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Platelets and coronary artery disease: Interactions with the blood vessel wall and cardiovascular devices

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Abstract

In view of the rare presence of studies concerning platelet function as risk factor in atherosclerotic patients, processes underlying thromboembolic events are reviewed in this paper. The morphology and the structural organization - membrane receptors, the open canalicular and dense tubular systems, the cytoskeleton, mitochondria, granules, lysosomes, and peroxisomes - of platelets are described. Platelet function under physiological conditions, in atherosclerosis and after implantation of cardiovascular devices is summarized.

Introduction

Cardiovascular science started with clinical observations and anatomical dissections emerging in the early 20th centuries. Hektoen reported that myocardial infarction was due to thrombi in

the coronary arteries already in 1892 [1]. Later, a range of epidemiological studies followed. The first was the Tecumseh study (starting in 1947) [2], which initiated lots of further epidemiological projects, among them the well-known and still ongoing Framingham study [3]. For such studies, the concept of the relation between "risk factors" and the incidence of coronary artery disease [2] was developed. In the following decades different epidemiological studies confirmed hypertension, diabetes, or serum elevated cholesterol levels as cardiovascular risk factors. Based on a profound understanding of risk factors, later including also physical activity, body weight, and glucose metabolism, preventive measures were tested. Thereafter, intervention studies revealed that the reduction of cardiovascular risk factors by different drugs decreased the frequency of cardiovascular events by almost half. These findings confirmed the significance and accuracy of this risk factor concept and led to both primary and secondary preventions.

While somatic and behavioral cardiovascular risk factors were deeply evaluated in the last 50 years, thrombotic events had been viewed traditionally as a biochemical process of the coagulation cascade characterized by the activation of factor X and the activation of thrombin, resulting in the formation of fibrin. Platelet dysfunction was not considered as a major risk factor for cardiovascular events. The first epidemiological study, in which platelet function was analyzed, was the Northwick Park Heart study. Neither measurements showed any association between platelet aggregation and ischemic heart disease incidences nor did similar measurements in 460 men, in whom epinephrine-induced aggregation was also carried out [4]. In the following years, it became clear that platelet aggregation tests performed with different agonists like adenosine diphosphate (ADP), collagen, epinephrine, arachidonic acid, or ristocetin indicate the risk for bleedings, however, did not predict future cardiovascular events. This hypothesis became evident when the Breddin´group published the prospective PARD study [5,6]. Here, the occurrence of new vascular occlusions (myocardial infarction, stroke and peripheral arterial occlusion) was significantly higher in those diabetic patients with enhanced

spontaneous platelet aggregation measured at entry as compared to those with normal values [7]. The results of this study have verified the hypothesis that spontaneous platelet aggregation is a major risk factor for future vascular occlusions in diabetic men.

Nowadays, it is well accepted that platelets are key elements of thrombotic processes leading to life-threatening cardiovascular diseases. Moreover, a growing body of evidence confirms that antiplatelet therapy is a clinically important entity, and most clinical studies have now shown that post-interventional platelet activity is a risk factor for thrombo-ischemic complications following cardiovascular procedures [8,9,10]. Sibbing et al. have conducted the first, prospective, large-scale trial assessing the relationship between responsiveness to an antiplatelet drug (Clopidogrel) and the risk of early stent thrombosis [11]. In a cohort of 1,608 patients they could show that platelet aggregation measurements could identify patients at increased risk of early stent thrombosis (≤ 30 days after percutaneous coronary interventions).

In summary, this shows that activated or hyperaggregable platelets are risk factors indicating future cardiovascular events and that antiplatelet therapies reduce this risk.

To understand the mechanisms underlying thromboembolic events, we firstly describe the morphology of platelets with its structural organization consisting of the membrane, the open canalicular and dense tubular systems, the cytoskeleton, mitochondria, granules, lysosomes, and peroxisomes. Thereafter, platelet functionality under physiological conditions but also in atherosclerosis or after implantation of cardiovascular devices is described.

Platelet morphology

Normal, non-activated platelets are small bi-convex disk-shaped anuclear cell fragments of 2 – 4 μm in diameter and 0.5 - 1 μm in thickness. They originate from the fragmentation of megakaryocytes (up to 8,000 platelets from one megakaryocyte) in the bone marrow. Extensions of dynamic protrusions into microvessels seem to be sheared from their

transendothelial stems by the flowing blood [12]. There are between 150,000 and 350,000 platelets per mm^3 of blood [13]. The structural organization of platelets can be subdivided in different systems: the envelope, internal membranes (open canalicular and dense tubular systems), a cytoskeleton (microtubules and microfilaments), mitochondria, glycogen granules, storage granules (α-granules and dense granules), lysosomes, and peroxisomes (Figure 1).

