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# Mechanical Properties Measurement of Sickle Cell (SS) Red Blood Cells Using Optical Tweezers

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## Abstract

Red blood cell deformability is a crucial factor in blood flow. Since sickle cell anemia is a disease characterized by a non-conforming deformation of red blood cells (RBCs) in oxygen-deprived conditions. This thread-like shape causes poor blood circulation. We measured, and compared, Sickle RBCs deformability by lateral indentation using optical tweezers. We used a camera to acquire the various videos. The trapped microbead distributions were processed using Boltzmann statistics to calculate the optical trap stiffness and trapping force. Finally, the Hertz model was used to determine the mechanical properties of sickle cell red blood cells. The mean values of shear modulus measured were (3.94 ± 0.71) μN/m for the sickle RBC Type I, (8.54 ± 1.7) μN/m for sickle RBC Type II and (11.72 ± 2.05) μN/m for sickle RBC Type III. These results confirmed that lateral indentation is becoming an almost indispensable technique for characterizing red blood cells.

## Keywords

Sickle Cell Disease, Red Blood Cell, Shear Stiffness, Optical Tweezers, Indentation

## 1. Introduction

Hemoglobin is the substance contained in red blood cells that transports oxygen throughout the body. This substance is affected by the blood disorder known as sickle cell disease. This genetic anomaly is caused by sickle cell hemoglobin (HbS),

a variant of the hemoglobin (Hb) molecule resulting from a mutation in the $\beta$-globin gene [1]. Red blood cells are normally discoid in shape, but under certain conditions and in sickle cell disease, they can take on a sickle or half-moon shape. These rigid, sickle-shaped red blood cells cause occlusions in the blood vessels (vaso-occlusion), preventing the normal flow of blood and oxygen to the organs, particularly the bones, and are responsible for the most frequent manifestation of the disease: painful attacks. These abnormally shaped blood cells also have a considerably reduced lifespan when they leave the bone marrow, leading to chronic anemia. For the majority of the population, the hemoglobin in red blood cells is normal A (from "adult", unrelated to blood groups); genetically, their hemoglobin type is AA (half A inherited from each parent). Carriers of the sickle cell gene produce normal hemoglobin A and the characteristic hemoglobin S characteristic of sickle cell disease: they are AS. They do not have the disease. In the most common and severe type of sickle cell disease, patients have two S genes, transmitted by both parents: they are known as SS, or sickle cell anemia [2]. In other forms of the disease, the S hemoglobin is associated with other hemoglobin abnormalities: this is the case of the SC form. Sickle cell sufferers are thus likely to develop chronic conditions affecting the lungs, brain, kidneys, heart and eyes. Because of these complications and the limited choices for medical treatments, life expectancy for SCD patients is short; only 50% of patients with SCD survive beyond their fifth decade [3]. Hydroxyurea (HU) has been successfully used to treat this disease. In addition to improving fetal hemoglobin production, hydroxyurea is known to have a crucial effect on red blood cell deformability [4]. The forces exerted by light have little effect on the macroscopic scale. One of the few macroscopic examples is the orientation of comet tails, as discovered by astronomer Johannes Kepler in the early 17th century. The tail always points away from the sun, because the particles in it are driven by radiative forces. In 1873, James Clerk Maxwell theoretically demonstrated that light can exert forces. Given their very low values, it wasn't until the early 1960 and the development of lasers that these forces were studied. A pioneer in these studies was Arthur Ashkin. By focusing a laser beam, he was able to move and even levitate particles just a few microns in diameter. This research forms the basis of atom trapping and cooling, as well as the development of optical tweezers. He demonstrated the possibility of optically manipulating biological species without damaging them, such as viruses, bacteria [5] or living cells [6]. These results paved the way for numerous research projects and applications in the fields of biochemistry, physics and medicine. Hee Su Byu's team had to fit the power spectral density function of the measured membrane fluctuations to quantitatively determine the mechanical properties of sickle cell RBCs [7]. Some have used the rotation speed of red blood cells to differentiate between sickle cell red blood cells from a treated patient and those from a patient not treated with the HU drug [8]. Recently, optical tweezers have been successfully used to characterize red blood cells [9]-[11]. With a view to making our contribution to research into sickle cell disease, we propose in this work the lateral indentation of sickle cell red blood cells to extract their mechanical properties.

