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Substrate-dependent cell elasticity measured by optical tweezers indentation

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ABSTRACT

In the last decade, cell elasticity has been widely investigated as a potential label free indicator for cellular alteration in different diseases, cancer included. Cell elasticity can be locally measured by pulling membrane tethers, stretching or indenting the cell using optical tweezers. In this paper, we propose a simple approach to perform cell indentation at pN forces by axially moving the cell against a trapped microbead. The elastic modulus is calculated using the Hertz-model. Besides the axial component, the setup also allows us to examine the lateral cell–bead interaction. This technique has been applied to measure 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. In addition, we have studied the influence of substrate stiffness on cell elasticity by performing experiments on cells cultured on two substrates, bare and collagen-coated, having different stiffness. The mean value of the cell elastic modulus measured during indentation was 26 ± 9 Pa for the bare substrate, while for the collagen-coated substrate it diminished to 19 ± 7 Pa. The same trend was obtained for the elastic modulus measured during the retraction of the cell: 23 ± 10 Pa and 13 ± 7 Pa, respectively. These results show the cells adapt their stiffness to that of the substrate and demonstrate the potential of this setup for low-force probing of modifications to cell mechanics induced by the surrounding environment (e.g. extracellular matrix or other cells).

1. Introduction

The pioneering work by Ashkin et. al. on the trapping of micro-particles and their manipulation by radiation pressure [1,2] led to the foundation of a new tool called optical tweezers (OT), which has found a multitude of applications in physics, chemistry and biology [3–5]. An important achievement for biology was the first demonstration that living micro-organisms (e.g. viruses, bacteria) could be manipulated by OT without being damaged [6]. This was followed by trapping and manipulation of single cells [7] and cell-organelles [8] using infrared (IR) laser beams.

Although the levels of intensity were high (typically tens of MW/cm^2, roughly corresponding to focusing 100 mW beams on an area of about 1 μm^2), the use of IR laser beams proved to be non-damaging to cells. Another important achievement was the measurement of the forces generated by organelle transport in vivo [9] and the use of a trapped microbead to probe forces in single-cell and single-molecule experiments [10–12]. OT versatility is highlighted by the wide range of applications which this technique has enabled: OT are now being used in the investigation of an increasing number of biochemical and biophysical processes, from the basic mechanical properties of biological polymers to the multitude of molecular machines that drive the internal dynamics of the cell [13]. Since OT forces are in the range from 1 to 200 pN and trap stiffness is in the range 0.001–1 pN/nm, it represents a complementary tool to other techniques for manipulation and force probing, such as atomic force microscopy (AFM) or magnetic tweezers [14,15].

Cellular processes as motility, adhesion, cell division and proliferation, involve mechanical forces in the range of the above-mentioned techniques, making them ideal tools for investigating the mechanisms of such processes. There is a continuous biomechanical interaction

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between the cells and their extracellular matrix (ECM), and with other cells, leading to the modification of the cell biomechanics [16–18]. Moreover, the hypothesis that cellular biomechanics may play a significant role in tumorigenesis, cancer invasion and metastasis gains more and more support [19–21], although it is not yet fully understood how the transformation from healthy to malignancy alters the mechanical properties within the tumor microenvironment [22,23]. Among the various properties of cell mechanics, viscoelasticity has been the most widely investigated, e.g., using membrane tether pulling, cell stretching and indentation, all of which can be implemented in AFM and OT setups [24–27]. AFM applies higher forces to the sample than does OT (tens of pN compared to tens of nN) and the probe is stiffer (cantilever stiffness > 10 pN/nm). Since the response of the cell to mechanical stress depends on the applied force and the stiffness of the probe, these parameters need to be taken into account when interpreting measurements. Moreover, since the mechanical properties of biological samples depend on the loading rate (measured in N/s), quantifying the viscoelastic properties of a cell makes little sense without also defining the loading rate at which this property was measured [28,29]. In fact, the combination of both techniques allows the investigation of single cells at small and large forces and/or loading rates, enabling a more complete characterization of cell mechanics. A first comparative study of cell elasticity measurements by vertical indentation using AFM and OT was reported for 3T3 fibroblasts [30]. For comparable loading rates, indeed, the elastic modulus determined by OT is much smaller than that calculated by AFM. So far, AFM has been more frequently applied to cell elasticity measurement, due to the faster development of this technology for cell biology. However, the lowest force that can be reliably controlled in AFM is of the order of tens of pN, and these forces can already lead to a strain large enough to enter the non-elastic deformation regime [18]. Therefore OT, can provide in many cases a more appropriate and useful tool for a detailed understanding the properties of the cellular composite material. Another implementation of axial cell indentation using an OT setup was recently reported for the measurement of localized cell stiffness of Balb3T3 cells [31]. In both OT implementations [30,31] a trapped microbead is axially pushed against the cell by moving the trap, and cell indentation is determined by measuring the axial displacement of the bead.