The envelope can be divided in two substructures: the plasma membrane and the glycocalix [14]. The plasma membrane has a thickness of ~8 nm and consists of two lipid layers joined back to back containing phospholipids; the negatively charged phosphatidylserine and phosphatidylinositol residues are primarily confined to the cytoplasmic side, where they may serve as substrates for phospholipases (PLs). An alternation of phospholipid and cholesterol molecules is present in each layer. The hydrophobic parts of these molecules – fatty acids of the phospholipids and steroid nuclei of the cholesterol – are located opposite to each other, while their polar groups – glycerol esterified by a phosphate in the case of the phospholipids, and a hydroxyl group in case of cholesterol – are directed towards the extracellular and intracellular medium.

Figure 1

A range of proteins are embedded in the platelet membrane, comprising adhesive, stimulatory, and inhibitory receptors [15]. The intrinsic proteins are located in varying depths within the layer and can even pass through it. The extrinsic proteins are associated in a more labile manner. Most of the proteins with one part exposed to the outer surface of the membrane are glycoproteins with one or more branched oligosaccharide chains. These oligosaccharides form a coating of variable density and thickness. This glycocalix is up to 50 nm thick and is responsible for the negative charge of the membrane surface [14]. The ends of these oligosaccharide chains also serve as antigen determinants and can therefore be visualized by immunocytological techniques. Their main function is to act as receptor for specific molecules.

Receptors regulate the cell-cell or cell-substrate (extracellular matrix components or body foreign materials) attachment. They respond to ligands or counter-receptors and transmit signals intracellularly, thereby changing the adhesive receptor profile and density on the cell surface via activating signaling pathways. This process is associated with rearrangements of the actin cytoskeleton and platelet shape changes (including platelet spreading) as well as with the secretion of soluble signaling molecules [16]. Platelet adhesion is mediated by several receptors essentially. A wide variety of mobile transmembrane receptors covers the platelet membrane, including many integrins ($\alpha_{IIb}\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_3$), leucine-rich repeated (LRR) receptors (Glycoprotein [GP] Ib/IX/V, Toll-like receptors), G-protein coupled seven transmembrane receptors (GPCR, PAR-1 and PAR-4 thrombin receptors, P2Y1 and P2Y1$_2$ ADP receptors, TPα and TPβ TxA$_2$ receptors), proteins belonging to the immunoglobulin superfamily (GP VI, FcγRIIA), C-type lectin receptors (P-selectin), tyrosine kinase receptors (thrombopoietin receptor, Gas-6, ephrins and Eph kinases) and a miscellaneous of other types (CD63, CD36, P-selectin ligand 1, TNF receptor type, etc). Many of these receptors are shared by other cell types, but some are only expressed on platelets (Figure 2).

Important for the adhesion process are: GP Ia/IIa facilitates adhesion to collagen [17], whilst GP Ib is important in the attachment of platelets to von Willebrand factor (vWF) [18] and the vascular subendothelium. In particular, the GP IIb/IIIa (integrin $\alpha_{IIb}\beta_3$) contributes towards adhesion to fibrin and the binding of soluble ligands (e.g. fibrinogen) that facilitate platelet-platelet interactions [19].

Figure 2

Internally, platelets contain a cytoskeleton, a dense tubular system, few mitochondrias, glycogen granules, dense (δ) and α storage granules and peroxisomes. The actin cytoskeleton is essential for the maintenance of platelet morphology and for the rapid change in shape following platelet activation. It is further involved in the regulation of the platelet surface

glycoprotein signaling. The dense tubular system (DTS) is derived from the smooth endoplasmic reticulum from the parent megakaryocyte [20]). The DTS is the major calcium sequestrating organelle in platelets and maintains the resting free calcium concentration ($[Ca2^{+}]_i$) of ~90 nM [21]. Furthermore, the DTS is the location of a pair of calcium-liberating inositol 1,4,5-trisphosphate (IP3) receptors (IP3RI and IP3RII) [22]. IP3, generated after agonist-induced platelet activation, acts to liberate calcium from the DTS and elevates the $[Ca2^{+}]_i$. Additionally, DTS plays a major role in arachidonic acid metabolism: the liberation of the enzymes PL A2 and diglyceride lipase [23]. These enzymes are involved in the stepwise conversion of arachidonic acid to thromboxane (TX) A2, prostaglandin H-synthase-1, and TX synthase [24]. TXA2 is an important inhibitor of platelet function, and conversely inhibition of this pathway serves as a primary target for anti-platelet therapy.

