Chapter 5
Platelet Function and Coronary Microvascular Dysfunction

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Introduction

The ability of platelets to activate and aggregate to form blood clots in response to endothelial injury is well established. They are therefore critical contributors to ischaemia in atherothrombosis [1]. However, their role in cardiovascular disease is not limited to end-stage thrombosis in large vessels [2]. Abundant experimental evidence has established that activated platelets are also important mediators of microvascular thrombosis and promote the inflammatory response during ischaemia-reperfusion (IR) injury [3–5]. While platelets do not physically interact with the healthy endothelium, they can bind to the wall of hypoxic microvessels and release a plethora of inflammatory mediators that further enhance the activation of the endothelial monolayer and the recruitment of circulating leukocytes

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(monocytes, neutrophils, T-cells) [2]. In addition, deposition of platelets to the dysfunctional endothelium can lead to vasoconstriction which accelerates microvascular occlusion, thereby impairing tissue perfusion [3]. In this chapter, we discuss the role of platelets in promoting microvascular dysfunction and inflammation during IR injury. Focus is placed on the cross-talk between platelets and other cell types (endothelial cells [ECs] and leukocytes) via platelet adhesion receptors and platelet-derived proinflammatory mediators. We also consider new paradoxical functionalities of platelets promoting cardiac recovery after myocardial infarction (MI).

Thrombus Formation in Coronary Arteries and Microvessels

Coronary Artery Disease and Plaque Rupture

Atherosclerotic plaques forming inside the vessel's intima layer lead to endothelial dysfunction which is the first trigger for coronary artery disease. An increased blood level of plasma lipids, especially low-density lipoproteins (LDL) [6–8], in combination with other risk factors including hypertension, diabetes mellitus, smoking and male gender [9] induce vascular damage. The binding of LDL to intimal proteoglycans is an important step for disease initiation [10]. Once sequestered in the intimal microenvironment, LDL particles may undergo extensive oxidation, aggregation/fusion or enzymatic fragmentation which may potentially contribute to their enhanced pro-atherogenic properties [11–13]. Modified LDL particles activate ECs, inducing the expression of adhesion receptors and disrupting intercellular junctions. These modifications potentiate the recruitment and infiltration of leukocytes (monocytes and lymphocytes) into the subendothelial space [7, 14]. Once in the intima, monocytes differentiate into macrophages which express scavenger receptors (SRAI and SRAII, CD36, LOX-1 or CXCL16), leading to the internalization and cytosolic accumulation of cholesterol molecules and cholesterol esters from modified LDL particles [15, 16]. Such macrophage-derived cells, called foam cells, release a multitude of inflammatory cytokines, chemokines, growth factors, metalloproteinases (MMPs) and reactive oxygen species (ROS) that perpetuate inflammation [17]. Apoptosis and secondary necrosis of foam cells and smooth muscle cells (SMCs) are an important cause of necrotic core development [18, 19]. The necrotic core material may act as a nucleus for calcium deposits in the vascular wall [20]. In vulnerable plaques, the lipid-rich atheromatous core is hypocellular and devoid of supporting extracellular matrix proteins due to reduced SMC amounts combined with high levels of MMPs [21, 22]. These plaques are at high risk of rupture. With plaque rupture, thin collagen cap and the highly thrombogenic lipid core are exposed to circulating platelets which initiate thrombotic occlusion of the coronary vessel leading to myocardial ischaemia and infarction [22].

