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Human Eosinophil Cell Manipulation by Optical Tweezers

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Abstract

In this work, lateral deformation of human eosinophil cell during the lateral indentation by an optically trapped microbead of diameter 4.5 μm is studied. The images were captured using a CCD camera and the Boltzmann statistics method was used for force calibration. Using the Hertz model, we calculated and compared the elastic moduli resulting from the lateral force, showing that the differences are important and the force should be considered. Besides the lateral component, the setup also allows us to examine the lateral cell-bead interaction. The mean values of the properties obtained, in particular the elastic stiffness and the shear stiffness, were Eh = (37.76 ± 2.85) μN/m and Gh = (12.57 ± 0.32) μN/m. These results show that the lateral indentation can therefore be used as a routine method for cell study, because it enabled us to manipulate the cell without contact with the laser.

Keywords

Optical Tweezers, Human Eosinophil Cell, Indentation, Shear Stiffness

1. Introduction

Apart from red blood cells function which is to transport oxygen to body cells and deliver carbon dioxide to the lungs, the eosinophil role in human health and disease has received considerable attention [1]. The eosinophil has a vital role in allergic inflammatory processes that include asthma [2] [3]. Evidence implicates the eosinophil and its granule proteins in host resistance to parasites, particularly helminths, but also antimicrobial activities toward bacterial, viral, and protozoan pathogens, and as mediators of hypersensitivity diseases. The migration of

eosinophils from the circulation into tissues involves a stepwise interaction between eosinophils and endothelial cells [4]. The steps are mediated by adhesion molecules on endothelial cells and counter-ligands on eosinophils and are followed by the passage of eosinophils between endothelial cells (**Figure 1**). The eosinophilic undergoes a deformation at the time of its transmigration. It should be noted that, apart from the important role of eosinophils, eosinophilic is defined as a high number of peripheral blood eosinophils. Patients who sometimes have very severe eosinophilic, usually in the case of chronic eosinophilic leukaemia, develop complications when the eosinophils form aggregates that occlude small blood vessels, causing tissue ischaemia and microinfarctions [5].

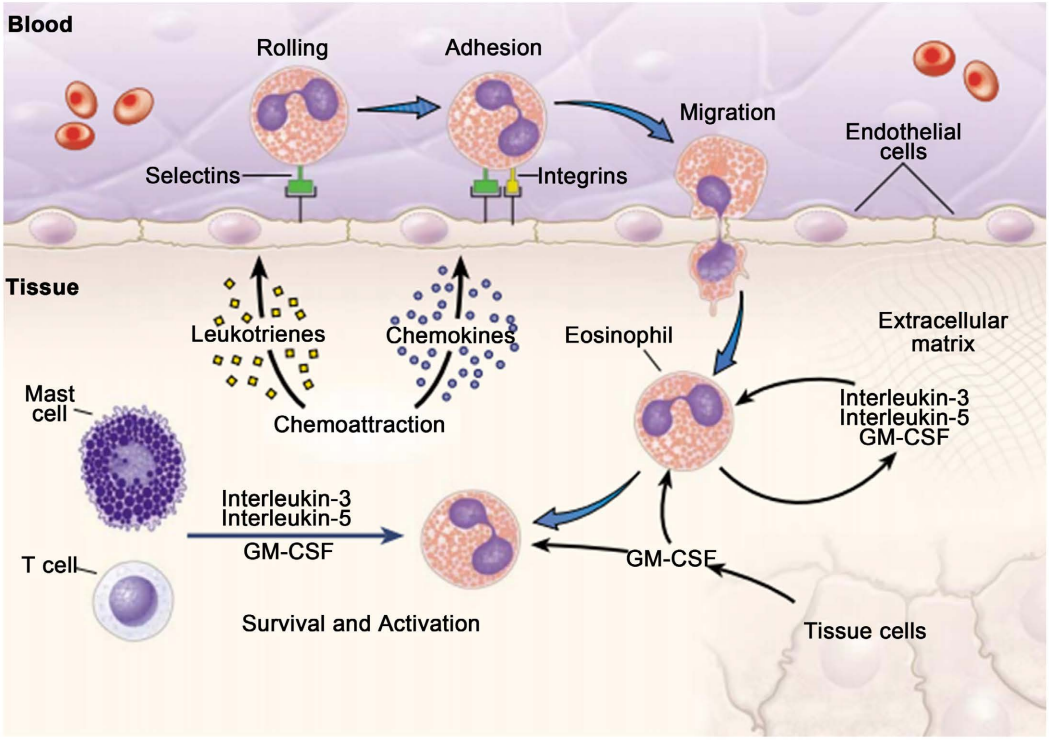

Figure 1. Processes involved in eosinophilia [4].

