Interaction of red blood cells in the environment inducing their aggregation: laser tweezers study

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Outline

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Introduction: Red blood cell aggregation

- The red blood cells (RBC) aggregation is a reversible process of clumping of cells that determine the low-shear viscosity of the blood and therefore significantly affect microcirculation of the blood.
Example of relationship between blood microcirculation and RBC aggregation

- Aggregated RBC have to disaggregate to flow through different blood vessel geometries (e.g. bifurcations).
- During pathologies, enhancement of RBC aggregation might inhibit cells disaggregation and cause impairment of microcirculation.
Clinical significance of RBC aggregation

- The RBC aggregation is enhanced during most of socially important pathologies (e.g. diabetes, cardiovascular diseases)
- This research is of great clinical significance as it can help improve the diagnostic and treatment procedures

Microphotography of healthy (top) and pathological (bot) RBC diluted in plasma, on microscope slide.

Clinical significance of RBC aggregation is substantiated in O. Baskurt and H. Meiselman, Clin Hem Microcirc (2013)
RBC aggregation inducing media

RBC aggregation occurs only in the environment with relatively big macromolecules and is dependent on their concentrations.

- Aggregation occurs in the plasma environment where the main aggregation inducing proteins are: fibrinogen, immunoglobulin, haptoglobin, C-reactive protein and few others.
- It also occurs in solutions of macromolecules: proteins or synthetic neutral-macromolecules

O. Baskurt et al., Red Blood Cell Aggregation, Cell Press (2012)
Motivation and the aim of study

• Currently, the RBC spontaneous aggregation is mainly studied, while only little is known about the enforced RBC disaggregation. Commonly these processes are considered to be directly reversed.

• Present study aims to assess the difference between the RBC spontaneous aggregation and the enforced RBC disaggregation processes using laser tweezers to measure the interaction forces between individual cells.
Method: Laser tweezers

- The main method used in the work is laser tweezers (LT).
- LT allows to trap and manipulate single cells and measure pN-ranged forces of cells interaction.

Principle of optical trapping

\[ F = k_{\text{trap}} x \]
Schematic layout

- ND:YAG 1064 nm 350mW
- Motorized steering mirror
- Halfwave plate
- Lens
- Dichroic mirror
- Power meter
- Olympus
- 100x, NA = 1.00 Water-immersion objective
- XY translation sample stage
- Motorized steering mirror
- Polar. cube
Force calibration

Viscous drag force:

\[ F_{\text{visc}} = 3\pi \eta dv \]

The trapping force applied on the RBC was calibrated by matching it with the trapping force applied on the microsphere.

\[ F_{\text{esc}} = aP \]

\[ a = 1.7 \pm 0.1 \text{ pN/mW} \]
Materials

• Aggregation inducing environments:
  – Autologous plasma
  – PBS solutions of protein: gamma-globulin, fibrinogen, albumin

• Blood samples:
  – The blood was drawn from 10 healthy male donors. Experiments were performed within 4 hours, at room temperature.
Trapping of red blood cells

The sequences of RBC lifting and trapping. (a) single cell is rest on glass surface, (b-c) optical trap is turned on, and cell is slowly lifted while rotating to the side, (d) the cell is lifted and trapped.

Measurements were carried out on over than two hundred RBC aggregates, each consisting by two initially non-interacting RBCs
RBC spontaneous aggregating force ($F_A$) was measured according to the following procedure:

1) RBCs are trapped by two independent LT, attached to each other and held with the trapping force ($F_{OT}$) > $F_A$ to not allow the cells spontaneous aggregation.

2) $F_{OT}$ is decreased slowly until it becomes equal or lower than $F_A$. The cells start to spontaneously aggregate by crawling one to another. At this moment $F_A$ is considered to be matching $F_{OT}$.

Schematic sequences of $F_A$ measurement. (X) mark shows position of laser tweezers, and arrows show direction of applied forces.
Measurement results for RBC spontaneous aggregation

Dependence of $F_A$ on interaction area. $F_A$ is linearly dependent on interaction area in plasma. No spontaneous aggregation was detected in protein solutions of fibrinogen, albumin and gamma-globulin, up to twice of physiological norm (blue line)
Measurement procedure
(enforced RBC disaggregation)

Set of frames demonstrating the steps of the measurement procedure. The cross marks show the positions of OT and the arrow show the direction of pulling. (1) two RBCs were trapped by OT; (2) brought to each other and attached with ~30 µm² of the interaction area; (3) held still for 20 seconds; (4) pulled one from another by moving the OT position away slowly at a defined trapping force ($F_1 - F_3$). During disaggregation more force was required to further separate cells as interaction area is diminishing. The step 4 was repeated multiple times with increasing trapping force. For every step, the minimal achievable cells linear overlapping distance ($\Delta X$) was measured for a certain trapping force.
Measurement results for enforced RBC disaggregation

- The reverse dependence of interaction force between cells on interaction area was observed.
- It indicates accumulation of interaction between cells.
- Note that measurement procedure is from right to left

Dependence of the minimum linear overlap distance achievable on the trapping force.
Estimation of RBC interaction energy in plasma

\[ U = \int dF \text{d}A \]

- Illustration of the method used to calculate the work required to separated cells RBCs on example of enforced RBC disaggregation. We consider it matching the energy of interaction between. (In the case of RBC spontaneous aggregation process is reversed)
- Thus, energy of interaction between RBCs calculated by summing up the work required to move the cells at all steps of measurement.
Interaction energy density

• The mean value of interaction energy density – $<E>$ (a parameter commonly used for characterizing RBC aggregation) was obtained by dividing work required to disaggregate or aggregate cells by the initial area of interaction between RBCs.

• For the enforced RBC disaggregation process, $<E>$ was found to be $2.7 \pm 0.9 \mu J/m^2$ (plasma).

• For the RBC spontaneous aggregating process $<E>$ was found to be $0.6 \pm 0.4 \mu J/m^2$ (plasma).
Energy per $\mu m^2$ shift of cells

- Energy required to shift cells by $\mu m^2$ was calculated for each step of measurement to describe the interaction kinetics.
- Interaction energy was divided by corresponding shift in interaction area and plotted, gray dots refer to the RBC spontaneous aggregation and black dots refer to the enforced RBC disaggregation.

Note that measurement process is going from right to left.
Conclusions

• We have shown that the spontaneous RBC aggregation and enforced RBC disaggregation are not directly reversed processes and exhibit different properties:
  – The absolute values of forces differ approximately three times.
  – Fibrinogen`s role in each processes exhibited differently. While no RBC spontaneous aggregation was observed, they interacted strongly within local interaction area artificially made by LT.

• Results consider that the factors determining spontaneous RBC aggregation and enforced RBC disaggregation processes might be different.
Thank you for attention!