Mechanical Properties of Stored Red Blood Cells Using Optical Tweezers

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ABSTRACT

We have developed a method for measuring the red blood cell (RBC) membrane overall elasticity μ by measuring the deformation of the cells when dragged at a constant velocity through a plasma fluid by an optical tweezers. The deformability of erythrocytes is a critical determinant of blood flow in the microcirculation. We tested our method and hydrodynamic models, which included the presence of two walls, by measuring the RBC deformation as a function of drag velocity and of the distance to the walls. The capability and sensitivity of this method can be evaluated by its application to a variety of studies, such as, the measurement of RBC elasticity of sickle cell anemia patients comparing homozygous (HbSS), including patients taking hydroxyrea (HU) and heterozygous (HbAS) with normal donors and the RBC elasticity measurement of gamma irradiated stored blood for transfusion to immunosupressed patients as a function of time and dose. These studies show that the technique has the sensitivity to discriminate heterozygous and homozygous sickle cell anemia patients from normal donors and even follow the course of HU treatment of Homozygous patients. The gamma irradiation studies show that there is no significant change in RBC elasticity over time for up to 14 days of storage, regardless of whether the unit was irradiated or not, but there was a huge change in the measured elasticity for the RBC units stored for more than 21 days after irradiation. These finds are important for the assessment of stored irradiated RBC viability for transfusion purposes because the present protocol consider 28 storage days after irradiation as the limit for the RBC usage.

Keywords: optical tweezers, red blood cells, elasticity

The red blood cells (RBC) deformability is the combined result of several mechanical and geometrical properties, such as internal viscosity, surface area to volume ratio, membrane elasticity and viscosity. The laser optical tweezers[1] provide a sensitive tool that allows individual, cell-by-cell, measurement of elasticity. The optical tweezers is based on photon momentum transfer and has a variety of biological applications such as the trapping of cells and organelles, micromanipulation of gametes, biomolecular genetic assay and study of membrane mechanical properties. We have developed a method based on optical tweezers for measuring overall elasticity of red blood cells. In this method, the elasticity μ was obtained by measuring the deformation of the cells when dragged at a constant velocity through the plasma fluid.

We demonstrated the capability and sensitivity of this technique by applying it to study irradiated stored erythrocytes[2] and sickle cell anaemia erythrocytes of patients homozygous (HbSS), including patients taking hydroxyrea (HU), and heterozygous (HbAS)[3]. Transfusion in immunosupressed patients can bring fatal complications because the white blood cells from the donors blood can kill the cells of these patients. Gamma irradiation of blood components is an effective way to prevent this kind of complications, however it induces hematologic and biochemical RBC changes and reduces RBC survival[4, 5]. Storage of RBC units for an extended period after irradiation is a regular procedure in blood banks without immediate access to a blood irradiator, where they can, usually, be stored up to 28 days. The sickle mutation substitutes thymine for adenine in the β-globin gene, thereby encoding valine instead of glutamic acid in the sixth position of the haemoglobin β-chain [hemoglobin S (HbS)]. This mutation causes a defect in the haemoglobin structure which is responsible for profound changes in molecular stability and solubility. In sickle cell anaemia, the RBC exhibit reduced deformability and the abnormalities in the erythrocyte rheology are of clinical importance this disease. Patients homozygous for this

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mutation (HbSS) present haemolytic anaemia and vaso-occlusive crises leading to the damage of most of the tissues[6]. On the other hand, heterozygous individuals for this mutation (HbAS or sickle cell trait) are asymptomatic. Hydroxyurea (HU) has been successfully used for the treatment of this condition. It is known that, in addition to the improving fetal hemoglobin production, HU has a great effect on whole cell deformability[7]. So, the reasons for developing this work were evaluate the properties of these kinds of erythrocytes and show that the optical tweezers is very sensitive tool than can be applied for clinical and research purposes.

