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Using Optical Tweezers to Study the Friction of the Red Blood Cells

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Abstract

In the last two decades the study of red blood cell elasticity using optical tweezers has known a rise appearing in the scientific research with regard to the various works carried out. Despite the various work done, no study has been done so far to study the influence of friction on the red blood cell indentation response using optical tweezers. In this study, we have developed a new approach to determine the coefficient of friction as well as the frictional forces of the red blood cell. This approach therefore allowed us to simultaneously carry out the indentation and traction test, which allowed us to extract the interfacial properties of the microbead red blood cell couple, among other things, the friction coefficient. This property would be extremely important to investigate the survival and mechanical features of cells, which will be of great physiological and pathological significance. But taking into account the hypothesis of friction as defined by the isotropic Coulomb law. The experiment performed for this purpose is the Brinell Hardness Test (DB).

Keywords

Friction, Indentation, Optical Tweezers, Tribology, Red Blood Cells

1. Introduction

The essential function of the red blood cell is the transport of gases throughout the body: oxygen to the cells and carbon dioxide to the lungs [1]. It has very high resistance to shearing thanks to the elasticity of its membrane and its biconcave shape, such a possibility allows it to pass through the capillary vessels without

however being altered and to return to its initial shape. Indeed, any disease or infection that affects the red blood cell membrane has an impact on its physical properties [2] [3] [4].

Given the important role it plays in the body, several studies have been carried out to determine certain mechanical parameters such as modulus of elasticity, shear modulus [5] [6] [7]. This has been possible because of the technological innovations recorded in recent decades in the field of micropipette aspiration [8], microfiltration [9], electric field [10], and atomic force microscopy [11]. Among these technological innovations we have: the optical tweezers which are positioned today as the indispensable tool for micromanipulation. This was justified by many works already done after the first works of Arthur Ashkin [12] [13]. It also owes its success in part to the work already done in the field of physics, biochemistry and biology, the biophysics of medicine, industry [14]. It should be noted that in the last two decades. the study of the elasticity of the red blood cell using the optical tweezers has experienced a boom in scientific research in light of the various work done [15] [16] [17]. The commonly used method is the indentation and the tensile test. Because of these different studies, no study has been done so far to study the influence of friction on the red blood cell indentation response by optical tweezers. However, measurements of interfacial forces of human red blood cells (RBCs) are extremely important to investigate the survival and mechanical features of cells.

However, the determination of the adhesion force is extremely complex due to the experimental variables commonly encountered [18]-[23]. But with the advent of new technologies and progress in Van des Waals (electrodynamic) theory [24] [25] based on the method of Lifshitz [26] have made it possible to calculate the forces of long-range attraction [27] and to make predictions about cell adhesion forces. As part of this study, we set up an experiment which allowed us to simultaneously carry out the indentation and traction test [28]. This experiment first allowed us to determine the tangential friction force of the human red blood cells as a function of the applied force or optical tweezers force via a microbead trapped. Then to directly quantify the adhesion force of the human red blood cell responsible for the attractive electrodynamic and electrostatic repulsive forces of biological cells as well as the friction coefficient of the silica red blood cell couple.

This allowed us to determine parameters such as the adhesion force (4.58 ± 1.08) pN, the deformation force (4.20 ± 1.16) pN as well as the friction coefficient (0.29 ± 0.01) of the red blood cell.

2. Materials and Methods

2.1. Description of the Experimental Device

As part of this work we used a photonic microscope in an inverted microscope configuration of the Thorlabs Optical Gripper Kit (**Figure 1**). This device is equipped with a near infrared laser diode (PL980P330J, λ = 980 nm), this diode

