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The redox physiology of red blood cells and platelets: implications for their interactions and potential use as systemic biomarkers

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The blood is the only tissue in contact with all other tissues of the body. Red blood cells (RBCs) and platelets (PLTs) are very abundant, highly specialized, short-lived, circulating cells. Though both these cell types are anucleate and seemingly simple in their structure, there is accumulating evidence that these cells are actually characterized by very complex redox physiology, regulating their canonical and non-canonical functional properties, as well as their interactions. In this review, we aim to provide a perspective on the redox physiology of RBCs and PLTs, and discuss their interaction and potential impact on hemostasis and systemic redox physiology. Moreover, we discuss the potential for the analysis of redox physiology of these small cells to serve as markers for systemic redox metabolic state and redox metabolomics.

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Introduction

The blood may be considered as the most peculiar tissue in multicellular organisms. It appears to be very simple in composition and consists mainly as a suspension of cells in a protein-rich solution (the plasma). The main cellular components of blood are red blood cells (RBCs), platelets (PLTs), and leukocytes. All blood cells are short lived and undergo rapid renewal; therefore, they are continuously produced in the bone marrow and derive from the same precursor cells. Furthermore, in blood circulate a heterogeneous population of membrane microparticles and/or exosomes of diameter between 1–100 μm; these membrane particles are thought to be originated from blood cells, vascular endothelial cells and other cell types (depending on the surface markers they carry). Microparticles are produced by membrane shedding, active vesicle release/degranulation or apoptosis and were discovered to play both physiological and pathological roles [1,2].

The blood continuously circulates in the vessel system carrying respiratory gases and nutrients to the tissues and back. Therefore, blood cells like vascular cells are constantly subjected to dynamic forces [3]. There is accumulating evidence that all blood cell subpopulations are equipped with mechanosensing and mechanotransducing systems, which are fundamental for blood cell physiology and pathophysiology. While these systems are well known for platelets, the mechanosensing and transduction system in leukocytes and RBCs are less well understood [4•,5,6].

Being in direct contact with all other tissues, the blood plays a fundamental role in inter-organ communication and systemic homeostasis [7••]. These characteristics, together with the fact that blood sampling is less invasive than other tissues and that the blood is relatively abundant (as compared to other tissues), has made blood a prime source in which to look for diagnostic markers to test the health status of an individual for decades. Most recently markers such as microRNAs and circularly snRNA, liquid biopsies of rare cells and microparticles/exosomes have been under evaluation (see e.g. Refs. [8–10]).

The blood is also discussed as a way to analyze the systemic redox state of an individual and to measure oxidative stress [7••,11,12,13••]. Interestingly, inflammatory cells were the first cells where oxidative stress was postulated and described: the study of redox physiology began with the appreciation that reactive oxygen species (ROS) were fundamental mediators in the oxidative burst of macrophages, produced to kill infected cells. Now it is clear that production of reactive species (including ROS, reactive nitrogen species and reactive sulfur species) mediates intricate signaling pathways, is tightly controlled by enzymatic reactions, is regulated at multiple levels including endogenous antioxidant systems, and has specific targets [14••,7••]. Indeed these systems are very conserved, and several authors have put forward the hypothesis that redox physiology participated in the evolution of cells [7••,15•,16•].

The field of blood cell redox physiology is expanding rapidly and the consequence of these cells on systemic redox physiology is still being uncovered. While much has been historically published about white blood cell redox biology, relatively less is known about the anucleate RBCs and PLTs. In this review, we aim to give a perspective on the redox physiology of RBCs and PLTs, and discuss their interactions and their potential impact on hemostasis and systemic redox physiology. Further, we discuss the possibility to consider the analysis of redox physiology of these small cells as diagnostic markers for systemic redox and metabolic state.

Cellular redox physiology and signaling in RBCs

The main function of RBCs is transport of O_2 and CO_2 in the circulatory system [4•]. The most abundant protein in RBCs is hemoglobin, which is present in a concentration equivalent of 10 mM heme [17•]. Oxygen binds to hemoglobin only if the heme prosthetic group is found in the reduced (Fe^{2+}) form, yielding oxyhemoglobin (oxyHb). The oxygen-free Fe^{2+} form is called deoxyhemoglobin (deoxy). Oxidation of hemoglobin – occurring for example by reaction with oxidants or nitric oxide (NO) – leads to formation of Fe^{3+} methemoglobin (metHb). In the RBCs, oxidants are mainly formed from degradation of products of methemoglobin leading to formation of superoxide radical ($O_2^{\bullet-}$) [4•,18]. Other potential sources of oxidants are reactions involving hemoglobin and other molecules including nitrite [17•,19••] and sulfide [20,21]. The presence of oxidant enzymes like NADPH oxidase (NOX) [22•] and xanthine oxidase (xanthine oxidoreductase) [23] have been described in RBCs, but their function is not fully understood. Oxidation of hemoglobin leads to formation of Heinz bodies (via precipitation of Hb complexes), which are found concomitant with loss of cytoskeletal flexibility accompanied by loss of RBC deformability, increased membrane fragility, and hemolysis [24].

