Atomic force microscopy: Studying mechanical properties of a cell

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Atomic force microscopy (AFM) is a modern imaging technique that generates height representations of a sample surface with subnanometer resolution. Since 1986 it has evolved into a versatile tool which also provides maps of elastic and viscoelastic properties. AFM offers the opportunity to image samples with or without treatment in various environments. Aim of this work is a short description of the relatively new ways of studying mechanical properties of cells – Peak Force Tapping, Stiffness Tomography. Both represent new approaches to obtaining or analysing force-distance curve which are essential for calculating mechanical characteristic of a sample.

Keywords atomic force microscopy; mechanical properties; Young modulus; adhesion; Peak Force Tapping; stiffness tomography; nanoindentation

1. Introduction

It is a generally accepted statement that a cell's function is determined by its structure. However, the structure itself is not the sole determinant of its function. Chemical composition, electrical charge distribution and mechanical properties have to be taken into consideration. Under different physiological processes living cells respond to biological changes such as shape alternation of membranes and nuclei [1], cell-spreading [2], actin and microtubule reorganization or cross-linking under cell membrane [3] and overall mechanical characteristics are changing [4-6]. To study these subcellular processes it is important to find the right instrumentation. One of the most powerful tools is the atomic force microscopy (AFM) [7,8]. Apart from topographical images it allows the probing of mechanical properties of living cells under physiological conditions. Furthermore, AFM can be used to monitor dynamic changes in the shape and mechanics during pharmacological treatment [8]. Besides AFM there are other relevant techniques to measure mechanical properties such as micropipettes, microplates and optical tweezers [9,10].

AFM uses a flexible cantilever with a sharp tip mounted to its end. Images are obtained by scanning the surface of the sample with the tip. Force interactions between the tip and the sample occur during the rastering the surface, which are then recorded. For imaging cells or cellular compartments two AFM imaging modes are frequently used [11]. First, in contact mode the tip is in direct contact with the sample. Interaction between the tip and the surface leads to bending of the cantilever. Deflection is subsequently detected by a laser beam, which is reflected at the end of the cantilever onto a sensitive detector. The deflection signal is used to minimize the applied force on to the sample by moving the sample holder or the tip by a feedback loop. The topography is then reconstructed from the piezo movement. In tapping mode, also known as intermittent contact mode, the cantilever is oscillated close to its resonant frequency. The tip oscillation is progressively dampened during the scanning because of distance dependent force interaction between the sample and the tip. A feedback loop is restoring the cantilever amplitude by adjusting the piezo position. Again the height image is reconstructed from the piezo movement. In tapping mode, lateral forces are reduced because the tip contacts the sample briefly at the end of its downward movement. [11,12]

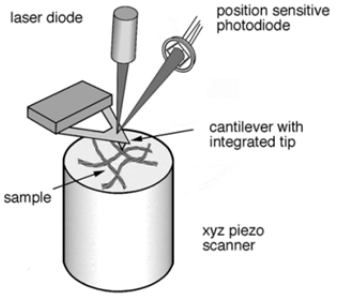

Figure 1: Principle of AFM. The sample is positioned by a xyz piezo scanner. The deflection is detected by a laser beam focused on to the end of the cantilever and reflected onto a position sensitive detector. (Edited from [13])

2. Mechanical properties

Mechanical properties are significant for functions of different structures such as bones, teeth or cartilages. From this, it is reasonable to assume that mechanical properties are equally important at the nanometric scale. Mechanical characterization is able to determine the effect of individual biomolecules and assemblies to overall properties. It is known that the physiological state of a cell is reflected to its characteristics. Hence it should be possible to correlate a change in a mechanical property with a structural change [14]. Nanoindentation belongs to commonly used methods for

probing mechanical properties. This procedure requires a sharp, rigid tip to probe a sample and records applied force and penetration depth [15]. Viscoelastic response of a sample for the indentation affects the measurement of the properties as well as indenter geometry, depth and loading rate. Such measured data – force-displacement curves (*F-d* curves) – are then analysed by various theoretical and empirical models. Widely used is Hertz theory [16]. This theory suggests an elastic contact of two spherical bodies and requires several assumptions for its validity: frictionless and non-conforming contact, small contact area relative to overall body dimensions and small deformations on contact. One of the most important approximations is the absence of adhesion or surface forces [17]. Loading force is defined as

$$F = \frac{4\sqrt{R}}{3}\frac{E}{1-\nu^2}\delta^{3/2},$$

where δ is the indentation depth, ν is the Poisson's ratio, R is the tip radius and E is the elastic modulus. Besides Hertz model, Sneddon's modification [18] is used for cone shape tip:

$$F = \frac{2}{\pi}\tan\alpha\frac{E}{1-\nu^2}\delta^2,$$

where α is the half opening angle of the tip. There are several models which bring corrections for adhesive forces to original Hertz model such as Johnson-Kindall-Roberts (JKR) [19], Dejaguin-Müller-Toporov (DMT) [20] and Oliver-Pharr [21]. For reliable estimation of elastic properties it is also important to pay attention to accurate identification of the contact point, calibration of the probe spring constant and accurate representation of the tip geometry.