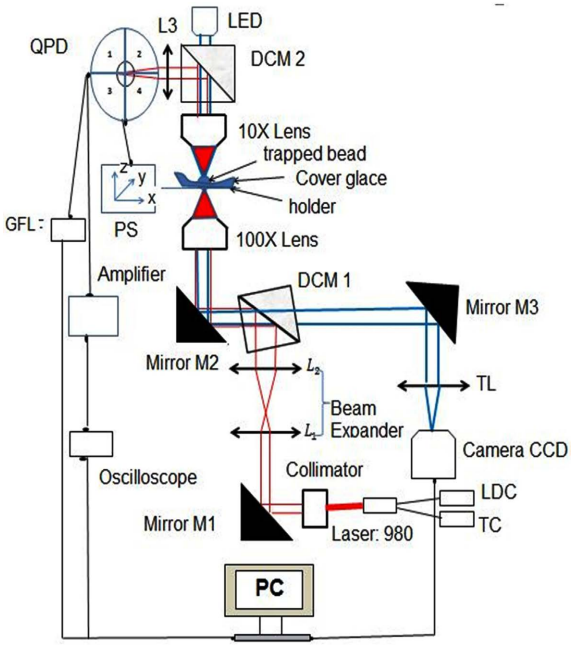

Figure 1. Optical tweezers setup for cell indentation and force measurement [15]. Laser trapping (980 nm) beam path (red) and bright-field imaging path (bleu). PS: 3-axis nano-piezo stage; TL: Tube Lens, L1 - L2 convergent lenses; DCM1-2 dichroic mirrors, TC: holder, QPD: Quadrant Photo Detector, Low-Frequency Generator (GFL), TC: temperature controller, LDC: Laser Diode Controller, PC: Computer (b) illustration of the different axes OXYZ.

laser is used for trapping through the immersion oil objective (Nikon 100X immersion, NA = 1.25 working distance 0.23 mm) with 330 mW power. The transmission of the laser beam is collected by an immersion air condenser (Nikon 10X, NA 0.25, WD 7) which transmits the signal to the QPD photodiode quadrant detector (Thorlabs, PDQ80A, detector size 7.8 mm). We have coupled this device with a digital oscilloscope and a low frequency generator, the signal produced by the photodiode is previously amplified by an amplifier [15].

2.2. Sample Preparation Protocol

As part of our study we used human blood obtained from the blood transfusion center in the Yamoussoukro district. This blood was first centrifuged at 3000 rpm in order to separate its different constituents. Then, we take 0.5 µl of this solution which contains humain red blood cells of 8 µm length and 0.4 µl of silica with a diameter of 2 µm; all in a 5 ml serum solution of Nacl. The solution resulting from the mixture formed the basis of our sample. For each measurement we take a volume of 5 µl of this mixture which we put in the sample holder for data acquisition. The experiment takes place at room temperature (ET, 25˚C).

2.3. Experimental Implementation

The implementation of this method does not require any particular provision. It

will be first to trap a microbead and attached to a moving red blood cell. Once the contact is established between the trapped microbead and the red blood cell in motion, then a sinusoidal voltage is applied to the sample holder (DC offset 3.5 Vpp) at 0.4 Hz frequencies [15]. As part of this study we chose to vary the intensity of the laser between 240 mA and 280 mA because of the stability of the trap and the safety of the laser because the laser used has a maximum power of 330 mW and a voltage 10 mV output connect to the laser intensity controller. For each intensity we acquire the microbead red blood cell pair by video microscopy. For video acquisition we record between 500 to 1000 frames. These measurements were carried out at the Instrumentation, Image and Spectroscopy Laboratory (L2IS), Institut National Polytechnique, Félix Houphouët-Boigny (INPHB), Yamoussoukro, Côte d'Ivoire.

The goal is to create sliding motion to the block and print a not insignificant speed. According to Bharat Bhushan in introduction to Tribology the sliding motion or any action to create sliding introduces a tangential force (or traction) called friction force T, active on both surfaces in a direction opposite to the sliding direction [28]. The direction of this force is opposed to the movement imposed by the sinusoidal voltage. This approach therefore makes it possible to create a sliding movement between the red blood cell and the trapped micro bead. Added to this is the fierce struggle between the already trapped micro bead and the red blood cell for the conquest of the focal point of the laser which will also allow the insertion of the micro bead into the red blood cell membrane when the red blood cell and the micro bead is found on the same axis.

