

Measuring Electrical and Mechanical Properties of Red Blood Cells with a Double Optical Tweezers

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ABSTRACT:

The fluid lipid bilayer viscoelastic membrane of red blood cells (RBC) contains antigen glycolproteins and proteins which can interact with antibodies to cause cell agglutination. This is the basis of most of the immunohematologic tests in blood banks and the identification of the antibodies against the erythrocyte antigens is of fundamental importance for transfusional routines. The negative charges of the RBCs creates a repulsive electric (zeta) potential between the cells and prevents their aggregation in the blood stream. The first counterions cloud strongly binded moving together with the RBC is called the compact layer. This report proposes the use of a double optical tweezers for a new procedure for measuring: (1) the apparent membrane viscosity, (2) the cell adhesion, (3) the zeta potential and (4) the compact layer's size of the charges formed around the cell in the electrolytic solution. To measure the membrane viscosity we trapped silica beads strongly attached to agglutinated RBCs and measured the force to slide one RBC over the other as a function of the relative velocity. The RBC adhesion was measured by slowly displacing two RBCs apart until the disagglutination happens. The compact layer's size was measured using the force on the silica bead attached to a single RBC in response to an applied voltage and the zeta potential was obtained by measuring the terminal velocity after releasing the RBC from the optical trap at the last applied voltage. We believe that the methodology here proposed can improve the methods of diagnosis in blood banks.

KEYWORDS: Optical Tweezers, Red Blood Cell, Agglutination, Membrane Viscosity, Adhesion, Zeta Potential.

INTRODUCTION:

The red blood cell (RBC) membrane contains proteins and glycoproteins embedded in, or attached, to a fluid lipid bilayer that confers to it the viscoelastic behavior. The fluid lipid bilayer is composed not only by

phospholipids, but also by glycolipids and cholesterols. The glycolipids the RBC membrane also leads to negatively charged surface, which creates a repulsive electric (zeta) potential between the cells and prevents their aggregation in the blood stream¹⁻². The first counterions cloud strongly bind moving together with the RBC is called the compact layer. There are techniques, however, to decrease the zeta potential to allow cell agglutination which are the basis of most of the tests of antigen-antibody interactions in blood banks. The identification of the antibodies against the erythrocyte antigens is of fundamental importance for transfusional routines because a mistake can lead in serious hemolytic reactions.

In this report we propose the use of a double optical tweezers for a new procedure for measuring: (1) the apparent* membrane viscosity (η_m), (2) the cell adhesion (a), (3) the zeta potential (ζ) and (4) the compact layer's size of charges (d) from the electrolytic solution formed around the cell. Optical trap rely on the radiation pressure to capture and manipulate microscopic particles. As the optical forces involved are very small, in the range of hundreds of femto to tens of pico Newtons, the optical tweezers has been used as a tool for measuring several kind of microscopic cell properties, where the forces involved are in the same scale³⁻⁵. We believe that the methodology here proposed to quantify these RBC parameters can give information about cell agglutination and improve the quality of the diagnosis immunohematologic tests in blood banks.

MATERIALS, METHODS AND RESULTS:

Optical tweezers: The double optical tweezers consisted of a Nd:YAG laser strongly focused through 100X oil immersion objective of an Olympus microscope (BH2, Olympus Optical CO., Ltd.) equipped with a CCD camera and a x-y-z motorized stage (Prior Scientific, model ProScan) controlled by a computer or a joystick. The laser beam is divided once and recombined using polarizer beam-splitters (BP1 and BP2), as shown in Figure 1. There is a half-wave plate (H1) before the first beam-splitter to distribute the power for each beam. Before the second beam-splitter there is another half-wave plate (H2) to control the power of one of the beams. A set of telescopes are used in the

* *It is apparent that different experimental techniques and theoretical models yield very different viscoelastic parameters even for the same cell type under similar conditions. A major reason for this is that the models used for data analysis are likely to be overly simplified, thereby lumping many unmodeled elements into the viscoelastic parameters. Consequently, they became model-dependent rather than intrinsic, despite their capability for reproducing a particular data set. As such, these viscoelastic parameters should be referred to as apparent properties⁶.*

system to capture particles in the microscope plane of view. An extra telescope (L1 and L2) is used for flipping the laser beams without disturbing the alignment. The movements of the beams of each optical tweezers are done by a joystick or computer using piezoelectric actuators (New Focus) of the gimbal mounts (G1 and G2).

