RED BLOOD CELL AGGREGATION STUDIED BY DOUBLE TRAP OPTICAL TWEEZERS

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Physiological aggregation, which is a reversible and dynamical process of mutual attraction between cell membranes, is a fundamental red blood cell (RBC) property. Research in this field continues to be of interest as RBC aggregation strongly determines blood microcirculation and therefore normal organism functioning. Physical mechanisms responsible for RBC aggregation still remain under discussion. There are two main co-existing theories describing this process, namely, bridging and depletion model. However, RBC aggregation is believed to be an effective finger-print of various diseases. System Lupus Erythematosus (SLE) is one of the basic examples. Patients with SLE have considerable enhancement of RBC aggregation properties.

The majority of contemporary methods of RBC aggregation research are dealing with an averaged behaviour of a large number of RBC rather than with reaction of a single cell. Optical tweezers technique is able to reveal qualitative parameters of single RBC pair aggregation in combination with exact visual control which can not be achieved using traditional methods. Using double-trap optical tweezers, aggregation forces and aggregation velocities in RBC pairs are obtained for healthy and SLE blood samples.

Two independent optical traps are formed by two orthogonally polarized Nd-YAG laser (1064 nm) beams focused by 100x oil immersion objective (N.A. 1.3). Laser power in each trap is estimated to be in between 15 to 20 mW. Samples consist of RBC suspension in plasma placed between two cover glasses with a gap about 0.15 mm between them. Setup allows an accurate two-coordinate sample positioning relative to optical traps. Erythrocytes were obtained from the venous blood of 30 healthy donors and 10 patients with System Lupus Erythematosus (SLE). Experiments are performed at room temperature.

The setup calibration was realized using the elastic force of single RBC. The maximal optical trap force F_{trap} is estimated to be about 20 pN. Being provided by quantitative characteristics of optical traps one can analyse the force between individual erythrocytes during aggregation and disaggregation. Experimental distance dependence of the interaction force for RBC during disaggregation was obtained.

It is revealed that this dependence has a qualitative growth with the distance between the centres of the cells.

Numerous experiments of RBC disaggregation induced by double optical trap were carried out to reveal the distinctions in aggregation properties for normal and pathological erythrocytes. Measurements were performed for 30 healthy donors and 10 SLE patients. Four different end-points of disaggregation were revealed during the experiments. The first one is a successful break of the aggregate while increasing the distance between the traps. The second is the break of the aggregate until two RBCs were fastened together by small tethers. The third one is aggregate destruction until a finite touch area. Finally, the fourth case is an abortive break of tight aggregates. Obtained statistics of the end-points of disaggregation shows that 2.5 times more abortive breaks of the aggregates were detected for SLE patients than for healthy donors, and 6 times more successful breaks were observed for normal erythrocytes. This demonstrates

that aggregation force for the SLE patients is in average higher comparing with healthy erythrocytes.

Direct measurements of aggregation speed were carried out for the quantitative comparative analysis of normal and pathological RBCs. The average speed value is appeared to be v~0.30±0.04 □m/s for normal erythrocytes and v~0.53±0.02 □m/s for pathological ones. Aggregation speed of the SLE erythrocytes is almost 2 times higher than aggregation velocity for normal cells which is showing strong difference in aggregation properties for pathologic and healthy donors.

Analysis of disaggregation scenarios, interaction force of erythrocytes and aggregation speed values can give an opportunity to make a conclusion about the reasons and mechanisms of RBC aggregation for normal a pathological blood.