To keep hemoglobin in reduced form, and overall to avoid oxidation of red cell components, RBCs are very well equipped with antioxidant systems, including the cytochrome b5 reductase (which converts metHb back into oxy/deoxyHb), antioxidant enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx)) and redoxins (thioredoxin and glutathredoxin), and their essential cofactors to donate reducing equivalents, including NADPH and GSH [4•]. The only source of ATP and reducing equivalents in RBCs is glucose via glycolysis (Figure 1a). Defects of enzymes of the glycolytic pathway (like glucose 6-phophate dehydrogenase (GP6DH)) and of antioxidant enzymes (like GPx, SOD) have been shown to cause hemolytic anemia in humans and animal models [4•].

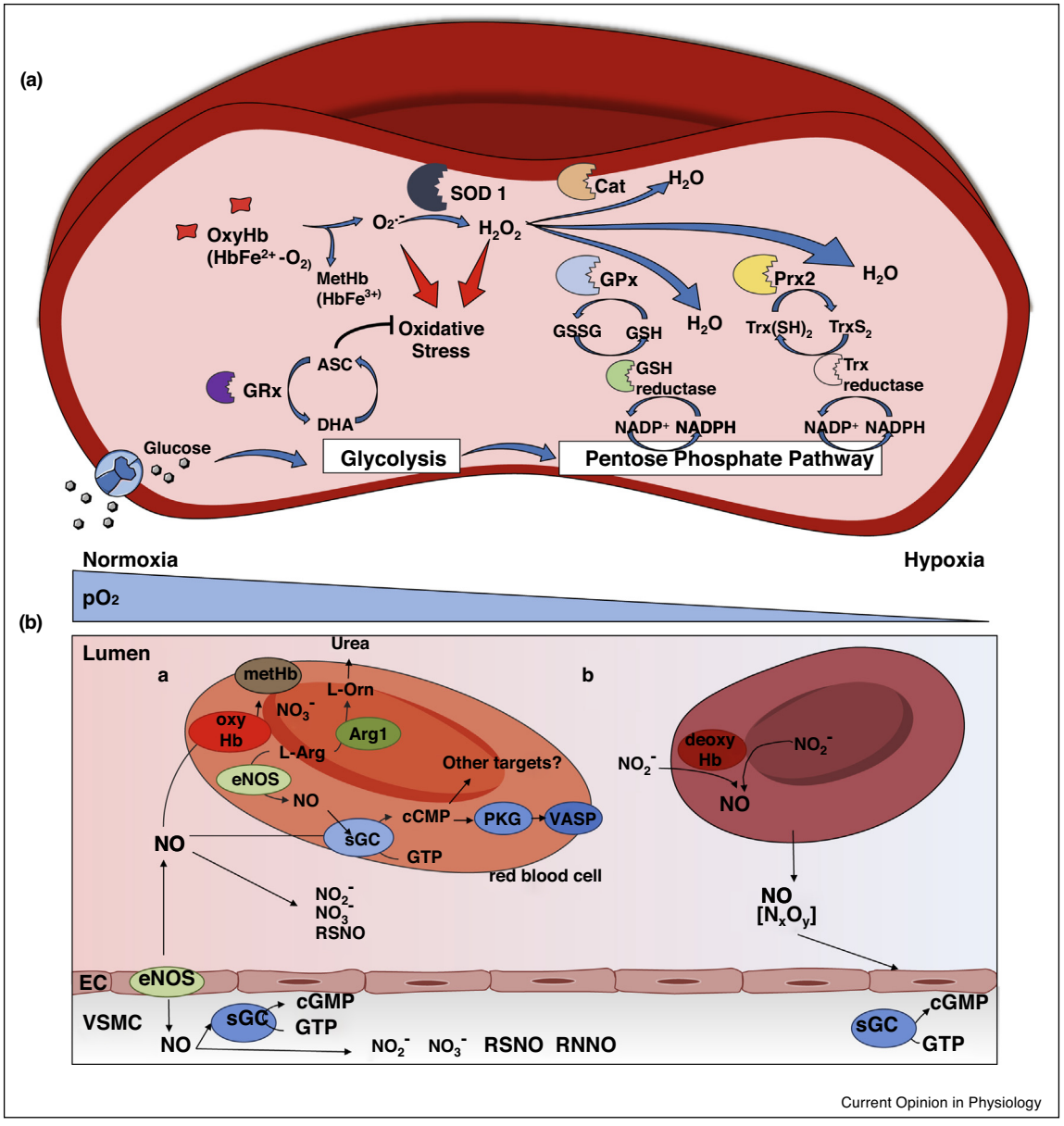
It is actually very difficult to oxidize healthy RBCs *ex vivo* if suspended in a buffer containing glucose [25•]. However, experimental manipulation of the redox state of RBCs *in vivo* can be induced by changes in iron availability or treatment with oxidants like phenyhydrazine, which rapidly induces intravascular hemolysis and hemolytic anemia [4•].

