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Review

AFM contribution to unveil pro- and eukaryotic cell mechanical properties

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ABSTRACT

Atomic force microscopy is nowadays a well-established technique that permits the investigation of numerous parameters of living matter. In particular, it allows the exploration of the mechanical properties of living organisms in almost physiological conditions. Here, we focus on the use of this technology to review recent contributions that relates the physiology and pathology of bacteria, yeast, plant and mammalian cells to their nano-mechanical properties.

Contents

1. Introduction

The atomic force microscope (AFM) was invented in 1986 by Binnig, Quate and Gerber [1]. It is a scanning probe technique that was developed immediately after the scanning tunneling microscope [2], essentially to overcome two of that instrument's most serious limitations, i.e. the impossibility to operate in liquids and its very limited capability to image non-conductive samples. Once the AFM became commercially available, it became increasingly popular among scientists working in the life sciences. A technique that permits imaging of living biological samples at high resolution in almost physiological conditions was very welcome by all the "bio" community of the 1990's. Very soon after its development, the AFM was demonstrated to be also capable of measuring numerous other parameters of the sample in addition to its topography. Indeed, the instrument can very efficiently measure interaction forces between single molecules, recognize individual molecular species present on any type of surface, measure adhesion forces between individual

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cells, or importantly for us, in the frame of this article, to explore the mechanical properties of the sample. This last capacity of the instrument dramatically simplified the access to the nano-mechanical properties of living mater and nowadays virtually, every category of living organism had his elastic properties measured by AFM. AFM revealed that elastic properties of living material are closely related to the function of organisms, and importantly that they are altered in pathological conditions. The instrument therefore opens novel research axes and the hope of the development of innovative diagnostic tools. After a short description of the AFM and the way it is used to measure the nano-mechanical properties of biological material, this review will focus on the AFM contribution in the exploration of the nano-mechanical properties of living material, starting from bacteria up to mammalian cells and tissues. The readers interested in the mechanical properties of molecular structures can refer to Kurland et al. [3].

2. The atomic force microscope

The instrument consists of a very sharp tip fixed at the end of a soft cantilever. During measurement, the tip is approached very close (nm or Å) to the sample and the short-range tip-sample interaction forces induce a bending of the cantilever. By moving the tip all over the sample and by simultaneously recording the cantilever deformations it becomes possible to obtain information about the sample's 3D surface topography. The tip and the cantilever are usually micro-fabricated of silicon or silicon nitride. The tip curvature radius, one of the most important parameters that determines the resolution of the instrument, is in the nanometer range. The cantilever can have a rectangular or triangular shape with a length that varies between 2 and 200 μm and a thickness in the order of one μm. These material and geometrical properties result in a spring constant in the range of 0.01–100 N/m. The spring constant determines the force that the cantilever applies onto the sample when its end is moved vertically at a given distance off its resting position. Different options exist to determine its bending, but the most widely used one consists of measuring with a multi-segment photodiode the angular deflection of a laser beam that illuminates the very end of the cantilever. Despite its simplicity, the method can easily reach sub-Å resolution [4].

The lateral and vertical movements of the cantilever (or the sample) are achieved by fixing the cantilever (or the sample) atop one or several piezo electric crystals. These crystals deform upon exposure to a potential difference. Typically, a potential difference of 400 V induces a displacement of the piezocrystal of 100 μm. Here again the displacement precision is in the range of the Å.

For imaging purposes the AFM tip is scanning the surface of the sample while the deformations of the cantilever are recorded and displayed on a computer screen. Numerous imaging modes exist that all have their advantages and drawbacks. The very first one was the so called constant force mode in which the tip was kept in contact with the sample during the scan while a feed-back loop maintained the deformation of the cantilever (i.e. the applied force onto the sample) constant. However, nowadays the most widely used imaging mode for biological samples is the so-called tapping mode. It consists of oscillating the cantilever above the sample and in letting the tip periodically interact with the surface; dedicated electronics monitors the cantilever oscillation's amplitude, phase and frequency. Any of these parameters can be used to set up a feedback loop that will hold the tip in (periodical) contact with the sample throughout the scan. Since the tip only applies a vertical force onto the sample, this mode is very convenient for imaging poorly attached samples. For mechanical measurement purposes other modes exist that are the subject of the following paragraph.

2.1. Measurement of mechanical properties by AFM

As for imaging, the measurement of the nano-mechanical properties by AFM can be carried out by using different strategies. Some are very straightforward, whereas others require dedicated electronics and specific data processing algorithms. The simplest technique consists of recording two different images of the sample at different forces and in measuring the differences in height in the two images. This technique was used by Kis et al. [5] to estimate the Young's modulus of microtubules. In these experiments the authors deposited microtubules atop filters and imaged their suspended segment several times with an increasing force. The stronger the tip pushed onto the microtubules, the deeper the suspended segments were pushed into the hole. By knowing the deformation of the microtubule as a function of the force applied on it and some other geometrical parameters the authors could calculate the stiffness (Young's modulus) of the microtubules.

2.2. Force distance curves

Another, somewhat more sophisticated method, consists of the analysis of force distance curves. Such curves are obtained by indenting (i.e. pushing) the AFM tip into the sample and by recording the deformation of the cantilever during the process. If the sample is hard and no indentation (i.e. deformation of the sample by an AFM tip) occurs, the slope of the in-contact region of the curve is linear and at 45°. This results from the fact that when the piezo crystal lowers the base of the cantilever (or raises the sample) by a given distance, the cantilever deforms vertically by the same amount. However, if the sample is soft, then indentation occurs, and the shape of the force distance curve in the contact region adopts a more complicated shape with a slope below 45° (Fig. 1).

The calculation of the sample's Young's modulus is conducted as follows: in a first step the force distance curve obtained on the soft sample is subtracted from another one that was recorded on a rigid substrate. The resulting curve is referred to as the indentation curve, that basically indicates the force that is required to push the tip to a certain depth into the sample. The shape of this curve depends not only on the sample's elastic properties but also on the geometry of the AFM tip as well [6]. To obtain the sample's Young's modulus numerical value one has to fit the newly obtained indentation curve with a function that takes into account also the geometry of the AFM tip. The two most widely used functions are those from Hertz [7] and Sneddon [8]. The Hertz model describes the elastic deformation of two spheres, whereas the Sneddon's model accounts for other geometries such as conical or paraboloidal tips against a flat sample. As an illustration, the equation that determines the force F required to indent the tip to a given depth (δ) for conical tips is

$$F\left(\delta\right) = \frac{2\tan\left(\alpha\right)}{\pi} E'\delta^2$$

and for paraboloidal ones

$$F\left(\delta\right) = \frac{4\sqrt{R}}{3} E'\delta^{1.5}$$

α is the opening angle of the tip and R its radius of curvature. E′ corresponds to the reduced Young's modulus that is related to the sample's Young's modulus E through

$$\frac{1}{E'} = \frac{1-\mu^2}{E}$$

if it can be assumed that the numerical value of E of the sample is much lower than the E of the AFM tip. μ is the Poisson ratio of the sample that is ranging from 0 to 0.5. This last value is usually used for living cells.

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