Platelet biology and functions

Platelet biology and functions: new concepts and clinical perspectives

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Abstract | Platelets — blood cells continuously produced from megakaryocytes mainly in the bone marrow — are implicated not only in haemostasis and arterial thrombosis, but also in other physiological and pathophysiological processes. This Review describes current evidence for the heterogeneity in platelet structure, age, and activation properties, with consequences for a diversity of platelet functions. Signalling processes of platelet populations involved in thrombus formation with ongoing coagulation are well understood. Genetic approaches have provided information on multiple genes related to normal haemostasis, such as those encoding receptors and signalling or secretory proteins, that determine platelet count and/or responsiveness. As highly responsive and secretory cells, platelets can alter the environment through the release of growth factors, chemokines, coagulant factors, RNA species, and extracellular vesicles. Conversely, platelets will also adapt to their environment. In disease states, platelets can be positively primed to reach a pre-activated condition. At the inflamed vessel wall, platelets interact with leukocytes and the coagulation system, interactions mediating thrombo-inflammation. With current antiplatelet therapies invariably causing bleeding as an undesired adverse effect, novel therapies can be more beneficial if directed against specific platelet responses, populations, interactions, or priming conditions. On the basis of these novel concepts and processes, we discuss several initiatives to target platelets therapeutically.

Developments in the field of platelet biology have led to new insights into platelet formation, function, heterogeneity, genetics, signalling (FIG. 1), and communication. Together, these advances open new horizons not only for better understanding of the multiple roles of these anucleated cells in healthy conditions, but also for new ways to target platelets in disease states. In this Review, we provide an overview of the latest developments in our understanding of platelet functions and populations in normal physiology and in haemorrhagic, thrombotic, and inflammatory disease processes.

Platelet formation, fate, and ageing

Platelets are anucleate blood cells (2–4 μm in diameter) with multiple functions and a short lifespan, circulating in blood for 7–10 days in humans and for a shorter time in mice, after which platelets are eliminated in the spleen and liver[1]. Thrombopoiesis (the production of platelets) occurs primarily in the bone marrow, and is preceded by the differentiation of haematopoietic stem cells into polyploid megakaryocytes (50–100 μm in diameter), which shed numerous long-branching cytoplasmic protrusions called proplatelets. Megakaryocyte homing has also been reported in the mouse lungs[2], but at a much lower density than in the bone marrow[3]. Thrombopoiesis is driven by the interplay of several transcription factors (see section on Platelet genetics, mass, and activation), with a negative feedback role of thrombopoietin in the final stage of platelet production. Thrombopoietin, which is produced in the liver, stimulates the thrombopoietin receptor in megakaryocytes to induce the formation of proplatelets via a mechanism that is activated at low platelet counts in blood[4]. In reactive thrombopoiesis, such as that occurring in inflammatory states, IL-6 enhances the process of proplatelet formation by increasing thrombopoietin levels[5].

Human megakaryocytes cultured from haematopoietic precursor cells or obtained from pluripotent stem cells are not all the same, and have major heterogeneities in their differentiation programme and glycoprotein expression profiles[6,7]. Common to designated megakaryocytes is the maturation process, during which these cells become polyploid through endomitotic cell cycles and their overall size increases[8]. In vivo observations show that the mature megakaryocytes shed proplatelets, where proplatelet elongation seems to rely on rearrangements of the microtubule cytoskeleton, involving the motor protein dynein[9]. This process ensures redistribution of organelles, vesicular structures, and granules from the megakaryocyte cell body to the proplatelets.

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Key points

- Multiomic approaches combined with functional testing of platelets have greatly advanced the understanding of genetic factors of platelet-related haemorrhagic disorders, but to a lesser extent the understanding of the causes of platelet hyper-reactivity.
- Negative and positive platelet priming alter the threshold for platelet activation in the circulation, with consequences for diagnostic assays.
- The diverse pathways of information transfer by platelets through release of bioactive molecules and extracellular vesicles are still incompletely understood.
- Platelets contribute to thromboinflammatory processes by their capacity to interact functionally with the activated endothelium, leukocytes, and coagulation proteins; the mechanisms are multivariate.
- Platelet populations and specific platelet responses are promising targets for new antithrombotic treatment of patients with cardiovascular disease.

Ex vivo studies have established that proplatelets further develop and divide into platelets.

In the traditional model of proplatelet formation, migration of megakaryocytes from osteoblastic to vascular niches in the bone marrow is needed to establish contact with the circulation[8]. However, studies using advanced microscopy approaches show that the bone marrow is highly vascularized and densely packed with low-motility megakaryocytes, the majority already residing at capillary sinusoidal vessels[3]. In addition, the early idea that platelet formation is a consequence of megakaryocytic apoptosis needs to be revised. In mouse, the combined loss of two apoptotic pathways (BAK–BAX-mediated intrinsic apoptosis and Fas ligand-inducible extrinsic apoptosis) did not affect thrombopoiesis, indicating that the apoptotic routes are dispensable for platelet production[10].

Several studies indicate that the megakaryocyte environment is a controlling factor in thrombopoiesis, a concept that supports the observed heterogeneity between these cells. A report established that type I collagen suppresses proplatelet formation (via glycoprotein VI (GPVI) and integrin α2β1), whereas type IV collagen, fibrinogen, and fibronectin stimulate this process[11]. Integrin-linked, mechanosensitive ion channels[12] and local shear stresses[8] might mediate the environmental effects on thrombopoiesis. In addition, proplatelet formation is controlled by proteasomal regulation of cytoskeletal elements, involving small GTPase proteins[13]. Taken together, these regulation pathways point to adaptations of certain megakaryocytes to the environment, thereby affecting the way and extent of proplatelet formation.

Platelets are eliminated from the circulation in different ways, such as seclusion in spleen and other organs, 'consumption' to maintain vascular integrity, deposition at the activated vessel wall, activation per se, microvesiculation, or ageing. However, which platelet fractions are cleared by each of these routes is unknown. Several mechanisms have been proposed for the clearance of activated or ageing platelets from the circulation. An early model is that activated or apoptotic platelets expose phosphatidylserine on their outer membrane surface and are cleared via scavenging receptors on phagocytic cells in the liver and other organs. Support for this model comes from the finding that platelet lifespan is determined by a balance in the cytoplasm between the pro-apoptotic BAX–BAK pathway and anti-apoptotic proteins such as BCL-2, with a change towards the pro-apoptotic pathway with ageing[14] (FIG. 2). Thus, administration of the BCL-2 inhibitor ABT-737 to mice resulted in shorter circulation times of platelets[15]. In this setting, apoptosis-induced exposure of phosphatidylserine serves as a recognition signal for phagocytic cells to mediate platelet clearance.

A second mechanism of platelet removal is loss of the negatively charged sugar moiety sialic acid (usually detected in the GPIb–V–IX complex, which is highly expressed in platelets) from the surface of senescent platelets by the action of sialidases, which can also be derived from platelets. Desialylated platelets are recognized by the hepatic asialoglycoprotein receptor 1. Of note, this receptor signals to increase thrombopoietin production by liver cells, thereby promoting platelet formation[1,4]. How the loss of sialic acid is linked to apoptosis is unclear. Another determinant of platelet lifespan might be — directly or indirectly — the gradual loss of RNA content in ageing platelets[16]. In addition to the liver, the spleen and lungs also act as platelet reservoirs and as potential sites for surface modification and platelet clearance[17]. However, the sorting pathways to these organs and subsequent platelet fates remain to be determined.

Heterogeneity in structure and function

Heterogeneity between human platelets was described in 1969 while platelet isolation procedures were being developed with the use of density gradients, showing that the larger, denser platelets had a greater metabolic potential than the smaller platelets[18]. Subsequently, a study using platelets from patients with platelet storage pool deficiency showed that the denser and more reactive platelets were enriched in α-granules[19]. Another study in baboons indicated that the heterogeneity in platelet density was not a consequence of age-related changes[20]. In patients with hereditary macrothrombocytopenia, this heterogeneity in platelet size can increase substantially, suggesting regulatory mechanisms at the level of transcription factors[21]. In the past decade, the concept of platelet structural heterogeneity in terms of size (small or large), content (light or dense), protein expression profile (low or high signalling or glycoprotein content), and age (young or old) has gained renewed interest, as reviewed elsewhere[22].