Very recently RBCs were discovered to carry a NO synthetic pathway, including arginase 1 (Arg1), endothelial nitric oxide synthase (eNOS), and its downstream pathway including the NO receptor soluble guanylate cyclase (sGC) and downstream target protein kinase G (PKG) [26–30] (Figure 1b). Similar to what has already been observed in other compartments, Arg 1 activity is increased in disease conditions linked to oxidative stress like diabetes type 2 [31]. The authors of this paper proposed that in analogy to what is described for endothelial cells, increased Arg1 activity may decrease L-Arg availability for eNOS in RBCs leading to eNOS uncoupling and eNOS-derived production of $O_2^{\bullet-}$ [31]. Although there are very powerful experimental data linking Arg1 activity to eNOS-dependent cardioprotection [29], the lack of biochemical data demonstrating a direct correlation between Arg1 activity, levels of L-Arg in RBCs, eNOS activity and NO production, particularly in a complex chemical environment like the RBC, urge more experimental work to confirm this hypothesis.

Interestingly, sGC may be also considered a redox sensitive enzyme and a redox indicator [32•,33]. It is now well accepted that sGC exists in tissues as the Fe^{2+} redox active form and Fe^{3+} inactive/or apo-sGC form [32•], which can be respectively targeted by NO/heme-dependent sGC stimulators (including YC-1, BAY 41-2272, BAY 41-8543, CFM-1571 and A-350619) and NO/heme-independent sGC activators (including BAY 58-2667 and HMR-1766) [32•,33]. Contrary to what is observed in platelets [34••] from patients with coronary artery disease (CAD) showing increased presence of the BAY 60-2270-responsive oxidized sGC form, in RBCs from patients with CAD the activity and reduced state of red cell sGC is fully preserved [26]. In RBCs this correlated to a fully preserved GSH levels, GSH/GSSG ratio and total thiol levels [26].

Oxidation of RBCs has fatal consequences on RBC physiology [4•]. Oxidative modifications are not only detrimental for hemoglobin, but are shown to strongly affect the cytoskeletal flexibility leading to formation of cross links of the spectrin/actin/ankirin nodes, the asymmetry of the cell membrane lipid bilayer with exposure of phosphatidylserine, and the overall cell flexibility and fragility [4•,35••]. RBC deformability is a fundamental property of RBCs allowing them to pass through the narrower vessels of the microcirculation. Loss of cytoskeletal flexibility leads to increased stiffness of RBCs and intrinsic deformability in response to deforming forces, decreased participation of RBCs in the flow with increase in viscosity and rheological behavior in the macro

Figure 1



Redox physiology and NO signaling in RBCs. **(a)** Redox regulation in RBCs. Oxyhemoglobin (OxyHb or Hb-Fe^{2+}-O_2) is oxidized into methemoglobin (metHb or Hb-Fe^{3+}) in RBCs by oxidant events naturally occurring in RBCs. If metHb (Hb-Fe^{3+}) is not converted back into Hb-Fe^{2+} by Cytb5 reductase (not shown), Hb-Fe^{3+} undergo further autoxidation reactions producing superoxide anion radical ($O_2^{\bullet-}$) and other reactive oxygen species. The enzyme superoxide dismutase (SOD)-1 reduce $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2). H_2O_2 can be further reduced into water by catalase (Cat), glutathione peroxidase (GPx) and peroxiredoxin (Prx)-2. The activity of GPx depends on reduced glutathione (GSH) and its recycling enzymes glutathione reductase (GR), which on turn depends on NADPH. Prx2 is associated to membrane of RBCs and its recycling depends on the oxidation of thioredoxin ($Trx(SH)_2$) into dimeric ($TrxS_2$); $TrxS_2$ is on turn recycled by the Trx reductase using NADPH as source for reducing equivalents. The only source of energy (ATP) or redox equivalents (NADPH) in RBCs is glucose. Glucose is taken up by the Glut-1 transporter and then channeled into the glycolytic pathway and (via glucose-6-phosphate) into the pentose phosphate pathway, which is the source NADPH. NADPH is used as a co-factor of GR to recycle oxidized GSH (GSSG) back into reduced GSH. Glutaredoxin (GRx) depends on the ascorbate (ASC)/dehydroascorbate (DHA) redox couple, which is also very abundant in RBCs. **(b)** NO signaling and in RBCs. (a) NO is synthesized in endothelial cells (EC) by an endothelial nitric oxide synthase (eNOS) and leads to activation of a soluble guanylate cyclase (sGC) in the vascular smooth muscle cell (VSMC) converting GTP into cGMP and leading to vasodilation. In the vessel lumen NO can be oxidized into nitrite, nitrate, nitrosothiols (RSNO) and nitrosoamines (RNNO) and nitro fatty acids (NO_2-FA) by enzymatic and non-enzymatic reactions. NO can be inactivated in RBCs under normoxic conditions by reacting with oxyHb leading to formation of metHb. RBC carry a full Arginase1/eNOS/sGC pathway and its downstream targets including protein kinase G and VASP. (b) Under hypoxic conditions nitrite is converted into NO by deoxyHb leading to hypoxic vasodilation.