Atherothrombosis: Platelet Recruitment, Activation and Aggregation

The mechanisms leading to platelet activation following plaque rupture closely resemble physiological haemostasis and can be divided into three main steps: (1) platelet rolling and adhesion on the subendothelium, (2) recruitment and activation of circulating platelets through the secretion of secondary platelet agonists and (3) platelet aggregation. Under elevated shear stress (e.g. found in coronary vessels), platelet tethering to the subendothelium is mediated by the interaction of platelet glycoprotein (GP) Ib-IX-V and the von Willebrand factor (vWF) bound to the exposed collagen. Platelets attached to vWF roll along the vessel wall following blood flow [23]. Stable attachment is provided by binding to other receptors, especially GPVI that binds collagen and induces a strong and sustained platelet stimulation [24]. Platelet-collagen interaction can shift αIIbβ3 and α2bβ1 integrins from a low to a high affinity state. While α2bβ1 binds directly to collagen, αIIbβ3 promotes irreversible adhesion by binding to vWF [25]. The stable integrin-dependent adhesion of platelets leads to further platelet activation, morphological changes via actin cytoskeleton remodelling, and release from three different platelet granules (α-, dense, and lysosomal) of a broad range of biomolecules which comprise over 300 distinct proteins, nucleotides (ADP, ATP), and neurotransmitters [26, 27]. These molecules act in both autocrine and paracrine manners to amplify platelet activation and consolidate the thrombotic process. In addition, degranulation alters the composition of the plasma membrane and results in surface exposure of P-selectin and formation of thromboxane A_2 (TxA_2) from arachidonic acid. TxA_2 binding to specific receptors (largely distributed in platelets and vascular cells) enhances platelet activation and vasoconstriction. Finally, the interaction of circulating platelets with adherent platelets proceeds via the extracellular region of αIIbβ3 expressing a high-affinity binding site for fibrinogen and allowing aggregation via the formation of stable bridges between platelets [28].

Platelets in Ischaemia-Reperfusion and No-Reflow

After MI, reperfusion therapy aiming to dissolve the thrombus inside the coronary artery is critical for the recovery of cardiac function. However, the abrupt restoration of blood supply following ischaemia is characterized by a significant burst of ROS production by ECs and macrophages, the release of various cytokines and chemokines, leukocyte recruitment and activation, vasoconstriction and embolization of thrombotic platelet-rich aggregates which are responsible for luminal obstruction of the microvasculature [3, 4, 29, 30]. This tissue hypo-perfusion at the level of myocardial and coronary microcirculation is characterized as no-reflow and

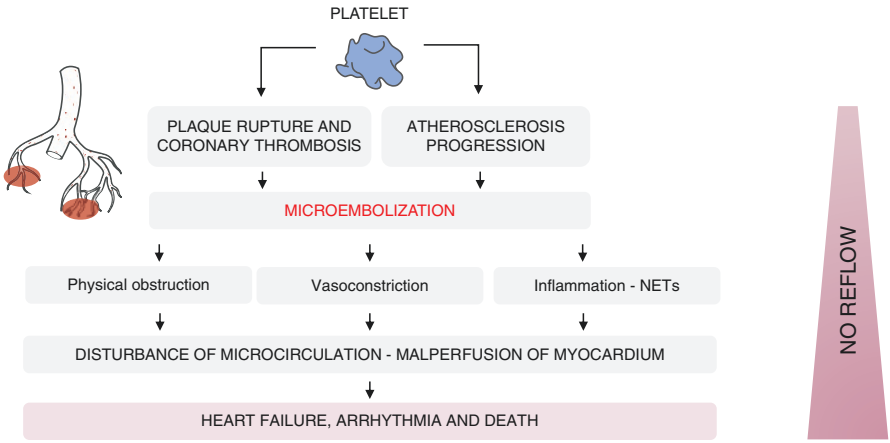

Fig. 5.1 Role of platelets in no-reflow. No-reflow refers to a state of myocardial tissue hypoperfusion after re-establishment of patency of an occluded epicardial coronary artery by reperfusion therapy. No-reflow is associated with a high risk of heart failure, arrhythmia and death and its aetiology is microvascular embolization by thrombotic and atherosclerotic debris. Activated platelets release vasoconstrictors and inflammatory mediators which promote the formation of microvascular thrombi responsible for no-reflow

is associated with a high risk of heart failure, arrhythmias and mortality [31] (Fig. 5.1). Platelets have a central role in the pathophysiology of no-reflow, notably via their capacity to adhere to ischaemic ECs and to cells of the innate and adaptative immune system.