Cultured megakaryocytes, even when derived from a single clone of pluripotent stem cells, have major differences in glycoprotein expression profiles[7]. Similarly, different platelet populations can be distinguished based on the levels of glycoprotein or signalling receptors, as observed by multicolour flow cytometry[22]. Whether platelet size (mean platelet volume) is the main determinant of glycoprotein expression levels is still unclear. Individual platelets can be enriched in certain receptors (for example, GPVI), but not in other receptors (for example, the GPIb–V–IX complex). Larger platelets are likely to be enriched in internal membranes (endoplasmic reticulum, mitochondria, lysosomes, and secretory granules) compared with smaller platelets. One can also speculate that, apart from being a result of the variability between

megakaryocytes, the heterogeneity in platelet density and size results from the stochastic process of membrane and organelle separation during proplatelet formation.

The distinction between heterogeneity in platelet structure and heterogeneity in platelet responses is important. However, how structural heterogeneity relates to variation in platelet responses is largely unknown. What is clear is that platelets from a given individual can greatly differ in their responses, such as agonist-induced spiking patterns of intracellular Ca^{2+} levels, the extent of cell spreading via small GTPase-regulated proteins, or the tendency to phosphatidylserine exposure[23]. Accordingly, individual platelets can differ from each other in the extent of signalling or in the formation of signalosomes that are responsible for classic platelet functions, such as adhesion, integrin αIIbβ3 activation, secretion, and procoagulant activity (FIG. 1).

Agonist-induced activation of platelets increases their heterogeneity in responsiveness, with evidence showing that distinct platelet populations with respect to almost

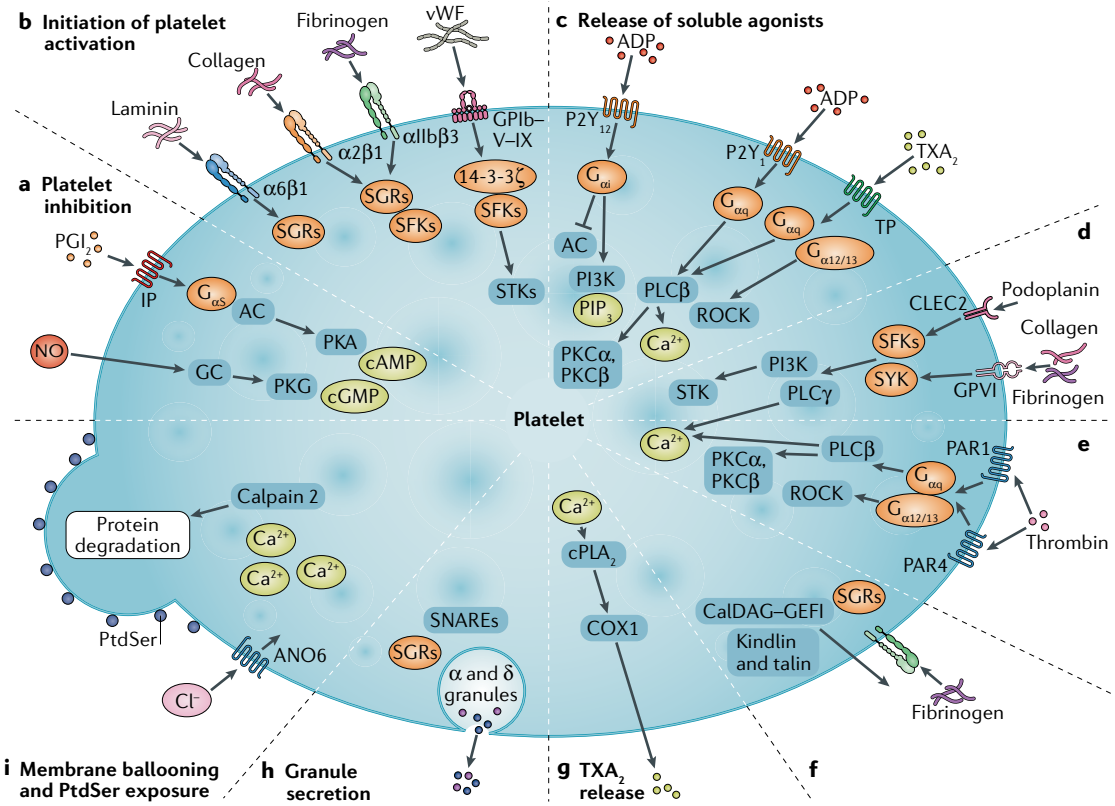

Fig. 1 | **Major signalling events and responses during platelet activation.** Platelet receptors are ordered clockwise according to stages of increasing platelet activation[38,154]. **a** | Platelet inhibition occurs via nitric oxide (NO) and prostaglandin I_2 (PGI_2; also known as prostacyclin) IP receptor, acting via guanylate cyclase (GC) and adenylyl cyclase (AC), with ensuing activation of protein kinase G (PKG) and protein kinase A (PKA), respectively. **b** |Platelet activation is initiated by interaction of adhesion receptors (integrins α6β1, α2β1, αIIbβ3, and glycoprotein (GP) Ib–V–IX complex) with their ligands, such as collagen and von Willebrand factor (vWF). Signalling through these receptors is limited at this stage and involves multiple small G-protein regulators (SGRs), SRC-family kinases (SFKs), and serine/threonine-protein kinases (STKs). **c** | Release of the soluble agonists ADP and thromboxane A_2 (TXA_2) leads to activation of the purinoceptors $P2Y_{12}$ and $P2Y_1$ and the TXA_2 receptor (TP). $P2Y_{12}$, acting through the G protein $G_{αi}$, inhibits AC but stimulates phosphoinositide 3-kinases (PI3Ks). $P2Y_1$ and TP signal via $G_{αq}$ proteins followed by phospholipase Cβ (PLCβ) stimulation, inducing Ca^{2+} release into the cytoplasm, protein kinase C (PKC) activation, and further downstream events. TP receptors also stimulate $G_{α12/13}$ proteins, triggering Rho-associated protein kinase (ROCK) activation, implicated in platelet shape change and spreading. **d** | Amplification of platelet activation. GPVI and C-type lectin-like receptor 2 (CLEC2) induce strong signalling via protein tyrosine kinase pathways involving SFK and SYK proteins that results in the activation of PI3K and PLCγ and Ca^{2+} release into the cytoplasm. **e** | Thrombin is a strong platelet agonist and triggers the $G_{αq}$-coupled receptors PAR1 and PAR4 (PAR3 and PAR4 in mouse). **f** | Conformational changes of integrins, including αIIbβ3, from a low-affinity to a high-affinity state for their ligands, in part downstream of PLC, PKC, and PI3K, and involving CalDAG–GEFI (Ca^{2+}- and diacylglycerol-regulated guanine nucleotide-exchange factor I). This pathway includes other SGRs (RAS3A and RAP1B), and cytoskeleton-linked signalling molecules (kindlin and talin isoforms). **g** | TXA_2 release is mediated by Ca^{2+}-dependent and protein kinase-dependent activation of cytosolic phospholipase A_2 ($cPLA_2$) and activation of cyclooxygenase 1 (COX1). **h** | Induction of α-granule and δ-granule secretion by strong agonists depends on Ca^{2+} and PKC and involves SGR and SNAP receptor (SNARE) complexes. **i** | Platelet membrane ballooning and phosphatidylserine (PtdSer) exposure occurs in response to combinations of high Ca^{2+}-mobilizing agonists, mediated by the ion channel anoctamin 6 (ANO6). Intracellular protein degradation is mediated by calpain 2. PIP_3, phosphatidylinositol 3,4,5-triphosphate.