and in the microcirculation. Contrary to what was published before [36•,37], there is accumulating recent evidence showing that neither NO donors nor red cell eNOS-derived NO affect RBC deformability per se [25•,38,39], but rather protects the RBC against oxidant-induced loss of RBC deformability [25•]. These effects may be particularly important in hemoglobinopathies associated with oxidative stress, release of free hemoglobin and decreased NO bioavailability like sickle cell disease [40••,41•] and warrant further investigation.

A major consequence of RBC membrane fragility, damage and intravascular hemolysis is the release of free hemoglobin and other proteins contained in RBCs (including Arg1) [4•,42], as well as hemoglobin-containing microparticles into the circulation [43••]. The presence of an excess of free hemoglobin in the blood may promote systemic NO scavenging and oxidative stress with effects on endothelial function and integrity [17•]. Also free Arg1 enzyme has been linked to decreased L-Arg availability in the endothelium, eNOS uncoupling and endothelial dysfunction [42]. Interestingly, a link between accumulation of RBCs in atherosclerotic plaque and calcification was recently proposed [44]

To summarize, redox physiology of RBCs is characterized by a very robust and complex system composed of strong antioxidant systems, which are optimized to counteract oxidation of hemoglobin and dependent on the energy and nutritional status of the organism. Pathological changes of hemoglobin (like hemoglobinopathies), antioxidant enzymes, or iron status lead to RBC membrane fragility, loss of deformability, release of microparticles, and hemolysis with systemic effects on the redox state and functionality of organs, specifically the endothelium.

Redox physiology of PLTs

Platelets are the second most abundant cells in the blood after RBCs and are central to vascular homeostasis and signaling. It is well established that platelets play a central role in hemostasis through their activation and aggregation to mediate thrombus formation at sites of vascular injury and prevent excessive bleeding [45]. However, it is also recognized that through their expression of specialized surface receptors, release of inflammatory mediators, and interactions with other leukocytes, platelets play a role in immunity and the inflammatory response [46,47].