2.4. The Brinell Hardness Test

The principle of this technique is to push through a trapped micro bead a normal force in the membrane of the red blood cell. Then extract the diameter of the spherical cap d to determine the radius a of this spherical cap (**Figure 2**).

The figure below shows the depression of the micro bead in the membrane of the red blood cell.

2.5. Adhesion to Friction

Figure 3 shows the case of a rigid sphere sliding on a deformable material. We will assume that this material is perfectly plastic. The contact pressure is therefore equal to the hardness H of the soft material. The forces express themselves thus:

$$F = HA_n \tag{1}$$

and

$$T = HA_t \tag{2}$$

where A_n and A_t are the contact areas projected in the normal and tangential direction, as shown in **Figure 3**. The coefficient of friction is then equal to the ratio of the two areas. The radius of contact a is smallest than the radius R of the sphere then [29]:

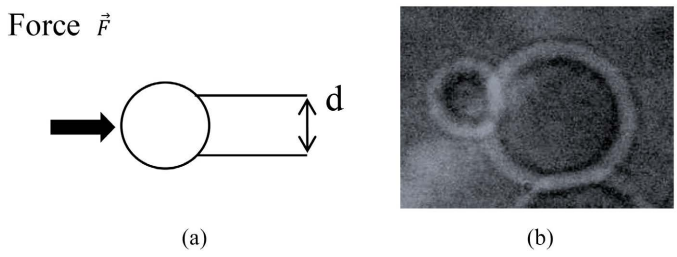

Figure 2. Illustration of the indentation: (a) experimental procedure of Brinell hardness; (b) pressing the micro beads of 2.5 μm in diameter in the red blood cell membrane.

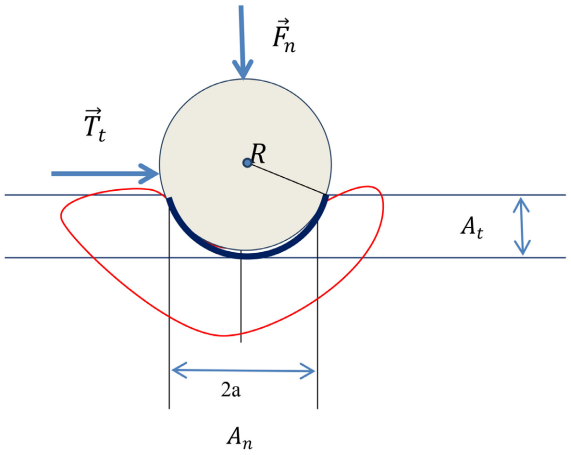

Figure 3. Illustration of the friction force of two spheres [29].

$$f = \frac{A_t}{A_n} \approx \frac{4}{3\pi}\frac{a}{R} \tag{3}$$

a: The radius of the spherical cap (radius of the embedded part).

It is through the ImageJ software that we directly extracted the different radii of the spherical cap from each recording. The determination of these values therefore allowed us to determine for each intensity the coefficient of the corresponding force friction.

2.6. Frictional Force

When the micro bead and the red blood cell moving will put in contact by the photonic force, it appears between the two particles a contact zone. Coulomb's law in mechanic expresses in a very simplified form the intensity of the friction forces exerted between two solids. Depending on whether these solids slide against each other, we talk about sliding (dynamic friction) or adhesion (static friction). Let $\boldsymbol{R}$ the force exerted by the red blood cell on the microbead in both cases, the reciprocal actions which are exerted between these solids comprise:

We have:

✓ A normal component $\boldsymbol{R}_n$ that presses them against each other,

✓ A tangential component $\boldsymbol{R}_t$ or T which opposes, or tends to oppose, sliding

$$\boldsymbol{R} = \boldsymbol{R}_n + \boldsymbol{R}_t \tag{4}$$

Consider the two solids in contact micro bead and red blood cell on the zone Γ (Figure 4(a) and Figure 4(b)). These solids are subjected to a normal force F on the border Γ_F.

