S11: Beyond Red cell stiffness

S11-1 RBC deformability: an exquisite homeostasis

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A large body of literature shows that red cell deformability is under the influence of the surrounding milieu. A comprehensive scheme of all this regulation is still missing. In particular, it will be important to delineate reversible changes in red cell rigidity that may be involved in physiological regulation, from the irreversible evolution toward cell death that occurs during eryptosis. For example, physiological reversible red cell rigidification during exercise has probably a completely different meaning compared to pathologic alterations of red cells observed in diseases such as diabetes or sickle cell disease. The major regulator of red cell deformability are the clearance of rigid red cells by the spleen, and the physicochemical characteristics of the surrounding milieu such as pH, osmolality, oxidant stress and thermal injury. An environment containing proteins (albumin) is required to avoid shape alterations such as echinocytosis. Divalent cations (magnesium, zinc, Fe++), as well as circulating molecules such as lactate, ketone bodies, and various hormones modify red cell deformability. Among them special attention has been given to purinergic and nitric oxide (NO) signaling, that are probably important regulators of red cell rheology. Blood lipid concentrations, but also meal composition (caloric and carbohydrate intake) are correlated with increased RBC rigidity. All that suggests that red cell deformability is a tightly regulated property sensitive to many factors. It is likely that some of these influences are involved in complex regulatory loops aiming at maintaining homeostasis, while others represent cell damage leading to erythrocyte destruction and removal from blood.

S11-2 Eryptosis or the death of a rigidified erythrocyte

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The hallmark of cardiovascular disease, including type 2 diabetes, is (systemic) inflammation, with an accompanying upregulated pro-inflammatory profile, characterized by circulating pro-inflammatory molecules, hydroxyl radicals and oxidative stress. These molecules and processes are key role players during inflammation, and results in both pathological clotting and biochemical changes to both erythrocytes (RBCs) and platelets. RBCs are extremely vulnerable in the presence of circulating inflammagens. However, notwithstanding this vulnerability, RBCs are exceptionally adaptable and react quickly to stabilize their membranes and structure in the presence of e.g. hydroxyl radical mopping agents (including treatment regimes). In this symposium, I will focus on using structure and function of RBCs and show their importance as health indicators. RBCs have a highly specialized and organized membrane structure, which interacts and reacts to inflammatory molecule insults, and undergo

programmed cell death, like apoptosis, known as eryptosis. Eryptosis in various cardiovascular diseases will be discussed, with special reference to membrane changes, aberrant rheology and pathological clot formation. Techniques to study eryptosis like flow cytometry, confocal microscopy and ultrastructural studies will be discussed. In conclusion, I will suggest novel ways how RBCs may be employed in an in vivo cell model system, for the early detection of the presence of inflammation and to track disease status, as well as compliance of patients to treatment regimes

S11-3 Erythrocyte deformability under nitric oxide Influence

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It is well evidenced that the degree of erythrocyte deformability changes as a consequence of alterations on either its membrane properties, or shape or internal composition. Erythrocytes are blood components acting as oxygen and nitric oxide (NO) sensor in the microcirculation. The mobilization of NO between its scavenger molecules S-nitrosohemoglobin, nitrosylhemoglobin, S-nitrosoglutathione and the NO efflux from erythrocyte are under dependence of type of several signal molecules and respective receptors and a variety of activators, or inhibitors of biomolecules belonging to the signaling transduction pathways. We will described the influence of NO donors or of internal or external stimuli which changes the red blood cells NO efflux, NO metabolism, and protein phosphorylation degree and redox thiol status on erythrocyte deformability. All data evidence the inclusion of NO as another influent parameter on erythrocyte deformability.

