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REVIEW

Platelets and physics: How platelets "feel" and respond to their mechanical microenvironment

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ABSTRACT

During clot formation, platelets are subjected to various different signals and cues as they dynamically interact with extracellular matrix proteins such as von Willebrand factor (vWF), fibrin(ogen) and collagen. While the downstream signaling of platelet–ligand interactions is well-characterized, biophysical cues, such as hydrodynamic forces and mechanical stiffness of the underlying substrate, also mediate these interactions and affect the binding kinetics of platelets to these proteins. Recent studies have observed that, similar to nucleated cells, platelets mechanosense their microenvironment and exhibit dynamic physiologic responses to biophysical cues. This review discusses how platelet mechanosensing is affected by the hydrodynamic forces that dictate vWF–platelet interactions and fibrin polymerization and network formation. The similarities and differences in mechanosensing between platelets and nucleated cells and integrin-mediated platelet mechanosensing on both fibrin(ogen) and collagen are then reviewed. Further studies investigating how platelets interact with the mechanical microenvironment will improve our overall understanding of the hemostatic process.

1. Introduction

Platelets are anucleate megakaryocyte fragments that circulate in the bloodstream and rapidly detect and respond to vascular injury [1–4]. Upon vascular injury, the subendothelium is exposed and von Willebrand factor (vWF) deposits on the exposed collagen. Platelets are arrested at the site of injury *via* interactions with these adhesive extracellular molecules, and once adhered, activated platelets aggregate and interact with the polymerizing fibrin network to form a hemostatic plug that stops bleeding. The biochemical cues that promote clot formation such as ADP, thromboxane A2, and thrombin have been extensively studied, less is known about how the physical environment affects platelet function. Indeed, biophysical cues, such as hydrodynamic forces and mechanical stiffness of the underlying substrate, also mediate those specific platelet–ligand interactions and affect the binding kinetics of platelets to those specific ligands (Fig. 1). For example, recent studies have observed that platelets also physiologically respond to the mechanical cues of their microenvironment, such as the stiffness of the extracellular matrix or the nascent fibrin network [5].

Numerous studies in the last several years have documented that nucleated cells interact dynamically with their mechanical microenvironment [6–9]. Specialized surface membrane receptors connect cells with the extracellular domain and allow them to detect the mechanical properties of the microenvironment. Mechanotransduction of these signals results in various cell responses, including morphological changes, altered proliferation rates, migration, and differentiation [8–10]. The group of proteins that mediates sensing of the mechanical microenvironment in nucleated cells is also expressed in platelets. Indeed, it was recently found that platelets detect mechanical changes in their microenvironment *via* similar mechanisms as nucleated cells [5]. As there are physiological differences between platelets and nucleated cells, additional comprehensive studies on platelet mechanosensing are necessary to fully understand how mechanical stimuli affect platelets. In this review, we will first discuss how hydrodynamic forces regulate vWF conformational changes and cleavage *via* enzymatic proteolysis to alter the platelet microenvironment, and thus ultimately regulate vWF–platelet interactions. We will then focus on the microenvironment's influence on the formation of a fibrin network that can exhibit a wide range of mechanical properties that will, in turn, affect platelet mechanotransduction. Finally, we will provide a framework for integrin-based mechanosensing by platelets, adhered to both fibrinogen and collagen that is based on the well-documented responses of nucleated cells. By comparing the

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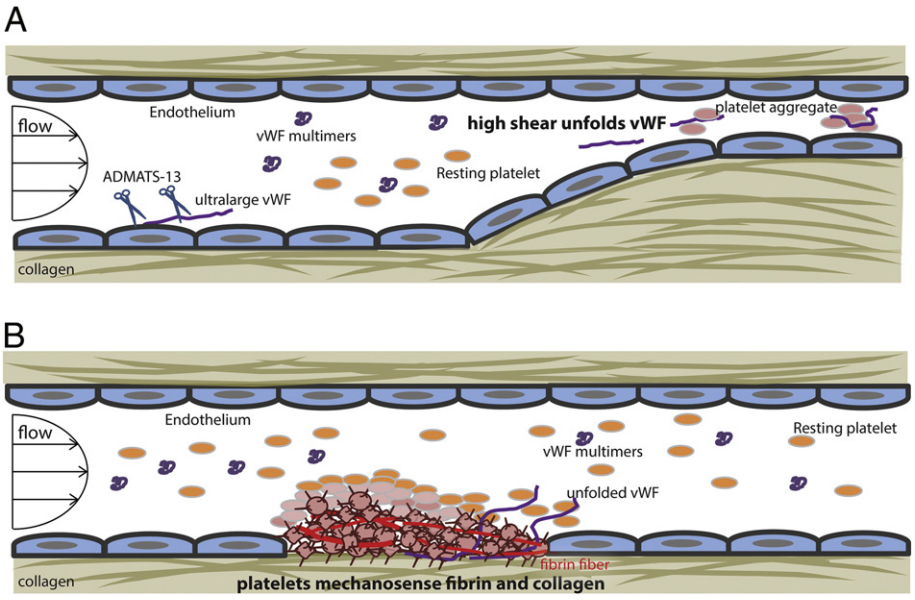

