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Elasto-mechanical properties of living cells

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ABSTRACT

The possibility to directly measure the elasticity of living cell has emerged only in the last few decades. In the present study the elastic properties of two cell lines were followed. Both types are widely used as cell barrier models (e.g. blood-brain barrier). During time resolved measurement of the living cell elasticity a continuous quasi-periodic oscillation of the elastic modulus was observed. Fast Fourier transformation of the signals revealed that a very limited number of three to five Fourier terms fitted the signal in the case of human cerebral endothelial cells. In the case of canine kidney epithelial cells more than 8 Fourier terms did not result a good fit. Calculating the correlation between nucleus and periphery of the signals revealed a higher correlation factor for the endothelial cells compared to the epithelial cells.

1. Introduction

All living organisms are dynamic systems, driven by well defined and synchronized mechanical processes between different parts of the whole [1–3]. The smallest living unit, with a complex function, is the cell. Uncountable types of cells, each with different well determined functions can form an organ or organism. To understand the function of a highly complex organism first we have to know its basic unit, the cell. Distinctive role have the barrier forming cells, which separate and link selectively important parts of a body.

Regarding the proper homeostasis of the Central Nervous System (CNS) the importance of the cerebral endothelial cells (CEC) cannot be questioned. They constitute the structural basis of blood-brain barrier (BBB), having crucial role in the control of trafficking substances across their membrane [4,5] to and from the CNS. While cerebral endothelial cells have a principal role in the maintenance of the homeostasis of the CNS, epithelium of the renal distal tubule contributes to the ion homeostasis of the organism. Although they are intensively studied, still limited information is available about their function, or how their internal molecular alterations manifest in mechanical properties such as in elasticity coefficient or molecular adhesion [6].

A rather new tool for determining mechanical properties, such as the elasticity of a microscopic object, is the atomic force microscope (AFM) [7,8]. Besides atomic resolution surface topography, the AFM can provide quantification of micro-mechanical parameters as well. A great advantage of it is that the measurements can be performed not only in vacuum, but in air or in liquid environment at human body temperature. The later is indispensable in case of living cells [9,10].

Mechanical properties of individual cells are strongly connected to biological functions, dynamically linked to both internal and external stimuli. Measuring the time dependence of mechanical properties of a biological system, spontaneous quasi periodical oscillation could be observed. Oscillation can appear in open nonlinear dynamic system. Biological systems fulfill these conditions [1,11]. The first documented biological oscillation was described by Luigi Galvani in 1780. Just to name few examples when oscillation was observed: mechanical and electrical oscillation in cardiac muscle of the turtle [12] drosophila tissue motion [13], oscillation of the elasticity and adhesion of vascular smooth muscle cell [14], shape oscillations of human neutrophil leukocytes [15], bronchial epithelial cells [16] elasticity oscillation of the cerebral endothelial cells [17]. The period of these oscillations show large variability, spanning from seconds to hours. Although more and more type of cells are intensively studied and several oscillating cells were investigated, apart of induced contractions e.g. muscle cells, the conditions when and why they emerge is mostly undeciphered.

In the present study the elastic oscillation measured on human brain micro-vascular endothelial and canine kidney epithelial cells were investigated and compared. Both cell lines are widely used as models mainly of the Blood-Brain barrier [18]. While the vascular endothelial cells are constantly exposed to mechanical forces from the blood stream the epithelial cells do not have to withstand

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shear forces. Hence their response to mechanical stimuli may result in completely different manifestation. All these information can help to understand the origin of emerging oscillations.

2. Materials and methods

2.1. Cell culture

The human cerebral micro-vascular endothelial cells (hCMEC/D3 - shortly D3) were grown on rat tail collagen-coated dishes in EBM-2 medium (Lonza) supplemented with EGM-2 Bullet Kit (Lonza) and 2.5% Fetal Bovine Serum (FBS) from Sigma [19,20]. MDCK (Madin-Darby canine kidney) cells were maintained in DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) (Lonza) supplemented with 5% FBS.

Cells were cultured at 37 °C, in 5% CO_2 atmosphere, seeded at $1.5*10^4$ cell/cm^2 in Falcon Petri dish (lid) with 3.5 cm diameter. MDCK monolayers were fed with fresh medium first after 24 h (post-seeding) than every second day until they reached confluence (3rd day).

All measurements were performed in serum free Leibovitz medium (Sigma) at 37 °C within 3 h after taking the cells out from the incubator. The temperature was kept constant using a home made heating stage at accuracy of 0.1 degree. According to our observations and to literature, in Leibovitz medium cells preserve their viability within this time period [21].

