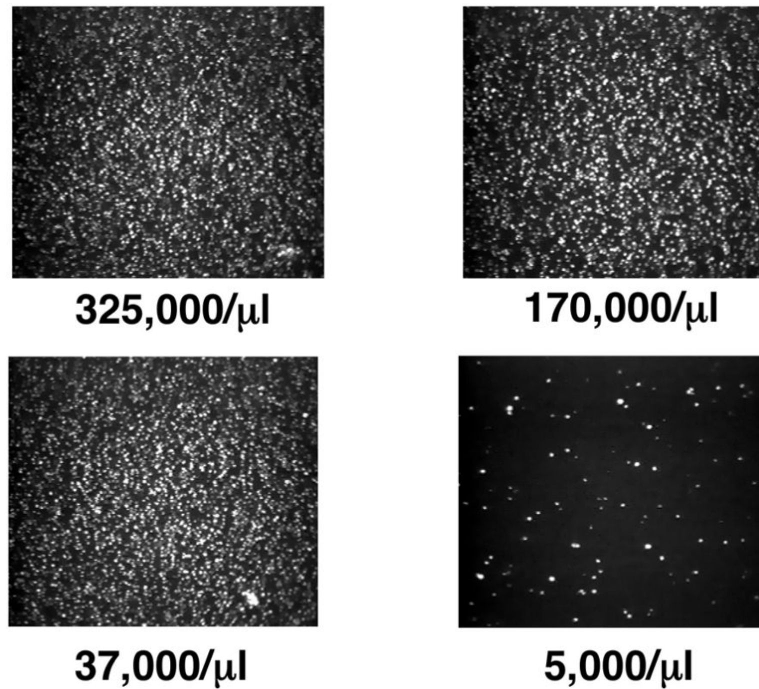

Figure 2. Platelet count and surface adhesion

These images are derived from a real time experiment recorded at the video rate of 30 frames per second. Whole blood containing the α-thrombin inhibitor D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone dihydrochloride (PPACK) as the anticoagulant was perfused over surfaces coated with immobilized VWF at the wall shear rate of 1,500 s^{-1}. Each fluorescent (white) particle is a single platelet tethered to the surface. The platelet count in the perfused blood was decreased by sedimenting the cells at low g force, removing the platelet rich plasma layer and replacing it with the same volume of homologous platelet poor plasma. Each image represents a single frame recorded one minute after initiating blood perfusion. Typically, surface coverage was maximal within 10 seconds from the beginning of flow. Note the decrease in surface coverage only at the lowest platelet count tested.