Platelets contain three types of granules: the contents of each are stepwise released following stimulation to further promote platelet adhesion and activation [25]. The α-granules retain relevant proteins for the hemostatic function of platelets, such as vWF, fibrinogen, P-selectin, PECAM-1, CD40 ligand (CD154), platelet factor-4, β-thromboglobulin, thrombospondin, platelet derived growth factor, Factor V, as well as further GP IIb/IIIa ($\alpha_{II}b\beta_3$) molecules. δ granules, on the other hand, are rich in nucleotides (ADP and adenosine triphosphate), serotonin, histamine, pyrophosphate, and calcium. Lysosomal granules contain acid proteases, acid glycosidases, acid phosphatases, and aryl sulphatases [26,27].

Physiological functions of platelets

Two thirds of the total number of platelets is circulating and the remaining one-third is sequestered in the spleen. Both pools are in equilibrium. The circulating platelets have a lifespan of 8 to 10 days [28] and they are replaced at the rate of ~35,000 / mm^3 per day. The major hormonal regulator of platelet production is thrombopoetin [29]. Recently, Battinelli reported

that nitric oxide (NO) can also stimulate platelet production from megakaryocytes [30]. Under physiological conditions, they circulate in a quiescent state before they are cleared by macrophages in spleen and liver. During their life time, most platelets never undergo firm adhesion.

Although platelets have no nucleus and thus no genomic deoxyribo nucleic acid, they contain gene transcripts (microribonucleic acid) [31], enabling them to produce various proteins including cytokines and interleukins [32,33]. In addition, platelets form microparticles by membrane budding, which contain a large array of bioactive compounds [34], possessing a procoagulatory action and are regarded as intercellular exchangers of biological signals [35].

Only when the endothelial cell monolayer is damaged or in case of nonendothelialized cardiovascular implants, the adhesive potential of platelets becomes evident, then performing their main function, which is primary hemostasis. This process involves the very rapid adhesion of platelets to the exposed subendothelium followed by platelet-to-platelet adherence. This ultimately culminates in the formation of a platelet plug, which temporarily seals the damaged vessel wall. To fulfil this task, an inhomogeneous distribution of the blood cells across the vessel is needed. Red blood cells accumulate in the center of the vessel, where the hematocrit can reach values up to 80%, while the main platelet population flows along the vessel wall [36], so that they can readily interact in case of a vessel wall injury [37]. The first phase of this process is called primary hemostasis, a very complex, well-orchestrated process. It starts with the agonist activation of G-protein coupled receptors and is followed by a cascade of intracellular steps. These lead to the so-called “inside-out-signaling, which is followed by integrin activation and binding to the extracellular matrix ligands (e.g. vWF, collagen, fibronectin, and laminin [38]). The initiated outside-in-signaling processes finally culminate in platelet activation. During activation, dynamic remodeling of the actin cytoskeleton network facilitates platelet shape changes from a discoid to a spherical shape with centralized granules

and pseudopods, and finally to a fully spread shape (Figure 3). The strong adhesion of the platelets and the contraction of the surrounding extracellular matrix by the platelet plug results in the sealing of the injury [39]. The adherence of platelets to the proteins of the subendothelium is receptor-mediated and depending on the proteins and the rheological conditions [40-42].

Figure 3

The shear rates in the blood stream determine, which proteins mediate the platelet adhesion via different receptors. *In vivo*, platelets are exposed to a broad range of hemodynamic conditions ranging from relatively low flow situations in venules and large veins (typical wall shear rates $< 500\ s^{-1}$) to small arterioles (shear rates up to $5{,}000\ s^{-1}$) to stenosed arteries with shear rates up to $40{,}000\ s^{-1}$ [43]. Platelets have the unique capacity to adhere firmly over all shear conditions and therefore to form hemostatic plugs also at elevated shear rates [40]. At very low or zero shear stress, platelets adhere to fibronectin, vitronectin, and/or fibrinogen. At medium shear rates, mainly fibrinogen mediates the adhesion process, and at high shear rates the vWF is favoured [44].

Membrane tethers in the initial phase of platelet adhesion

This first phase of platelet adhesion consists in the formation of tethers with the initiation of platelet-substrate and platelet-platelet interactions [45,46]. Membrane tethers are smooth cylinders of lipid bilayer that are pulled from the surface of platelets under the influence of shear force. One of the key features of membrane tethers is their ability to reduce the pulling forces imposed on adhesive bonds [47] and, as a consequence, the probability of sustaining adhesion in a shear field is increased. Recently, high-resolution imaging of platelets during thrombus development has provided new insights into this process [46]. This initial phase involves platelet activation and the adhesive function of both GPIb and integrin $\alpha_{IIb}\beta_3$. It does not require soluble agonists such as ADP, TXA2, or thrombin. However, tethering provides a

mechanism of facilitating autocrine/paracrine stimulation by locally generated agonists such as ADP from dense granules as well as the production of TXA2. Tethers are always reversible and seem to play an important role in maintaining close physical proximity between substrates or platelets. The key features provided by membrane tethers are mechanisms to sustain platelet interactions with a thrombus or platelet plug without the requirement for global platelet activation.

