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Laser tweezers technique in studies of impact of endothelium derived nitrogen oxide (NO) on red blood cell (RBC) aggregation

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Red blood cells (RBC) play a variety of roles in human body. Besides gas exchange function there are roles in immune response and hemostasis [1]. One of the important properties of RBC is their spontaneous aggregation, i.e. ability to reversibly form linear or more complex aggregates. RBC aggregation significantly impacts blood rheology by changing the blood viscosity and its alterations in some diseases may cause pathological complications [2]. RBC aggregation depends on different factors: from blood plasma osmolarity to protein concentration. One of these factors is the concentration of signaling molecules in the blood flow. Nitric oxide (NO) is a signaling molecule from gasotransmitters class. Its main target in blood flow are smooth muscle cells, NO causes vasorelaxation and systemic decrease in arterial pressure. The main sources of nitric oxide in the blood flow are endothelial cells, that produce NO from L-arginine by endothelial nitric oxide synthase (eNOS). There is evidence of NO changing RBC properties, in particular, their deformability and aggregability [3].

In this work, RBC aggregation in presence of endothelial cells monolayer was studied by laser tweezers technology. To stimulate NO production endothelial cells were treated with L-arginine solution. Measurements were taken in a microcuvette with the height of 300 µm and volume of 300 µl. On the bottom of the cuvette a monolayer of endothelial cells was placed, then cuvette was filled with human plasma containing a small number of RBC (0.1 % hematocrit). RBCs aggregation force is the minimal force needed to prevent spontaneous aggregation of two adjacent contacting RBC. RBC disaggregation force is the minimal force needed to disrupt the contact of two aggregated RBC. In order to decrease the data dispersion, the measured values were normalized by the mean value of the control (0 µM of L-arginine). Statistical significance of their alterations was calculated using the Mann-Whitney test.

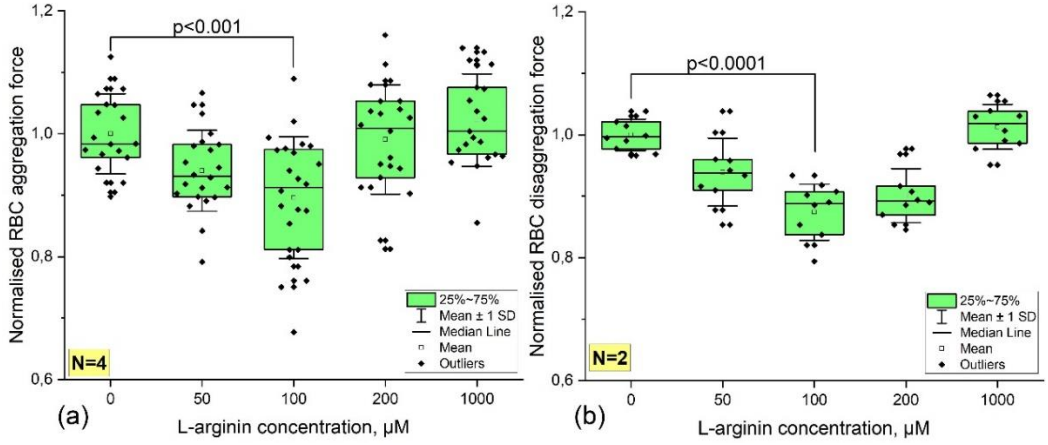

Figure 1. The dependence of RBC aggregation (a) and disaggregation (b) forces on L-arginine concentration

In our experiments, L-arginine caused a decrease in both RBC aggregation and disaggregation forces with a minimum at L-arginine concentration of 100 µM (Figure 1). In both cases, the dependence seems to be bell-shaped: the effect disappears at higher concentrations.

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