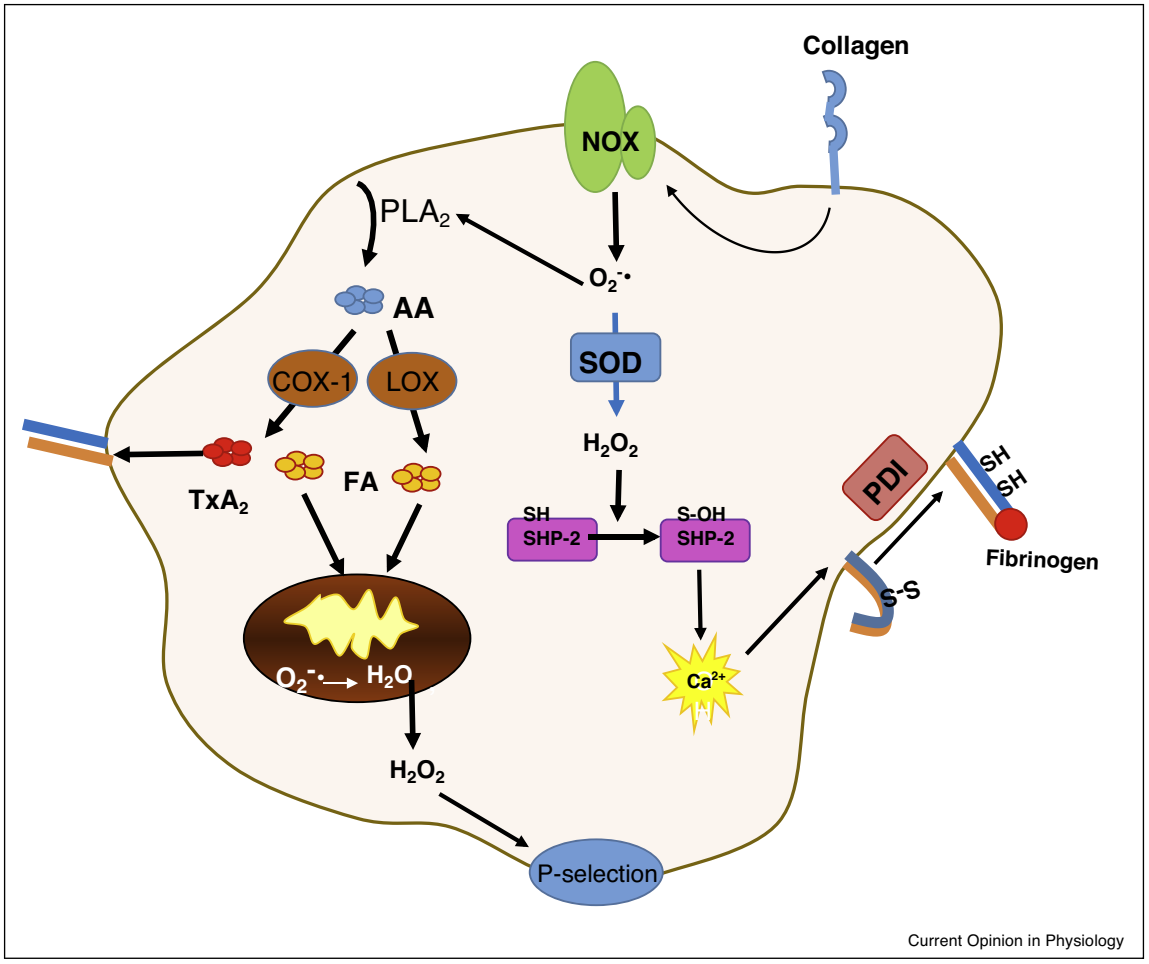
Platelets express several enzymes that generate oxidants including NOX1 and 2 [48,49], xanthine oxidase [50], and cyclooxygenase [51,52], and redox regulation has long been known to play a role in platelet activation (Figure 2). For example, activation and subsequent superoxide production by NOX2 in response to agonists such as collagen and oxidized low density lipoprotein propagates platelet activation. ROS generated by NOX2 reversibly oxidizes critical cysteine residues to sulfenic acid on the src-homology domain containing phosphatase (SHP-2) family of tyrosine phosphatases, leading to inhibition of the phosphatase activity and downstream signaling mediating calcium release, p-selectin surface expression, and α granule release [48,49]. Beyond free thiols that may be oxidized by ROS, thiol-disulfide exchanges are prominent in platelet activation and signaling. One example of this mechanism involves the disulfide bond in the αIIbβIII receptor on the surface of the platelet, which signals platelet activation when the disulfide bond is reduced. Reduction of this disulfide bond is catalyzed by protein disulfide isolmerase (PDI), which can be expressed on the surface of the platelet or released from the platelet to mediate intracellular signaling [53]. Notably, PDI also has been shown to upregulate NOX activity, suggesting feed-forward redox signaling pathway to platelet activation [54]. For a comprehensive review of platelet thiol and disulfide exchange reactions see Ref. [55••].

Beyond thiol modification, fatty acid metabolism represents another mechanism of redox regulation of platelet function. Calcium mobilization by oxidants and other thrombotic stimuli activates phospholipase A_2, which translocates to the plasma membrane to release arachidonic acid and other fatty acids into the cytoplasm of the platelet. Once released, these fatty acids are oxidized by cyclooxygenase and 15-lipoxygenase to prostaglandins and thromboxane A_2 and 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE) respectively [52]. These lipid products bind to G-coupled receptors to signal downstream thrombotic and inflammatory activation [56].

The mitochondrial electron transport chain (ETC) represents another point of redox regulation within the platelet. The platelet contains small numbers (7–10) of functional mitochondria, which were for many decades thought to be present solely to generate ATP to fuel platelet shape change upon activation and aggregation. However, recent studies demonstrate that the mitochondrion regulates platelet function through signaling mechanisms beyond ATP production [40••,57].

The ETC is a significant source of oxidants within the platelet. Production of superoxide from complexes I and III is regulated by electron flux through the ETC, with decreased rates of flux leading to greater inner mitochondrial membrane hyperpolarization and enhanced superoxide production. This superoxide, which is rapidly dismutated to hydrogen peroxide by SOD, can oxidize free thiols and lead to platelet activation. This increase in mitochondrial superoxide production has been associated with aberrant platelet activation in a number of pathologies including sickle cell disease and type II diabetes, but may also play a role in protective signaling such as platelet activation in the complement pathway for immune signaling [40••,58].

Figure 2



The redox physiology of platelet activation. NADPH oxidase (NOX)2 is assembled and generates superoxide in response to agonist binding (such as collagen). Superoxide is dismutated to hydrogen peroxide which can oxidize free thiols on Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (Shp2) (SHP-2) to propagate calcium release and lead to integrin expression. Integrin αIIβIII is inactive in its oxidized state (disulfide bridge), but is reduced by protein disulfide isomerase to become active for fibrinogen binding. Superoxide also stimulates phospholipase A2 (PLA2) to release arachidonic acid (AA) from the plasma membrane, which cyclo-oxygenase (COX-1) and lipoxygenase (LOX) converts to TxA2, leading to integrin activation or fatty acids (FA) that can be used for beta oxidation by the mitochondrion. Mitochondrial respiration (complexes I and III) generate oxidants that stimulate platelet activation through the surface expression of p-selectin.