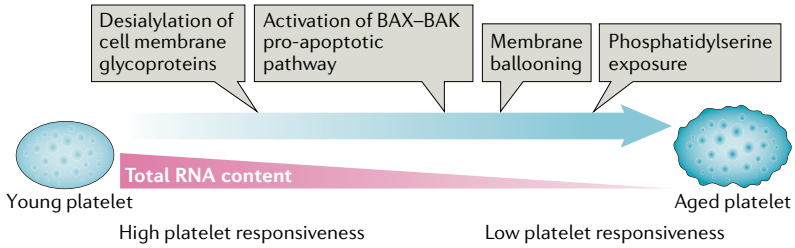

Fig. 2 | **Platelet alterations during ageing.** During their time in the circulation, platelets have a reduction in their RNA content and lose sialic acid residues of glycoproteins in the cell membrane, with the latter change promoting hepatic clearance of the platelets. Activation of the pro-apoptotic BAX–BAK pathway in aged platelets results in caspase-dependent surface exposure of phosphatidylserine, which serves as a recognition signal for phagocytic cells to uptake platelets. In terms of functionality, senescent platelets have impaired adhesion and aggregation responses.

every platelet response can emerge. These different populations include platelets with or without secretion, platelets with or without activated integrins, platelets carrying a fibrin coat, and platelets with membrane ballooning and exposed phosphatidylserine[24–26]. In addition, platelet populations can be distinguished on the basis of major functional impairments related to ageing, storage, or apoptosis[27].

Limited progress has been made to understand the causes and consequences of platelet functional heterogeneity. Examples of age-related platelet heterogeneity are shown in FIG. 2. Newly formed platelets (reticulated platelets containing rough endoplasmic reticulum with ribosomes) are enriched in multiple footprint RNA species, which in part degrade with platelet ageing[16,28]. As a consequence, old platelets have a relative increase in the more-stable circular RNA species compared with younger platelets. In mice, extended platelet age results in platelet exhaustion, a state characterized by a diminished capacity for secretion and thrombus formation[29,30]. In addition, during their time in circulation, platelets can process their microRNA cargo[31,32] and acquire new RNAs[33], which can alter protein expression levels to some extent. These processes can provide platelets with a high potential for versatility and adaptivity in structure and function.

In the whole platelet fraction in blood, sensitive proteomic analyses have revealed the presence (and for most proteins also the estimated copy numbers) of >5,000 platelet proteins, and have provided insight into post-translational modification patterns (phosphorylation, acetylation, and cleavage) that can regulate the functions of these proteins[34,35]. Just one example is the complete proteomic analysis of platelets from a patient with the rare Scott syndrome, which is characterized by the lack of the procoagulant platelet population; this study revealed new insights into the Ca^{2+}-dependent mechanisms of phosphatidylserine exposure and intracellular protein phosphorylation and cleavage[36]. In the near future, quantitative multi-omic analyses of isolated platelet populations will provide more detailed information on structure–function relationships. Furthermore, genetically modified mice can also provide new insights. For instance, the 14-3-3ζ protein, previously considered to mediate GPIb–V–IX signalling events, has recently been identified as a regulatory component linking mitochondrial respiration to the formation of the procoagulant, phosphatidylserine-exposing platelet population[37].

Fibrin coat
Fibrin-coated platelets are a subpopulation of phosphatidylserine-exposing platelets that bind fibrin via transglutaminase activity and activated integrin αIIbβ3. Fibrin is 'coated' on the platelet surface.

Membrane ballooning
Adherent platelets on a collagen surface form phosphatidylserine-exposing, balloon-like membrane structures as a result of salt and water entry into the platelets.

Procoagulant platelet
Platelet swollen to a balloon shape, with surface exposure of phosphatidylserine and displaying greatly increased capacity for coagulation factor activation.

Pseudopod formation
Cytoplasm-filled projection of the platelet membrane following platelet activation.

Platelets in haemostasis and thrombosis

The vascular endothelium continuously prevents platelet activation processes via multiple mechanisms, of which the best-studied are: ectonucleotidases (which degrade ATP and ADP), thrombomodulin (which inactivates thrombin), and the release of prostaglandin I_2 (PGI_2; also known as prostacyclin) and nitric oxide[38] (FIG. 1). PGI_2 and nitric oxide suppress most platelet activation processes, including adhesion, pseudopod formation, secretion, aggregation, and procoagulant activity. The prevailing concept is that this suppression is impaired at sites of vascular injury, by erosion or rupture of an atherosclerotic plaque, or by vascular inflammation (cytokine and von Willebrand factor (vWF) release)[39,40].

Ex vivo, whole-blood, flow studies have indicated that fibrillar collagens (type I and type III), which are present in the vascular intima and media as well as in atherosclerotic plaques[41], are among the most potent platelet-activating substances[42], thus explaining why deeper vascular injury or disintegration of an atherosclerotic plaque causes full, occlusive thrombus formation. Animal thrombosis models further indicate that collagen-induced platelet activation and thrombus formation are greatly increased by thrombin generated via tissue factor[43]. Tissue factor is highly expressed on subendothelial smooth muscle cells and macrophages, and to a limited extent also on the inflamed endothelium[43].

The cascade of reactions induced by collagen and tissue factor–thrombin is considered to be, at least in part, common in haemostasis and arterial thrombosis. In mice, both collagen and tissue factor have a role in tail bleeding and experimental thrombosis induced by arterial ligation or $FeCl_3$ application[44]; a process that in vitro can be mimicked in microfluidics chambers coated with collagen and tissue factor[45,46]. Mouse models further indicate that a reduction of the platelet count to <30% is needed to affect tail bleeding or large-vessel thrombosis, suggesting that platelet count alone is not a major determinant for thrombosis[47]. With regard to haemostasis, this study supports a multi-hit model in which, in addition to thrombocytopenia, another trigger (such as inflammation) is needed for bleeding to occur[48]. Of note, in models of deep venous thrombosis, triggering can be independent of collagen. Venous thrombosis is promoted by low shear rates in the venous part of the circulation, resulting in different types of red thrombus formed, thereby pointing to a more important triggering role of the coagulation process than in arterial thrombosis[38].

During the development of a thrombus, incorporated platelets are differently exposed to collagen and thrombin, which contributes to the heterogeneity in platelet responses (FIG. 3). This heterogeneity is observed in distinct aspects. In rabbit or mouse arteries, thrombi formed in vivo show an inner core of densely packed, contracted platelets that is surrounded by an outer shell of loosely adhered platelets with limited activation[49,50].