Manipulation of biological specimens using light microscopic is a subject of increasing interest, due to its applicability and relevance to fundamental research [6]. Optical tweezers has been demonstrated to be able to conveniently manipulate the single living cell as a noninvasive trapping method [7]. Two glass or polystyrene beads attached to a human red blood cell (RBC) are manipulated by two focused laser beams to stretch the cell and study its mechanical characteristics [8]. The local elasticity of HBL-100 cells, an immortalized human cell line, originally derived from the milk of a woman with no evidence of breast cancer lesions was studies using optical tweezers [9]. Recently, study of RBCs deformation using OT has been performed [10]-[12]. Cell elasticity can be locally measured by pulling membrane tethers, stretching or indenting the cell using optical tweezers. In this paper, we propose a study of human eosinophil cell deforma-

tion, to determine their mechanical properties.

2. Experimental Setup

In this section, we describe in detail the equipment and controls used for the experiment. Figure 2 shows the experimental setup. The optical tweezers setup consists of a Diode Laser (PL980P330J) at a wavelength of 980 nm with an output power of up to 330 mW. A large numerical aperture (NA 1.25) Nikon 100X oil immersion objective (MRP01902) was used to focus a laser beam and form an optical trap. A white LED source is mounted above the optical trap in order to illuminate a sample with light in the visible part of the electromagnetic spectrum. The forward-scattered light transmitted from the sample is collected by a Nikon 10X air condenser. The sample is mounted on a 3-axis piezo translation stage (MAX301) with strain gauge feedback. The particles used in these experiments are silica beads with diameters of 4.5 μm (bangs laboratories, Inc.), and human eosinophils cells. In order to prepare a sample for our experiment, 0.5 μl of blood was immediately suspended into 5 ml phosphate-buffered saline (PBS) and this solution was incubated with beads concentration of 4.5 μm diameter. The silica bead was attached to the eosinophil membrane. The images were captured using a CCD camera and recorded onto a videotape. The video images were then downloaded onto a computer and digitized for image analysis. The individual frames of the recorded movies were analyzed by using the Image-J software. The laser power measured after the objective used in our experiments covered the range from 62 mW to 76 mW.

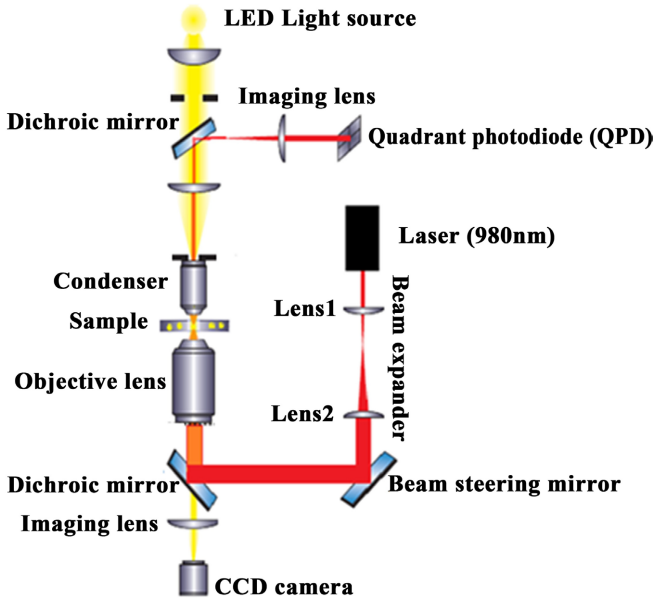

Figure 2. Setup scheme used for experiments.

3. Experimental Procedure

Cell elastic modulus, is measured locally by horizontal indentation using a trapped

microbead as probe. Before the measurement, the silica bead is trapped and a video in which, there is no contact between the trapped silica bead and the eosinophilic one is recorded. From this video the force which maintenance the silica bead in the trap is calculated. Two images resulting from this video are presented in Figure 3.

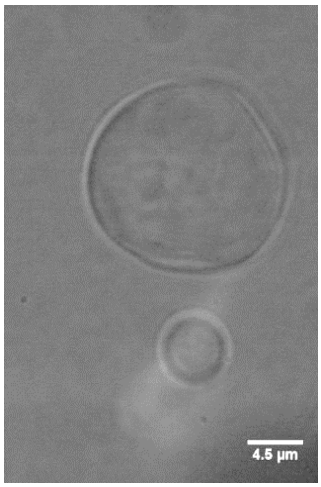

Figure 3. Optical microscope image of an eosinophil cell and a trapped silica microbead.

The experimental approach is illustrated in Figure 3. The silica bead is optically trapped above the cell, without touching it. Then the stage is displaced horizontally so that the cell comes into contact with the trapped silica bead. When the cell intercepts the bead, it exerts a force causing a displacement of the bead from the trap equilibrium position. The bead also begins to push the cell, inducing an indentation *Hc* in the cell membrane. With each time the distance between the silica bead and the cell decreases, the indentation force increases and the deformation becomes significant. Figure 4 shows some images of the cell deformation according to the force applied.

