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Review Article

Microvascular platforms for the study of platelet-vessel wall interactions

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ABSTRACT

Platelets interact with the endothelium to regulate vascular integrity and barrier function, mediate inflammation and immune response, and prevent and arrest hemorrhage. In this review, we describe existing tools to study the flow-dependent interactions of platelets with the vessel wall. We also discuss our work on building engineered microvessels to study the roles of platelets on endothelial barrier function, endothelial sprouting, and thrombus formation on both quiescent and stimulated endothelium. In particular, we will show the advantage of using a cell-remodelable system in the studies of platelet-vessel wall interactions.

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Introduction

Platelets are anulceate blood cells that circulate at the periphery of the blood stream, being displaced there by the larger and denser erythrocytes. From this position, they constantly survey the endothelium for defects, either larger injuries that would result in hemorrhage, or smaller defects that perturb the vessel's barrier function. Under normal circumstances, the platelets do not adhere to intact endothelium, attaching to the vessel wall only upon exposure of subendothelial proteins at sites of vessel injury. These adhesive interactions, coupled with exposure to platelet agonists generated at the site or released from other platelets, cause the platelets to attach to each other and form an occlusive plug. Under some unusual pathological circumstances, the platelets can also form thrombi on intact endothelium, sometimes leading to occlusion of small blood vessels [1].

Endothelium, another crucial component involved in hemostasis and homeostasis, serves as an interface between the blood and surrounding tissue [2]. If thought of as a single organ, the endothelium is one of the largest organs in the body, with a combined surface area of approximately 1000 m^2 in an adult human [3,4]. The endothelium is involved in many vital functions, including the regulation of tissue perfusion and blood pressure, fluid and solute exchange, hemostasis, inflammation, and angiogenesis. Healthy endothelium provides a non-adhesive and antithrombotic surface for the transport of blood through the vessel, largely by expressing molecules that inhibit platelet adhesion (prostacyclin, nitric oxide, and ecto-ADPase) and blood coagulation (*e.g.*, thrombomodulin). During inflammation or injury, however, the endothelial cells become activated, and release the contents of their granules, Weibel-Palade bodies (WPBs) [5], the major component of which is von Willebrand factor (VWF) [6], and express adhesion molecules such as P- and E- selectins

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on their surfaces. A portion of the newly released VWF remains attached transiently to the endothelial surface, and recruits platelets from the blood stream until the VWF is proteolytically removed from the endothelial surface by the plasma metalloprotease ADAMTS13 [7,8]. If removal of VWF from the endothelial surface is delayed, the formation of thrombi and subsequent shedding of relatively large emboli may occlude downstream vasculature. In larger vessels, this mechanism can contribute to pathologies such as myocardial infarction, stroke, and deep vein thrombosis; whereas microvascular thrombi contribute to organ dysfunction and failure [9], either in isolated organs, or systemically in conditions such as thrombotic thrombocytopenic purpura (TTP) [10].

The adhesion and aggregation of platelets largely depend on the blood flow and shear conditions at sites of vascular activation or injury [11]. The flow velocity in blood vessels is greater at the center than near the vessel wall, and the velocity difference creates a shearing effect between adjacent fluid layers. This shear stress, defined as force per unit area, is the greatest near the vessel wall and decreases to zero at the center of the vessel. For blood, the shearing effect is frequently represented by the shear rate, the ratio of shear stress to blood viscosity. This parameter is easier to determine for blood because blood is non-Newtonian, its viscosity changing as a function of shear rate. Typical wall shear rates are in the range of 300–800 s^{-1} in large arteries and of 500–1600 s^{-1} in arterioles, and can exceed 10,000 s^{-1} in stenotic vessels [12,13]. Platelets are thus exposed to a wide range of shear stresses as they circulate near the vessel wall, from 10–60 dyne cm^{-2} in the arteries and arterioles to 0.8–8 dyne cm^{-2} in the venous circulation [14].

At wall shear rates greater than 500 s^{-1}, the initial tethering and adhesion of platelets to the vessel wall is primarily mediated by the interaction of the glycoprotein (GP) Ib- IX-V complex to VWF [11]. At wall shear rates above 5000 s^{-1} or in the presence of sharp shear gradients, endothelial cells may be activated to secrete more VWF and further trigger GPIb-IX-V–dependent thrombus formation [15]. Platelet adhesion mediated by the GPIb-IX-V–VWF interaction is unstable, with platelets rolling on VWF-coated surfaces without stably adhering [16]. Stable adhesion requires platelet integrins to be activated to conformations capable of binding VWF or other immobilized ligands such as fibrinogen [17,18].