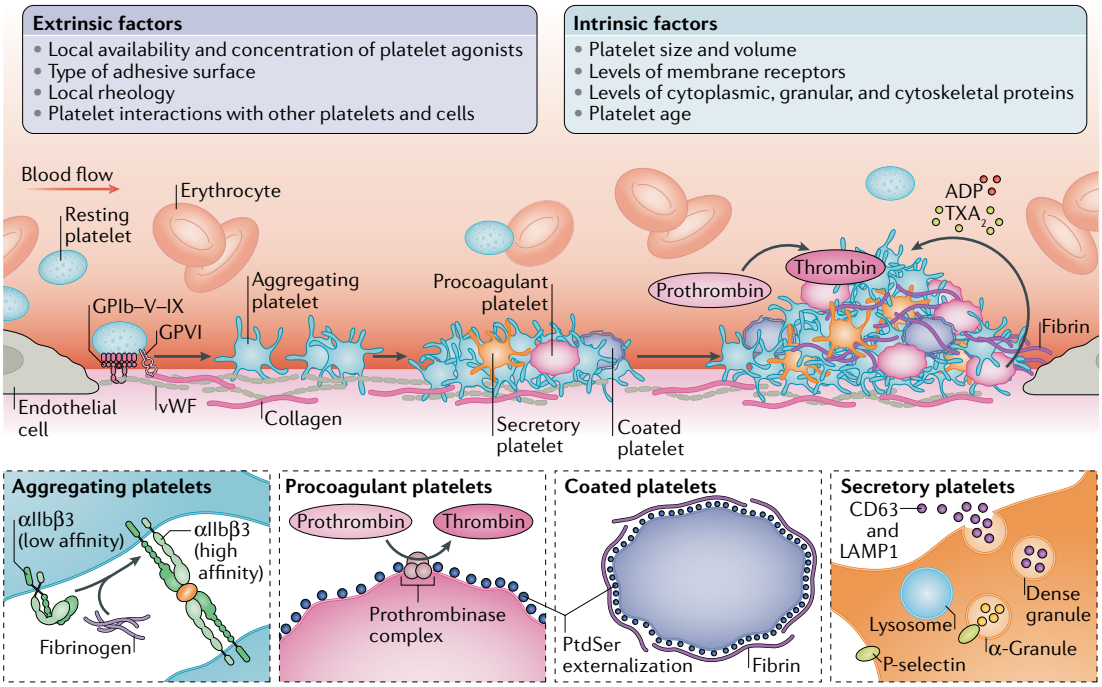

Fig. 3 | **Environmental and platelet factors influencing platelet heterogeneity in the thrombus.** Heterogeneity in thrombus composition is promoted by extrinsic (environmental) factors such as blood flow dynamics, vascular environment, and local availability of platelet agonists, and by intrinsic platelet-specific factors. Endothelial damage exposes the subendothelial matrix proteins von Willebrand factor (vWF) and collagen. Thrombus formation is triggered by platelet adhesion to collagen (via glycoprotein VI (GPVI) receptors) and vWF (via GPIb–V–IX). Different platelet populations can be distinguished on the basis of specific markers. In aggregating platelets, fibrinogen binding to activated integrin αIIbβ3 triggers aggregation; procoagulant platelets have exposed phosphatidylserine (PtdSer) on the membrane and can generate thrombin from prothrombin; coated platelets have exposed PtdSer and a fibrin coat on the surface; and full-secretory platelets express P-selectin and the glycoproteins CD63 and LAMP1 on the outer membrane. Activated platelets release mediators, in particular ADP and thromboxane A_2 (TXA_2), attracting circulating platelets to the growing thrombus. Environmental changes as a consequence of disease can affect platelet populations and responses, for instance by positive priming. Differences in these processes between young and aged platelets are not indicated.

Therefore, platelets in the outer shell are less influenced by collagen and/or thrombin activation, whereas the densely packed platelets in the inner layer might be more activated, and adjacent platelets can interact via contact-dependent signalling and gap junctions[51,52].

Both in vivo and ex vivo studies of collagen-dependent thrombus formation also revealed another form of platelet response heterogeneity. The aggregated platelets in the thrombus, presenting integrin αIIbβ3 (also known as GPIIb/IIIa) activation and a secretory phenotype (P-selectin expression), are surrounded by distinct patches of platelets with phosphatidylserine exposure and coagulation factor-binding properties[53] (FIG. 3). Procoagulant platelets are characterized by high Ca^{2+}-dependent signals and this platelet population in part overlaps with the so-called coated (or COAT) platelets, which serve as anchoring points for the formation of a fibrin coating[25]. Considering GPVI and the protease-activated receptors PAR1 and PAR4 as the main platelet receptors for collagen and thrombin, respectively, in humans, this setting implies potent positive interactions between platelet and coagulation activation pathways (FIG. 4). However, these interactions might differ between conditions of haemostasis (fast process and low shear stress), arterial thrombosis (fast process and high shear stress), and venous thrombosis (slow process and near stasis). The platelet population with phosphatidylserine exposure dramatically increases the generation of both factor Xa (tenase complex) and thrombin (prothrombinase complex)[43], and the generated thrombin can further increase this platelet population. GPVI can also serve as a signalling receptor for fibrin, thereby providing another link between coagulation and platelet activation[54]. Apart from the extrinsic coagulation pathway triggered by tissue factor, the intrinsic coagulation pathway (via factor XII and factor XI) can also contribute to the thrombus activity, for example, through factor XII activation at collagen fibres[55] or via platelet-produced polyphosphate clusters[56].

A comparison of platelet-adhesion ligands has shown that combined GPVI and GPIb–V–IX interacting surfaces (for instance, containing collagen and vWF) provide the most potent substrate for thrombus formation at high-shear stress conditions, followed by interacting surfaces for C-type lectin-like receptor 2 (CLEC2)[42]. Similarly, in vivo mouse models point to crucial roles for GPVI and CLEC2 in arterial thrombus formation and in haemostasis[48,57]. Platelet adhesion to collagen via GPVI, enforced by integrin α2β1, induces SRC family kinase (SFK) and SYK protein tyrosine kinase activation, which leads to stimulation of phospholipase Cγ, elevation in cytosolic Ca^{2+} levels, and stimulation of protein

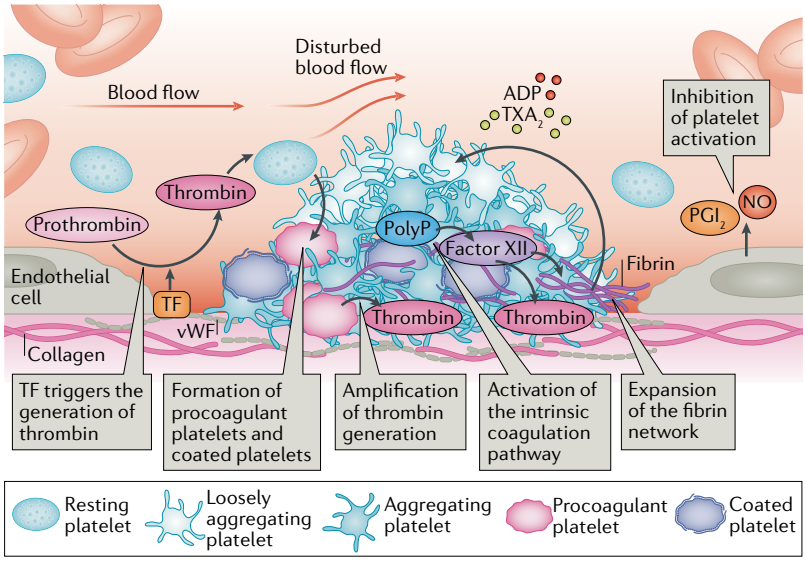

Fig. 4 | **Coagulation pathways contributing to the heterogeneous nature of thrombus formation.** Thrombus buildup under blood flow represented as the result of mutual enforcement of platelet activation and coagulation[43,50]. Vascular tissue factor (TF) triggers the initial generation of thrombin. Thrombin stimulates the formation of procoagulant (phosphatidylserine-exposing) and coated (fibrin-binding) platelets, which leads to the amplification of thrombin generation at procoagulant platelets. At later stages, the fibrin network expands outside of the thrombus (promoted by low shear stress) and the activation of the intrinsic coagulation pathway via factor XII, possibly through platelet-released polyphosphates (PolyP), increases the generation of thrombin and fibrin. Endothelial-derived nitric oxide (NO) and prostaglandin I_2 (PGI_2) suppress these processes. Highly activated platelets in the thrombus core are exposed to higher agonist concentrations than the loosely adhering platelets in the thrombus outer shell.

kinase C isoforms (FIG. 1). Via a series of downstream events involving small GTPase-regulating proteins[58], integrin αIIbβ3 opens up to bind fibrinogen, which acts as a bridge between platelets to form an aggregate or thrombus. Other events downstream of cytosolic Ca^{2+} elevation are granular secretion of secondary mediators (including ADP and ATP) and the release of thromboxane A_2 (REFS[38,59]). In particular, platelets that experience prolonged Ca^{2+}-dependent signalling will swell, undergo membrane ballooning, and expose phosphatidylserine at their membrane surface, owing to activation of the Ca^{2+}-activated ion channel–phospholipid scramblase, anoctamin-6 (ANO6) and mediated by the intracellular protease calpain 2 (FIG. 1)[36,60]. Membrane ballooning increases the surface of the phosphatidylserine-exposing platelets, probably serving to increase their capacity for coagulation factor binding[26].