Distinct from the production of oxidants, mitochondrial membrane potential in itself regulates platelet apoptosis. While acute treatment of platelets with agonists such as collagen, thrombin, and sheer stress enhance membrane potential to initiate activation, prolonged treatment with these agonists cause mitochondrial membrane collapse, an initial step in apoptosis. Collapse of membrane potential leads to opening of the mitochondrial permeability pore and release of cytochrome c, which propagates a signaling cascade ultimately resulting in the activation of caspase 9 and apoptotic death [59]. Though anucleate, mitochondrial-induced, platelet apoptosis is prominent in the platelet; likely apoptosis plays a physiological role in close proximity to a forming thrombus, in which additional platelet recruitment is unnecessary. Pathologically, increased platelet apoptosis has been implicated in the progression of diseases, such as malaria and immune thrombocytopenia [60–62].

Notably, recent studies demonstrate that measurement of platelet bioenergetics may provide useful information on systemic energetic alterations in disease [40••,63,64]. For example, specific mitochondrial respiration profiles have been defined for platelets isolated from subjects with several pathologies. In some cases these alterations in mitochondrial function have been shown to modulate downstream platelet function as well [40••,65–70]. Notably, recent studies show that platelet mitochondrial oxidant generation is stimulated by NOX2 activation and arachidonic acid metabolism leads to the production of

lipid products that are further metabolized by the mitochondrion through β-oxidation [54,71]. Thus, the multiple redox regulatory systems within the platelet are linked in function and form a strong network of modulation of platelet function (Figure 2).

Redox physiology of RBC and PLT interactions

Very little is known about the interaction of RBCs and PLTs in the circulation and the vessel wall. There is accumulating evidence that humoral and physical cell–cell interactions exist between RBCs and PLTs, which may play a fundamental role in physiology and pathology of the cardiovascular system. Many of these interactions are linked to the redox physiology of these cells.

NO is a well-known and very potent inhibitor of PLT aggregation acting via activation of sGC/PKG pathway in PLTs [72,73]. Experimental studies demonstrated that PLTs can be activated via pharmacological inhibition of endogenous NO production in humans [74], leading to hypercoagulability and thrombotic complications. RBCs are a well-known scavenger for endothelial NO and limit the NO-mediated PLTs inhibition, contributing to hemostasis (Figure 3a). Intriguingly, anemic conditions without concomitant hemolysis are linked to increased bleeding complications [41•]; thus, a decreased number of RBCs may decrease NO scavenging and increase NO bioavailability, thereby inhibiting PLT aggregation and leading to bleeding complications. A further mechanism proposed recently is that nitrite-derived NO release by RBCs under hypoxic conditions may contribute to hemostasis via inhibition of PLT aggregation [75••]. On the contrary, in hemolytic anemia increased NO scavenging by free Hb might decrease NO availability and therefore increase PLT aggregation and thromboembolism [41•] (Figure 3b). Accordingly, the risk of thromboembolism is elevated after transfusion of packed/damaged RBCs and was shown to correlate with impaired outcome and survival of patients [76].

Both PLTs and RBCs are optimized to have low adhesively to their self, other blood cells and the vascular wall. Activation of PLTs by collagen exposure on a damaged vessel or by soluble factors (such as thrombin or ADP), accompanied by changes in flow characteristics, lead to increase adhesiveness of PLTs to other PLTs and to the vessel wall by exposure of adhesive molecules (integrins, fibrinogen and von Willenbrand factor) to their surface, allowing thrombus formation. Thus, according to the mainstream model, platelets are the first cells being recruited on the site of vascular damage and RBCs are, though, to be trapped during thrombus formation. However, a recent study was carried out to follow the cellular events occurring after vascular damage by electronic microscopy in a model of iron-induced atherothrombosis. By using this elegant approach the authors demonstrated that RBCs were the first cells adhering to the damaged endothelium/vessel wall and then PLTs were recruited from the circulation [77•]. They proposed that adhesion between RBCs and PLTs took place immediately after iron-mediated oxidative damage and that was mediated by exposure of PS and/or by enhancing α(IIb)β integrin receptor activation and p-selectin expression on PLTs [77•]. Indeed, PS on the outer leaflet of RBCs was identified to be a target mediator of PLT activation, resulting in enhanced aggregation [78]. Interestingly, PS exposure on RBCs is increased in sickle cell disease [78].

In PLTs also increased oxidative stress may play a role in disturbance of hemostasis and/or hyperaggregability. There is compelling evidence that PLTs and RBCs from patients with different types of anemia such as sickle cell disease, β-thalassemia, and myelodysplastic syndrome show a dysregulation of redox systems [79–81] and altered PLT–RBC interactions. In particular, experimental studies indicate that elevated oxidative stress can be found in PLTs when incubated with thalassemic RBCs compared with normal RBCs, leading to increased PLT activation [79].