Stable binding of platelets

The conversion to a stable binding is mediated by further ligand/receptor interactions and the concomitant generation of soluble agonist most notably ADP. These substances further activate platelets in an autocrine and paracrine manner, acting on the ADP receptor $P2Y_{12}$ [48] and thromboxane receptor TP [49] causing a shape change of the platelets. Platelets undergoing a shape change show pseudopods, membrane blebbing and microvesiculation [50]. This shape change results in the exposure and activation of the GP IIb/III receptor, which binds fibrinogen leading to firm platelet adhesion and aggregation. Activated platelets support coagulation via activation of the contact phase by factor XII [51]. The subsequent production of thrombin and fibrin stabilizes the adherent platelet plug. Thrombin activates platelets by a stimulation of protease activated receptors (PARs) expressed on the platelet surface [52].

The central event for the stabilization of adherent platelets and platelet-platelet contacts is the maintenance of high-affinity GP IIb/IIIa adhesion bonds. Initiation of its activation is controlled by well-characterized signaling events operating downstream of soluble agonist (G_q and $G_{12/13}$) and adhesion (non-receptor tyrosine kinase) coupled receptors [42]. Sustaining GP IIb/IIIa activation is critically dependent on signals operating downstream of G_i coupled receptors, principally the purinergic $P2Y_{12}$ receptor [53]. Thus, ADP plays a key role in both initiating (through the G_q-linked $P2Y_1$ receptor) and sustaining GP IIb/IIIa activation. There is growing evidence that the $P2Y_{12}$ signals do not operate in isolation. Once engaged by a ligand, the

GP IIb/IIIa initiates the formation of membrane-proximal signaling complexes, which induce cytoskeletal changes that promote GP IIb/IIIa clustering and increased receptor avidity [54]. Recent evidence suggests that members of the tetraspanin family, CD151 [55] and TSSC6 [56], play an important role in regulating GP IIb/IIIa outside-in signaling. The development of close platelet-platelet contacts also enables the juxtaposition of ligands on one platelet with receptors on adjacent platelets. Examples of this include various members of the immunoglobulin superfamily (PECAM-1 [57], JAM-A [58], JAMC [59], ESAM [60], and CD226 [61]), Eph kinases/ephrins [62], and Gas6 and its receptors, Axl-Tyro3-Mer [63]. The role of these individual components in regulating the stability of platelet aggregates is only beginning to be addressed, although there is evidence that Eph kinases/ephrins [64] and Gas6 [63] and its receptors play an important role in this process. The exodomains of various platelet surface proteins, including P-selectin [65], CD40L [66], GPIb [67], GPV [68], GPVI [69], and Sema4D [70], are also shed from the surface of platelets with evidence that the soluble form of CD40L promotes thrombus stability by engaging GP IIb/IIIa [71].

Under normal physiological conditions missing EC or groups of EC are sealed by a delimited layer of platelets. This process can derail and overshoot. The continuous adhesion and aggregation of platelets can lead to an ongoing thrombus growth. Such thrombi can occlude the vessel or can be detached by the blood flow as an embolus; processes, which occur e.g. in patients with atherosclerotic diseases.

Platelets in cardiovascular diseases

Endothelial dysfunction and atherosclerosis

Atherosclerosis is a chronic inflammatory disease with complex pathophysiology and the development of atherosclerotic plaques over decades. Beyond well-known functions in thrombosis and hemostasis, platelets are considered to play also a role in the early phase of

atherosclerosis associated with endothelial dysfunction but also in later states facilitating vascular plaque formation via P-selectin-dependent mechanisms [72,73,74].

Under physiological conditions platelets do not adhere to endothelial cells *in vivo*. In healthy vessel, the endothelial glycocalyx determines vascular permeability, attenuates blood cell-vessel wall interactions, mediates shear stress sensing, enables balanced signaling, and fulfils a vasculoprotective roll. But when it is disturbed, as e.g. in atherosclerosis or after implantation of cardiovascular devices, these properties are diminished or even lost. Under such conditions significantly elevated levels of activated platelets are present. Activated platelets start secreting a plethora of inflammatory mediators, inducing a low-grade inflammation of the endothelium leading to glycocalyx and cellular dysfunction [75,76,77]. Inflammation of the endothelium is associated with an altered capacity of responses to endothelium-dependent vasodilators and vasoconstrictors and so impairs the vasomotor response called “endothelial dysfunction” [78,79]. It is widely accepted that the enhanced production of reactive oxygen species and especially the diminished bioavailability of NO - that accompanies an inflammatory response - play a pivotal role in mediating the vascular dysfunction [80].