Other platelet-adhesive receptors (for instance, integrin α6β1, interacting with laminin, or platelet glycoprotein 4 (CD36), interacting with thrombospondin) are less potent in leading to thrombus formation than GPVI, GPIb–V–IX, and CLEC2, but still mediate adhesion under flow conditions in vitro[42]. These receptors cause platelet adhesion to the subendothelial matrix, for example, for the maintenance of vascular integrity. In mice, integrin α6β1 deficiency impairs arterial thrombus formation but does not affect haemostasis, suggesting an enforcing role for subendothelial laminins together with collagens[61].

α-Granules
Platelet secretion granules containing multiple stored proteins including growth factors.

δ-Granules
Platelet secretion granules with dense appearance in electron microscopy, containing Ca^{2+}-bound nucleotides (ADP, ATP, and polyphosphates).

Plateletcrit
Product of mean platelet volume and platelet count in blood.

Platelet genetics, mass, and activation

Gene-sequencing analysis of patients with a family history of bleeding is a common way to identify the underlying causes of platelet-function defects. In these patients, multiple mutations have been identified in genes encoding platelet receptors, signalling proteins upstream and downstream of second messengers, and cytoskeletal proteins[62,63]. In the majority of cases, roles of these genes in thrombosis and haemostasis have been confirmed in mouse or zebrafish studies. In addition, abnormalities — usually linked to changes in platelet count — have been identified in genes encoding transcription factors implicated in haematopoiesis and megakaryocytopoiesis, such as *FLI1*, *GATA1*, *NFE2*, *RUNX1*, and *TAL1* (REFS[64,65]). Risk of bleeding can also be linked to mutations in several genes encoding proteins regulating the biogenesis of α-granules or δ-granules, such as for the grey platelet syndrome (*NBEAL2* and *GFI1B*) and Hermansky–Pudlak syndrome (*HPS1*, *HPS3*, *HPS9*, and other genes)

Conventional Sanger sequencing techniques have fairly low success rates of <50% in identifying possible genetic causes in patients with defects in platelet function or coagulation function. Despite this limitation, variants associated with a bleeding tendency have been identified in >51 genes that are mainly related to platelets or coagulation[66]. Newer approaches using high-throughput and full-genome sequencing techniques have so far provided higher success rates of >70%[67,68]. A more integrative approach is needed for further completion of this list of platelet disease-related genes. For optimal support of patient diagnosis and management, a combination of genomic, epigenomic, and phenotypic data sets, as well as protein–protein interaction networks and standardized clinical phenotyping, is advised[69].

A different approach to find genetic variance that is linked to haemostasis comes from the search of healthy individuals with extreme platelet traits (such as high or low platelet count, mass, and plateletcrit), with the rationale that these traits are indicative of an altered megakaryocytopoiesis or platelet clearance[70]. In 2011, a first meta-analysis of genome-wide association studies, including >66,800 individuals, identified 68 genomic loci associated with platelet count or volume, with the majority related to regulation of platelet formation[71]. An extension of this work by identifying megakaryocyte-matched epigenomic data and finding promoter, long-range, transcription factor interactions (super-enhancers) resulted in a much larger set of 423 non-coding, genome-wide variants, all associated with platelet count or plateletcrit[72]. Several of these variants were linked to changes in platelet activation, as shown by multiparameter tests of platelet function. An important continuation of this work will be the identification of altered super-enhancer sites in patients with haemostatic or thrombotic disorders.

Despite the progress in genetic analyses made so far, linking genetic variations or mutations to dysfunctional platelets or altered coagulation properties for the prediction of bleeding is not easy. Reasons are the often multigenetic causes of haemorrhagic disorders, the confounding effects of acquired factors, and the insight that

even in patients carrying the same mutation (for example, in the Ca^{2+}-signalling protein STIM1 or ORAI1), platelet functionality can differ[73].

Some progress has been made in the elucidation of genetic factors that contribute to platelet hyperreactivity. Gain-of-function mutations have been described in only a few genes, for instance, genes encoding platelet GPIbα (*GP1BA*) or ORAI1 (*ORAI1*)[62,73]. These gain-of-function mutations can lead to positive priming of platelets and thereby even to activation-induced thrombocytopenia (see section on Priming to modulate responsiveness). Mutations have been identified in several genes associated with a high platelet count (thrombocytosis) and which can be thrombogenic; for example, genes encoding components of the thrombopoietin-induced signalling pathway such as thrombopoietin (*THPO*), its receptor (*MPL*), and the downstream kinases (*JAK2*)[64]. Also, deficiencies in several genes encoding anticoagulation proteins are associated with a prothrombotic propensity[67]. An intriguing finding is that a polymorphism in *GP6* is linked with an increased risk of venous thrombosis[74]. Of note, correlations between common genetic variants of platelet glycoproteins and thrombosis came from early, low-powered studies[75].

Platelets in bidirectional communication

As secretory cells, activated platelets release multiple substances from different storage granules: α-granules, δ-granules, and lysosomes. The secretion products can influence many physiological and pathophysiological processes beyond haemostasis, such as inflammation, immunity, angiogenesis, and tumour growth[76]. Platelets also produce other bioactive components such as eicosanoids and extracellular vesicles. Conversely, platelets can uptake plasma and even cellular components, thus providing another mechanism by which the environment influences platelets (TABLE 1).

Table 1 | **Overview of platelet–environment communication mechanisms**

Platelet response	Mechanism	Physiological and pathophysiological consequences
Platelet influence on environment		
α-Granule and δ-granule secretion	Single-vesicle or compound exocytosis	Release of bioactive mediators and exposure of leukocyte interactors
Lysosomal secretion	Late-stage exocytosis	Release of endoplasmic reticulum-resident proteins
Eicosanoid release	Cyclooxygenase and lipoxygenase mediated	Release of short-lived mediators
Extracellular vesicle shedding	Ca^{2+}-dependent and Ca^{2+}-independent	Coagulation support and communication
Influence of environment on platelets		
Sensitization or desensitization to activation	Negative or positive priming	Exhausted or pre-activated platelets
Uptake of plasma components	Endocytosis via endosomes	Adaptation to plasma conditions
Uptake of cellular components	Unknown	Information transfer (tumour adaptation)

Platelet exocytosis or secretion requires fusion of intracellular granules with the plasma membrane, a process that is regulated by SNAREs (SNAP receptors) and connected regulatory proteins, via the interaction of vesicle SNAREs (vSNAREs) with target-membrane SNAREs (tSNAREs). Mouse and cellular experimental models have shown that both vSNAREs (VAMP2, VAMP3, and VAMP8) and tSNAREs (syntaxin 8, syntaxin 11, and SNAP23), together with small GTPases and MUNC18 and MUNC13 proteins, whose functions are not well understood, are required for the secretion of α-granules and δ-granules[77]. A study published in 2018 points to a regulatory role of microtubules, with the motor protein kinesin 1 contributing to α-granule and δ-granule secretion[78]. Lysosomal secretion has been studied more incidentally, especially in the context of Hermansky–Pudlak syndrome (which is characterized by a combined defect of δ-granules and lysosomes), describing a role for the lysosome-related complex BLOC1–BLOC3 in this process[79]. Lysosomal secretion can be responsible for the surface expression of resident reticular proteins in platelets, such as protein disulfide isomerases[80].

An initial report suggesting that individual α-granules in platelets have differences in their protein cargo[81] can be understood from the finding that the protein-sorting process can differ during the formation of individual granules[82]. However, results from proteomic studies have pointed to a similar release pattern of all granule cargo proteins, whereas the release kinetics depend on the type and strength of the applied stimulus[76]. Detailed ultramicroscopic analysis has demonstrated that, at lower levels of stimulation, α-granules and δ-granules tend to exocytose individually, whereas at high agonist levels, the α-granules tend to fuse first, a process called compound exocytosis[83].

In both humans and mice, defects in α-granules (grey platelet syndrome), δ-granules (Hermansky–Pudlak syndrome), or both (platelet storage pool deficiencies) can result not only in bleeding, but also in impaired wound healing, reduced inflammation, or aberrant vascular remodelling, which emphasizes the multiple functions of platelet-stored granule contents[84,85]. The neurobeachin-like 2 (NBEAL2) protein is essential for the biogenesis of α-granules. Mice deficient in NBEAL2 phenocopy the grey platelet syndrome in humans and are protected from arterial thrombosis and thromboinflammatory stroke[85]. In addition to the cyclooxygenase 1 product thromboxane A_2 (whose formation is suppressed by aspirin intake), activated platelets produce a whole range of other bioactive eicosanoids, as described in detail elsewhere[86].