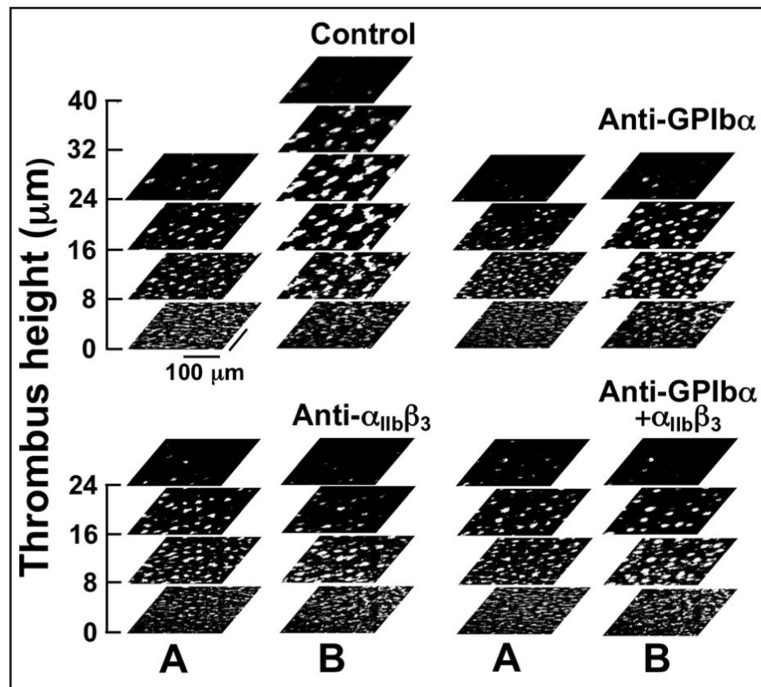

Figure 3. Role of GP Ibα in platelet aggregation during thrombus growth

Blood containing PPACK as the anticoagulant was perfused over collagen type I fibers for 100 seconds at the wall shear rate of 1500 s^{-1}. At this point, the height of thrombi was measured from confocal (*z*) sections, as shown in the stacks labeled A, while the perfusion continued for an additional 740 seconds with blood containing either buffer or function-blocking monoclonal antibodies directed against GP Ibα or αIIb-β3, or both, as indicated. The flow rate was decreased such that the calculated shear rate was 300 s^{-1} at the collagen coated surface but >1000 s^{-1} at the surface of thrombi with a height >20 mμ. After a total perfusion time of 840 seconds, thrombus height was measured by confocal sections, as shown in the stacks labeled B. The results demonstrate that the adhesive function of GP Ibα is as necessary as that of αIIbβ3 to sustain platelet aggregation at the edge of a growing thrombus. Modified from Ruggeri ZM et al [151] and reprinted with permission.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

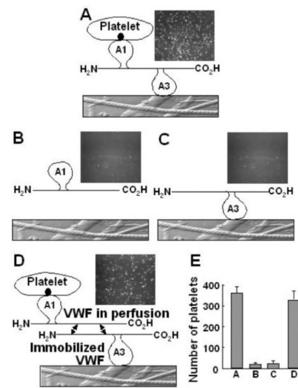

Figure 4. Functional self-association of VWF multimers

A washed blood cells suspensions devoid of plasma proteins and containing EDTA to block integrin function and prostaglandin E_1 to block platelet activation was perfused over immobilized collagen type I fibers at the wall shear rate of 1500 s^{-1}. (A) Control experiment with normal multimeric VWF added to the cell suspension. The A3 domain mediates VWF binding to collagen, and the A1 domain interacts with platelet GP Ibα. Tethered platelets are seen rolling on the surface, which is represented by an electron micrograph of collagen fibrils. (B, C) Experiments performed after adding to the cell suspension, respectively, recombinant VWF devoid of the A3 domain (ΔA3-VWF), which cannot bind to collagen, or devoid of the A1 domain (ΔA1-VWF), which binds to collagen but cannot interact with platelet GP Ibα. In either case, no platelets are seen tethered to the surface. (D) Collagen was pre-coated with ΔA1-VWF multimers, which cannot initiate platelet tethering, and then exposed to the blood cell suspension containing ΔA3-VWF. Although the latter cannot bind directly to collagen (see b), it could compensate for the lack of A1 domain in the surface-bound VWF and restore platelet tethering. The association of VWF multimers with one another can explain this result; the two-sided arrows between multimers indicate that the association is reversible. The images are single frames from a real time recording representing an area of 65,536 μm^2. The bar graph (E) shows the number of platelets tethered to the surface under the different experimental conditions described above (mean ± SEM of two separate experiments). Modified from Savage B et al [128] and reprinted with permission.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

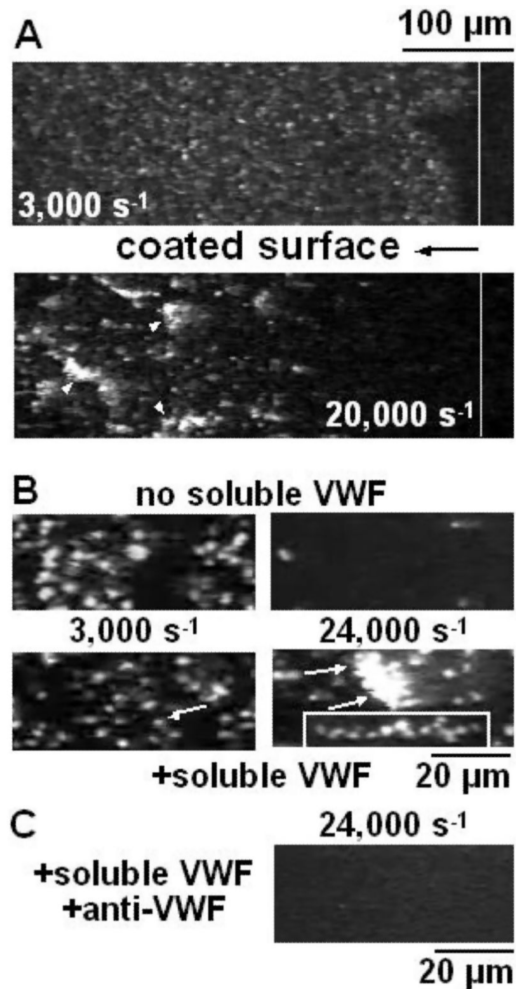

Figure 5. Activation-independent platelet adhesion and aggregation at the interface of immobilized and soluble VWF