Crosstalk Between Platelets, Endothelial Cells and Leukocytes During IR injury

Platelet-Endothelium Interaction

Healthy arterial ECs limit thrombosis by releasing nitric oxide (NO) and prostacyclins [32, 33]. ECs also express the exonucleotidases CD39 and CD73, that hydrolyze ATP and ADP (both platelet agonists) to AMP and ultimately to the platelet antagonist adenosine [34]. Upon ischaemic conditions and oxygen deprivation, the normal EC function is dysregulated. ECs switch to anaerobic glycolysis, accumulating metabolic intermediates and ROS. The excess generation of ROS results in the loss of NO availability. In addition, oxidative stress and proinflammatory cytokines downregulate CD39, promoting ADP accumulation and lowering AMP levels [35]. NO and adenosine depletion combined with the accumulation of proinflammatory mediators also increase expression of adhesion molecules, leading to enhanced crosstalk between platelets and ECs [36]. Hence, endothelial disruption is not a prerequisite to commit platelet attachment to the vasculature.

The Adhesion Molecules

Different surface molecules have been involved in the platelet-EC interaction during acute IR. They comprise P-selectin, GPIb-IX-V, αIIbβ3, intercellular adhesion molecule-1 (ICAM-1) and vitronectin [2, 37] (Fig. 5.2).

P-selectin is a glycoprotein stored in granules of platelets and in Weibel-Palade Bodies of ECs, and exposed at cell surface upon activation. Endothelial P-selectin has been demonstrated to mediate platelet rolling in inflammatory processes via the binding of P-selectin glycoprotein ligand-1 (PSGL-1) expressed on platelet surface [38]. PSGL-1 is also responsible for leukocyte adhesion to the vessel wall and for the formation of platelet-neutrophil aggregates at sites of endothelial injury [39]. Accordingly, anti-PSGL-1 antibodies markedly inhibited platelet-leukocyte interaction and the effect of platelets on neutrophil intravascular migration, both in vitro and in vivo, in a model of limbal microvessel injury [38].

Platelet attachment to intact but dysfunctional endothelium also involves GPIb-IX-V. The main ligand of GPIbα is vWF but this receptor has also been identified as counter-receptor for P-selectin [40]. GPIbα blockade has been reported to decrease infarct volumes and improve neurological functional outcomes in a model of transient middle cerebral artery occlusion [41–43], a protective effect attributed to a reduction in microvascular obstruction [44]. Furthermore, GPIbα can facilitate leukocyte recruitment via the leukocyte-expressed integrin Mac-1 [45].

The tight platelet adhesion on the inflamed endothelium is achieved via αIIbβ3 and intracellular adhesion molecule-1 (ICAM-1) (37). The latter plays a crucial role as it strongly binds to fibrinogen-platelet αIIbβ3 complexes [46, 47], extending the role of the integrin αIIbβ3 beyond platelet-platelet aggregation. In addition to interacting with ICAM-1, αIIbβ3-fibrinogen interacts with $\alpha_V\beta_3$, an integrin upregulated upon endothelial activation [48]. Interestingly, αIIbβ3 antagonists have been used in

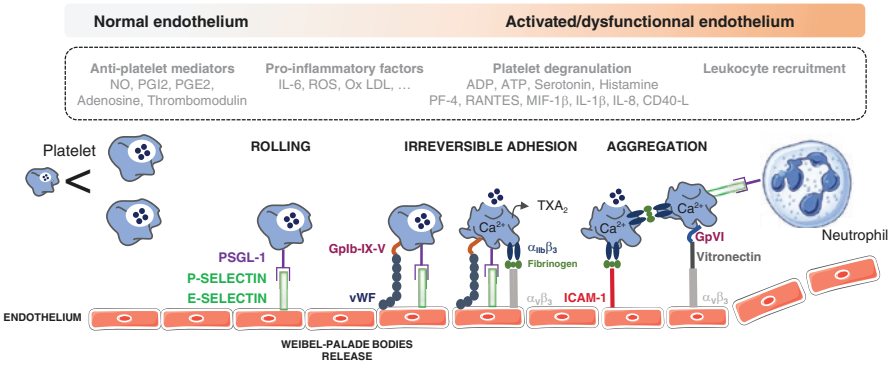

Fig. 5.2 Crosstalk between platelets and activated endothelial cells. Different surface molecules have been involved in the platelet-endothelium interaction: P-selectin, GPIb-IX-V, αIIbβ3, intercellular adhesion molecule-1 (ICAM-1) and vitronectin. "Servier Medical Art" was used for the illustration

