Studying Red Blood Cell Agglutination by Measuring Membrane Viscosity with Optical Tweezers

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ABSTRACT:

The red blood cell (RBC) viscoelastic membrane contains proteins and glycoproteins embedded in a fluid lipid bilayer that are responsible for cell agglutination. Manipulating RBCs rouleaux with a double optical tweezers, we observed that the cells slide easily one over the others but are strongly connected by their edges. An explanation for this behavior could be the fact that when the cells slide one 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. Therefore, in a first step of this work, by measuring the force as a function of the relative velocity between two cells, we confirmed this assumption and 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 about cell agglutination. Methods related to cell agglutination induced by antigen-antibody interactions are the basis of most of tests used in transfusion centers. Then, in a second step of this work, we measured the apparent membrane viscosity using antibodies. We observed that this methodology is sensitive to different kinds of bindings between RBCs. Better comprehension of the forces and

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bindings between RBCs could improve the sensibility and specificity of the hemagglutination reactions and also guides the development of new potentiator substances.

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

INTRODUCTION:

The red blood cell (RBC) viscoelastic membrane contains proteins and glycoproteins embedded in a fluid lipid bilayer that are responsible for cell agglutination. In cell agglutination some proteins from one cell link to other red blood cells. Mechanical measurements performed in agglutinated RBCs can provide valuable information about the type of cell agglutination and the number of bonds between the cells.

It was shown in previous work that when the RBC cells are dragged in a shear movement one over the other using a double optical tweezers, the proteins and/or the antigens that link the cells are dragged through their lipidic membrane and the force involved in this movement is of viscous nature [1]. Because the viscous forces are proportional to the drag velocity, an apparent membrane viscosity can be extracted from the curve force vs drag velocity. From this measurement it is possible to have information not only on the number and type of the bonds between the cells, but also on the whole membrane protein/antigen network that are dragged together. In this work, we show that we can use the measurement of the apparente membrane viscosity to differentiate RBC agglutination obtained using antibodies against erythrocyte antigens of the Rh system and control cells (RBCs).

Methods related to cell agglutination caused by antigen-antibody interactions are the basis of most of immunohematologic tests used in transfusion centers. Besides the protein binding, however, there are a set of barriers to prevent that the agglutination process would happen spontaneously in the blood stream. Due to the presence of the glycolipids, the RBC membrane surface is negatively charged and creates a repulsive electric (zeta) potential between the cells that prevents them to come close and, therefore, their aggregation [2, 3]. Better comprehension of the forces and bindings between RBCs could improve the sensibility and specificity of the hemagglutination reactions and also guides the development of new potentiator substances.

MATERIALS AND METHODS:

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). The performed test was also analyzed using antibodies against erythrocyte antigen (system Rh), samples without antibodies were analyzed as control. Silica beads (Bangs Laboratories, Fishers, IN, USA) diluted in physiological serum was added to 10 μL of RBC solution. These silica beads act as a pico-Newton force transducer after a calibration using the displacement of the bead from the equilibrium position assuming a geometrical optics model. The displacement of the center of the bead under the presence of external forces was quantified with the software Image Pro Plus (Media Cybernectics, Baltimore, MD, USA). Previous calibration of this procedure against hydrodynamic force showed good results [4]. All the measurements were carried out at room temperature (25 ^{o}C). All the measurements were recorded in real time and captured by the computer.

The double optical tweezers consisted of a Nd:YAG laser strongly focused through a 100X oil immersion objective (NA = 1.25) of an upright Olympus microscope equipped with a CCD camera and a x-y-z motorized stage controlled by a computer or a joystick. The laser beam is divided once and recombined using polarizer beam-splitters. Two sets of telescopes are used in the system to capture particles in the same focal plane. Two gimbal mounts are used to steer the beam position in the focal plane and the extra telescope is used to translate the steer pivot to the back aperture of the objective, avoiding power losses. This means that the gimbal mounts and the back aperture are conjugated optical planes.

For the measurement of the apparent membrane viscosity the spatialy fixed optical tweezers trap a silica bead binded to one RBC of a set of two cells agglutinated by nonspecific and specific bonds, while the second optical tweezers trap directly the other RBC. By moving the second optical tweezers with a computer controlled piezoelectric actuator it was possible to inprint any velocity between the RBCs and, at the same time, to measure the displacement of the traped silica bead, as shown in figure 1. With this procedure we measured the optical force as a function of the velocity between the RBCs.

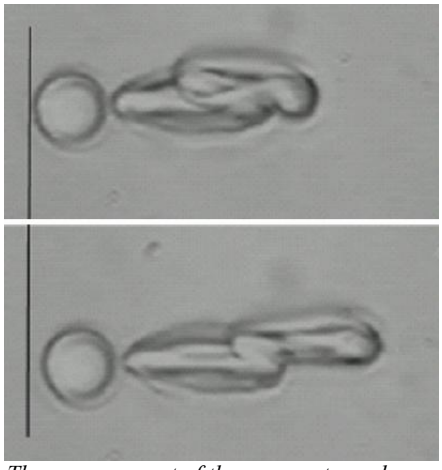

Figure 1. The measurement of the apparent membrane viscosity.

RESULTS:

The apparent membrane viscosity was determined using the Saffman Theory [5 - 6]. Basically, Saffman modeled a protein in a membrane as a cylindrical inclusion in a continuous film and computed the drag force on the cylindrical particle undergoing translational and rotational motion. 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 intrinsic membrane shear surface viscosity, η is the fluid viscosity, a is the radius of the cylinder, C =0.58 is the Euler-Mascheroni Constant and u is the velocity. Considering that there are N proteins involved, we have:

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

The unit of the parameter that incorporate the number of proteins and the other factors η_m, called apparente membrane viscosity, is poise. Figure 2 shows the plot of the force versus velocity that confirms the expected viscous behavior for the movement of one cell on top of the other. From this chart we can obtain the apparent membrane viscosity. Figure 3 shows the apparent membrane viscosity using antibodies against erythrocyte antigens - Rh system and control cells. From this figure, we observe that this methodology is sensitive to different kinds of bindings between RBCs. The result obtained for control RBC membrane viscosity median was $1x10^{-3}$ poise.cm and the samples analyzed with antibodies showed $2x10^{-3}$ poise.cm (Table 1).