Several indications suggest that platelets might significantly contribute to the inflammatory processes that promote atherosclerotic lesion formation. Beyond the functional effects on vasomotion, platelets can activate or stimulate endothelial cells [81]. During adhesion, platelets become further activated, then releasing pro-inflammatory cytokines and chemoattractants (e.g., IL-1β, or RANTES) and express surface CD40 ligand (CD40L) [66,82,83]. In this manner, the adhesion of platelets to the endothelial surface is discussed to generate signals for leukocyte recruitment and extravasation of monocytes, a process of paramount importance for atherogenesis [84]. The platelet-endothelial cell interaction is mediated by platelet receptor GP IIb/IIIa, involving platelet-bound fibrinogen, fibronectin, and vWF, as well as endothelial receptors, such as intercellular adhesion molecule-1, integrin $\alpha_v\beta_3$, and GP Ib [85,86,87,88].

The interaction between platelets and the vascular wall involves different steps. After their initial interaction, both platelets and endothelial cells release chemoattractants, like P-selectin, and provide an adhesive surface for leucocytes [89,90]. Platelet-leukocyte aggregates are formed, which activate leucocyte adhesion receptors and serve as a bridge between leucocytes and the endothelium. The following putative mechanisms whereby platelets may promote atherosclerosis were discussed: 1) releasing chemokines and their precursors, which trigger the atherogenic recruitment of vascular cells or modulate processes such as angiogenesis or lipoprotein metabolism; 2) inducing chemokine secretion by endothelial and other vascular cells; and 3) binding and presenting vascular cell-derived chemokines to trigger arrest of circulating mononuclear cells [42,75,86,91,92].

Thrombus formation in atherosclerosis

A number of studies have demonstrated that platelet function (aggregation and reactivity) is pathologically altered in atherosclerotic patients [10,93,94,95,96,97]. Already in 1994, Bach et al. could show that elevated spontaneous platelet aggregation prior intervention is a risk factor for stent restenosis in the following months [10]. With the development of antibodies against platelet membrane receptors responsible for aggregation and adhesion, flow cytometry allowed the detection of activated (CD62P-positive) platelets. In healthy subjects, about 2% of the circulating platelets are CD62P-positive, while this percentage is markedly increased in CAD patients to about 30% [98,99]. In the 1990s, Neumann et al. demonstrated that patients with enhanced platelet activation have an increased risk of stent thrombosis [100]. Beyond that, Murakami et al. reported that CD62P-expression was significantly higher in patients with coronary artery disease compared to controls and that the CD62P expression increased with progressing atherosclerosis [98].

The combination of hyperaggregable platelets and a dysfunctional or pathologically altered blood vessel wall constitutes a high risk situation for thrombotic events. In the initial state of

atherosclerosis an endothelial dysfunction develops, which is not associated with significant platelet deposition. Few platelets may interact with subtly injured endothelial cells, contributing to a mild intimal hyperplasia by the release of growth factors. In case of endothelial denudation and mild intimal injury, one or a few layers of platelets seal the lesion with or without mural thrombus formation depending on the size of the denuded area. The increasing amount of platelet growth factors may then contribute markedly to an accelerated intimal hyperplasia accelerating the atherosclerotic processes. In severe injuries, with exposure of components of deeper layers of the vessel wall - e.g. in plaque rupture or transluminal interventions - significant activation with mural thrombus formation follows. The severity of the platelet reaction depends on the plaque content: a foam cell rich matrix in fatty streaks, collagen-rich matrix in sclerotic plaques, collagen-poor matrix without cholesterol crystals in fibrolipid plaques, atheromatous core with cholesterol crystals in atheromatous plaques, and highly cellular plaques. Ruptures of plaques with atheromatous cores have the highest risk for thromboembolic events [101]. Immediately after exposition of the thrombogenic tissue factor rich core to the lumen, platelets become activated, adhere and the growing thrombus becomes stabilized by fibrin [102]. Platelet deposition and thrombus formation on the lipid-rich atheromatous core exposed to flowing blood is up to sixfold greater than that on other substrates, including collagen-rich matrix [101]. As outlined, these processes are considerably more pronounced in vessel areas with stenosis of 70% or more because of the high shear rates, which induce additional shear rate-activation of platelets.

