

## **O4: Red Blood Cell Deformability**

### **O4-1 Beta-Estradiol and Ethinylestradiol enhance RBC deformability dependent on their blood concentration.**

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**BACKGROUND:** Natural and synthetic estrogens seems to have opposite effects on thrombosis and female cardiovascular system, since natural estrogen was supposed to be protective against cardiovascular diseases and synthetic estrogen has been related to thrombosis and cardiovascular diseases. In this work we have investigated if these differences could be related with the effects on those hormones on some hemorheological parameters.

**OBJECTIVE:** The objective of this work was to investigate the hemorheological changes of different concentrations of beta-estradiol and ethinylestradiol, on RBC aggregation and RBC deformability.

**METHODS:** Samples of blood of healthy donors were added with different concentrations of natural beta-estradiol or synthetic ethinylestradiol and were analyzed for red blood cell (RBC) aggregation and RBC deformability.

**RESULTS:** There were no significant changes in RBC aggregation. Both beta-estradiol and ethinylestradiol increase the RBC deformability in shear stresses above 3.0 Pa accordingly with the hormone's concentration.

**CONCLUSIONS:** Beta-estradiol and ethinylestradiol enhance RBC deformability dependent of their concentration. These findings may explain the different patterns of thrombotic and cardiovascular effects in different phases of the menstrual cycle or different dosages of oral contraceptive or hormonal replacement therapy.

### **O4-2 Dual mechanical characterization of red blood cells: role of surface area, internal viscosity and membrane rigidity**

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Osmotic gradient ektacytometry is the gold standard to assess red blood cell (RBC) deformability. It has been proposed that, when measured in iso-osmolar condition, RBC deformability under 3 Pa would depend on membrane elasticity while it would be influenced by internal viscosity above 3 Pa, but this hypothesis remains to be tested.

Healthy RBCs were treated by (i) lysolecithine (LPC), (ii) diamide or (iii) nystatine associated with hyperosmolar solutions, which reduces membrane surface area, decreases membrane elasticity or promotes cell dehydration, respectively. In iso-osmolar condition, LPC reduced RBC deformability at 30 Pa only, diamide decreased RBC deformability at all shear stresses and nystatine decreased RBC deformability above 3 Pa. The modification of the surface area to volume ratio and cell dehydration affected RBC deformability mainly at high shear stresses whereas a reduction in membrane elasticity affected RBC deformability at both low and high shear stresses.

The consequences of these rheological modifications on the dynamic behavior of RBCs were then evaluated by perfusing them in a microfluidic channel implementing a series of restrictions and enlargements, which dimensions were chosen to deform significantly the cells. Mechanical response was measured through RBCs amplitude of deformation  $\Delta D$ , their elongation at the exit of the last constriction  $D_{out}$  and their relaxation time ( $t$ , i.e. the time necessary to recover a stationary shape). Diminution of membrane elasticity and surface area reduced  $\Delta D$ ,  $D_{out}$  and  $t$ . However, the dynamic response of RBC was insensitive to internal viscosity.

Combining results from both techniques allowed discriminating the effects of different RBC rheological properties on the flow dynamics of RBCs.

### **O4-3 Proteomic analysis of the role of adenylyl cyclase-cAMP pathway in red blood cell mechanical response**

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Red blood cell (RBC) cytoskeleton plays a key role in the modulation of cell deformability which is structured by several membrane proteins. The goal of this study is to investigate the regulation of RBC deformability in response to shear forces and the molecular changes in RBC membrane proteins via cAMP/Protein kinase A (PKA) mediated signaling pathway.

Blood from healthy donors were treated either with or without SQ22536, Pentoxifylline or H89 which are known to inhibit adenylyl cyclase, phosphodiesterase and PKA, respectively. Shear stress (SS) at 5 Pa level was applied by (I) a capillary tube system (0.05 cm radius, 1 m length) connected to a syringe pump and (II) an ektacytometer with a shearing system (LORRCA) at 37 °C. RBC deformability were measured by LORRCA and matched results were included in the study. Phosphorylative changes in serine and tyrosine residues of membrane proteins and expression profiles were studied by immunoblotting and two dimensional gel electrophoresis, respectively. Differentially expressed proteins were identified by mass spectrometry (LC MS/MS).

Inhibitors act on cAMP/PKA pathway significantly decreased SS-induced improvements of deformability upon 5 Pa SS compared to controls ( $p < 0.05$ ). Inhibitors increased both serine and tyrosine phosphorylation. Mass spectrometric analysis revealed that differentially expressed proteins are mostly belong to cytoskeletal and proteasomal protein families.

SS-induced improvements of deformability are diminished by the inhibitors of signaling molecules in cAMP/PKA pathway. The manipulation of this pathway may be responsible for altered expression and phosphorylation status of membrane proteins that determines the associations within the cytoskeleton and further regulates SS-induced RBC deformability.