In the past decade, attention has been focused on the release of membrane vesicles from platelets. These extracellular vesicles comprise exosomes (40–100 nm in diameter) and microvesicles (100–1,000 nm in diameter). The majority of vesicles in blood circulation are platelet-derived and/or megakaryocyte-derived ($CD41^+$)[87], suggesting a role in blood-related processes. Such a role is confirmed by the presence of bioactive cytokines, eicosanoids, coagulation factors, and RNA species in platelet-derived extracellular vesicles. Interactions between these vesicles and other cells are

Negative or positive platelet priming
Suppression or promotion of platelet activation by bioactive molecules in the blood.

Exhausted platelets
Also known as refractive platelets; platelets with reduced secretion capacity owing to previous activation.

possible through ligand presentation and/or membrane fusion[87,88]. Interactions of platelet-derived extracellular vesicles with leukocytes are mostly reported in the context of inflammation[89,90]. Interestingly, depending on the platelet state (ageing or activation), the extracellular vesicles seem to diverge in glycoprotein expression levels and differ in modulating monocyte functions[91]. An inflammatory role of platelet-derived extracellular vesicles (formed by platelet activation) is observed in rheumatoid arthritis, stimulating cytokine production from synovial fibroblasts[92]. However, to what extent platelet-derived extracellular vesicles contribute to platelet communication with their surroundings is still unclear.

A small number of studies also describe that platelets can take up plasma-derived or cell-derived components, for instance, RNA species from tumour cells — which leads to the formation of what has been designated, perhaps improperly, as tumour-educated platelets — with a potential for cancer diagnostics[93]. The physiological and pathophysiological importance of this communication from the environment to platelets needs to be established. Specific unanswered questions with regard to the bidirectional environment–platelet interactions are how platelet microRNAs, for instance, carried in extracellular vesicles, are transferred and functionalized to and from other cells. The literature so far is confined to observations of microRNA transfer from platelets to endothelial cells[94] or tumour cells[95].

Priming to modulate responsiveness

Circulating platelets are subjected to a balanced spectrum of activating and inactivating biomolecules and conditions. Under physiological conditions, when vascular activation or injury is absent, the net result is inhibition of spontaneous platelet adhesion and activation. This insight has led to the concept of negative or positive platelet priming (FIG. 5) to describe physiological and pathophysiological conditions in which the threshold for platelet activation is increased (negative platelet priming) or lowered (positive platelet priming)[22].

Several components circulating in the blood are unable to trigger platelet activation, but can act as positive primers and potentiate the activation process in the presence of stronger agonists. For example, adrenaline acts as a positive primer by binding to the $G_{\alpha i}$-coupled α_2-adrenergic receptor, which lowers cytosolic cAMP levels and thus augments platelet activation by other agonists[38,96]. Other positive primers are insulin-like growth factor I and thrombopoietin, both of which enhance platelet activation via phosphatidylinositol 3-kinase (PI3K) signalling pathways[97]. Several positive primers present in plasma (such as the vitamin K-dependent protein GAS6) or derived from platelets (such as thrombomodulin and soluble CD40 ligand (sCD40L)) increase collagen-dependent platelet responses and thrombus stability[98,99]. These priming events will add to the activating effects of platelet-derived autacoids (thromboxane A_2 and ADP). Positive priming can also be induced locally by high wall-shear stresses, for example, at vessel stenotic sites with increased vWF activity, thereby promoting

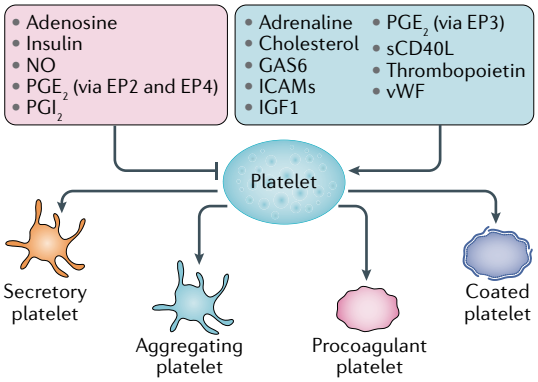

Fig. 5 | **Negative and positive priming factors influencing platelet responses.** Negative (pink box) and positive (blue box) priming substances can alter the thresholds for platelet activation responses induced by agonists. EP, prostaglandin E_2 receptor; GAS6, growth arrest-specific protein 6; ICAM, intercellular adhesion molecule; IGF1, insulin-like growth factor I; NO, nitric oxide; PGE_2, prostaglandin E_2; PGI_2, prostaglandin I_2 (also known as prostacyclin); sCD40L, soluble CD40 ligand; vWF, von Willebrand factor.

thrombus formation[39,100]. Such positive platelet priming has clinical relevance; for example, it can override the inhibitory effects of aspirin and P2Y purinoceptor 12 ($P2Y_{12}$) blockade on platelet function[101].

Negative platelet-priming substances include bioactive mediators released from endothelial cells. PGI_2 and prostaglandin E_2 (PGE_2) bind to the platelet IP and EP2 and EP4 receptors, respectively, causing elevation of cytosolic cAMP levels, which increases the threshold for platelet activation (FIG. 1). Of note, at low dose, PGE_2 can also act as a positive primer by interacting with the PGE_2 receptor EP3 subtype[102]. Endothelial cell-produced nitric oxide impedes platelet activation mediated by elevation in cytosolic cGMP levels[103]. A 2017 study indicated that polyunsaturated fatty acid products of 12-lipoxygenase can also hamper platelet activation via elevation of cytosolic cAMP levels[104].

In disease conditions, the excitability of platelets can be modified by systemic and local changes in the balance between negative and positive priming factors[22]. For instance, diabetes mellitus, hyperglycaemia, and states of increased vascular stress might prime platelets to increase their responsiveness to agonists (hyper-reactive platelets)[105]. In atherosclerosis, platelet responsiveness can be modulated by feedback of platelet-derived chemokines, with C-X-C motif chemokine 12 (CXCL12; also known as SDF1) promoting and C-C motif chemokine 5 (CCL5) suppressing platelet aggregation[106]. Taking this concept further, extensive positive priming can lead to further activation of (a population of) circulating platelets, which subsequently causes refractoriness to activation (so-called exhausted platelets). This platelet phenotype is seen in blood samples from patients with solid tumours, sepsis, or stroke, and can explain the low platelet activation responses in vitro[22]. Whether this impairment affects all platelets or only certain platelet populations, such as the low-responsive ageing platelets, is unclear. Plasma from patients with coronary artery

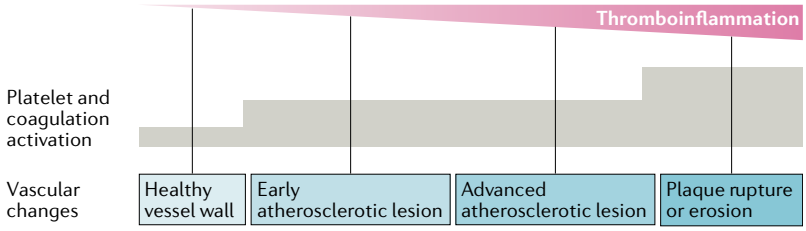

Fig. 6 | **Platelets in vascular thromboinflammation.** Platelets are attracted to the early inflamed vessel wall, where they interact with leukocytes and promote coagulation. These interactions with leukocytes trigger vascular outside-in signalling processes that, in more advanced stages of thromboinflammation, promote atherosclerosis progression and vascular remodelling. Rupture or erosion of a vulnerable atherosclerotic plaque results in nonocclusive or occlusive formation of a platelet–fibrin thrombus followed by leukocyte invasion of the thrombus.

disease frequently has elevated levels of platelet products (such as platelet factor 4, β-thromboglobulin, and sCD40L) compared with plasma from healthy individuals[107], most likely as a consequence of in situ platelet activation. Although evidence exists for altered platelet responses in other systemic diseases such as chronic kidney disease and renal insufficiency[22], the responsible priming factors are unknown.