Shear-dependent platelet–vessel wall interactions have been studied using various *in vivo* and *in vitro* models. Animal models are fully physiological, and genetically modified mice represent very useful tools to dissect the molecular mechanisms underlying the complex multistep process leading to the formation of a stable platelet plug [19,20], particularly employing intravital video microscopy [21]. In spite of these advantages, the complexity of whole organisms is such that dissecting the contributions of individual molecular or cellular components to the process of thrombus formation is exceedingly difficult [22]. Ex vivo studies are in some cases a good alternative for the study of platelet–vessel wall interactions. Large blood vessels can be isolated from an animal and the vessel can then be perfused with blood components and other substances [23]. This model has the advantage that native blood vessels with the proper cellular and matrix composition are being studied, and the interactions of blood components and the vessel intima can be examined under physiological flow. The model is suitable for examining thrombus formation in large blood vessels due to its accessibility, easy of study, and the fact that large arteries are involved in common and relatively easily definable diseases. Unfortunately, a similar system for studying small blood vessels is currently not feasible. Small blood vessels are hard to isolate in a way that maintains their architecture, and often contain bifurcations and junctions, resulting in complex profiles for blood flow and shear stress. Systems that model small blood vessels are in great need as there is increasing appreciation for the role of the microvascular thrombosis in a large number of human diseases.

Engineered platforms that mimic the vasculature have been developed as an alternative way to study flow-dependent thrombus formation and platelet–vessel wall interactions *in vitro*, particularly targeting processes occurring in small blood vessels. One such approach has employed parallel-plate flow chambers [24,25]. These flow chambers usually have depths of 0.1 - 0.5 mm, widths of 2 - 10 mm, and lengths of 20 - 50 mm, and therefore require large blood volumes (in the order of 10 mL) for each experiment. The endothelial cells used in the chambers are usually cultured as a monolayer on glass or plastic, sometimes with an added underlying matrix. These types of flow chambers allow for the assessment of the interactions of platelets with stimulated or unstimulated endothelium under flow, and, to a certain extent, can be used to probe the role of hydrodynamic forces on these interactions. However, these chambers generally use fixed conditions of shear stress and substrate conditions within individual experiments, and have relatively low throughput to allow study of different experimental parameters across the full range of physiological and pathological shear stresses. Furthermore, the flow chambers usually have simple geometries, *i.e.* straight channels with unidirectional flow, making it impossible to mimic the complex flow patterns found at vessel bifurcations and in regions of high curvature that occur *in vivo*. Parallel-plate chambers have largely been eclipsed over the past ten to fifteen years by advances in microfluidic technologies, which offer new possibilities to study shear-dependent phenomena related to platelet-vessel wall interactions.

Conventional Microfluidics For The Study of Platelet-Vessel Wall Interactions

Microfluidic devices have several advantages over conventional flow chambers, including the very small volumes they require, their capacity for high-throughput testing resulting from the possibility of using multiple channels within a single device, and the ability to use several geometries in one small chip. Numerous studies have been carried out with these types of devices to study platelet adhesion and aggregation and blood coagulation under flow, although generally the role of the endothelium has not been taken into account [26,27], until recently [15,28–30]. Most relevant microfluidic devices have been made of glass or polydimethylsiloxane (PDMS), a silicone elastomer, which is transparent and biocompatible. The microfeatures were usually created by silicon or SU-8–based lithography, with the size of features ranging from several microns to a few hundred microns. Features that have been incorporated into microfluidic devices include parallel microchannels of uniform size [28,29], microchannels with varying dimensions, including incorporation of “stenotic” regions [15], or channels with bifurcations and junctions [30]. Once these microfluidic chambers are made, they can be seeded with endothelial cells, which can be grown under flow until they reach confluence. Isolated platelets, platelets in plasma, or whole blood can then be perfused through these chambers to evaluate platelet adhesion and aggregation on the endothelialized surfaces under different flow conditions [15,31] and stimuli [28]. These studies can be valuable to screen for drugs to promote or inhibit platelet adhesion to an endothelium [29]. They can also be used to study the biophysical mechanisms of microvascular occlusion in diseases such as sickle cell disease and hemolytic-uremic syndrome [30].

Though much progress has been made using microfluidic chambers, the chambers have limitations in their ability to mimic real blood vessels. One disadvantage is that conventional microfluidic chambers have square or rectangular cross sections resulting from the limitations of the fabrication procedures. These features result in inhomogeneous and unphysiological shear conditions on the vessel wall, as the shear stresses are very different at corners than at the center of the walls. This inhomogeneity creates large artifacts that hinder interpretation of studies of shear-dependent platelet-vessel wall interactions. Development of microfluidic chambers with cylindrical cross sections [32] would provide more physiological tools for vascular research, particularly for the study of flow-dependent