## 2. Experimental Procedure

The description of experimental setup has been well detailed in our previous articles [9] [12]. The experimental approach used in this work is shown in **Figure 1**. First, the silica bead was optically trapped without coming into contact with the RBC. The stage was then translated horizontally so that the red cell came into contact with the trapped microbead. Once contact has been made between the two, the microbead receives a force from the red blood cell, causing the microbead to move from its initial equilibrium position. In turn, the trapped microbead deforms the red cell membrane, inducing an Id indentation in the RBC membrane.

Each time the contact between the microbead and the RBC increases, the indentation force increases and membrane deformation becomes significant. It is therefore possible to measure the distance the microbead is pressed into the RBC membrane (the indentation Id) using this relationship [12].

$$I_d = \left(D - \sqrt{D^2 - d_i^2}\right) \tag{1}$$

where **D** is the diameter of the microbead and $d_i$ is the touch diameter between the microbead and the RBC in micrometers. Another parameter essential for calculating the shear modulus is the contact force between the RBC and the trapped bead. Parameters such as the trapping force and the contact force between the trapped microbead and the red blood cell were obtained using the Boltzmann statistical method [13]. The various steps are well detailed in our previous articles [12].

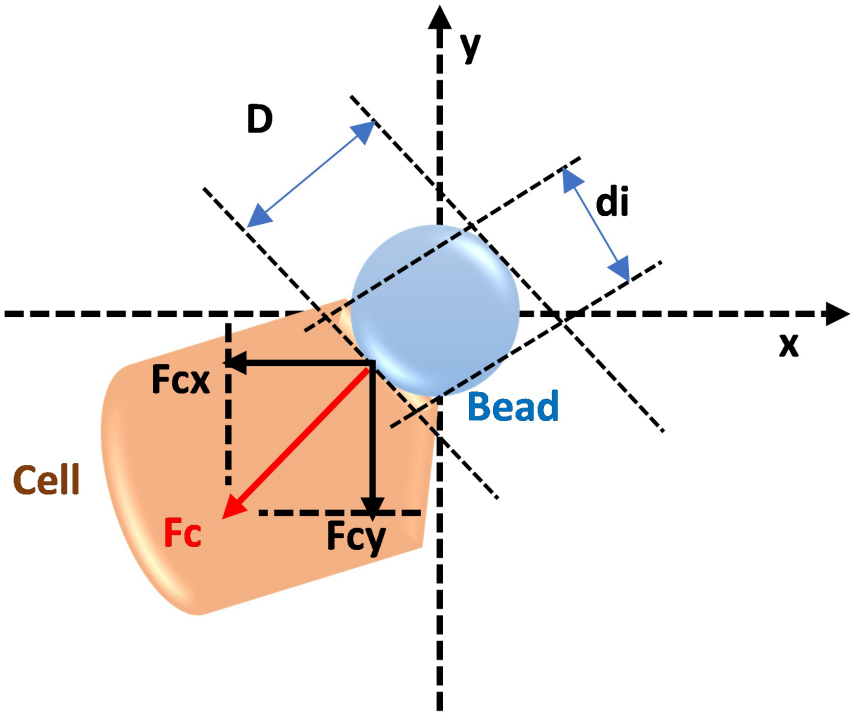


**Figure 1.** Interaction illustration between trapped bead and cell.

In this work, we determined the various parameters mentioned above, taking into account the two horizontal directions.

### 2.1. Cell Preparation

The particles used as indenters in these experiments were silica beads with a diameter of 3 μm. We used blood from a sickle-cell anemia patient (a single donor), type SS. To carry out the experiments, the sample was prepared as follows: 0.5 μl of blood was suspended in 5 ml of phosphate-buffered saline, then this solution was incubated with a dilute solution of microbeads.

### 2.2. Elastic Stiffness and Shear Stiffness

In our work, Hertz-model was used to obtain the elastic stiffness [12] [14] [15]. *Eh* is given by

$$Eh = \left[\frac{3\left(1-v^2\right)}{4\cdot\sqrt{I_c R}}\right]\cdot F_c \tag{2}$$

where $R$ is the microbead radius, $F_c$ the contact force between trapped bead and cell, $I_c$ the indentation and $v$ the Poisson ratio. For these experiments, $v = 0.5$ was used [15]. In the literature, usually the cortical shear modulus $Gh$ is given, rather than the elastic stiffness $Eh$. The Shear stiffness are related by:

$$Gh = \frac{Eh}{2\left(1+v\right)} \tag{3}$$

The Hertz model was used because for large deformations, the Hertz model leads to large prediction errors, and because the contact surfaces are small and flat.

