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# RAPID ASSESSMENT OF AGGREGATION ABILITIES AND SURFACE PROPERTIES OF PLATELETS AND RED BLOOD CELLS

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## ABSTRACT

Rheological properties of erythrocytes and platelets define blood rheology in general. The role of their aggregation and surface geometry is highly essential. The screening remains in demand in practical biology. Erythrocyte aggregation activity can be recorded quite quickly and easily with an optical microscope by counting in a hemocytometer of rouleaux, aggregated and non-aggregated erythrocytes in a washed erythrocyte suspension in blood. Erythrocyte cytoarchitectonics can be determined with a light phase contrast microscope. Assessment of erythrocyte microrheological properties should be added with platelet aggregation evaluation, which can be carried out visually by a simplified method. The percentage of active forms of platelets and aggregates in blood can be assessed with a phase contrast microscope. No expensive equipment is required to assess the main microrheological properties of erythrocytes and platelets for all details of their dynamics at multiple testing. These morphofunctional methods help determine that in norm a changed form is noted in blood only in an insignificant number of red blood cells and platelets. Under pathology, these indicators can be substantially changed due to altered rheological properties of red blood cells and platelets, significantly impairing the microcirculation.

**KEY WORDS:** **red blood cells, RBCs, erythrocytes, platelets, aggregation, cytoarchitectonics, determination methods.**

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## INTRODUCTION

Being the main part of the mammalian blood cell population, red blood cells and platelets affect its rheological properties[1,2]. In this respect, the role of their aggregation and surface geometry is considered to be highly essential, resulting in demand in methodological approaches to screening of microrheological properties of red blood cells and platelets in humans and animals[3,4]. They will allow promptly and adequately evaluate these parameters at any age[5,6,7], correcting if necessary and preventing dyscirculatory violations in vital organs[8]. Definitely the development of microrheological dysfunctions of erythrocytes and platelets has a genetic component[9,10,11]. Their deterioration, affected by environmental factors, is observed in the pathogenesis of many diseases[3,12,13]. Under pathology, their rheological properties deterioration may cause functional disorders of various internal organs, determining the state of humans or animals, and further prognosis[14,15,16]. All this underlines the diagnostic importance of rapid and easy lab assessment of aggregation and cytoarchitectonics of erythrocytes and platelets[17,18].

## MATERIALS AND METHODS

***Assessment of erythrocyte aggregation***

Venous blood is to be taken in the morning after a 14-hour starvation through a thick needle by gravity into a test tube with 3.8% of sodium citrate in 9:1 ratio and be centrifuged for 10 min at 3 000 rpm. Two wells 96-well reaction plate are to be filled with 0,2ml of the patient's plasma in a 96 lunula reaction plate. All plasma and a leucocyte layer are to be removed from the tube. Erythrocytes are resuspended in standard phosphate buffer in 1:4 ratio, followed by 10-min centrifugation at 3000 rpm, allowing to wash them from plasma residues at the supernatant removal. Then 0,02ml of erythrocytes are taken and resuspended in the first well filled with autologous plasma in the 96-well reaction plate to provide 10% hematocrit. Then 0,02ml content is to be taken out of this well with a clean dry pipette and placed into the second filled well to provide 1% hematocrit. Thereafter, one grid in a hemocytometer is to be filled with obtained erythrocyte suspension, kept for 3 minutes till the spontaneous aggregation, and then free erythrocytes (including two erythrocytes together) and aggregates are counted, starting from 3 erythrocytes, combined as "rouleaux") in 2 large squares cameras (lens x 40, ocular x 10). Aggregation activity of erythrocytes can be recorded with a light microscope by counting in a hemocytometer of erythrocyte aggregates, aggregate and non-aggregate erythrocytes in the washed red blood cells suspension in plasma followed by the calculation of an average aggregate size (AAS):

AAS = EAA / AQ, where
EAA - an amount of all erythrocytes in the aggregate;
AQ - an amount of aggregates.
An aggregation value (AV) is calculated as follows:
AV = (AAS x QA + CSE) / (QA + FEA), where
FEA - an amount of free erythrocytes.
The percentage of non-aggregated erythrocytes (NAP) is determined as follows:
NAP = (FEA x 100) / (AAS x QA + FEA) [19].

***Assessment of erythrocyte cytoarchitectonics***

To test the surface geometry of erythrocytes, blood is fixed by 1% glutaraldehyde in 199 medium (pH 7.4) at 4°C for one day, and then a "crushed drop" is prepared and a light phase contrast microscope is applied. Cells are counted by percent per 200 erythrocytes. Recorded erythrocytes are typed by ten groups: discocytes; single spur discocytes; thorn discocytes; spiculated discocytes; berry-shaped erythrocytes; spherocytes (stomatocytes); smooth spherocytes; crenated spherocytes; elliptical-shaped erythrocytes; degenerative erythrocytes. The first five categories of red blood cells (discocytes, including those with ehinocyte transformation signs) are considered to be reversibly deformed due to their ability to spontaneous recovery. The other red blood cells categories are related to the group of irreversibly deformed or pre-hemolytic forms[19].