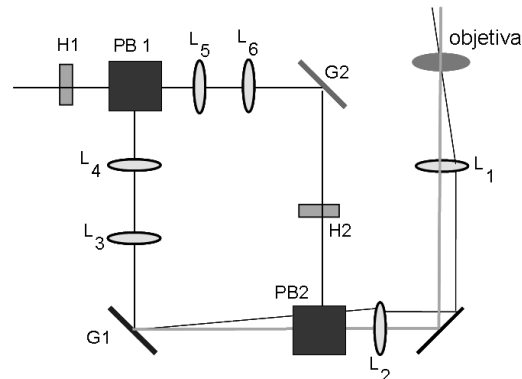


Figure 1: Double Optical Tweezers system.

General considerations: The RBC units were obtained from Hematology and Transfusion Center UNICAMP. Samples were diluted in plasma ABO compatible (0.5:1000 μL) with known refractive index (Abbe refractometer) and viscosity (Ostwald viscometer). Silica beads (Bangs Laboratories) diluted in physiological serum was added to 10 μL of RBC solution. Cell samples and measurements were carried out at room temperature (25 $^{\circ}\text{C}$). All the measurements were recorded in real time and captured by the computer. At least five measurements of each property (η , a , ζ , d) were done. The analysis of the images was performed with the Image Pro Plus Software (Media Cybernetics, Baltimore, MD, USA).

Calibration: For this experiment, an optical tweezers trap a silica bead that binds strongly and non-specifically to a RBC. At the same time, after calibration through its displacement from the equilibrium position using geometrical optics regime, this silica bead acts as a pico-Newton force transducer. In the geometrical optics regime the optical force is generated by the momentum transfer due to refraction of the laser light at the boundaries of the particles that have a higher index of refraction than their surrounding medium and several different methods have been developed for trapping force strength calibration⁷. One method consists in dragging a microsphere at known velocities through a fluid with known viscosity and observing the microsphere displacement from the original position at null velocity. The optical force could then be accessed by the calculated hydrodynamical force. Therefore in our calibration we checked the geometrical optics force model against the hydrodynamical models in the vicinity of two parallel plane

walls. We obtained, after careful measurement of the parameters involved, a plot of the optical versus hydrodynamic force as a straight line at 45 degrees. Among the parameters used by the models the laser power measurement has been the most difficult to obtain, because the usual power meters readings are not accurate for the very high numerical aperture of the beam after the objective. We solved this problem using an integration sphere after the objective to assure that optical rays in any angle are taken into account. The measurements have been performed for a wide range of parameters such as fluid viscosity, refractive index, drag velocity, wall proximities and laser power.

The adhesion (a) and the apparent membrane viscosity (η_m): Manipulating RBCs rouleaux with the double optical tweezers, as shown in figure 2, we observed that the cells slide easily one over the others but are strongly connected by their edges. An explanation for this behavior is the fact that when the cells slide over the others, proteins are dragged through the membrane. It confers to the movement a viscous characteristic that is dependent of the velocity between the RBCs and justifies why is so easy to slide them apart. By measuring the force as a function of the relative velocity between the cells, we confirm this assumption. On the other hand, it is difficult to detach the cells at the end of the movement. The reason for that it is because now it is necessary to break the bonds between the proteins of the cell membranes to separate the RBCs. We used this viscous characteristic of the RBC rouleaux to determine the apparent membrane viscosity of the cell. As this behavior is related to the proteins interactions, we can use the apparent membrane viscosity to obtain a better understanding of cell agglutination.



Figure 2: Manipulating RBCs rouleaux with the double optical tweezers. The cells slide easily one over the others but are strongly connected by their edges.

For the measurements of adhesion and apparent membrane viscosity, one of the optical tweezers trap a silica bead that binds to a RBC at the end of a RBCs rouleaux of two cells that is formed spontaneously. To measure the membrane apparent viscosity, the second optical tweezers trap directly the RBC at the other end of the rouleaux and the optical force is measured as a function of the relative velocity between the RBCs, as shown in figure 3 (a). To measure the adhesion, silica beads captured by the optical tweezers are attached to cells of the rouleaux and are slowly displaced apart until the RBCs disagglutination happens, as shown in figure 3 (b). The movements of the

second optical tweezers were done respectively by joystick and by computer controlled piezoelectric actuators (New Focus) for adhesion and apparent membrane viscosity experiments.