the context of no-reflow and their infusion improves myocardial perfusion, involving the presence of microvascular platelet thrombi in this phenomenon [49]. In addition, αIIbβ3 inhibitors prevent the shedding of cell differentiation 40 ligand (CD40L) from platelet surface, thereby attenuating the inflammatory process [50]. Lastly, another mechanism involved in platelet-endothelium interaction is mediated by the binding of vitronectin to platelet GPVI [51]. Vitronectin is a plasma glycoprotein that binds to upregulated endothelial molecules, including the urokinase plasminogen receptor and various integrins such as $\alpha_V\beta_3$ [52].

Once deposited in the microvasculature, platelets participate to thrombo-inflammation via multiple mechanisms including (1) amplification of coagulation cascade leading to thrombin and fibrin generation, (2) secretion of a variety of pro-inflammatory and vasoconstricting molecules which further exacerbate the physical obstruction of microvessels and (3) interaction with neutrophils and generation of neutrophil extracellular traps (NETs) serving as a prothrombogenic matrix inciting platelet aggregation and growth of microvascular thrombi.

Cooperation of Platelets and the Coagulation Pathway

The classic blood clotting cascade can be triggered via either the tissue factor (TF) pathway or the contact pathway. Intravascular TF is present on microparticles (MPs) and exosomes (Es) produced by activated platelets. Initially, it lays in an encrypted silent form that needs to be activated by redox modifications involving the disulfide isomerases (PDI, ERp5, ERp57) that are secreted by platelets and activated ECs [53]. The contact pathway also clearly participates in thrombosis and can be triggered by inorganic polyphosphates (PolyP) abundantly present in platelet dense granules [54, 55]. PolyP enhance the amplification steps of the coagulation cascade via the binding of multiple coagulation enzymes [56]. Furthermore, activated platelets interact with the coagulation pathway by various other mechanisms [57]. They secrete (anti)coagulation factors (prothrombin, FV, FXIII, fibrinogen, antithrombin, various serpins, etc) and expose phosphatidylserine (PS) on cell surface. PS exposure is triggered by strong agonists via prolonged elevated cytosolic calcium. PS-containing membranes have a high affinity for coagulation factors such as the tenase and prothrombinase complexes and their binding at platelet surface enhances their activity. Thus, platelets support local thrombin and fibrin formation [58]. Under flow conditions, thrombin initially binds to PS-exposing platelets via protease-activated receptors (PAR1 and PAR4), and then relocalizes to the newly-formed fibrin fibers which confine the protease to the thrombus proximity [59]. Early findings suggest that fibrin-bound thrombin is protected from inactivation by antithrombin [60]. Platelets also bind coagulation factors via the glycoprotein complexes GPIb-V-IX, integrin αIIbβ3 and GPVI. The

platelet GPIb-V-IX complex interacts with thrombin, which potentiates platelet activation through protease-activated receptors (PAR) [61]. The integrin αIIbβ3 binds fibrinogen and induces outside-in signalling which is required for the retraction of the platelet-fibrin thrombus [62]. Finally, GPVI has been recently identified as a receptor for fibrin. GPVI-fibrin interaction leads to formation of a GPVI signalosome involved in the continued growth of platelet-fibrin thrombus, independently of αIIbβ3 [63]. Altogether, this indicates that platelets and the coagulation system are interconnected processes and interact in multifaceted ways, not only during the phases of thrombus formation, but also in specific areas within a formed thrombus. Accordingly, strong evidence suggests that the thrombus is composed of a heterogeneous population of activated platelets forming a very hierarchical structure with distinct regions defined by the level of platelet activation and packing density which depend on the nature of the agonist and its distribution [64].