2.2. AFM

All experiments were carried out with an Asylum Research MFP-3D atomic force microscope (Asylum Research, Santa Barbara, CA; driving software IgorPro 6.32A, Wavemetrics), mounted on a Zeiss Axiovert 200 optical microscope. The experiments were performed with gold coated silicon nitride rectangular cantilevers, nominal spring constant of 0.03 N/m, resonant frequency 37 kHz in air which drops to 10 kHz in liquid, with a "V" shaped tip (Olympus, Optical Co. Ltd). The spring constant of the cantilever was determined each time by thermal calibration [22]. All images were recorded in Alternate Contact (AC) mode having 256 lines by 256 points, tip velocity of 60 μm/s. Trace and retrace images were both recorded and compared for internal accuracy. Noteworthy differences could not be found which underlines their reliability. On both cell lines more than 20 experiments were run. A representative set is reported in this paper.

2.3. Force measurements

After taking an image three cells were chosen, with selected points on nuclear and peripheral region respectively (Fig. 1). Force curves were measured consecutively on each pre-defined spot on cells, having equal time elapsed between two consecutive measurement, which was typically 30 s. The force curves were recorded in contact mode at constant loading speed (2 μm/s) and sampling frequency (0.5 kHz). Total force distance was kept at 3 μm and maximum load below 0.3 nN. Through the whole text we use elasticity which is directly measured. A constant multiplication factor could convert in elasticity constant or Young's modulus. Probing any material with a hard indenter (AFM tip) leads to the theory of indenting an elastic half-space with a stiff object, based on the work of Heinrich Hertz [23] and Ian Sneddon [24] later modified for AFM tips [25]. This theory is used for indentation tests, regardless of length scale. Elastic characterization was based on calculating the sample's elastic modulus [17,26] from each force curve. Fig. 2 shows the Force-Indentation curves recorded on both cell types, and the black lines represent the Hertzian fit. Both the upper and lower 10% of data beyond 0 indentations (contact point) were omitted in order to avoid internal errors of contact point determination and high force induced uncertainty. Data extraction and elasticity calculations were performed with the AFM driving software (IgorPro 6.32A, Wavemetrics).

2.4. Data analysis

Periodical oscillations can be described with three different parameters: amplitude, frequency (period) and phase. In our case the phase was neglected, since it is meaningful if a reference time point is available. A home made MatLab (Math Works Inc., Natick, Massachusetts) routine was implemented to calculate the amplitude and frequency spectra of the elastic variations and their reproduction based on sum of sine functions. Discrete Fourier Transform (DFT) method using a Fast Fourier Transform algorithm is a widespread method to quantify and convert periodic signals to frequency domain [27]. The spectra can be represented in function of both frequency and period. In our case the latter being more straightforward. The obtained frequency spectra were smoothed to avoid fragmentation. This does not induce any change in peak

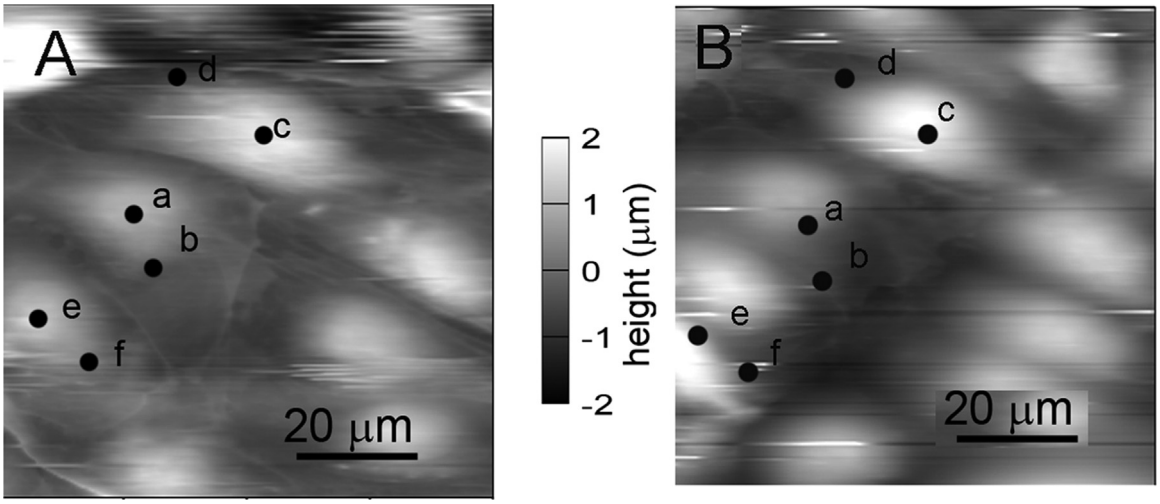

Fig. 1. Image of endothelial D3 cells (panel A) before and (panel B) after the force measurements. The dots with letters (a-b-c-d-e-f) show the locations where the forces were measured cyclically.