2.7. Data Acquisition Method

Our experimental data was acquired by video microscopy using the CCD camera. It should be noted that microscopic video acquisition is better suited to determine the behavior of several particles in a field. It consists of filming the trapped micro bead and the red blood cell that tries to enter the trap. The different positions of the micro bead were extracted by ImageJ.

Calculation of the applied force, the applied force here represents the force of the optical tweezers, first before acquiring the images by video microscopy we have, using a calibration target, convert the pixels of the CCD camera into measurable unit. After extracting the different positions of the trapped microbead we used "Optimization Toolbox" and "Statistics Toolbox" by Iva Marija [30]: to calculate the stiffness constant of the optical tweezers. This toolbox uses the equitation theorem given by the following relation as well as Boltzmann's theorem.

$$\frac{1}{2}K_B T_\theta = \frac{1}{2}k\left\langle x^2 \right\rangle \tag{5}$$

$$k = \frac{K_B T_\theta}{\left\langle x^2 \right\rangle} \tag{6}$$

Or K_B is the Boltzmann constant and T_θ is the temperature. Then Hooke's law for the calculation of the force exerted by the micro bead on the red blood cell.

$$F_x = k_x \cdot \Delta x \tag{7}$$

With Δx the displacement along the Ox axis.

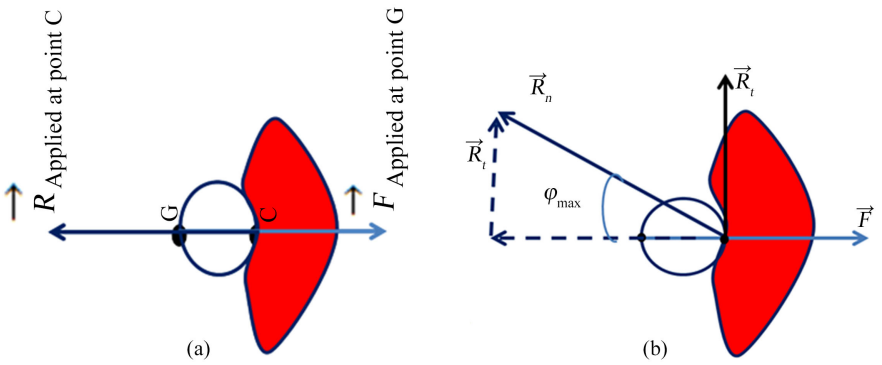

Figure 4. (a) No slip between the micro bead and the red blood cell (v = 0); (b) sliding motion after applying the sinusoidal voltage: sliding between the micro bead and the red blood cell (v ≠ 0).

During friction, the dissipated power is:

$$\mathcal{P} = TV = fFV \tag{8}$$

Coulomb's law uses a threshold that is proportional to the normal effort:

$$f = \left|\frac{T}{F}\right| \tag{9}$$

Two situations are deduced from Equation (9):

When $V \neq 0$, we speak of dynamic friction and

$$|T| = f|F| \quad \text{(slip)} \tag{10}$$

When $V = 0$, we speak of static friction and

$$|T| < f|F| \quad \text{(adhesion)} \tag{11}$$

In what follows, we will study the influence of friction on the response in indentation of the red blood cell. However, it should be noted that the friction force is the sum of the adhesive forces T_a and the plastic deformation forces T_d [29]:

$$T = T_a + T_d = (f_a + f_d)F \tag{12}$$

Indeed, when two objects A and B are in contact, there appears a phenomenon of adhesion. The force that results from this phenomenon is called the adhesion force. The adhesion force is the sum of three forces which are: the Van Der Waals force (F_{VDW}), the electrostatic force (F_{el}), and the capillary force (F_{cap}).

$$T_a = F_{VDW} + F_{cap} + F_{el} \tag{13}$$

3. Result and Discussion

The experiment described above allowed us to obtain the following results (**Figure 5**).