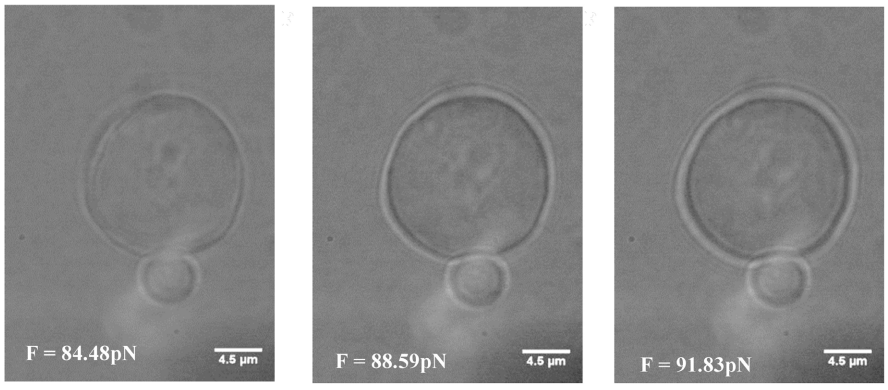

Figure 4. Image of indented eosinophil at different applied forces.

It is possible to measure the bead movement into the cells (the indentation *Hc*) by this relation:

$$Hc = \frac{1}{2}\left(D - \sqrt{D^2 - d^2}\right) \tag{1}$$

where D is the microbead diameter and d is the measured mean diameter of the indentation in micrometer (See Figure 5).

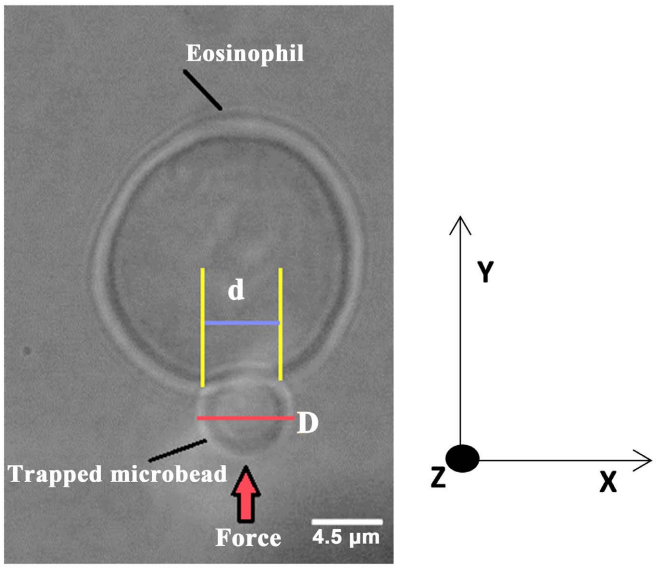

Figure 5. Image of eosinophil indentation using a trapped microbead of 4.5 µm diameter.

Another parameter required to calculate the hardness, elasticity is the contact force between the cell and the microbead: $F = K \cdot \Delta l$ where K is the trapping stiffness and Δl the microbead displacement. Video analysis is used to locate the microbead center of mass for each frame, and reconstruct the microbead path. Image-J software has been used to reconstruct the XY-path of the microbead from a video recording of its Brownian movement.

We used the Boltzmann statistics to obtain the microbead trap stiffness. The optical potential reconstruction using Boltzmann statistics can be used to determine any continuous trapping landscape in the accessible region by thermal agitation [10]. In equilibrium the probability density $\rho(x)$ of the 1D particle position is given by:

$$\rho(x) = C \exp\left(\frac{-U(x)}{K_B T}\right) \tag{2}$$

where C is a normalization constant and $U(x)$ is the trap potential. The shape of $U(x)$ can be obtained from the normalized histogram of the trapped bead positions as

$$U(x) = -\ln\left[\rho(x)\right] \tag{3}$$

In the case of the commonly used TEM_{00} Gaussian trapping beam, which results in a harmonic trapping potential, one can fit a parabola $y = ax^2 + b$ to the data in the central region of the potential to extract the trap stiffness and check for possible deviations from the perfect harmonic shape. The stiffness coefficient

$K = 2a/K_B T$ obtained in such manner is more accurate than (2). Another advantage of such calibration is that it also gives the information about the potential in the region away from the trap center where the optical potential is non-harmonic.