S11-4 The sickle cell: far more than a rigid erythrocyte

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Sickle cell anemia (SCA) is a genetic disease characterized by the presence of abnormal hemoglobin (HbS) that polymerizes under deoxygenated conditions causing a mechanical distortion of red blood cells (RBC). SCA patients are characterized by a severe reduction in RBC deformability, which contributes to the occurrence of frequent painful vaso-occlusive crises. In addition, this reduction in RBC deformability is at the origin of the increased cell fragility leading to enhanced hemolysis. While the high hemolytic rate explains why patients are anemic, recent studies demonstrated a key role of hemolysis in the development of vascular dysfunction. In addition, we reported that increased oxidative stress in SCA decreases the bioavailability of nitric oxide and promotes the formation of peroxinitrite, which in turn participates to the reduction of microvascular reactivity. Because the vascular reactivity is reduced, patients with the highest blood viscosity may develop frequent vaso-occlusive crises. More recently, we observed a negative correlation between sickle RBC deformability and the level of circulating microparticles (MPs) released by sickle RBCs. MPs are phospholipid microvesicles with a diameter ranging from 100 to 1,000 nm that are derived from the cytoplasmic membrane of cells submitted to various stresses. We found that the increased oxidative stress in SCA could be at the origin of the important release of MPs from sickle RBCs. In turn, these MPs, known to express a high amount of phosphatidylserine and to carry heme, participate to the genesis of vascular dysfunction in SCA. In conclusion, RBC physiology is severely impaired in SCA, which leads to chronic vascular dysfunction.

S11-5 Signaling pathways in regulation of RBC microrheological properties by catecholamines

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In circulation RBC are in contact with biologically active substances dissolved in plasma. Traditionally, RBC have been considered as simple reservoirs for oxygen transportation, but now it is evident that erythrocytes have a number of signaling molecules and participate in regulatory processes, which integrate body functions. Catecholamines act as stress hormones ensuring an effective adaptation to environmental factors, by regulating oxygen transport and cell metabolism. They may modify blood flow in nutritive capillaries under stress by alteration of red blood cell aggregability and deformability. Red blood cell microrheological properties connected with oxygen transport efficiency may be regulated through activation of cellular molecular signaling pathways. Likely regulatory pathways causing rheological responses in RBC include: extracellular ligands (hormones, prostaglandins), membrane-receptors coupled with G-proteins, adenylyl cyclase, cAMP, protein kinase A and phosphorylation of membrane proteins (band 3, band 4.1). Specificity of catecholamine action is proved by the experimental evidences of the presence of alfa- and beta-adrenoceptors on RBC membrane and dose-dependent changes of erythrocyte aggregation within elevation of adrenalin concentration. Activation of ligand specific receptors induces local increases in cAMP that are regulated by specific phosphodiesterases which are associated with individual signaling pathways. The subcellular location of PDEs is critical for coupling these enzymes to

specific signal transduction pathways. Our experimental data have demonstrated the involvement of ubiquitous second messengers – cAMP and ionized calcium – in the regulation of RBC aggregability and deformability. Work was supported by RFBR grant 18-015-00475.

S11-6 Complete Dynamics of Erythrocytes in Shear Flow: the story behind the term of deformability

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Physiological blood flows being shear-dominated, the dynamics of an erythrocyte subjected to an external shear flow constitutes one of the basic configurations for the understanding of hemorheology. In this configuration, the deformability of the red blood cells can be probed by applying an external shear stress and measuring the cells deformation, which is the principle of ektacytometry, for instance. However, the term of deformability often masks the complexity of the red blood cell mechanics. In particular, both the viscosity of the cytoplasm and the stiffness of the membrane resist to external stress and prevent red blood cells deformations.

Thanks to recent numerical, theoretical and experimental works, we will review the different motions of a red blood cell under shear flow to identify how the properties of the membrane and of the cytosol control its dynamics. We will notably show how the term deformability includes a variety of mechanical properties that need to be unraveled to understand the motion of erythrocytes and blood rheology. In particular, we will show that membrane and cytoplasm properties may have similar effects on the dynamics at low shear stress. However, at high shear stress, two different regimes appear depending on the ratio of viscosity between the internal and the external media. We will discuss the consequence of these recent findings on our understanding of blood shear-thinning at high shear rates, where aggregation effects are negligible.