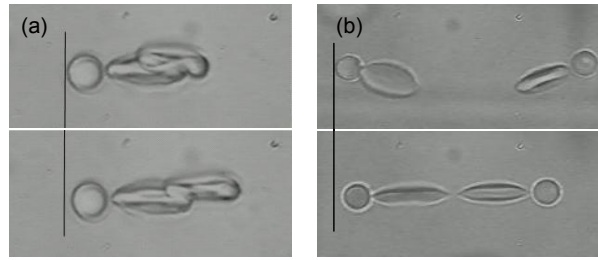


Figure 3: (a) The measurement of the apparent membrane viscosity. (b) The measurement of cell adhesion using the double optical tweezers.

We obtained a average value of $a = 14 \text{ pN}$ for the adhesion of RBCs rouleaux of two cells. This measurement was directly quantified using the silica bead displacements from the equilibrium position.

The apparent membrane viscosity was determined using the Saffman Theory⁸⁻⁹. Basically, Saffman modeled a protein in a membrane as a cylindrical inclusion in a continuous film and computed the drag on a cylindrical particle undergoing translational and rotational motion in a model lipid layer. He found for the force of a protein in translational movement:

$$F = 4\pi\eta_s \frac{1}{\ln(\eta_s/\eta a) - C} u \quad (1)$$

where η_s is the membrane shear surface viscosity, η is the fluid viscosity, a is the radius of the cylinder and $C=0.58$ is the Euler-Mascheroni Constant and u is the velocity. Considering that there are N proteins involved:

$$F = 4\pi\eta_s N \frac{1}{\ln(\eta_s/\eta a) - C} u = \eta_m u \quad (2)$$

where η_m is the apparent membrane viscosity and its unit is poise. As the optical force is equal the viscous force, we obtained $11 \times 10^{-4} \text{ poise} \cdot \text{cm}$ for this preliminary result. As we have two cells in each measurement, $\eta_m = 5.5 \times 10^{-4} \text{ poise} \cdot \text{cm}$, that is of the same order of the values for membrane viscosity found in the literature¹⁰. Figure 4 shows the plot of the force versus velocity for one rouleaux that confirms the expected viscous behavior for the movement of one cell on top of the other.

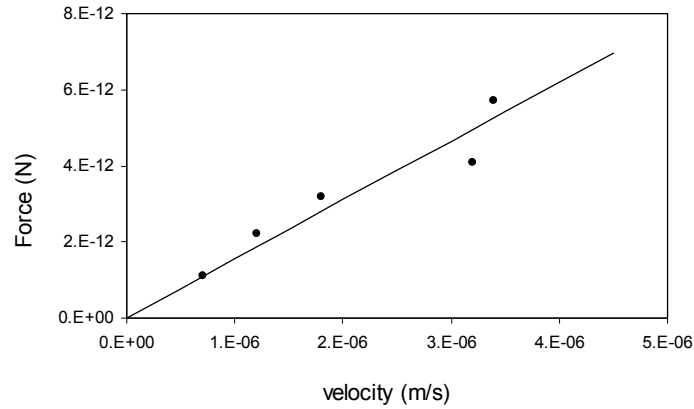


Figure 4: Plot of the optical force in function of the velocity between the cells of the one roleaux.

The zeta potential (ζ) and the size of compact layer of charges (d): A negatively charged RBC suspended in an electrolytic solution induces a cloud of oppositely charged ions surrounding the cell. This cloud, usually referred as the electrical double layer, extends outwards from the surface of the RBC with decreasing density and merges finally with the electrolytic solution arranged in such a way to maintain electrical neutrality. The most of this diffuse cloud of ionized electrolyte will be arranged in a way to maintain electrical neutrality. Immediately next to the charged cell surface there is thin layer of counter ions strongly electrostatically attached to it, called compact layer¹¹. Counter ions outside this compact layer are mobile and form a diffusive layer. The zeta potential is the electrostatic potential at the boundary between the compact layer and the diffusive layer.

When an external electrical field E is applied to the cell, there is an electrical force $F_{elet} = \Delta q E = \rho(x) A \Delta x E$ acting on the thin double-layer of area A and thickness Δx . As the velocity of the ions increases, there is also a viscous force $F_{vis} = -\eta A d/dx [dv/dx] \Delta x$ balancing the electrical force and keeping constant the velocity of the ions. Thus, for the equilibrium condition,

$$\rho(x) A E \Delta x = -\eta A \frac{d^2 v}{dx^2} \Delta x \rightarrow \rho(x) E = -\eta \frac{d^2 v}{dx^2} \quad (3)$$