Proinflammatory Mediators

Platelet activation results in the release of multiple proteins and other biomolecules that can influence the function of endothelial or circulating cells [26]. Many of these molecules are preformed and stored in granules and notably include chemokines, growth factors, fibrinolytic proteins, coagulation factors, adhesion molecules, nucleotides, ions and agonists. Others are synthesized by platelets (TxA_2, ROS, IL-1β) or shed from the cell surface (sCD40L, sP-selectin) upon activation [65]. Some of these mediators induce the expression of adhesion molecules on the endothelial surface, enhance neutrophil recruitment at the site of injury, and promote the formation of platelet-leukocyte aggregates [2]. When attached to platelets, the leukocytes can achieve a more activated state and so, are able to produce more ROS than their platelet-free counterparts [66]. As it is recognized that the oxidative burst increases cell permeability, platelets can indirectly contribute to endothelial barrier dysfunction via their interaction with leukocytes [67]. However, the role of platelets in vascular integrity remains unclear. Indeed, some reports suggest that platelets can exert opposing effects on endothelial permeability, notably in the tumour microvasculature [68]. This controversy probably reflects the diversity of mediators that can be released by platelets in different models of thromboinflammation.

Platelets also contain microRNAs (miRNAs) retained from megakaryocyte-derived mRNAs [69]. Recent advances in platelet biology demonstrate that activated platelets release MPs or Es containing abundant amounts of miRNAs. They can be internalised and modulate gene expression and function of recipient cells, e.g. ECs, leukocytes or macrophages [70, 71]. Therefore, the horizontal transfer of platelet miRNAs represents a novel form of cell-cell communication, which may participate in the inflammatory process.

Platelets and Neutrophil Extracellular Traps

As mentioned above, monocytes and neutrophils recruitment is supported by activated platelets via the granule release of chemokines and expression of adhesion receptors such as P-selectin [36]. One way neutrophils may promote thrombus stability is by producing neutrophil extracellular traps (NETs) [72], a process that is enhanced by activated platelets presenting high mobility group box 1 (HMGB1) protein to neutrophils [73]. NETs are extracellular DNA fibers coated with histones and proteases and catapulted out of neutrophils. They are known for their antimicrobial function but recent evidence indicates that NETs may have a role in non-infectious diseases including atherosclerosis and thrombosis [74]. Mechanistically, NETs provide a scaffold for platelet adhesion and concentrate effector proteins involved in thrombosis and fibrin generation. The interaction with platelets can be mediated via vWF and fibrinogen immobilized on NETs, or alternatively by direct binding to DNA and histones [72]. NETs can also initiate fibrin formation more directly by activating the contact pathway of coagulation which is initiated by the activation of factor XII to factor XIIa [75]. Finally, NETs also promote a strong procoagulant response by forming a catalytic platform that stimulates the proteolytic activity of neutrophil elastase which induces the degradation and inactivation of natural anticoagulants such as thrombomodulin or TF pathway inhibitor (TFPI) [76] (Fig. 5.3).

Recent studies have demonstrated that, in a rat experimental model of IR, both platelets and NET-mediated microthrombosis contribute essentially to no-reflow [77]. Accordingly, a reperfusion strategy based on deoxyribonuclease I (DNaseI)