The 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 (TK1085-U, JVC - Victor Company of Japan, Ltd.). The images were recorded in real time and captured by a computer. The analysis of the images was performed with Image Pro Plus Software (Media Cybernectics, Baltimore, MD, USA) and the mathematical calculation was performed using MS Excel (Microsoft Corp., Richmond, CA, USA). The analysis was performed in a 100 μm depth Neubauer chamber attached to a computer-controlled motion control (Prior Scientific, Rockland, MA, USA). The RBC units were obtained in the Hematology and Hemotherapy Center of Campinas and diluted (0.5:1000 μL) in AB human plasma with Rh positive and known viscosity. Cell samples and measurements were carried out at room temperature (25^o C). At least 10 cells of each sample were submitted to six velocities varying from 150 to 250 μm/s with an addition of 20 μm/s on each step. We studied the RBC deformability of 25 HbS subjects ageing from 23 to 51 yr old (15 homozygous for HbS including five patients taking HU for at least 6 months and 10 subjects with sickle cell trait) and 35 normal controls. For the irradiated RBC studies, 5 units from healthy donors were collected and each one split into two portions (split units) via a sterile connecting device. One bag from each split unit received a gamma irradiation dose of 25 Gy (IBL 437C Irradiator, Cis Bio International, Gif sur Yvette, France), and a second bag reserved as a control and was not irradiated. The elasticity of RBCs was examined during storage at 4^{o}C on days 1, 14, 21 and 28. Statistical analyses were performed using Whitney–Wilcoxon.

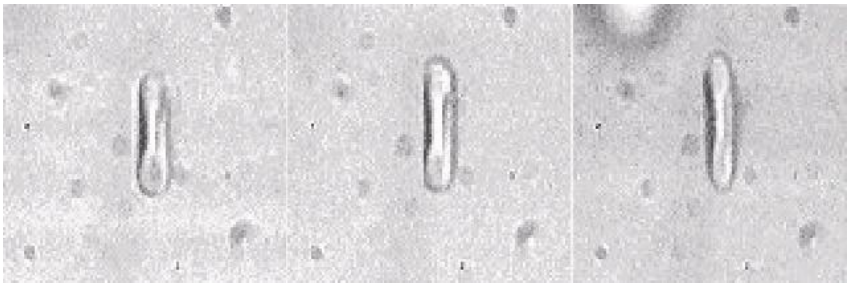

Figure 1. Deformed red blood cells with velocities varying from 150 to 210 μm/s.

In order to evaluate the red cell membrane elasticity and viscosity, the cell model was assumed as a parallelepiped with length L, width W and negligible thickness d. The cell was located at a distance Z_1 from the bottom of a Neubauer chamber and Z_2 from the cover slip. Optical tweezers dragged it with six constant velocities (V) through a fluid (human AB plasma) with viscosity η (Figure 1). To define the drag force F, we assumed a simple model of two surfaces with W and L dimensions, one surface standing still (cell surface) and the other moving with velocity V in a viscous fluid with viscosity η. This resulted in the mathematical expression,

$$F_{Drag} = \eta \frac{W L_0}{Z_{eq}}$$

where, η is the plasma viscosity and $1/Z_{eq} = 1/Z_1 + 1/Z_2$. We assumed that the overall cell elastic response to an applied force F is given by

$$F_{Elastic} = \left(\frac{\mu W}{L_0} \right) \Delta L$$

where μ is the overall elasticity and $\Delta L = L - L_0$ the cell length deformation, adopting L_0 as the cell length in the absence of any force. Equilibrium occurs when the elastic force cancels the drag force. At equilibrium, the cell length L is given by

$$L = L_0 + \left(\frac{\eta L_0^2}{\mu Z_{eq}} \right) V ,$$

independent of the cell width W. Therefore, the measurement of the cell length as a function of the drag velocity can be used to extract a value for μ, once the plasma viscosity η, the initial length L_0 and Z_{eq} are known. The slope of this curve $(\eta L_0^2 / \mu Z_{eq})$ is also a function of the equivalent depth Z_{eq}, and can be used to test this model.