Considering a RBC as a disc with the diameter much larger than its thickness, we can describe the dependence of the potential with the density of charges of an electrolytic solution by the Poisson equation in one dimension, $d^2\Psi(x)/dx^2 = -\rho(x)/\varepsilon$, which leads to

$$\frac{d^2v}{dx^2} = \frac{\varepsilon E}{\eta} \frac{d^2\Psi(x)}{dx^2} \rightarrow v = \frac{\varepsilon E}{\eta} \Psi(x) \quad (4)$$

The thin compact layer of cations formed around the cell moves together with the same velocity of the cell (v_p). Therefore the zeta potential (ζ) is given by:

$$v_p = (\varepsilon E / \eta) \zeta, \quad (5)$$

that is known as Smoluchowski equation¹², where η is the viscosity and ε is the electrical permittivity of the electrolytic solution. It is also possible to show that the zeta potential and the electrical potential can be written as:

$$\zeta = \sigma_s d / \varepsilon \quad \text{and} \quad \Psi(x) = (\sigma_s / \varepsilon k) e^{-kx} \quad (6),$$

where σ_s is the surface charge density of the compact layer, $d = 1/k$ is the size of the compact layer, $k^2 = (2n_0 z^2 e^2 / \varepsilon k_B T)$, n_0 and ze are the concentration and the ionic force of the ion, T is the temperature and k_B is the Boltzmann constant¹³. Using viscous force and equations 4 and 6 we obtain:

$$F_{visc} = -\eta A [dv/dx] = -A \varepsilon E [d\Psi(x)/dx] = A \varepsilon E k \Psi(x) \quad (7),$$

Now, at the boundary between the compact layer and the diffusive layer, $\Psi(x) = \zeta$ and in the equilibrium the optical trap force F_{op} is equal to the viscous force F_{vis} , which leads to:

$$F_{op} = (A \varepsilon \zeta / d) E \quad (8),$$

For the measurements of zeta potential (ζ) and compact layer's size (d) we build a special chamber consisting of two electrodes of platinum separated by a pool of $l = 170$ mm (length), $w = 30$ mm (width) and $h = 75$ μm (depth). The external electrical field was applied with a voltage power supply (HP 712 C) connected to the electrodes. The compact layer's size was measured by the force on the silica bead attached to a single RBC in response to an applied voltage from 50 - 100 V. The zeta potential was obtained by the measurement of the terminal velocity of the RBC after released from the optical trap for the last applied voltage. The RBC area used was $A = 50$ μm^2 and the electrical permittivity¹⁴ was $\varepsilon = 1.06 \times 10^{-9} \text{C}^2 / \text{N m}^2$. In this way, using the equations 5 and 8 we could get simultaneously both measurements (ζ and d) for the same RBC. We found $d = 0.85$ μm for the compact layer's size and $\zeta = -12.5$ m V for the zeta potential. The result obtained for the zeta potential agrees to the data of the literature^{2,13}. Figure 5 shows an example of measurements done in this experiment.

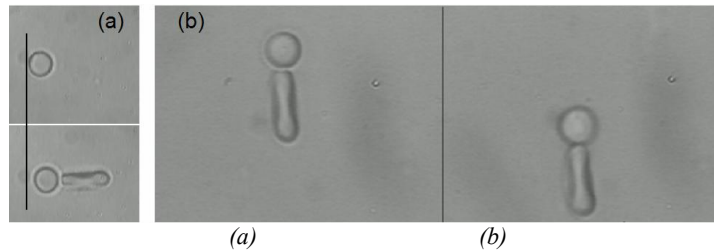


Figure 5. . A example of measurements done in the same cell of the compact layer's size using the optical force (a) and of the zeta potential using the terminal velocity (b).

CONCLUSION:

The results presented in this article demonstrated a new methodology using a double optical tweezers to determine RBC properties such as apparent membrane viscosity, adhesion, zeta potential and the size of the compact layer. The measurements of the zeta potential, as well as the preliminary results of the apparent membrane viscosity, are in agreement with values found in the literature. As far as we know, this is the first time that the size of the compact layer and the RBC adhesion are measured using optical tweezers. The ability of the optical trap to capture and manipulate single particles allowed us to characterize cell by cell individually and also to obtain the zeta potential and the size of the compact layer for the same RBC. Individual cell analysis methods are always more sensitive to small differences than those based on average values. There are various conventional electrophoresis methods to measure cell zeta potential¹⁵⁻¹⁶. However, the data acquisition of such methods are not straightforward due to the complex motion of cells inside closed vessels, specially because the velocity of the cell depends on its distance from the walls of the vessels. In conclusion, we believe that the methodology here proposed can provide information about cell agglutination that helps to improve the immunohematologic tests usually performed in blood banks.

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