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Title: The impact of endothelium-derived nitric oxide on RBC aggregation studied *in vitro* by laser tweezers

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WORKSHOPS: OPTICAL TECHNOLOGIES IN BIOPHYSICS & MEDICINE XXV

Red blood cells (RBC) play one of the key roles in blood microrheology which is mainly defined by two processes in which RBCs involved. RBC aggregation determines blood viscosity, while RBC deformability and adhesion to endothelium impacts both vessel resistance to the blood flow and viscosity [1]. Alterations of RBC aggregation, deformability and their adhesion to the endothelial cells may cause negative consequences in number of diseases such as type 2 diabetes, arterial hypertension and sickle cell anemia [2]. In turn, RBC properties depend on various factors, one of which is concentration of signaling molecules in the blood. One of such molecules is nitric oxide (NO) which is mainly produced by endothelial cells via endothelial nitric oxide synthase (eNOS) enzyme in circulatory system. The only substrate of this reaction is L-arginine amino acid. There are multiple data suggesting that NO increases deformability and reduces aggregation of RBC [3]. Thus, study of NO, RBC and endothelium interplay represents both fundamental and clinical interest.

The main goal of this work was to study RBC aggregation and adhesion to endothelium at different NO conditions using the laser tweezers *in vitro*. To induce NO production by endothelium, endothelial cells were preincubated with L-arginine in concentration of 100 µM. To prove the hypothesis of endothelium-derived NO, endothelial cells were first incubated with eNOS inhibitor, L-NAME (in concentration of 200 µM), and then with L-arginine. There are data suggesting that eNOS is able to produce NO only if there is significant extracellular L-arginine concentration in the sample. To verify this hypothesis, we studied samples with and without presence of L-arginine in the endothelium extracellular medium.

Whole blood was drawn from the cubital vein of healthy donors. The lithium heparin anticoagulant to prevent blood clotting was used. Endothelial cells represented a monolayer of cells grown on a round glass plates. They were kept in a CO2 environment at 37°C before the measurements. The samples to be measured comprised platelet poor blood plasma and a small amount of blood (1:1000). Samples (highly diluted RBC with platelet poor blood plasma) were placed in cuvettes consisting of slide, endothelial cell monolayer and coverglass. The cuvettes were isolated from the air to prevent oxidative stress. Measurement of each sample took approximately 30 min and was performed under room temperature. Laser tweezers allow to trap and manipulate single cells hence measure their interaction forces in the range of pN. In this work homemade double beam laser tweezers setup with two traps based on Nd:YAG laser (1064 nm) was used [4].

Our results show statistically significant decrease in RBC aggregation in the samples with endothelium preincubated with L-arginine in comparison with ones without L-arginine (RBC aggregation force was 4.8 ± 0.4 pN in the control samples and 4.1 ± 0.2 pN in the sample pretreated with L-arginine, $p < 0.0001$, RBC disaggregation forces were 5.9 ± 0.1 pN and 5.5 ± 0.1 pN, respectively, $p < 0.00001$). Samples pretreated with L-NAME, as inhibitor of NO producing, show no significant impact on RBC aggregation, which witness in favor of endothelium-derived NO hypothesis. Basing on the obtained data we can conclude that there is no need in L-arginine extracellular concentration during measurements for effective NO production by endothelium. Considering this we can suppose that L-arginine is accumulated by endothelial cells. No significant alterations of RBC adhesion to endothelium between samples were obtained. These results improve our understanding of RBC-NO-endothelium interplay.

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