In order to verify the previsions of the parallelepiped model, the cell length was measured as a function of the drag velocity for various Z_1. The camera of the optical tweezers microscope registered the cell images. These images were captured in a computer where its length was measured. We used the Neubauer chamber squares for the length calibration. The depth Z_1 was measured by focusing the bottom of the Neubauer chamber and then lowering the chamber by the desired amount (10 – 90 μm) while keeping the cell fixed with the optical tweezers. Figure 2 shows the result of the cell length measurement as a function of the drag velocity and Figure 3 shows the slope of the cell length vs. velocity as a function of depth Z_1. The slope of this straight line (L vs. V) shows a dependence on Z_1 given by the expression $1/Z_{eq} = 1/Z_1 + 1/Z_2 = 1/Z_1 + 1/(d - Z_1)$, assuming $Z_2 = d - Z_1 - \delta = d - Z_1$. This slope tends to infinity as Z_1 tends to zero or d, producing the odd shaped curve of Figure 3. With this proposed technique, not only the intrinsic membrane elasticity but the entire cell is analysed.

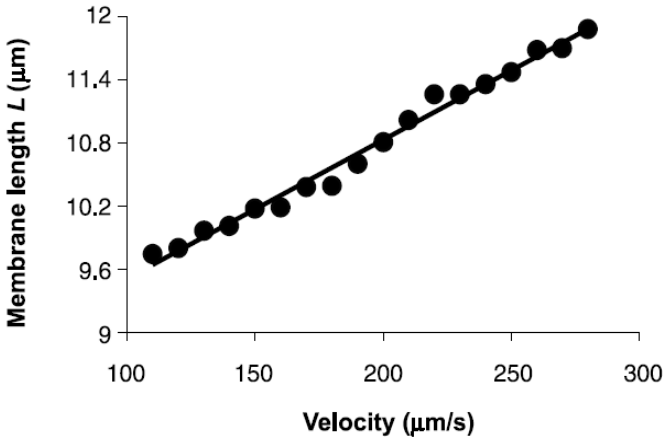

Figure 2. Red blood cell length plotted as a function of the drag velocity.

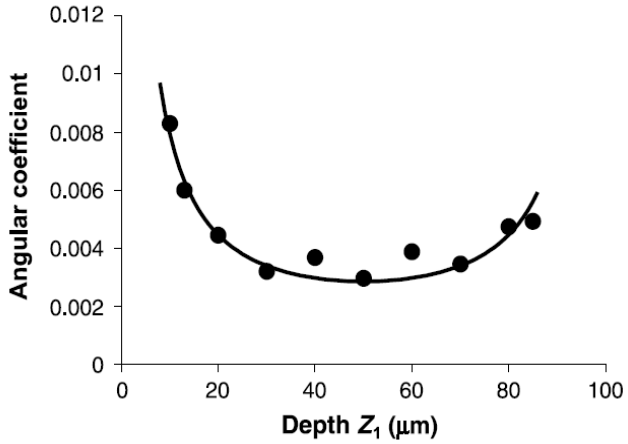

Figure 3. Slope obtained from the length vs. velocity, plot as function of the depth Z_1.

Our results showed that the deformability of RBC was lower in subjects with HbS than normal controls. On the other hand, the RBC deformability of HbS homozygous taking HU was similar to that observed in controls. The results are presented in Figure 4. We found that the red cell deformability was not different in HbS subjects (homozygotes and heterozygotes), but that both were significantly different from normal cells. After testing this methodology in several normal RBC, we observed that the data are distributed in a very narrow range and are very reproducible. On the other hand, in the HbSS and HbAS groups, a very heterogeneous RBC population was detected, with regard to their deformability. The elasticity of 50 RBCs from 5 irradiated and control units were analyzed during storage up to 28 days. RBCs from irradiated units stored for 21 days $(3.54 \pm 1.3)\times10^{-3}$ dyn/cm and for 28 days $(14 \pm 3.2)\times10^{-3}$ dyn/cm presented significantly lower elasticity when compared with control cells stored for 21 days $(0.37 \pm 0.03) \times10^{-3}$ dyn/cm and 28 days $(0.5 \pm 0.09) \times10^{-3}$ dyn/cm. The elasticity of RBCs irradiated and stored for 14 days $(0.43 \pm 0.038) \times10^{-3}$ dyn/cm presented no significant difference when compared with nonirradiated units $(0.34 \pm 0.02) \times10^{-3}$ dyn/cm. These results can be seen in the figure 5 below.