## 3. Results and Discussion

We worked on five cells: one cell of almost spherical biconcave RBC (Type I), three cells of square biconcave RBC (Type II) and one cell of non-biconcave RBC (Type III).

### 3.1. Mechanical Properties of RBC (Type I)

For this first type of red blood cell, a microbead with a diameter of 3 μm was optically trapped with a force of 20.60 pN. By translating the sample holder in 5 μm steps in a horizontal direction, the contact diameter between the microbead and the red blood cell increases. As the contact between the two increases, so does the deformation of the red blood cell membrane. A number of different images from the videos recorded during each measurement are shown in **Figure 2**.

Under the action of a contact force of 14.36 pN, an indentation of 0.31 μm was measured. We then used the Hertz model to obtain the mechanical properties: (11.83 ± 2.51) μN/m for the elastic modulus and (3.94 ± 0.71) μN/m for the shear modulus.

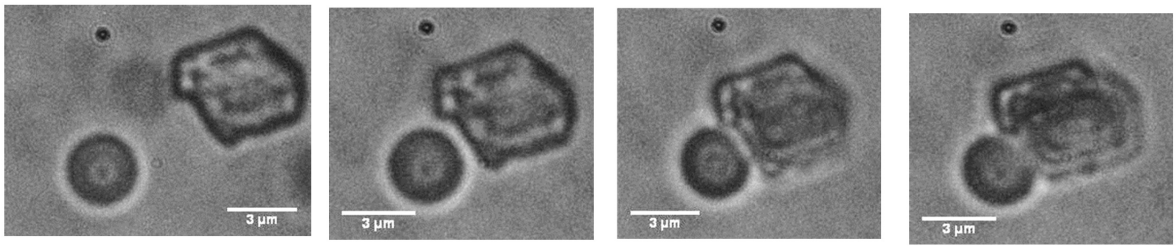


Figure 2. Images of RBC Type I indented at different contact forces.

### 3.2. Mechanical Properties of RBCs (Types II and III)

This time, the bead trapping force was 29.87 pN for RBC type II and 59.25 pN for RBC type III. Different images from the videos recorded during each measurement are shown in Figure 3.

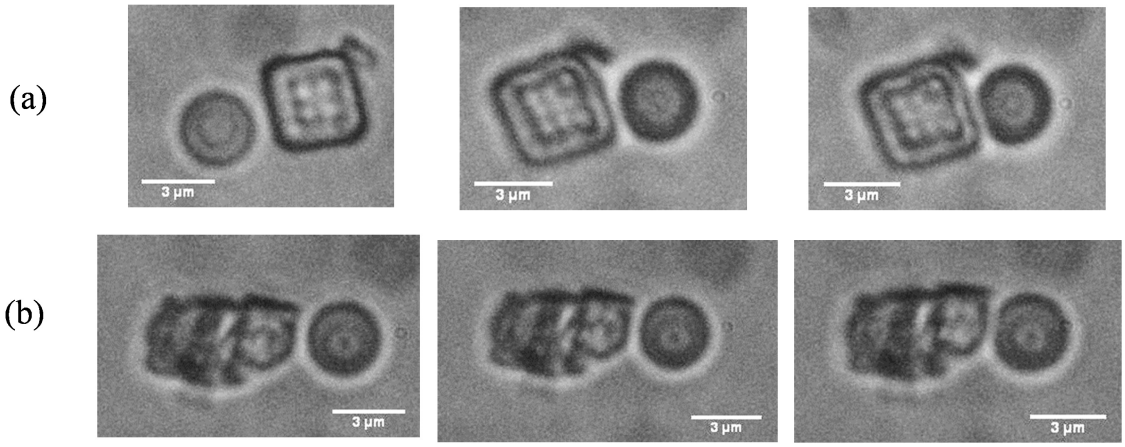


Figure 3. Images of RBCs indented at different contact forces: (a) RBC Type II, (b) RBC Type III.