Platelets in vascular inflammation

Studies dating back to the 1960s demonstrated that platelets have a key role in maintaining the confluent endothelial structure and the endothelial barrier function[108]. In the context of blood transfusion, this process is known as platelet consumption. Platelet adhesion also has a role in the development of blood vessels (with a pro-angiogenic effect) and lymphatic vessels, as well as in the 'leaky' blood vessels of tumours[48]. Platelets adhered to the endothelium can further assist in the recruitment of progenitor cells to the vasculature[109]. For these multiple physiological functions, one can safely assume that platelets cause essential changes in the vessel wall through the release of biomediators (growth factors, lipid products, and cytokines). Nevertheless, further mechanistic research is needed.

Limited progress has been made in understanding how platelets alter the vessel wall in conditions of inflammation[39,110]. Thromboinflammation describes the interaction of platelets, leukocytes, and the coagulation system at sites of inflamed vessel wall. This concept unites various vascular conditions with a certain inflammatory component, such as systemic inflammation, atherosclerosis[111], ischaemic stroke[112], and ischaemia–reperfusion injury[113]. In vivo studies in mice have demonstrated that at the inflamed vessel wall, platelets roll and adhere to the activated endothelium[110,112]. The adhered platelets can prevent inflammation-induced bleeding events in skin, brain, lungs, and other organs. Mouse studies also indicate roles of the receptors involved in arterial thrombosis, GPIb–V–IX, GPVI, and CLEC2, in inflammation-linked haemostasis and ischaemia, as well as redundant roles of the common G protein-coupled receptors and integrin αIIbβ3-dependent aggregation of platelets[110,114].

Multiple mechanisms (with undefined relative importance) contribute to the bidirectional interactions between platelets, leukocytes, and the vessel wall during the progression of atherosclerosis, vascular remodelling, inflammation, and ultimately thrombus formation (FIG. 6). These mechanisms involve, for example, platelet trapping by ultralarge vWF multimers deposited on endothelial cells, which are secreted from Weibel–Palade bodies[115]. Other examples are multi-molecular platelet contacts with leukocytes (monocytes, neutrophils, and T cells), in particular via the GPIb–V–IX–integrin, CD40L–CD40, and P-selectin–PSGL1 (P-selectin glycoprotein ligand 1) axes[116–118]; the multiple proteins released from the platelet α-granules[85]; bimolecular interactions between platelet-derived and leukocyte-derived chemokines[106,119]; contributions of platelet-derived extracellular vesicles[120]; and processes involving coagulation factors, complement factors, and the fibrinolysis system[121]. Of current interest is the involvement of neutrophils in thromboinflammatory processes in part via late formation of neutrophil extracellular traps (NETs)[110,122]. Taken together, the existence of these multiple mechanisms indicates that the communication of platelets and platelet-derived mediators with components of the inflammatory pathways is complex, especially because in haemostasis and inflammation the roles of platelets partly overlap. How platelets regulate inflammation and immunity has been reviewed in detail previously[123–125].

Arterial thrombotic events are commonly induced by rupture of an atherosclerotic plaque (most commonly plaques with a thin-cap fibroatheroma and a thrombogenic necrotic core), and these frequently, but not always, result in the formation of vaso-occlusive platelet thrombi mixed with red clots[126]. Mouse models of experimental atherosclerotic plaque rupture or damage point to potent thrombogenic roles of collagen and tissue factor (plaque-derived or media-derived), which trigger persistent platelet and coagulation activation[127,128]. An alternative cause of thrombus formation is plaque erosion[129]. This process is characterized by superficial damage of atherosclerotic plaques with a small necrotic core, the exposure of subendothelial layers (leaving the fibrous cap more or less intact), and the formation of mostly white, platelet-rich thrombi[130]. A proof-of-concept study suggests that antiplatelet therapy alone (without stent implantation) is beneficial in patients with plaque erosion, in accordance with the high platelet content of thrombi formed in these patients[131].

Current platelet-targeted therapeutics

Antiplatelet drugs are the first-choice therapy for the treatment of cardiovascular disease and the prevention of atherothrombosis[132]. However, growing evidence indicates that also the coagulation system has a substantial role in disease progression[133]. A limitation of current treatment options is that the number of antiplatelet medicines in clinical use is fairly limited. The same holds true for prohaemostatic therapies, that is, therapies to prevent platelet-related bleeding, where treatment options are restricted to drugs that increase platelet count (such as thrombopoietin) or to transfusion of platelet preparations in the case of pathological thrombocytopenia.

Weibel–Palade bodies
Storage granules of endothelial cells that store ultralarge von Willebrand factor multimers.

Table 2 | **Current and potential antiplatelet drugs**

Target (drug)	Inhibitory mechanism	Platelet-function assay
Current targets		
COX1 (aspirin and NSAIDs)	Blocked TXA_2 formation (autocrine)	LTA, PFA-100
$P2Y_{12}$ (clopidogrel and prasugrel)	Irreversibly inhibited ADP receptors (autocrine)	LTA, VerifyNow[a], VP
$P2Y_{12}$ (ticagrelor)	Reversibly inhibited ADP receptors (autocrine)	LTA, VerifyNow, VP
Integrin αIIbβ3 (abciximab, eptifibatide and tirofiban)	Blocked integrin αIIbβ3 (aggregation)	LTA, VerifyNow
PAR1 (vorapaxar)	Blocked thrombin receptors (coagulation)	LTA, VerifyNow
Potential targets		
Factor Xa or thrombin	Suppressed coagulation (anticoagulants)	TEM, coagulation tests
GPIb-V–IX	Blocked adhesion to vWF (flow-dependent)	PFA-100, microfluidics
GPVI and CLEC2 (revacept)	Blocked ITAM-like signalling receptor	LTA, microfluidics
TKIs (>20 drugs)	Blocked ITAM-like signalling (SFK, BTK, and SYK)	LTA, microfluidics
PI3Kβ and PDI	Inhibited integrin αIIbβ3 (aggregation)	LTA, microfluidics
Ecto-nucleotidases	Inactivated autocrine ADP and ATP	LTA, microfluidics
PAR1 and PAR4 (pepducins)	Blocked thrombin receptors (coagulation)	LTA, VerifyNow

BTK, Bruton tyrosine kinase; CLEC2, C-type lectin-like receptor 2; COX1, cyclooxygenase 1; GP, glycoprotein; LTA, light transmission aggregometry; $P2Y_{12}$, P2Y purinoceptor 12; PAR, protease-activated receptor; PDI, protein disulfide isomerase; SFK, SRC-family kinase; TEM, thrombo-elastometry; TKI, tyrosine kinase inhibitor; TXA_2, thromboxane A_2; VP, VASP phosphorylation; vWF, von Willebrand factor. [a]Other point-of-care platelet aggregation tests available.

Aspirin (acetylsalicylic acid) is the drug most commonly prescribed in cardiovascular disease (TABLE 2). Aspirin irreversibly inhibits platelet cyclooxygenase 1, thereby blocking the formation of thromboxane A_2. Expert position papers still support the use of low-dose aspirin for primary prevention of cardiovascular disease in individuals at increased risk of cardiovascular disease, despite the non-negligible risk of bleeding associated with aspirin therapy[134,135]. NSAIDs, although targeted at inflammatory processes, also reversibly inhibit cyclooxygenases and, therefore, have the same adverse effect on bleeding as aspirin.

Dual antiplatelet therapy (aspirin in combination with $P2Y_{12}$ blockage) is the standard treatment for secondary prevention of atherothrombotic events in patients with acute coronary syndrome or after coronary stent implantation[133]. Platelet $P2Y_{12}$ receptors can be irreversibly targeted with the prodrugs clopidogrel and prasugrel, and more recently, with the reversible receptor antagonists ticagrelor and cangrelor, which do not require metabolization and, therefore, have a faster antiplatelet effect[136]. In patients with symptomatic peripheral artery disease, single antiplatelet therapy with either aspirin or clopidogrel is indicated, with a more favourable benefit-to-risk ratio than the use of dual antiplatelet therapy, although the risk of thrombosis recurrence remains particularly high in this patient population[133].