We observe through these images a change in the morphology of the human red blood cell. The red blood cell which before the measurement had a relatively biconcave shape for an intensity of 240 mA gradually took on the spherical shape for an intensity of 280 mA. This morphological change could be explained by the oscillatory movement imposed on the sample gates which favored the exchange of the red blood cell with its external environment due to its membrane permeability. This osmosis phenomenon led to the stiffening of the red blood cell membrane, which explains the depth of penetration which is high as the laser intensity increases before decreasing and remaining constant regardless of the increase in laser intensity. Indeed, the normal force applied or the force of the trap being a function of the intensity of the laser [31], we observe that as we increase the normal force applied, the electric field created by the laser beam also increases which leads to an increase in the force of attraction between the trapped microbead and the red blood cell which is attracted by this electric field towards the waist. It is through the force of action and the reaction that we witness the sinking of the microbead into the membrane of the red blood cell.

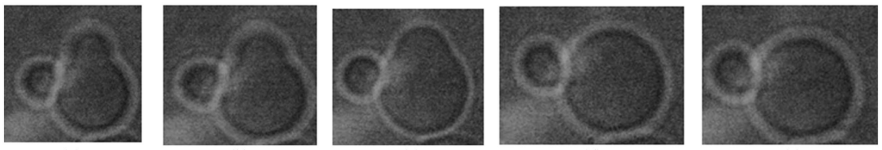

Figure 5. Acquisition of the microbead red blood cell couple as a function of different intensities: (a) 240 mA; (b) 250 mA; (c) 260 mA; (d) 270 mA; (e) 280 mA.

Laser intensity as a function of the penetration radius of the microbead into the red blood cell membrane. Determining the values of the radius of insertion of the microbead into the membrane of the red blood cell corresponding to each intensity value therefore allowed us to obtain the curve of the tangential force as a function of the applied force.

This graph below illustrates the evolution of the friction force T as a function of the trapping force applied to the red blood cell. Through **Figure 6**, it can be seen that the friction force can be static, maximum static or kinetic.

In this zone **AB**, the red blood cell is perfectly stuck to the microbead does not move, so the friction force increases to match the applied force. This is what results in the increase of the graph. In the linear part of the graph, the static friction force is also adhesive force. In this case, the sliding speed between the red blood cell and the micro bead is zero. The red blood cell and the micro bead are in equilibrium. The friction force scales with the force applied to the red blood cell until it reaches the maximum friction force. It should be noted that in this part, it is not really possible to say that the red blood cell is immobile but there is no sliding movement between the red blood cell and the microbead. Since the microbead is at the center of the trap, when the cell comes to stick to it after a while the two particles find themselves in a relatively stable state. In this zone, the friction force is less than the maximum friction force so the adhesive friction forces thus remain inside the Coulomb cone.

$$T_a < f_s F \tag{14}$$

Indeed, when the friction force reaches the maximum value, ie the limit equilibrium (strict).

At point B the friction force reaches a maximum, it is this force which represents the adhesion force of the red blood cell. It is from this force (friction force equal to applied force) that the red blood cell begins to slide relative to the microbead. The sliding phase is the phase during which the applied force remains constant according to Coulomb.

$$T_a = f_s F \tag{15}$$

In the case where the equality is obtained then one is at the limit between the adhesion and the slip:

$$f_s = \tan\varphi = \frac{\Delta T_a}{\Delta F} = 0.29 \pm 0.01 \tag{16}$$

And f_s is the adhesion coefficient of the microbead red blood cell couple, with φ angle of the friction cone.