Upon formation of intraluminal thrombi, arteries can become occluded ending up in myocardial infarction, stroke, or critical limb ischemia, but more often thrombi detach, move into the circulation, and eventually occlude smaller downstream branches causing thromboembolism.

Cardiovascular devices

In this scenario of hyperaggregable platelets and atherosclerotic vessels, the implantation of cardiovascular devices such as vascular grafts, stents, heart valves, or occluders with thrombogenic surfaces promote the activation of the complex system of platelets, contact and complement system and subsequently of the plasmatic coagulation [103]. Though there are efforts to prevent thrombosis on blood-contacting medical devices, results *in vivo* have been insufficient up until now [104]. In principle, three major strategies exist: 1) rendering the surface inert (passivation) 2) modification of the implant surfaces with glycocalyx components (or analoga), and 3) the rapid endothelialization of the implant surface. Advances in biomaterial sciences and an increased understanding of the interphase processes have resulted in the design of inert coatings for implant surfaces that are more resistant to protein adsorption and cell adherence, such as ethylene glycols and glycerols [105,106,107,108,109,110,111]. In the field of bioactive surface coatings, anticoagulants such as heparin or platelet inhibitors (adenosine diphosphate or GPIIb/IIIa receptor antagonists, nitric oxide donors) have been studied, which are able to inhibit platelet responses and activation of coagulatory [112,113]. However, additional studies are needed to assess their applicability in complex devices. Another strategy for improving the hemocompatibility of implant surfaces is the *in vitro* or *in vivo* endothelialization [107]. To support this process, metallic surfaces have been coated with varying polymers (with or without drug or growth factor loading) [114]. But also polymeric implants, such as PTFE-based vascular grafts, have been seeded with autologous endothelial cells, resulting in improved graft patency [115]. The most desirable design concept for a cardiovascular implant is a hemocompatible and degradable polymer, which facilitates the rapid adherence, migration, and proliferation of vascular cells, particularly of endothelial cells, enabling a complete regeneration of the vascular tissue [116]. Perspectively, new approaches for the hemocompatibility testing are needed, which allow to assess the *in vivo* predictability of biomaterials studied *in vitro.* At present, such studies are lacking [117]. This might further lead

to a better understanding and advanced design of biomaterials [118]. In the meantime, the standard therapy after implantation of cardiovascular devices (antiplatelet/antithrombotic therapy) is further needed, bearing the risk of major bleedings [119-123].

The development of devices being absolutely hemocompatible with blood cells/components would likely help to prevent thromboembolic events. It would also limit the need for antiplatelet medications and may therefore reduce drug induced bleedings or drug interactions in patients requiring complex treatment strategies with a number of medications.

Conclusion

There is now a strong rationale to expect that platelet activation may be associated with endothelial dysfunction, with atherosclerotic plaque burden or ultimately with thrombotic processes depending on the state of the disease and the platelet function (or the antiplatelet therapy, respectively). These processes might be aggravated when cardiovascular devices e.g. stents or synthetic vascular grafts are implanted. A perfect strategy therefore is the design of slowly degrading and hemocompatible cardiovascular devices, such as stents. These should support the rapid formation of a functionally confluent and shear resistant endothelial monolayer, leaving behind - after being completely degraded - a regenerated and functional vascular vessel wall.

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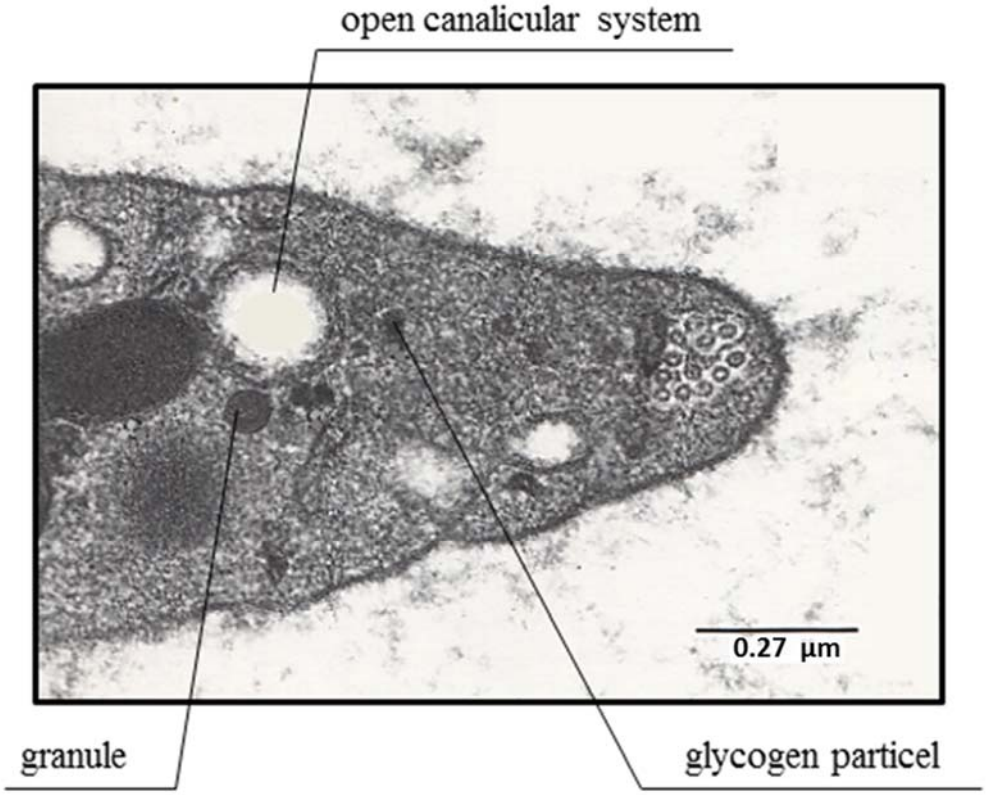