The mean values of mechanical properties, in particular elastic modulus and shear modulus were (25.61 ± 3.12) µN/m and (8.54 ± 1.7) µN/m for RBC Type II and (35.17 ± 2.74) µN/m and (11.72 ± 2.05) µN/m for RBC Type III. Measurements of contact forces, indentations and elastic stiffness were presented in Table 1.

Table 1. Different values of measurements.

| Parameters | RBC (Type I) | RBC (Type II) | RBC (Type III) |
|---|---|---|---|
| Contact force (pN) | 14.36 | 26.74 | 31.85 |
| Indentation (µm) | 0.31 | 0.23 | 0.17 |
| Elastic stiffness (µN/m) | (11.83 ± 2.51) µN/m | (25.61 ± 3.12) µN/m | (35.17 ± 2.74) µN/m |

The values presented in the table show that for a low contact force, the microbead sank further into the RBC Type I than in the other two cases. The values of shear modulus for the sickle RBCs are shown in Figure 4.

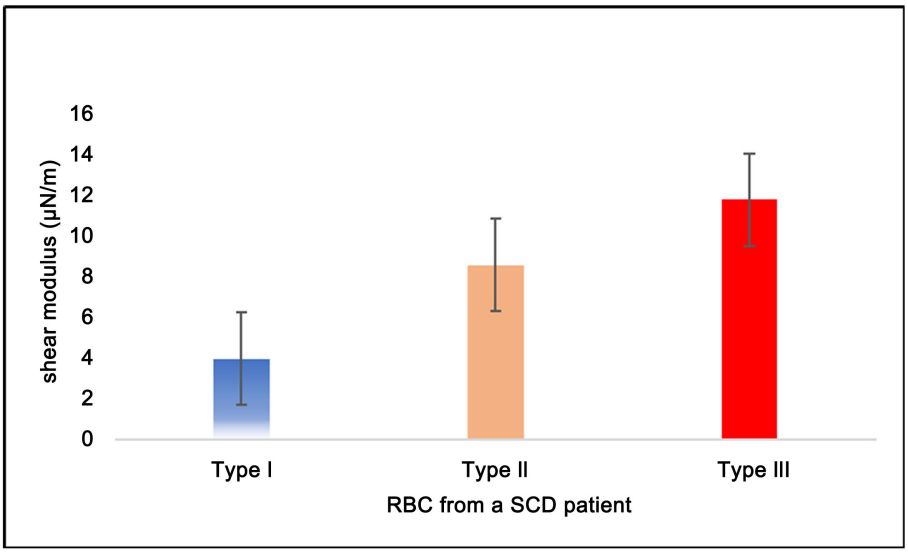


**Figure 4.** Mean values of shear modulus.

For sickle RBCs Types II and Type III, the values obtained are higher than that for the sickle RBCs Type I. These results show that RBC Type I are more elastic than RBCs Type II and Type III. This difference may be explained by the different membrane conditions of sickle cell RBCs. The values obtained for RBC Type I are supported by the results of previous work carried out on healthy red blood cells [9]. This can also be explained by the fact that when HbS-containing red blood cells are oxygenated, they can function relatively normally and retain a biconcave shape, although they are generally stiffer than normal red blood cells. Sickle RBCs Types II and III showed significantly decreased cell deformability compared to healthy RBCs [9] [16]. These results are consistent with previous works on sickle RBCs [17]. Note that, when the sickle RBCs releases oxygen into the tissues, hemoglobin S tends to polymerize, forming rigid fibers that deform the cell into a characteristic sickle shape. This process is reversible up to a point, but repeated cycles of polymerization and depolymerization progressively damage the cell membrane. With repeated polymerization of HbS, red blood cells become increasingly rigid and fragile. This rigidity hampers their ability to pass through small capillaries, leading to blood vessel obstruction.

## 4. Conclusion

In this paper, we used the lateral deformation of sickle cell red blood cells. Using the linearized Hertz model, we obtained the indentations and mechanical properties of the cells studied. These results showed us that lateral indentation remains an appropriate technique for characterizing any type of RBCs.

## Authors' Contributions

P. Yale., A. A-B. N'guessan., J.-M. E. Konin conceived and designed the experiments; P. Yale, A. A-B. N'guessan performed the experiments; P. Yale analyzed

the data; P. Yale wrote the paper. P. Yale, M. A. Kouacou and J. T. Zoueu revised the paper.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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