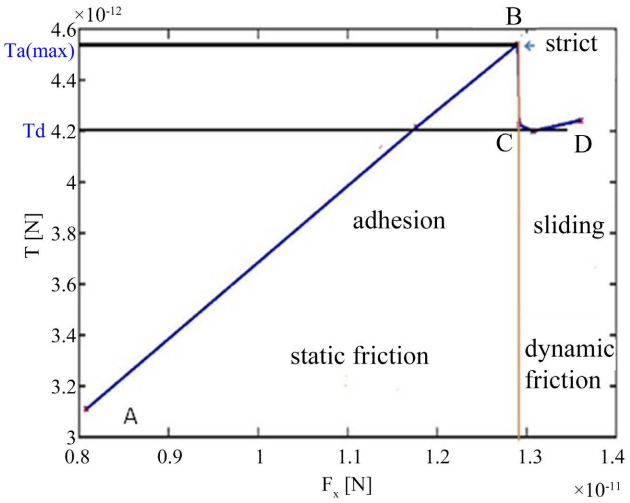

Figure 6. Force friction as a function of normal force applied.

The value of the adhesion force is obtained by projecting the maximum point of the curve onto the axis (Oy) (4.58 ± 1.08) pN and the force applied along the axis (Ox) corresponding to (12.6 ± 0.13) pN.

The method commonly used for the determination of the interaction force or adhesion is the Derjaguin-Landau-Verwey-Overbeek (DLVO) method. This method requires the knowledge of the mechanical properties of the material such as the Zeta potential, the Debye length, the Hamaker constant. But thanks to recent work by S. K Sainis [32], it is now possible to determine the interaction force of two particles intimately in contact in a liquid without knowing all these parameters [32]. But also through the graph, friction force as a function of the normal force applied, one can geometrically estimate the value of the adhesive force and the deformation force. This new approach also allows us to obtain these values without however knowing these parameters mentioned above.

Zone BC: The frictional force decreases as the object begins to slide relative to the microbead. There is at this point the contact breakage due to the oscillatory movement imposed on the block containing the red blood cell and the micro bead. This maximum value corresponds to a transient regime which represents the setting in motion of the two particles. It could be included in the sliding phase. This imposed oscillatory motion creates a sliding movement between the two surfaces. The red blood cell is set in motion relative to the micro bead and vice versa. There is thus a sliding speed between these two particles. This friction force is opposed to the oscillatory movements imposed on the system.

CD zone: The friction force is relatively constant in this zone, which reflects the sliding movement of the red blood cell and the microbead. This force represents the deformation force of the red blood cells which is graphically estimated at (4.20 ± 1.16) pN corresponding to the applied normal force which is also (13.0 ± 0.1) pN.

The linear part of the graph thus allowed us to determine the value of the coefficient of friction of the red blood cell which is 0.29 ± 0.01. We compared

this value to that of Gillian M Gunning, 2016 [33]. The latter during his work showed that when the percentage of the globule is between 20% and 80% the average value of the coefficient of friction varies very little, it is between 0.258 ± 0.093 and 0.247 ± 0.046. The study done by Toshiyuki HAYASE using the centrifugal microscope inclines confirmed that the friction characteristics of red blood cells were modeled as the sum of Coulomb friction with a coefficient of friction of 0.206. This result is in agreement with the one we obtained with the optical trap [34].

4. Conclusion

The method developed during this work has a considerable advantage. It makes it possible to determine the adhesive force without however knowing the mechanical properties of the material such as the potential Zeta, the length Debye, the constant of Hamaker. It allowed us to determine the deformation force of the red blood cell as well as the adhesive force. The coefficient of friction of the red-micro bead pair obtained is substantially equal to those of the literature. It can therefore be said that this approach will be welcome and could be used to characterize the surface properties of the pairs of materials. But doing a similar experiment requires a lot of patience and measuring the small contact area is an additional problem.

Author Contributions

Author Contributions (Appuyez-vous sur l'article de Pavel pour les contributions): P.Y., J.-M.E.K., M.A.K. conceived and designed the experiments; P.Y., J.-M.E.K. performed the experiments; P.Y. analyzed the data; P.Y., J.-M.E.K., M.A.K. and E.M. wrote and revised the paper. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data can be made available upon request to the corresponding authors.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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