Figure 1. Perpendicular cross-section trough a discoid platelet. Trilaminar plasma membrane layer with glycocalyx; open canalicular system; clusters of small circles surrounded by a halo represent transverse sections through microtubules; dark dense circles represent granules and beta-glycogen particles. (Transmission electron microscopy, with kind permission of Moriau et al. Blood Platelets (Hologramme, Neuilly-sur Seine, 1988).)

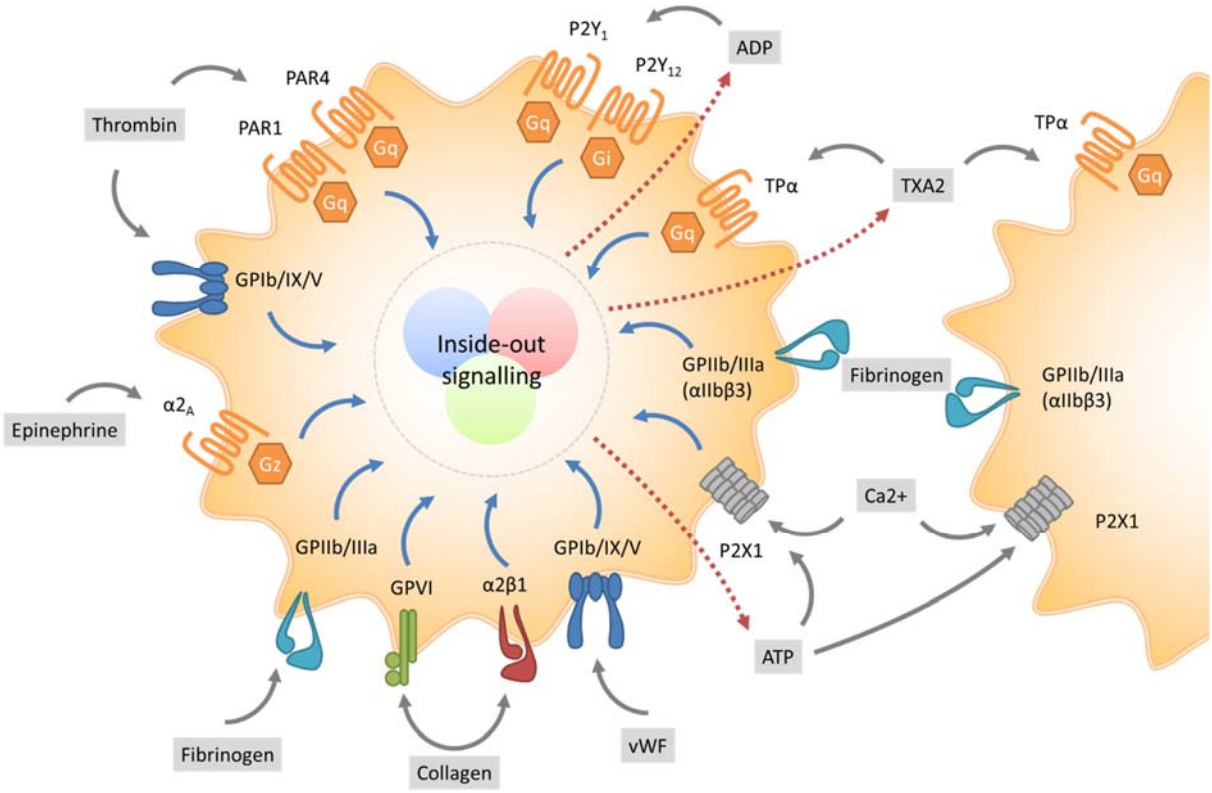

Figure 2. Major platelet receptor-ligand interactions.