(A). Blood containing 93 μM PPACK as anticoagulant, the fluorescent dye mepacrine (10 μM), prostaglandin (PG) E_1 (10 μM) to inhibit platelet activation, and EDTA (5 mM) to prevent ligand binding to integrins, was perfused over immobilized VWF (20 μg/ml coating concentration). The white line delimits VWF-coated (to the left) from uncoated glass. Single platelets adhere when the shear rate is 3,000 s^{-1} (top); rolling aggregates (some identified by arrowheads) form at 20,000 s^{-1} (bottom). (B). Perfusion over immobilized VWF of washed blood cells suspended in buffer (20 mM Hepes, 150 mM NaCl, pH 7.4). In the absence of soluble VWF, single platelets adhere when the shear rate is 3,000 s^{-1} (upper left), and fewer single platelets adhere at 24,000 s^{-1} (upper right). After adding soluble VWF (20 μg/ml), single platelets adhere at 3,000 s^{-1} (lower left; an arrow points to a single platelet shown for reference), but aggregates form at 24,000 s^{-1} (lower right; arrows point to a rolling aggregate and an inset highlights a stretched aggregate during stationary adhesion). (C). Perfusion over immobilized VWF of washed blood cells with added soluble VWF and anti-VWF A1 domain monoclonal antibody (NMC-4, 20 μg/ml).[133] No platelet adhesion is detected. Modified from Ruggeri ZM et al [169] and reprinted with permission.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

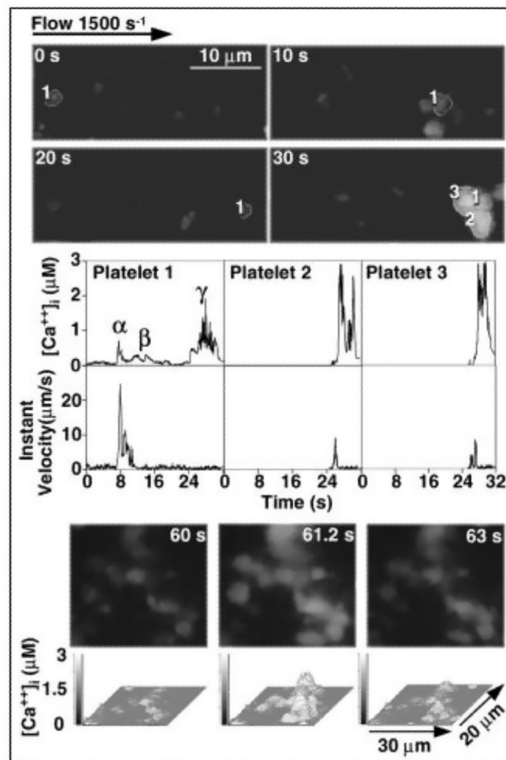

Figure 6. Real time analysis of $[Ca^{++}]_i$ during platelet translocation and aggregate formation on immobilized VWF

Platelets loaded with fluo-3 AM (2×10^7/ml) were suspended with washed erythrocytes in homologous plasma and perfused over immobilized VWF for 3 min at the shear rate of 1500 s^{-1}. The sequence of images at the top shows an example of aggregate formation. At 0 s, platelet 1 appears in the optical field; at 10 s, it has moved in the direction of flow by approximately 20 µm; at 20 s, it has moved by an additional few µm; at 30 s, it is in the same position, and two new platelets (2 and 3) are attached in close proximity forming a small aggregate. The diagrams in the middle show $[Ca^{++}]_i$ and instant velocity of platelets 1, 2 and 3. The translocation of platelet 1 occurs mostly during a few seconds of relatively rapid movement, coincident with the appearance of transient $[Ca^{++}]_i$ peaks (α/β); a higher and longer lasting increase in $[Ca^{++}]_i$ (γ) develops while the platelet is stationary. Cytosolic Ca^{++} oscillations appear also when platelets 2 and 3 arrest on the surface, without a clear sequence from α/β to γ. The images at the bottom, captured between 60 and 63 s after the appearance of platelet 1 in the field, show the long lasting synchronous increase of $[Ca^{++}]_i$ in platelets forming a large aggregate. The 3D diagrams below each image show the measurement of $[Ca^{++}]_i$ in all the platelets in the field. Modified from Mazzucato M et al [190]and reprinted with permission.