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RBC aggregation dynamics in autologous plasma and serum studied with double-channel optical tweezers

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ABSTRACT

Red blood cells aggregating and disaggregating forces were measured in the autologous plasma and serum using the double-channelled optical tweezers. A significant, three-fold decrease of the both forces was observed in the serum compared to the plasma. The results of this study help to better assess the RBC aggregation mechanism.

Keywords: red blood cells, aggregation, disaggregation, forces, plasma, serum, optical tweezers.

1. INTRODUCTION

The red blood cell (RBC) aggregation is one of the main factors that determine the fluidity of the blood[1]. This process is currently of the significant scientific and clinical interest for a potential application in clinical diagnostics and monitoring of the microcirculation of blood[2]. The RBC aggregation is mainly determined by the concentration of different proteins in plasma like fibrinogen, globulins, C - reactive protein, haptoglobin, etc. Among them the dominating factor is the fibrinogen[3-5]. In a number of works an importance of the synergetic effect of proteins in the RBC aggregation is mentioned[4-8]. However, the synergetic role of different proteins in the RBC aggregation is still to be assessed.

In a number of works, the optical tweezers (OT) were shown to be an effective tool for studying the RBC interaction kinetics during their aggregation[9-13]. OT allow for measuring the interaction forces between the individual cells by freely manipulating them with a tightly focused laser beam[13]. Detailed assessment of the factors affecting RBC aggregation became possible using OT.

In this work, we study the role of fibrinogen by deduction to assess its role while many types of proteins are present in the solution. The parameters characterizing the RBC spontaneous aggregation and disaggregation processes were measured in the autologous plasma and serum using OT and compared. We believe that the results obtained in this paper improve our understanding of the proteins role in RBC aggregation, and therefore open up a perspective of better account for the hemorheologic disorders in clinical practice.

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2. MATERIALS AND METHODS

2.1 Sample preparation

The blood for experiments was drawn by venipuncture from a single clinically healthy male donor. In this way we could avoid an unnecessary deviation of the results between the donors. The ethylene-diamine-tetra-acetic acid (EDTA) was used as an anticoagulant for plasma; serum was obtained using the BD Vacutainer tube, containing serum-clot separation gel and thrombin based clotting activator. Plasma for the measurements was obtained by centrifugation of the whole blood sample according to the recommendations for hemorheological measurements[14]. Additional procedure of centrifugation at 12,000g for 10 minutes was performed to obtain platelet free plasma (further we refer to it as plasma). The absence of platelets was crucial for the experiments because platelets being trapped along with RBCs hinder the precise force measurements. The sample was prepared by adding a small portion of whole blood to the plasma or serum to achieve the final concentration of RBC of about 0.5%. The high dilution was necessary to perform measurements at the individual cells level. The sample was put into a special chamber, made of a microscope slide and a glass coverslip separated by a 100 μm width adhesive tape. The cells were allowed to settle down and then a pair by pair the initially non-interacting cells were lifted up from the surface and used for the force measurements. Detailed experimental procedures are described further with results. All measurements were done at the room temperature (22°C) during 4 hours after drawing the blood.

2.2 Experimental setup

The schematic layout of the double channeled optical tweezers used in for experiments is shown in the Fig. 1. The main elements of the setup are the single-mode Nd:YAG laser (1064 nm, 250 mW) and a 100× water-immersion objective with high numerical aperture (Olympus, ∞-corrected LumPlanFi, N.A. 1.00) used to form the optical traps. Single laser beam was expanded using telescope and separated using polarizing cube, the ratio of intensities in each of optical traps was controlled using half-wave plate. The motorized mirror was used to control position of the optical trap and 1:1 telescope was used to form a conjugate plane on objective` back aperture. The laser beam power used for trapping the live cells did not exceed 30 mW. This laser power does not cause changes in cells during the time of trapping and measurements, which did not exceed 5 to 10 minutes[12]. The calibration of the setup was done by utilizing the viscous drag force. The external viscous friction force was matched with the maximum returning force (further trapping force) of OT.