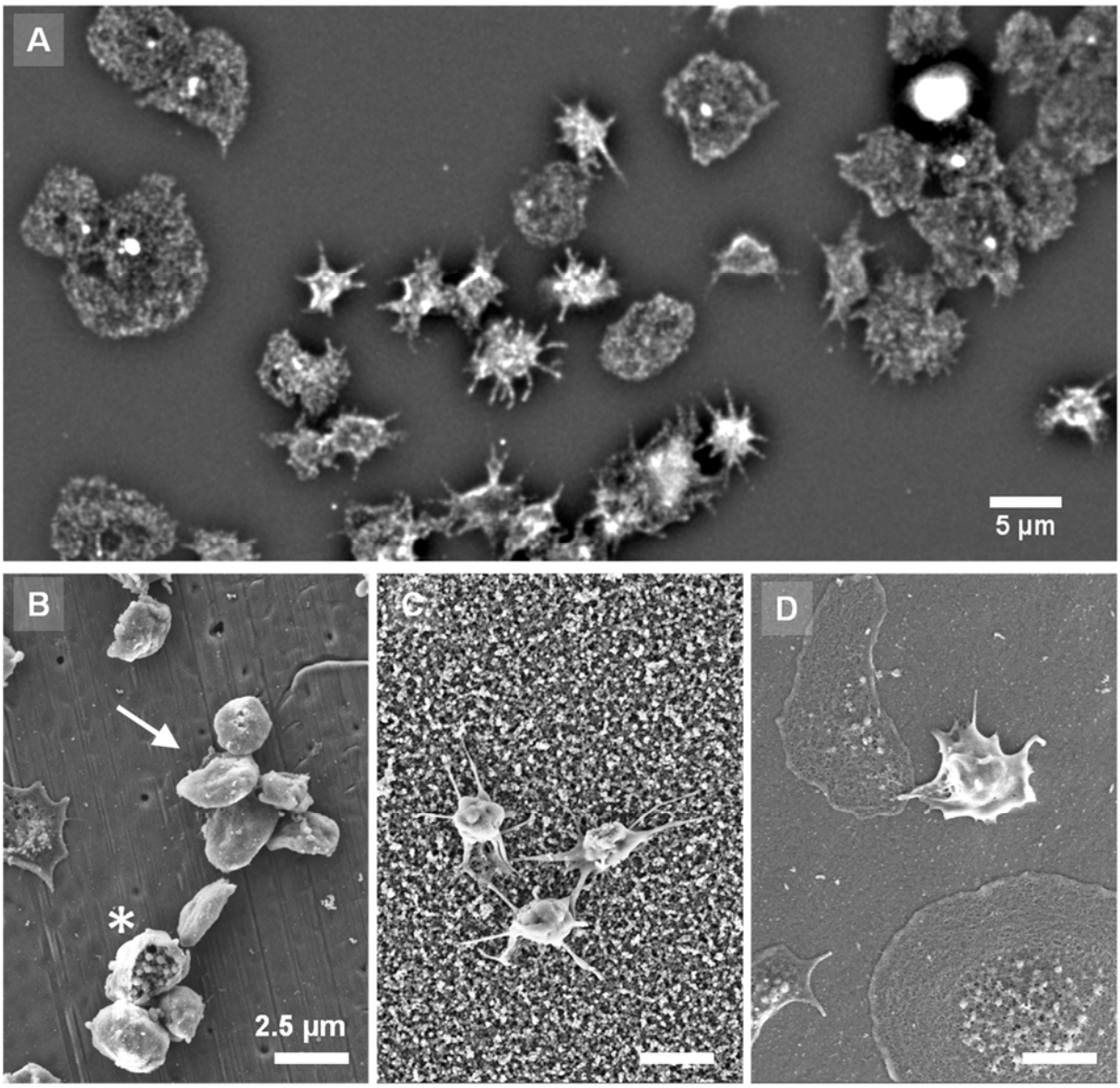

Figure 3. Different morphologies of platelets adherent to biomaterials. A) Heterogeneous states of activation on a poly (tetrafluoroethylene) film; B) Arrow: normal, non-activated small bi-convex discoid platelets, *: view on granules stored in the platelet (platelet membrane dislocation as drying artifact from the Scanning electron microscopy (SEM) sample preparation); C) Activated and spreading platelets with intermediate pseudopodia formation, D) Activated and fully spread platelets. (A: anti-CD42a antibody staining; stimulated emission depletion microscopy, scale bar = 5 µm; B-D: SEM, scale bar = 2.5 µm).