Fig. 1. Platelets interact with ECM proteins during clot formation. Hydrodynamic forces and the mechanical microenvironment affect each of these platelet–ligand interactions. A) vWF regulation and vWF-dependent platelet aggregate formation at high shear rates. Ultralarge vWF molecules (purple) are released from the Weibel–Palade bodies of activated endothelial cells and attach to the endothelium. A2 domain exposure on adhered ultralarge vWF leads to cleavage of ultralarge vWF by ADAMTS-13 into vWF multimers. vWF multimers adopt a globular conformation, which prevents both platelet binding and cleavage by ADAMTS-13. However, under pathological high shear stress, for example at the site of stenosis, vWF multimers are unfolded by hydrodynamic forces to switch to an extended conformation, thus causing platelets to bind and form aggregate. B) Clot formation at the vessel injury site. In vascular injury, the endothelium is damaged and underlying collagen (brown lines) is exposed to the bloodstream. vWF multimers adhere to exposed collagen and adapt an unfolded conformation due to wall shear stress. Platelets are arrested at the injury site *via* interactions with both collagen and vWF, both of which initiate platelet activation and recruit additional platelets. At the injury site, platelets interact with fibrin fibers (red), which display a wide range of mechanical properties, to form a clot to stop bleeding. The mechanical properties of the underlying collagen and fibrin, in and of themselves, affect platelet physiology and function.

similarities and differences in mechanosensing and mechanotransduction between platelets and nucleated cells, we hope to provide insight into platelet mechanosensing that promotes better understanding of platelet physiology during clot formation.

2. von Willebrand factor (vWF)

vWF is a large plasma glycoprotein that plays an integral role in platelet adhesion and activation (Fig. 1A). It consists of 275 kDa disulfide-linked subunits, and each subunit contains domains that specifically interact with platelets, the extracellular matrix, and plasma proteins [11]. A1, the major functional domain, contains the binding site for platelet membrane receptor glycoprotein (GP) Ib [12]. In addition, A2 domains contain an enzyme cleavage site to ADAMTS13, a plasma metalloprotease, which regulates the size of vWF multimers *via* proteolysis. Further, A3 domains bind collagen and C1 domains contain an RGD sequence that interacts with platelet receptors integrin αIIbβ3 and αvβ3 [12]. Hydrodynamic forces regulate the exposure of these domains by changing the conformation of vWF multimers, thereby modulating both the affinity and avidity of vWF for platelet binding and vWF-mediated platelet aggregation. In this section, we will first discuss how hydrodynamic forces, enzyme proteolysis, and collagen collectively regulate vWF size and conformation, which in turn regulate its interaction with platelets. We will then focus on the role of GPIb-A1 binding kinetics and GPIb-mediated mechanosensing in regulating platelet recruitment, adhesion, and signal transmission.

2.1. vWF production, storage and ultralarge vWF

vWF is synthesized in megakaryocytes and stored in their α-granules (which are later partitioned amongst platelets); vWF is also synthesized in endothelial cells where it is stored in Weibel–Palade bodies and released upon endothelial cell stimulation. Stored and newly released vWF primarily exists as ultra-large multimers, which have a total molecular weight of greater than 50, 000 kDa [13]. Ultra-large vWF multimers are highly prothrombotic and bind to platelets with high avidity even at low shear rates [14]. Upon release into the bloodstream, ultra-large vWF attaches to the cell surface. ADAMTS13 then cleaves vWF at the Tyr 1605–Met1606 scissile bond in the central vWF A2 domains to form smaller, less adhesive vWF multimers. Cleavage reduces the high avidity of ultra-large vWF, thereby preventing platelet clumping and regulating vWF-mediated clot formation [13,15,16]. Importantly, cleavage of ultra-large vWF into smaller multimers functionally shifts the shear regime in which vWF-mediated platelet activation occurs, as higher forces are required to expose platelet binding domains in the smaller multimers.

2.2. Shear-induced conformation change and unfolding of vWF

In circulation at low shear rates, cleaved vWF multimers adopt a globular conformation, in which the A1, A2, and A3 binding domains (sequentially located near the center the monomer) are covered [11,15]. vWF unfolds above a critical shear rate, both in free flow and when one end is surface bound. Mechanistically, unfolding is thought to proceed from hydrodynamic forces acting on small extensions projecting from the tumbling globular vWF [17]. This process is rapid and reversible, and simulations suggest that the critical shear rate for unfolding is more dependent on the multimer radius than on its total length [17]. Additional simulations suggest that elongation flow, such as that occurring in stenosis, more readily unfolds vWF at physiological flow rates [18].

Experiments on individual A2 domains showed that cleavage by ADAMTS13 only occurs when locally applied tension is above a threshold value [19]. As the force exerted on a vWF multimer is dependent on shear rate and multimer size, ADAMTS13 should not be able to cleave vWF multimers that remain below a critical length [14]. In addition, physiological levels of calcium play an important role in stabilizing the native conformation of vWF, and thereby increase the force needed for unfolding [20]. Careful kinetic measurements predict that at physiologic ADAMTS13 concentrations, cleavage occurs on time scales of 200 s,