3. New approaches to obtaining mechanical properties

3.1 Peak Force Tapping

Peak Force Tapping is a new operating mode of AFM that was developed at Bruker. Unlike its previous mode HarmoniX it can operate with a wide variety of standard AFM probes. It provides images at a relatively high speed (similar to Tapping mode) and a high resolution. In Peak Force Tapping the probe is oscillated at a frequency of 2 kHz with peak-to-peak amplitudes of 300 nm. Figure 2a represents the measured force on the probe during approach of the tip and the retraction. When the tip is at a distance from the surface (point A) there is little or no force on the tip. As it is approaching to the surface the cantilever is pulled down by attractive forces such as van der Waals, electrostatics or capillary forces. At point B the tip touches the surface and stays on the surface until the Z position reaches its lowest position (point C) while the force is increasing. The peak force arises at point C. The probe starts to withdraw and the force decreases until its minimum at point D. The adhesion is determined by the force at that point. The tip leaves the surface and only long range forces affect the tip. When the tip-sample separation is at its maximum (point E) there is a very small or zero force. A constant force at point C is maintained by adjusting the extension of Z piezo through the feedback loop. Dependence of the force against the Z-position can be compared with force-displacement curves that have usually been used in measuring mechanical properties of a sample. This method is faster than nanoindentation where an approach-retract cycle is performed at rate of 0.5 to 10 Hz [22]. Figure 2b illustrates the information which can be obtained. The most commonly used characteristics are elastic modulus, adhesion, energy dissipation and maximum deformation. When the force curve is complete it is then analysed to obtain the properties of the sample and the information is sent to one of the image data channel. The result is maps of material properties in user-defined false colours. [23,24]

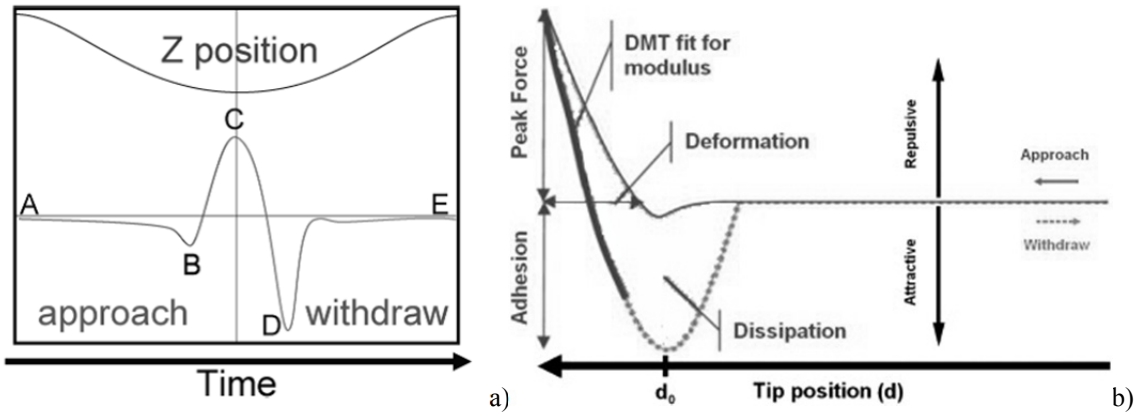

Figure 2: Force curves for a single cycle of peak force tapping a) a force and the Z position as a function of time b) plot of the force vs. z-displacement and how the different material properties are extracted (Edited from [23]).

As far we know, Peak Force Tapping on biological samples was used to investigate mechanical properties of amyloid fibrils [22,25], living diatoms [26], living and fixed cells [27], epidermal cells [24] and *S. aureus* bacteria [28]. Sweers et al. [22] compared different operation modes for evaluating mechanical properties of α-synuclein amyloid fibrils – nanoindentation, HarmoniX and Peak Force Tapping, and resulted in similar values of elasticity for all the methods. The independence in extracting mechanical characteristics such as adhesion, topography, dissipation etc. according to the phase signal in tapping mode and high resolution for all properties according to force volume are Peak Force Tapping main benefits. In addition, quantitative data can be directly obtained and do not require postprocessing, the applied force can be minimized to several pN and the mode can be applied on a variety of samples [25].