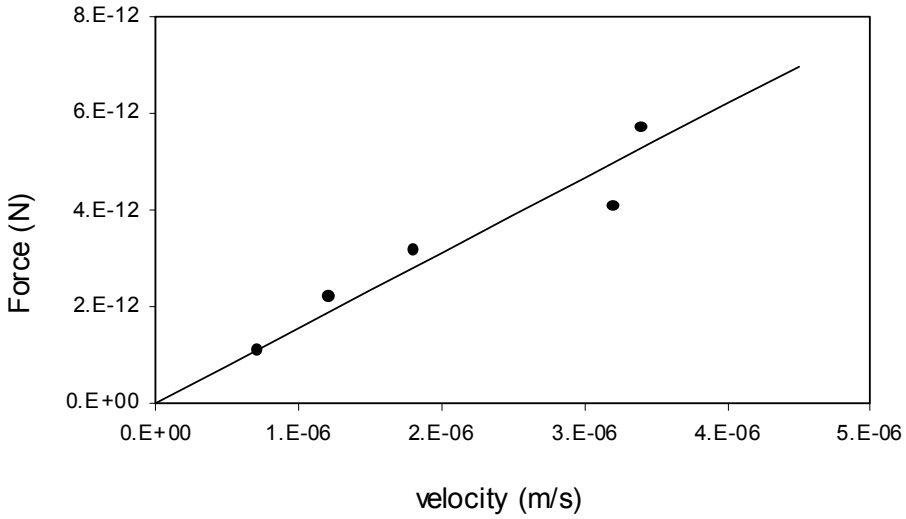

Figure 2. Plot of the optical force in function of the velocity between the cells.

Table 1. Results obtained for membrane viscosity for control RBCs and RBC plus antibodies.

	Number of cells analyzed	Membrane viscosity (poise.cm x 10^{-4})	Median (min-max)
Control	**20**	**10**	**(0.1 - 2.5)**
Antibodies	**15**	**20**	**(1.0 - 4.0)**

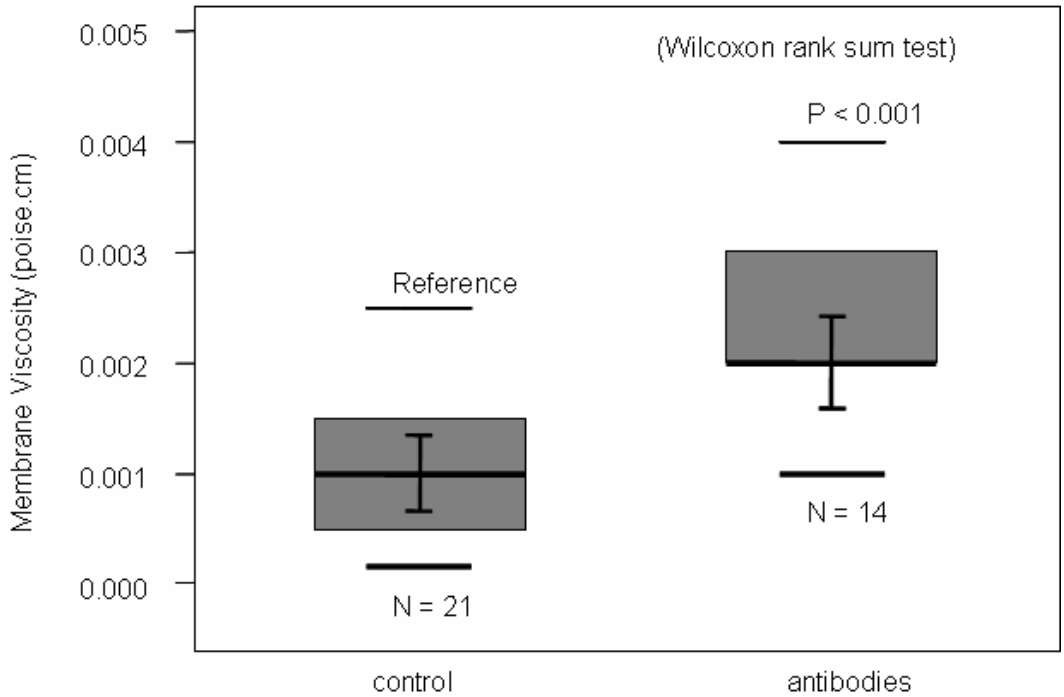

Figure 3. Plot of the results obtained for membrane viscosity for control and RBC plus antibodies.

CONCLUSION:

The results presented in this article demonstrated a new methodology using a double optical tweezers to determine the apparent membrane viscosity. The measurements of the apparent membrane viscosity are in agreement with values found in the literature. We observed that this methodology is sensitive to different kinds of bindings between RBCs. We were able to differentiate the apparent membrane viscosity measurements when compared control cells and RBCs linked to antibodies. The results showed that when antibodies were used on reaction, the membrane viscosity increased comparing to the control measurement (without antibodies). This demonstrates that these methodology is sensitive to a variety of factors that can interfere in agglutination reactions performance. Better comprehension of the forces and bindings between RBCs could improve the sensibility and specificity of the hemagglutination reactions and also guides the development of new potentiator substances.

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