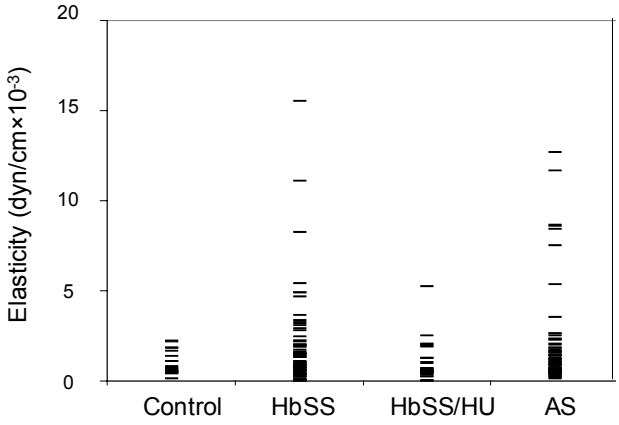

Figure 4. Distribution of red cell elasticity for sckicle cell anaemia study.

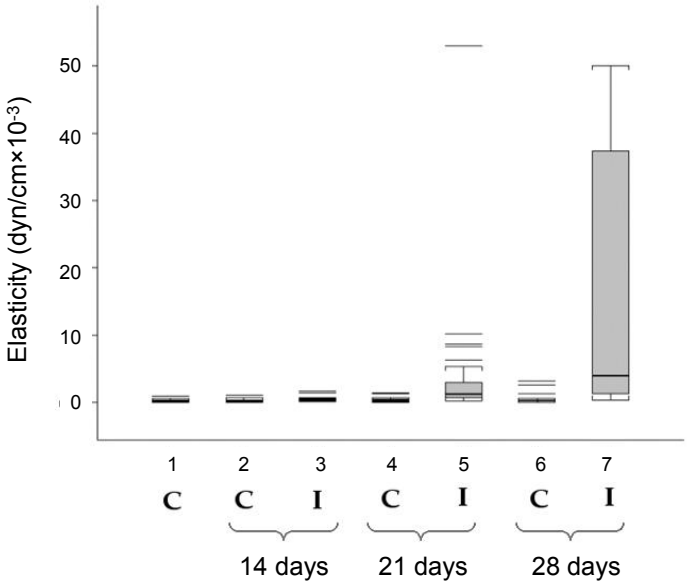

Figure 5. Distribution of red cell elasticity in all the subjects analyzed for irradiated and nonirradiated cells. Box plot, the black line represents the median and single lines over the boxes indicate the out liners.

Other techniques such as ektacytometry[7, 8, 9] and micropipette aspiration[10, 11] have been successfully used for analysis of HbSS red cell deformability. The micropipette technique, and optical tweezers, has the advantage of quantifying the rheological properties of individual cells rather than the average rheological behaviour of a cell population[12]. This individual cell analysis is necessary to detect differences between normal and abnormal populations. The micropipette technique measures, mainly, the cell membrane deformability[11] whilst the optical tweezers measures the entire cell deformability (overall elasticity) and not only the intrinsic membrane elasticity. In fact, Evans and colleagues[13] demonstrated that the rheological behaviour of the erythrocyte is provided by both membrane and cytoplasm properties; therefore, the overall elasticity is very sensitive to any change in the cell environment, such as the osmotic pressure, changes because of molecules attached to the cell surface or hemoglobin defects[14].

In conclusion, we showed that the laser optical tweezers technique was able to detect differences in the deformability of RBC from HbS subjects. Therefore, it was also able to detect an improvement in cell deformability after treatment with HU, indicating that laser optical tweezers is a very sensitive method and can be applied for detection of drug-response in sickle cell disease. The laser optical tweezers methodology also allowed us to conclude that there is no significant change in RBC elasticity over time for up to 14 days of storage, regardless of whether the unit was irradiated or not. In addition, units stored for more than 21 days after irradiation presented loss of elasticity properties. This modification could interfere in the mechanical behavior of the RBC in microcirculation. Taken together, our data suggest that irradiated RBC units stored for 21 days seem to be impaired, and the transfusion of these components may be more effective during the first 14 days of storage. Biochemical and morpholologic effects of irradiation on RBCs have been reported using doses from 15 up to 30 Gy and these effects can be accentuated by blood storage[15].

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