### **O4-4 The oxygenscan: continuous measurement of red blood cell deformability with oxygen gradient ektacytometry to monitor disease severity and treatment effect in sickle cell disease**

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In Sickle Cell Disease (SCD) hemoglobin S (HbS) polymerizes upon deoxygenation, resulting in sickling of red blood cells (RBCs). In this study we validated the Oxygenscan: a method to measure RBC deformability as a function of oxygen tension.

RBC deformability (Elongation Index – EI) was measured as a function of oxygen tension using the Laser Optical Rotational Red Cell Analyzer (Lorrc, Zwaag, The Netherlands) during deoxygenation (using nitrogen) and reoxygenation, under fixed shear stress. Read out parameters are: EImax: maximum EI, EImin: minimum EI,  $\Delta$ EI: difference between EImax and EImin, Point of Sickling (POS): pO<sub>2</sub> (mmHg) at which >5% decrease in EI is observed during deoxygenation.

Oxygenscan curves were highly reproducible (CV<5%). POS likely reflects an individual patient's hemoglobin dissociation curve. Upon ex vivo exposure to anti-sickling agents, currently in clinical development, that alter the oxygen affinity of hemoglobin, a left-shift of the POS was observed, indicating improved deformability at lower oxygen tensions. In addition, a substantial decrease in  $\Delta$ EI was observed, suggesting less cells are able to sickle. When RBCs from 19 SCD patients with different genotypes and treatment regimens were analyzed the POS was highest in untreated HbSS patients. Treatment with either Hydroxyurea or transfusion caused a decrease in the POS, and an increase in EImax and EImin. Notably, RBCs from healthy control blood samples show no change in EI during the assay.

The Oxygenscan brings the sickling assay to a new level with unparalleled repeatability, and with multiple parameters that quantify different aspects of sickling biology. We suggest that the Oxygenscan can be used to assess an individual patient's disease severity and monitor treatment effect.

#### **O4-5 Nitric Oxide Regulates Human Erythrocyte Deformability through Adjusting Band 3 Phosphorylation Status in Hypoxia**

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Hypoxia is an often seen problem in diverse conditions; systemic adaptations to hypoxia permit people to adjust to the hypoxic environment at high altitudes and to disease processes. In addition to the cardiopulmonary and hematologic adaptations that support systemic oxygen delivery in hypoxia, RBCs assist through newly described NO-based mechanisms, in line with their vital role in oxygen transport and delivery. Furthermore, to increase the local blood flow in proportion to metabolic demand, NO regulates membrane mechanical properties thereby modulating RBC deformability and oxygen carrying-releasing function.

But the clear mechanisms of NO regulate RBC deformability remain unknown. Here, we have carried out a systematic study to find the mechanisms of NO regulate RBC deformability under hypoxia. NO levels, RBCs membrane elongation index (EI), band 3 and membrane bound haemachrome were determined with an NO donor (sodium nitroprusside) or an NO synthase inhibitor (1-nitro-arginine methylester) under hypoxia.

In the present article, it is determined that NO plays a potential role in maintaining RBC deformability in hypoxia through altering band 3 tyrosine phosphorylation by maintaining the activity of SH-PTP2 and reducing band 3 crosslinking, which may occur during hypoxic ischaemia diseases, and at high altitudes. This study may provide insights into the molecular mechanisms of RBC adaptation to hypoxia.

## **O4-6 Hypoxia: The Best Stimulator that Increases Shear-Induced Response of Red Blood Cells**

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Red blood cells (RBC) carry and deliver oxygen (O<sub>2</sub>) to peripheral tissues through different microcirculatory regions where they are exposed to various levels of shear stress (SS). O<sub>2</sub> affinity of hemoglobin (Hb) decreases as the blood enters to the microcirculation. This phenomenon determines Hb interactions with RBC membrane proteins that can further regulate the structure of cytoskeleton and affect mechanical properties of cells. The goal of this study is to evaluate shear-induced RBC deformability and simulate RBC dynamics in blood flow under oxygenated and deoxygenated conditions.

Venous blood samples from healthy donors were oxygenated with ambient air or deoxygenated with 100% nitrogen gas for 15 minutes and immediately applied into an ektacytometer (LORRCA). RBC deformability was measured before and after the application of continuous 5 Pa SS by LORRCA and recorded as elongation index (EI) values. RBC deformability significantly increased in deoxygenated blood compared to oxygenated samples both before and after 5 Pa SS implementation ( $p < 0.01$ ). A computational model was generated for the simulation of blood flow in an artery section. Distribution of EI was calculated during oxygenation/deoxygenation which is 5-10 times higher around the vessel wall compared to the center of the lumen for sections of the pulsatile flow profile.

Deoxygenation substantially improves RBC deformability that these improvements are also significant with shear exposure. Oxygenation status of RBC may modulate its membrane properties in the microcirculation. Although the extent of RBC deformability increases as RBCs approach to the vessel wall for both oxygenated/deoxygenated conditions, this increase is higher for deoxygenated condition compared to oxygenated condition.