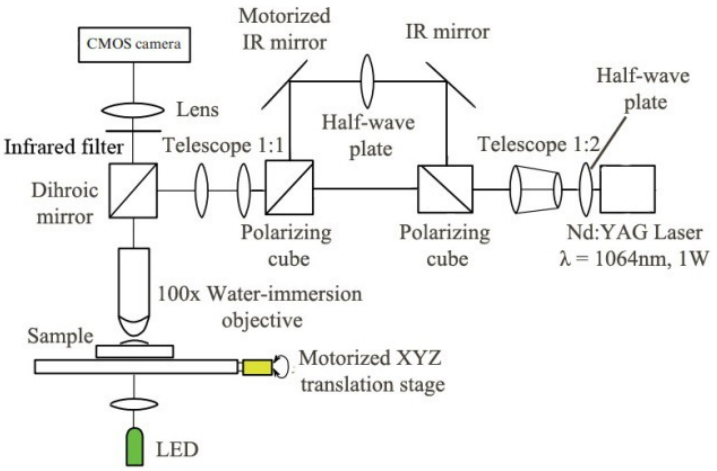

Fig. 1. Schematic layout of the optical tweezers setup.

2.3 Measurement procedures

The parameters of RBC aggregation were measured using the methods used in the previous work[13]. The aim of the present work was to clarify the role of fibrinogen by deduction in the RBC spontaneous aggregation and disaggregation processes. We refer to the RBC spontaneous aggregation as the process of cells spontaneous overlapping each other after a point (small area) interaction is enforced with the OT. The RBC spontaneous aggregating force (F_A), velocity of aggregation (V_A) and disaggregating force (F_D) were measured. The measurement procedure for the spontaneous aggregation process – F_A and V_A is shown in the Fig. 2; and for disaggregation process - F_D is shown in the Fig. 3.

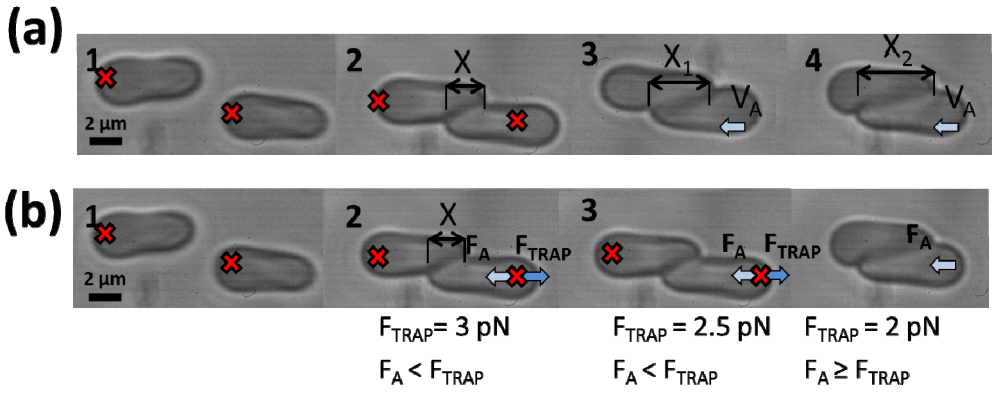

Fig. 2. (a) The measurement procedure of the RBC spontaneous aggregating velocity (V_A): (1) the cells are trapped by optical traps; (2) attached to each other with defined linear overlap distance (X), approximately 1 µm and released from the trap; (3) the cells spontaneously overlap each other with the velocity V_A depending on the suspending medium; (4) V_A was measured by dividing the change in X by the time of the cells movement. (b) The measurement procedure of the RBC spontaneous aggregating force (F_A): (1) the cells are trapped by the OT; (2) they are attached to each other with a defined initial X and held with the OT from further overlapping; (3) the trapping force (F_{TRAP}) is slowly decreased; (4) at a certain moment the cell escapes from the trap and the cells spontaneously overlap each other. At that moment the F_A is considered to be matching F_{TRAP}. The cross marks show the positions of the OT and the blue arrow shows the direction of pulling, the white arrow shows the direction of the cells movement during spontaneous aggregation.

Measurements of each parameter were carried on at least 20 pairs of individual cells. The standard deviations of the measured parameters were calculated and shown in the corresponding figures. The main contribution to the error was introduced by the individual differences between cells. It is known that even within the blood of the defined donor, the RBC aggregation parameters differ significantly between the cells due to such factors like the age difference between cells[15].