In this paper, we propose an alternative OT approach to perform cell indentation at pN forces by axially moving the cell against the trapped microbead and measuring its displacement. Since the position of the trap is fixed, the displacement of the microbead directly reflects its interaction with the cell, avoiding possible interference with drifts during trap axial displacement. Therefore, this solution is conceptually more precise than the trap displacement solution used in previously mentioned works [30,31]. The optical setup and the measurement approach are presented in Section 2. The technique has been applied to measure the elastic modulus of HBL-100 cells. HBL-100 has already been used as a non-neoplastic model when studying the properties of breast cancer cell lines, such as MCF-7 or MDA-MB-231, but it has not been characterized from the mechanical point of view. Cell preparation is described in Section 3. In Section 4 we report measured cell elasticity for cells cultured on two different substrates: bare and collagen coated glass cover slips, and demonstrate the adaptability of cells to the substrate stiffness (knowing that cells sense their mechanical environment and change their response accordingly).

2. Apparatus and protocol for OT cell indentation

2.1. Optical tweezers setup

A modular Thorlabs optical tweezers kit [32] with some modifications has been used in this work. We also performed multiple trapping experiments with this setup (using dynamic arrays of traps generated by diffractive optical elements [33]) and for that reason we replaced the original laser trapping source (single mode laser diode, 975 nm, max 300 mW) by a more powerful IR laser (single mode Yb fiber laser YLM-5, 1064 nm, max 5 W, IPG Photonics GmbH), as shown in Fig. 1. The laser head has a built-in collimator providing a TEM00 laser beam with a diameter $D=5$ mm. After reflection by mirror M1 (which helps for alignment) the beam passes through a 2X beam expander, increasing its diameter to slightly overfill the entrance pupil of the microscope lens (Nikon 100X, NA 1.25 oil immersion, WD 0.3). The laser beam is focused into the sample chamber by the microscope lens, where a silica microbead is trapped at the point of focus. A home-made temperature controlled holder [24] is connected to the sample chamber (a Petri dish) to keep the cells at the physiological temperature, $T=37\ ^{\circ}C$ during the experiments. This is mounted on a nano-piezo cube, PS, (Thorlabs, NanoMax 3-axis flexure stage) allowing 5 nm control of the sample displacement. A second microscope lens (Nikon 10X, NA 0.25, WD 7) collects the laser light scattered by the trapped bead. The scattered light interferes in the back focal plane (BFP) of the second lens. The interference pattern (IP) is imaged by lens L3 ($f=40$ mm) onto the quadrant photo detector, QPD, (Thorlabs, PDQ80A, detector size 7.8 mm) which senses the lateral and axial displacement of the trapped bead, as indicated. When the bead is in the equilibrium position, the IP is centered on the QPD. A lateral displacement of the bead is indicated by an IP lateral displacement, while an axial displacement is indicated by the change in size of the IP. The lateral and axial differential signals (ΔX, ΔY, ΔZ) are obtained combining the signals from the quadrants 1–4 as follows:

$$\Delta X=[(1+4)-(2+3)]\ ;\ \Delta Y=[(1+2)-(3+4)]\ ;\ \Delta Z=[1+2+3+4] \tag{1}$$

The differential signals are acquired through a digital acquisition card (DAQ – NI USB 2561) and a custom LabView code running on a PC. As the QPD has a large bandwidth (150 kHz), it can measure very well the thermal movement of the bead in the trap, characterized by a maximum bandwidth of 1–2 kHz. The sample is illuminated by the light from a LED through the second microscope lens. The sample is imaged by the first microscope lens and the tube lens (TL) on the sensor of a CMOS camera (Thorlabs, DCC 1240C).

2.2. Experimental procedure.

Cell indentation is observed by moving axially the cell against the trapped bead, as shown in Fig. 2. When contact is made, the bead will try to resist cell advancement, producing an indentation of the cell. As the stage displacement (SD) is known and bead displacement (BD) can be measured by BFP interferometry as previously shown, it is possible to measure the bead movement into the cells, i.e. the indentation, Id:

$$Id = SD - BD \tag{2}$$

Another parameter required to calculate the elasticity is the force, F, exerted by the cell on the bead. This is given by the linear relation:

$$F = k \cdot BD \tag{3}$$

where k is the stiffness of the optical trap. This linear relation for the force is valid for a limited range of BD (± 500 nm) [14].

At the beginning of each single cell experiment, a bead is trapped and a cell is positioned slightly below it, preventing cell–bead contact. The microscope image of a HBL-100 cell under a trapped bead is shown in Fig. 2c. The PS is then vertically displaced with a sinusoid signal (amplitude $A=1.14$ μm, one period $T=5$ s) as shown in Fig. 3, and the vertical displacement of the bead in the trap is acquired at a 10 KHz sampling frequency (dark blue curve).