The achievements (such as better efficacy) and limitations (such as metabolic conversion of prodrugs and the risk of bleeding) obtained with dual antiplatelet therapy are discussed in detail elsewhere[136,137]. Of note, current antiplatelet drugs target autocrine release mechanisms (thromboxane A_2 and ADP), rather than receptors of primary platelet agonists, and are aimed to reduce one particular response of platelets, namely platelet aggregation. The use of integrin αIIbβ3 inhibitors is restricted to patients at high risk of cardiovascular disease, owing to the fairly high risk of bleeding associated with the investigated drugs. Vorapaxar, a competitive inhibitor of the PAR1 receptor for (plasma-generated) thrombin, reduces ischaemic events in different patient groups, but again at the expense of an increased risk of bleeding[136]. In summary, an unmet clinical need remains for antiplatelet agents that effectively prevent arterial thrombosis with minimal risk of bleeding.

In the field of interventional cardiology, many studies have focused on the optimal antithrombotic treatment regimens for the prevention of thromboembolic events. Owing to the progress made by the introduction of drug-eluting stents (which release anti-proliferative agents) and by new stent materials and designs, the rate of in-stent thrombosis has now been greatly reduced[138,139]. Nevertheless, further optimization of antithrombotic treatment is required in patients with coronary artery disease[139]. Current guidelines recommend personalized treatment and adjusted duration of dual antiplatelet therapy on the basis of the individual risk profile[140]. However, dual antiplatelet therapy has meanwhile become the standard of care in patients (without anticoagulation) undergoing transcatheter aortic valve implantation[141,142]. Given that the primary mechanism of thrombus formation after transcatheter aortic valve implantation is still unclear, ongoing trials need to reveal whether antiplatelet or anticoagulant therapy is the preferred treatment strategy[143]. The same holds true for the treatment of patients with implanted left ventricular assist devices, in whom the risk of bleeding due to loss of high-molecular-weight vWF multimers in the plasma complicates antithrombotic treatment[144].

Novel platelet-targeted therapeutics

While most of the approved antiplatelet drugs suppress autocrine events involved in platelet aggregation, as measured by light-transmission aggregometry, novel drugs in development are frequently directed against other platelet-activation processes, such as adhesion, signalling, secretion, procoagulant activity, and interaction with coagulation components (TABLE 2). These new targets imply that new tests for other platelet functions, and maybe for platelet populations, are needed to evaluate drug efficacy in vitro and ex vivo. In addition to platelet function tests, the search for genetic determinants of non-responsiveness to current and novel antiplatelet drugs, for instance, with the use of genome-wide association studies and candidate-gene approaches, might also aid in improving treatment efficacy and the development of personalized treatment strategies[145].

Targeting GPVI and GPIb–V–IX. Efforts are underway to target the primary adhesive receptors GPVI (for collagen) and GPIb–V–IX (for vWF), given that these glycoproteins are expressed only on platelets and megakaryocytes. Clinical trials targeting GPVI and GPIb–V–IX performed so far are described extensively elsewhere[136]. Novel drugs directed against platelet populations with the active conformation of integrin αIIbβ3 are also being tested.

PI3Kβ inhibitors. The targeting of platelet signalling proteins has as a general disadvantage that similar signalling processes in other cells will also be affected. Nevertheless, such drugs provide noteworthy treatment options. Given the strong thrombo-protective phenotype of PI3Kβ-deficient mice, mimicked by the use of selective PI3Kβ inhibitors[146], it seems that these inhibitors in combination with aspirin show a greater antiplatelet potency but less risk of bleeding compared with the conventional combination of aspirin and clopidogrel[147].

Tyrosine kinase inhibitors. Tyrosine kinase inhibitors are widely used as targeted strategies for the treatment of cancers. Tyrosine kinase inhibitors commonly act by competition with ATP in the conserved catalytic domain of the protein tyrosine kinase superfamily. More than 20 of the currently prescribed tyrosine kinase inhibitors also suppress platelet responses or reduce plateletcrit, with several inducing mild bleeding[148]. The assumed targets in platelets of many of these tyrosine kinase inhibitors are SYK, BTK (Bruton tyrosine kinase), and SRC family members, although some inhibitors can also affect mitogen-activated protein kinase (MAPK) signalling[148]. Of note, these tyrosine kinases are crucial for platelet signalling through the GPVI, CLEC2, and FcγRIIA receptors (FIG. 1). Indeed, BTK inhibitors were shown to substantially suppress GPVI-dependent thrombus formation on human atherosclerotic plaque tissue ex vivo[149]. This observation implies a potential use of tyrosine kinase inhibitors for antiplatelet therapy, although adverse effects on other cells and organs are inevitable. Low dosing and short-term use of tyrosine kinase inhibitors might provide the solution to prevent adverse effects.

Targeting the platelet-secretion process. Preclinical data indicate that elements of the platelet-secretion process can be targeted for antithrombotic therapy. Potential targets are lysosome-derived protein disulfide isomerases[136], platelet-secreted ADP (degradation is promoted by recombinant ectonucleotidases)[150], and platelet P-selectin[151]. Another approach is inhibition of the process of granule secretion, for example, by interfering with SNARE complexes, which might have both antithrombotic and anti-inflammatory effects. In this context, targeting the population of highly activated platelets with secreted granule content might be an attractive therapeutic strategy.

Targeting the platelet–coagulation pathway interaction. The potent mutual interactions between platelets and coagulation pathways (FIG. 4) provide largely unexplored opportunities for a targeted antithrombotic intervention, although caution is required because of the risk of bleeding. Especially promising in this respect will be interventions aiming to suppress the population of procoagulant platelets. For instance, suppression of procoagulant platelets might normalize the fairly high fractions of this platelet population reported in patients with coronary artery disease[152]. An important question is whether treatment with thrombin receptor inhibitors or with antiplatelet plus anticoagulant drugs indeed abolishes the formation or the function of procoagulant platelets.

In addition to vorapaxar, other small molecules against the thrombin receptors PAR1 and PAR4 were shown to be effective in inhibiting thrombus formation in preclinical settings and in non-human primates[136]. With adequate dosing as a prerequisite, improved efficacy in thrombosis prevention can be reached by combining antiplatelet and anticoagulant drugs. For example, results from a large clinical trial published in 2017 showed that aspirin together with a low dose of the factor Xa inhibitor rivaroxaban is beneficial in patients with stable atherosclerotic vascular disease[153]. This topic has been extensively reviewed elsewhere[133].

Conclusions

Writing a broad, up-to-date Review on platelet biology and clinical targets is challenging in that choices must be made on the main discussion items, superfluous details need to be left out, and many primary research papers cannot be mentioned. In this Review, we focused on a number of new and lesser-known concepts and mechanisms of platelet biology and function that might direct future research. These new concepts and clinical perspectives are the presence of platelet populations with heterogeneity in formation, structure, and properties, and the consequences of this heterogeneity for functional specialization; the advances of genetic and proteomic approaches, providing information on an increasing number of platelet proteins, which can contribute to quantitative or qualitative platelet disorders as well as non-platelet-based diseases; important roles of platelets in thromboinflammatory processes in interaction with leukocytes and the vessel wall; evidence on how circulating or adhered platelets can communicate with

their environment via lipids, bioactive peptides, proteins, RNA species, and extracellular vesicles; and the response adaptation of platelets to their environment by negative or positive priming. All these new insights will stimulate the development of new medications for the treatment of cardiovascular disease and related conditions. New intervention approaches can include targeting the strong positive interactions between platelets and coagulation components, the populations of procoagulant or secreting platelets, positive priming conditions, for instance, those giving rise to pre-activated platelets, and platelets in patients with specific genetic disorders.

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P.E.J.v.d.M. is a consultant at Bayer AG. J.W.M.H. is a founder and shareholder of FlowChamber BV.

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