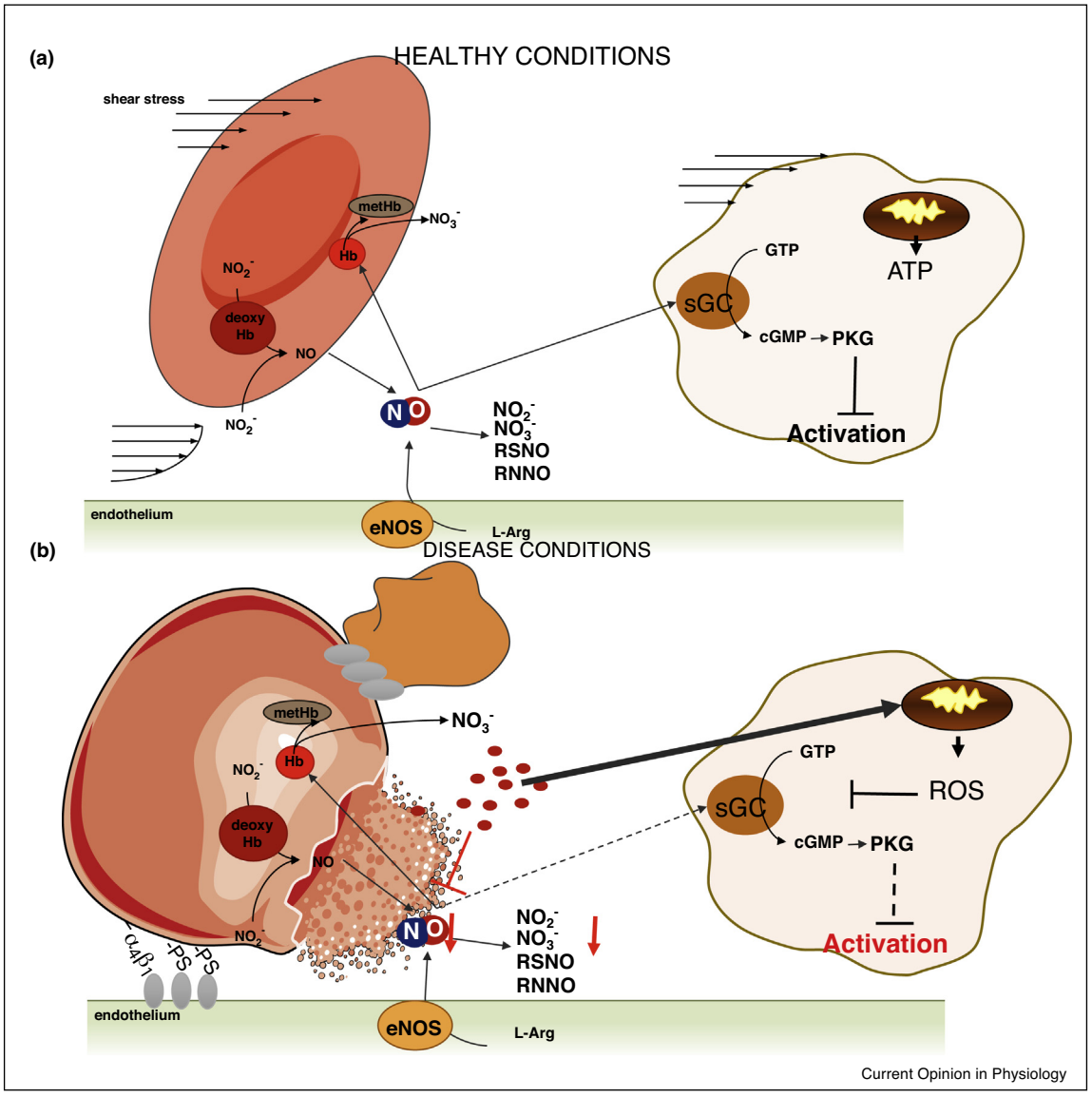
It has long been recognized that patients with sickle cell disease show increased platelet activation, aggregation and increased risk of thrombosis [82–84]. Interestingly, the degree of basal platelet activation correlates significantly with the rates of RBC hemolysis in these individuals [40••,41•]. We recently showed that hemoglobin released from the RBC by hemolysis mediates signaling within the platelet to inhibit mitochondrial ATPase enzymatic activity, leading to increased ETC oxidant production and subsequent oxidant-induced platelet activation in the form of surface p-selectin expression [40••]. Notably, many pathologies that encompass a component of hemolysis also show characteristic platelet activation suggesting that this mechanism of hemolytic RBC dependent platelet activation is a common pathogenic mechanism.