3.1.1 Elastic modulus

The retract curve in Peak Force Tapping is fit with Derjaguin-Müller-Toporov (DMT) model [20] to obtain Young's modulus:

$$F - F_{adh} = \frac{4}{3} E^* \sqrt{R(d - d_0)^3}$$

where $F - F_{adh}$ is the force on the cantilever relative to the adhesion force, R is the tip end radius, $d - d_0$ is the deformation of the sample and E^* is the reduced modulus. If Poisson's ratio is known, the software can calculate the Young's modulus according to

$$E^* = \left[\frac{1 - \nu_s^2}{E_s} + \frac{1 - \nu_{tip}^2}{E_{tip}} \right]^{-1},$$

where ν_s is the Poisson's ratio of the sample, ν_{tip} is the Poisson's ratio of the probe, E_s is the Young's modulus of the sample and E_{tip} is the Young's modulus of the probe. The Poisson's ratio is usually established to 0.5 for cells (perfectly incompressible).

3.1.2 Adhesion

Besides the elastic modulus, the adhesion forces are frequently ascertained. As mentioned above, adhesion is determined by the minimum force in figure 2a. The source could be any attractive force between the tip and the sample. If the tip is functionalized, the origin becomes more specific and reflects the chemical interaction between the tip and the sample.

3.1.3 Dissipation

Energy dissipation is given by the force times the velocity integrated over one period of the vibration:

$$W = \int \vec{F} \cdot d\vec{Z} = \int_0^T \vec{F} \cdot \vec{v} dt ,$$

where W is energy dissipated in a cycle of interaction, F is the interaction force vector and dZ is the displacement vector. Because the Z motion and the velocity reverse direction, the integral is zero if the loading and unloading curves coincide. The dissipation is therefore the hysteresis between the loading and unloading curves.

3.1.4 Deformation

Deformation is defined as the indentation of the tip into the surface at the peak force. The measured deformation may contain both elastic and plastic contributions. This can be recalculated to the hardness with known tip parameters and contact area.

3.2 Stiffness tomography

A curve displaying the force needed to indent the AFM tip into given depth of a sample is referred to as a force-indentation curve (F-I curve). The shape of this curve allows estimating Young's modulus, when some parameters such as the shape of the tip and the Poisson's ratio of the sample are known. The systematically divided F-I curve with applied mathematical model on each segment provides information about the mechanical properties and the depth of inclusion. This procedure shows stiffness differences along the indentation path. If this method is applied to force volume scan, an image in which every pixel consists F-I curve, it gives information about the surface topography and the interior mechanical characteristics of the sample.

Firstly the acquisition of force-displacement curve (*F-d* curve) is needed to determine the required attributes of the sample. *F-d* curves are obtained by indenting the AFM tip into the sample and recording the deflection of the cantilever during the process. This curve is subsequently subtracted from an *F-d* curve of undeformable substrate such as glass or

sapphire in order to get the F-I curve. The last step of the analysis involves fitting the F-I curve with theoretical model in order to calculate the Young's modulus.

The standard force-curve analysis involves fitting the whole F-I curve with mathematical model, on the other hand, in stiffness tomography, the F-I curve is divided in individually chosen long segments. There are afterward separately fitted with the theoretical model. The obtained stiffness value is then inserted in a 3D matrix that represents the stiffness values of the whole indented section of the sample. This procedure therefore is able to highlight the structure that the tip encountered during its scanning and that would be flattened out by the standard procedure.[29,30] Roduit et al. [30] used stiffness tomography to locate different structures underneath a macrophage's membrane and their contribution to cell's stiffness. And Longo et al. [31] investigate stiff structures under bacterial membranes.

4. Conclusion

Over the last decade, AFM has become a broadly used tool for studying mechanical properties of living cells. Nevertheless, complexity of biological structures represents a challenge in the fitting of measured data to an appropriate theoretical model. In addition many external factors have to be taken into account, including the tip geometry and the contact area. Also with the growing body of evidence related to connection between cell mechanics and chemical and biological functions there is a need for an improvement in data acquisition and its analysis for detailed and exact knowledge. AFM modes have already provided an insight in various fields like cancer research [32] or tissue engineering [28]. There are also indications that AFM may be a potential diagnostic tool. The development of new techniques will lead to faster and easier acquisition of information on biological structures.

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