4. Elastic Stiffness and Shear Stiffness Calculation

In our experiments, we used the Hertz-model to obtain the elastic stiffness [10]. *Eh* is given by:

$$Eh = \left[3\left(1-\nu^2\right)/\left(4\sqrt{H_c.R}\right)\right]\cdot\left(F/H_c\right) \tag{4}$$

where R is the microbead radius, F the force, H_c the indentation and ν the Poisson ratio. For these experiments, we used ν = 0.5. In the literature, usually the cortical shear stiffness *Gh* is given, rather than the Young's modulus *Eh*. The quantities are related by:

$$Gh = Eh/2\left(1+\nu\right). \tag{5}$$

5. Results and Discussion

We investigated the elastic modulus of eosinophil cell lines c onsidering the optical tweezers indentation experiment described in Section 2. In order to measure their elasticity, the cells were indented in the horizontal direction by a 4.5 μm diameter silica bead that was held in the optical trap. By moving the cell against the trapped bead in the horizontal direction, the bead displacements in lateral direction were measured. Using the Hertz model, we calculated then the elastic moduli corresponding to the total force *F*. We considered the indentation interaction between cell and bead (see **Figure 3**) and calculated the indentation elastic modulus *E*.

We obtain the Force-indentation (F-Id) curves, shown in **Figure 6**. As one can see from this figure, the curve is almost linear, indicating that the behavior of the eosinophil at low indentation forces is elastic.

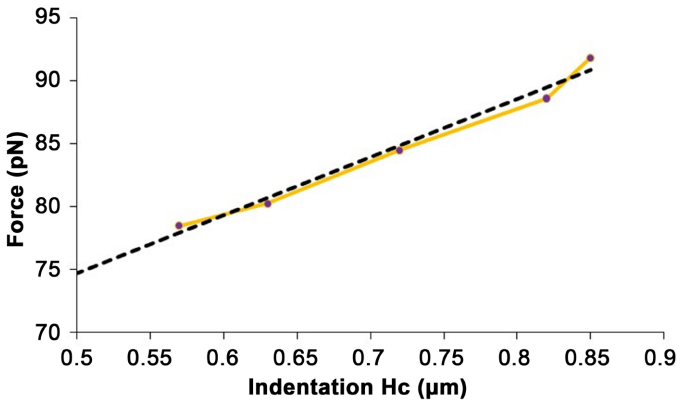

Figure 6. Example of force-indentation plots taken for indentation intervals.

In this work, we worked on two cells of the same sample. Five series of mea-

surement were carried out on each cell and a minimum time 3 minutes to pass from a series to another.

Table 1 shows the whole of measurements of Elastic stiffness and Shear stiffness obtained on the 2 cells. Each value presented is the value resulting from the maximum force applied for each cycle of deformations carried out to the same cell. The number of cycles of deformations carried out is indicated between brackets.

Table 1. Mechanical properties values of the studied eosinophils.

Measure	*Elastic stiffness* *Eh* (pN/m)	*Shear stiffness* *Gh* (pN/m)
(1)	37.26	12.42
(2)	37.33	12.44
(3)	37.90	12.63
(4)	36.62	12.21
(5)	38.97	12.99
(6)	41.79	13.93
(7)	37.38	12.46
(8)	41.40	13.80
(9)	31.56	10.52
(10)	37.39	12.46
Mean	**37.76 ± 2.85**	**12.57± 0.32**

The mean values of the properties obtained, in particular the elastic stiffness and the shear stiffness, were Eh = (37.76 ± 2.85) μN/m and Gh = (12.57 ± 0.32) μN/m. The shear stiffness value calculated for the eosinophils is of the same order of magnitude as those obtained by the method of the aspiration in a micropipette [13] ($4 < Gh < 10$ μN/m), only higher. Micropipette aspiration is the most established technique for measuring cellular elasticities, and the value for the shear stiffness of the RBC membrane has been confirmed many times and is well accepted. Before migrating into infected tissues, eosinophils undergo excitation, allowing them to acquire a high degree of elasticity to enable them to migrate. These results confirm that the eosinophils studied in this work were in their fundamental state.

6. Conclusions

In this work, we used a simple optical tweezers setup to measure eosinophil-bead interaction by lateral displacement of the eosinophil against a trapped bead. The linearized Hertz model was used to calculate the lateral indentations resulting from lateral forces.

The use of this indentation technique enabled us to manipulate the cell without it not being in contact with the laser and the glass surface. Our future work

will focus on characterizing eosinophils excited or extracted from tissues.

Author Contributions

P. Yale, J.-M. E. Konin, A. A-B. N'guessan conceived and designed the experiments; P. Yale, J.-M. E. Konin, A. A-B. N'guessan performed the experiments; P.Yale analyzed the data; P. Yale, J.-M. E. Konin, M.A. Kouacou and J. T. Zoueu wrote and revised the paper.

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Conflicts of Interest

The authors declare no conflicts of interest.

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