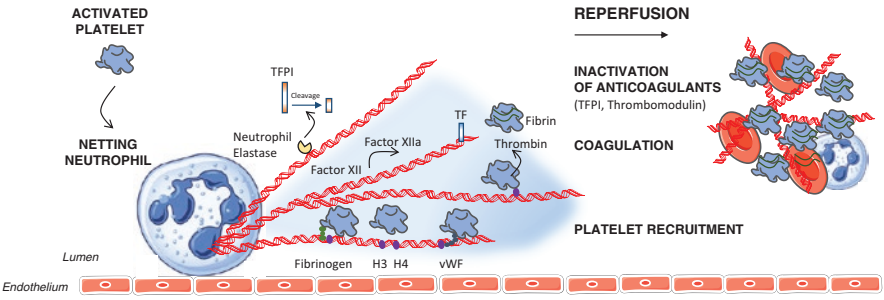

Fig. 5.3 Role of platelets and Neutrophil extracellular traps in IR-induced microvascular thrombosis. Neutrophil extracellular traps (NETs) provide a scaffold for platelet adhesion and concentrate effector proteins involved in thrombosis and fibrin generation. The interaction with platelets can be mediated via von Willebrand factor (vWF) and fibrinogen immobilized on NETs, or alternatively by direct binding to DNA or histones. NETs can also initiate fibrin formation more directly by activating the contact pathway of coagulation which is initiated by the activation of factor XII to factor XIIa. Finally, NETs also promote the degradation and inactivation of natural anticoagulants such as thrombomodulin or tissue factor pathway inhibitor (TFPI); *NE* Neutrophil elastase. "Servier Medical Art" was used for the illustration

used in combination with recombinant tissue-type plasminogen activator (rt-PA), a thrombolytic agent, has proved to be capable of attenuating significantly myocardial IR-induced no-reflow, suggesting that this combination might be a promising therapeutic option [77].

Double-Edged Sword Functionality of Platelets in IR Cardiac Injury

Resolution of Cardiac Inflammation

While platelets and their secreted cargo are widely accepted as crucial players in the inflammation-associated injury inflicted on the myocardium during IR, a growing body of evidence implicates them in inflammation resolution via the release ofpro-resolving mediators such as lipoxin A4, maresin 1, and annexin A1 [78, 79]. Interestingly, it has been shown that maresin-1 can decrease the level of proinflammatory mediators in platelet releasates [80]. The mechanisms underlying this differential secretory pattern need to be further characterized.

Cardiomyocyte Survival and Fibrotic Remodelling After Myocardial Infarction

A recent study shows that platelets and their secretome can exert a cardioprotective activity on ventricular cardiomyocytes during myocardial IR injury, independently of their role in coronary thrombosis. This protective effect requires α-granule components such as stromal cell-derived factor-1α (SDF-1α) and transforming-growth factor-β1 (TGF-β1), and is significantly attenuated in the presence of specific platelet antagonists [81]. Following MI, fibrotic scarring in the left ventricular (LV) necrotic area can trigger deleterious LV remodelling, when distending forces cause excessive volume and pressure load on non-infarcted areas. Myofibroblasts (MFs) are crucial components of this fibrotic response. Indeed, multiple regulators of the myodifferentiation process, including TGF-β1 [82], serotonin [83] and thrombospondin-1 (TSP-1) [84] are present in platelet granules. In addition, numerous miRNAs are enriched in platelet MPs/Es and can promote (miRNA-21 and miRNA-199) or attenuate (miRNA-29 and miRNA-101) the fibrotic response in cardiac tissue [85]. The high heterogeneity of the platelet secretome and MPs/Es challenges the clear characterization of platelet contribution in the cardiac repair process. Further studies using conditional (PF4/GPIba-Cre) transgenic mice with specific platelet defects will be required to assess the complex role of platelet secretome in the post-MI fibrotic remodelling.

Conclusion

The central role played by platelets and platelet-derived mediators in promoting microvascular thrombus formation during IR injury is increasingly well recognized. The underlying mechanisms involve endothelial activation, release of proinflammatory factors, vasoconstriction, leukocyte recruitment and NETosis (Fig. 5.3). All these events can—in turn—increase further platelet activation, leading to a vicious cycle that exacerbates tissue malperfusion and cardiac injury. Beyond thrombosis-mediated ischaemic damage, platelets may also exert additional "non-classical" functions within the infarcted myocardium, in particular through the release of bioactive cargo that can paradoxically facilitate cardiac repair processes. Therefore, considering the high functional diversity of the platelet secretome, much more attention should be paid to the efficacy of antiplatelet therapies in the management of cardiac recovery after MI.

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