***Assessment of platelet aggregation***

Blood is to be taken with 3.8% sodium citrate in 9:1 ratio, centrifuged for 5 min at 1000 rpm to obtain platelet-rich plasma. Part of the plasma is taken, and the rest is to be centrifuged at 3000 rpm for 20 minutes to obtain platelet-poor plasma. Platelet-rich plasma is standardized by the platelet number to 200 × $10^9$/L. From resultant standardized plasma, 0.02 ml plasma is taken per each analyzed inductor and their combination. The remaining plasma can be used for other hematological and biochemical tests. 0.02 ml plasma is taken on a slide from collected standardized plasma, and 0.02 ml inductor solution with different pipettes. Adenosinediphosphate (0,5 × $10^{-4}$ M), collagen (1:2 diluted primary suspension), thrombin (0.125 u/ml), adrenalin (5,0 × $10^{-6}$ M), ristomycin (0.8 mg/ml), hydrogen peroxide (7,3 × $10^{-3}$ M) can be applied as agonists. Plasma is to be mixed with inductors with a glass rod, and then a stopwatch is to be started. The mixture is stirred so that to held the liquid within a circle of 2 cm diameter. When moving circularly the slide in transmitted beams of the illuminator, aggregates appearance is observed through a magnifying glass on a black background. Once the aggregates are clearly evident, the solution is clarified, and some aggregates are stuck to the glass, the stopwatch is stopped and the time of platelet aggregation is recorded. The reaction is repeated 2-3 times with each inductor and an arithmetic mean is found from the obtained resultants[19]. Optimum values of platelet aggregation at platelet concentration of 200 × $10^9$ /L are for ADP – 37-50 sec, collagen – 27-36 sec, ristomycin – 38-50 sec, thrombin – 48-59 sec,

adrenaline – 81-106 sec, hydrogen peroxide – to 40-60 sec.

***Recording of intravascular platelet activity***

2 ml blood from the median cubital vein are to be taken into a siliconized centrifuge tube with 8 ml of 0.125% glutaraldehyde solution, followed by immediate centrifuging for six minutes at 1000 rpm. The supernatant is to be diluted with glutaraldehyde by four times (0.1 ml + 0.3 ml solution) and stirred 5 times with a pipette and poured into a hemocytometer, placed in a humidified Petri dish for 20 minutes[20]. The percent distribution of the above platelet forms is to be determined with a phase contrast microscope per 200 cells. The first visible platelet activation is their changed forms. This manifestation can be used to adequately assess the process both induced in vitro, and developed in the body. In the bloodstream with no pathological activating effects, most intact platelets, referred to as discocytes, have a typical disc-shape or oval form and smooth surface. A typical sequence of changes is developed at platelet activation: from discocytes to activate cells – discocytes, i.e. discoechinocytes with burrs on the surface, and further to spherocytes or spheroehinocytes. The latter has not only the more spherical form, but also the increased number of burrs. The degree of aggregation is assessed also by the relative number of all platelets involved in the aggregation. The aggregation can be detected by the percentage ratio of the number of aggregated platelets to the total number in the drug (i.e. to the sum of free-lying cells and involved in the aggregation) by the formula:

$$\frac{2x+3y+4z+....}{500+2x+3y+4z+...}\times 100\%,$$

***where x, y, z, etc. - The number of aggregates of appropriate size per 500 free platelets[21].***

## RESULTS

These methods of assessment of the main microrheological properties of red blood cells and platelets do not require expensive equipment and are able to provide sufficient information[22,23]. Long-term practical application of these methods allows to record changed shapes of insignificant part of red blood cells and platelets in healthy people and animal blood[5,6,7,22,24], which was confirmed by more complicated tests[8,25,26]. Under pathological conditions[27,28], the same methods confirmed that changes of microrheological indices of erythrocytes and platelets could be much more pronounced[29,30,31,32]. This indicates the great practical importance of the above methods, able to provide accurate information on pathological conditions, when microcirculation can significantly deteriorate[33,34,35,36,37] because of significant amount of blood of erythrocytes and platelets with modified shapes and a large number of their aggregates[38,39,40]. Provided that adequate perfusion of internal organs of humans and animals in many respects defines their overall functional state[41,42], rapid assessment of aggregation activity and of the surface geometry of the red blood cells and platelets is practically needed to be easy and affordable[25]. As previous studies revealed, these data are very important in the evaluation of the functional state of human and animals in early stages of individual development[5,43]. It is clear that the state dynamics in animal or human depend on dynamics of microrheological properties of red blood cells and platelets, including at the pathology development[44], as well as the recovery duration and completeness and degree of genetically certain qualities or pathology process[45,46,47]. All this proves that microrheology properties of red blood cells and platelets, on the one hand, are a major marker of the functional state of the body, on the other hand, an important target of the corrective impact on it[24,48].

## CONCLUSION

Thus, the rheological properties of erythrocytes and platelets largely determine the rheological properties of whole blood. In this regard, the role of aggregation and surface geometry is very high; their screening remains very popular in practical biology. Assessment of these microrheological properties of red blood cells and platelets does not require expensive equipment and is able to provide accurate information in pathological conditions, when microcirculation can significantly deteriorate. By applying these morphofunctional methods, it is confirmed that only a small portion of red blood cells and platelets is characterized by changed shapes in norm in blood. In pathological conditions, changes in these indicators can be much more pronounced, significantly impairing the microcirculation.

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