Taken together, clinical and experimental evidence reveals a complex role RBC–PLT interactions in homeostasis and thrombosis, which involve NO, reactive species and probably other humoral factors (ATP, ADP, ET2 etc.) as well as membrane adhesion molecules and redox equilibria (Figure 3). Further research should address how anemic conditions with or without hemolysis may be linked to alterations of RBC–PLT interactions and thrombotic and hemostatic complications.

Summary and perspective

Both RBCs and PLTs have been considered for a long time as simple, highly specialized circulating cells carrying out a very limited albeit important physiological functions — being respectively transport of respiratory gases and hemostasis. However, both cell types play a number of non-canonical roles and interact actively with

Figure 3



Redox Physiology of RBC–PLTs interactions. **(a)** Healthy conditions. NO produced by the endothelium can be oxidized into nitrate by the reaction with oxyhemoglobin (OxyHb) or be converted into nitrite, nitrate, nitrosothiols (RSNO) or nitrosoamines (RNNO) or nitro-fatty acids by enzymatic or nonenzymatic reactions. Endothelial NO leads to inhibition of platelet aggregation by activating the soluble guanylate cyclase (sGC) in platelets. Mitochondria are functional and produce ATP. **(b)** Disease conditions. Damage to the membrane of RBCs leads to loss of membrane integrity and hemolysis, release of Hb-carrying microparticles, and/or expression of adhesion molecules/surface molecules on the surface of RBCs like phosphatidylserine (PS) or the $\alpha 4\beta 1$ integrin. This leads to increase of adhesivity of RBCs to platelets and endothelium. Free hemoglobin (freeHb) or Hb-containing microparticles leads to NO scavenging and decrease in NO bioavailability, alteration of mitochondrial function and release of superoxide anion radical and other reactive oxygen species (H_2O_2), to inactivation of redox-sensitive enzymes (e.g. of sGC), overall inducing platelet activation.

each other participating in physiological and pathophysiological processes in the cardiovascular system. Specifically, redox physiology of both RBCs and PLTs assume a prominent role for understanding these non-canonical functions and their dysfunction occurring in disease.

Both cells are very well equipped with antioxidant enzymes and are rich in small molecular weight and protein thiols playing a central role in keeping them functional.

RBCs are highly resistant to redox changes and oxidative damage. Energy (ATP) and redox equivalents (NADPH) to feed the antioxidant and redox recycling enzymes (regenerating the cofactors GSH and ascorbate/dehydroascorbate) (GSH, ascorbate/dehydroascorbate)

are produced exclusively from glucose. Hemoglobin is its self a very efficient radical scavenger and antioxidant present in millimolar concentrations in RBCs. Redox changes occur only in pathological conditions including hemoglobinopathies (sickle cell disease, beta-thalassemia, etc.), genetic defects of enzymes of the glycolytic pathway (G6PDH) and antioxidant enzymes (SOD, GPx, GRx). There is some evidence of a role of Arg1/eNOS/sGC/PKG dependent pathway in redox physiology of RBCs, but it needs further confirmation.

PLTs contain an intricate and interdependent balance of oxidant and antioxidant systems that are central to their thrombotic and inflammatory functions. Oxidant producing enzymes are central to regulation of platelet activation and degranulation through the oxidation of critical thiols, and this is regulated by thiol reducing enzymes as well as a counterbalance of cellular antioxidants [55••,79]. Mitochondria represent a significant source of oxidants within the platelet. Importantly, this organelle critically links ROS production to lipid metabolism and energy production, and thus measurement of platelet bioenergetics may provide insight into not only systemic energy production but also redox status [63,71,85].

The redox physiology of the interaction of RBCs and PLTs seems to involve both humoral factors and cell–cell interactions. One example is the complex role that NO plays in regulation of hemostasis: NO is a potent inhibitor of platelet activation, is scavenged by RBCs in normoxic conditions, while in hypoxic conditions is produced by RBCs form nitrite. This pathway is under control of sGC, which in conditions of oxidative stress is not responsive to NO. Oxidation of RBCs or PLTs promote their adhesiveness to each other and to the vascular wall, participating in atherothrombosis. Both cells express NADPH oxidase, but whether and how redox signaling may participate to regulation of RBC or PLT function and/or interaction is still unknown.

RBCs and PLTs are relative abundant and easily accessible cells. As already put forward by us in independent work [40••,27,25•] a characterization of the redox/metabolic state of RBCs and PLTs may provide a strong indicator of the systemic redox/metabolic state of the organism. Parameter to test would be (1) thiol redox state; (2) L-Arg metabolic pathway; (3) NO metabolites; (4) metabolic activity (glycolysis and/or mitochondria) in plasma, RBCs, PLTs. These parameters can be directly correlated with functional properties of the cells like oxygen transport capacity (RBCs), platelets activation and their interaction with RBCs. This approach can be tested both in animal models and human healthy/diseased cohorts and may provide a novel understanding of the role of RBCs and PLTs in redox disease and at the same time diagnostic and prognostic markers and druggable targets for these diseases.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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