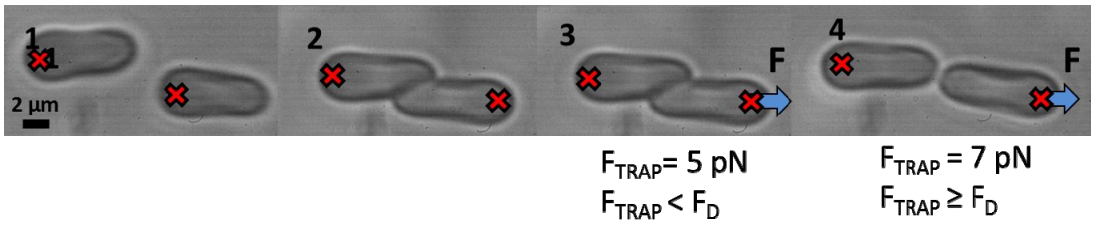

Fig. 3. The measurement procedure for the RBC disaggregating force (F_D): (1) The cells are trapped by the OT; (2) they are attached to each other with defined initial linear overlap distance (X), approximately 2 µm, and held for 10 seconds; (3) they are pulled away slowly with a defined trapping force (F_{TRAP}); (4) F_{TRAP} is increased slowly until the cells could be separated completely. Thus, the minimum disaggregating force (F_D) is found.

3. RESULTS

The measurement results for the V_A is shown in the Fig. 4 (a), a significant decrease was observed in serum compared to plasma. This indicates that for fibrinogen to play the dominating role in RBC spontaneous aggregation. The measurement results for the RBC aggregating and disaggregating forces are shown in the Fig. 4 (b) the F_A is significantly weaker than F_D in serum as well as in plasma.

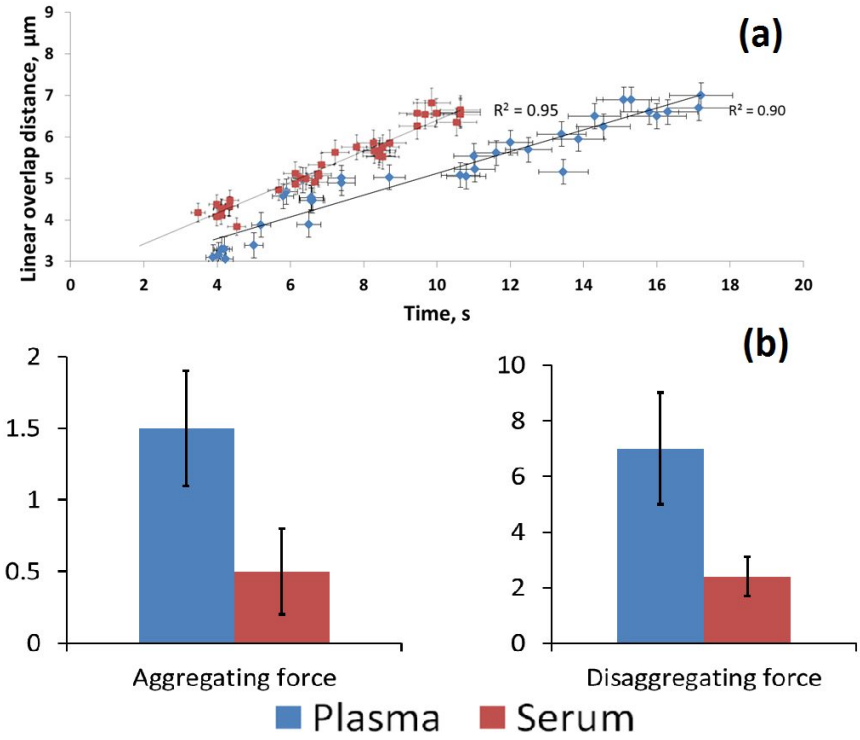

Fig. 4. (a) RBC aggregating velocity as dependence of linear overlap distance on time for plasma (red) and serum (blue). Note that RBC aggregation is significantly weaker in serum compared to plasma. (b) The RBC disaggregating force (F_D) and aggregating force (F_A) in serum and in plasma Note the significant decrease in the forces measured in serum. The linear overlap distance in all cases is about 2 μm.

We performed the study of the RBC spontaneous aggregation and the disaggregation processes in serum and plasma using the optical tweezers. It allowed us to directly compare these two processes, and assess the role of fibrinogen by deduction. The significant role of fibrinogen was observed in determining both F_A and F_D. For the RBC spontaneous aggregation, it plays the dominating role when other types of plasma proteins are present in the solution. The F_D was significantly higher that F_A in serum as well as in plasma. Overall our study allows for assessing the role of fibrinogen in the RBC aggregation at the individual cells level. Further studies would allow for the advances in the application of RBC aggregation as a biomedical marker.

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