REVIEWS

Medicina (Kaunas) 2013;49(4):155-64

Atomic Force Microscopy as a Tool for the Investigation of Living Cells

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***Key Words**: atomic force microscopy; living cells; Young's modulus; elasticity; cell imaging; cancer cells.*

***Summary.** Atomic force microscopy is a valuable and useful tool for the imaging and investigation of living cells in their natural environment at high resolution. Procedures applied to living cell preparation before measurements should be adapted individually for different kinds of cells and for the desired measurement technique. Different ways of cell immobilization, such as chemical fixation on the surface, entrapment in the pores of a membrane, or growing them directly on glass cover slips or on plastic substrates, result in the distortion or appearance of artifacts in atomic force microscopy images. Cell fixation allows the multiple use of samples and storage for a prolonged period; it also increases the resolution of imaging. Different atomic force microscopy modes are used for the imaging and analysis of living cells. The contact mode is the best for cell imaging because of high resolution, but it is usually based on the following: (i) image formation at low interaction force, (ii) low scanning speed, and (iii) usage of "soft," low resolution cantilevers. The tapping mode allows a cell to behave like a very solid material, and destructive shear forces are minimized, but imaging in liquid is difficult. The force spectroscopy mode is used for measuring the mechanical properties of cells; however, obtained results strongly depend on the cell fixation method. In this paper, the application of 3 atomic force microscopy modes including (i) contact, (ii) tapping, and (iii) force spectroscopy for the investigation of cells is described. The possibilities of cell preparation for the measurements, imaging, and determination of mechanical properties of cells are provided. The applicability of atomic force microscopy to diagnostics and other biomedical purposes is discussed.*

Introduction

Atomic force microscopy (AFM) is a valuable and useful tool for the imaging of biological samples in a liquid medium where composition and temperature replicate natural conditions. AFM has the greatest resolution among many other tools applied to the imaging of living cells, since it is capable of subnanometer resolution and can be used not only for the imaging of bacteria (1), viruses (2), microbes (3), and cultured mammalian cells, but also for the evaluation of many other phenomena including molecular binding (4), elastic properties of the membrane, and rigidity at the submicron level (5). Similarly, the AFM-based techniques can be applied in order to get additional information about the physical properties of biological samples, e.g., the AFM force spectroscopy mode is used for the determination of cell mechanical properties, such as Young's modulus and adhesion forces (6). The force spectroscopy mode can be applied not only for the measurement of one point of interest, but also for getting the spatial maps of elasticity across the cell surface and for representing the topographical Young's modulus (6–8). Mechanical properties can be mapped (8, 9) by recording force-volume images and stiffness tomography (10). Using this method, the Young's modulus of the whole cell can be measured, and the statistical distribution analysis of elasticity can be performed and compared with height analysis (11).

Structural changes induced in the cell membrane, the cytoskeleton, and the cytosol, changes in the cell shape and size, and changes in cell deformability show cancer cell metastasis (12, 13). Studies on the mechanical properties of cells of different cancer types (bladder, melanoma, prostate, breast, and colon) show that the Young's modulus of cancer cells is lower compared with that of healthy cells (13).

The most important part of AFM is a nanomechanical probe (tip), which is placed on the cantilever. The probe interacts with a sample in a selected physical way. The end of the probe can have vari-

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ous forms, such as conical, pyramidal, and spherical. In order to expand the scope of AFM, the tip can be modified by attaching some cells, nucleotides, proteins, lipids, or DNA (14). Modified probes are used for scanning a cell surface in order to get a topographical image and for the indentation of cells in order to determine the elasticity of various types of cells, e.g., metastatic lung, breast, and pancreatic cancer cells (15, 16). For imaging of cells, unsharpened AFM tips (radius ~20–50 nm) frequently yield better images than ultrasharp tips (radius <10 nm) (6).

The investigations of living cell elastic properties, adhesion, hydrophobicity of the membrane and morphological studies are performed in order to estimate differences between healthy cells and cells affected by some diseases (17, 18), e.g., hydrophobicity of cells, affected by respiratory syncytial virus, decreases during infection (19). AFM can be applied to study cancer cells before and after treatment with anticancer drugs. After treatment, increased fluctuations of the surface components of the cell membrane, an increase in shrinkage, or even the appearance of the pores in the image are observed (20). These parameters are associated with cancer cell apoptosis, e.g., cells are damaged and roughness is increased when cancer cells are treated with paclitaxel (2α,4α,5β,7β,10β,13α)-4,10-bis(acetyloxy)-13-{[(2R,3S)- 3-(benzoylamino)-2- hydroxy-3-phenylpropanoyl]oxy}- 1,7-dihydroxy-9- oxo-5,20-epoxytax-11-en-2-yl benzoate) (21). It has been demonstrated that all the registered changes depend on treatment time and drug concentration.

Elastic properties, such as Young's modulus and indentation depth, have to be calculated from measured force-distance curves. A different Young's modulus can be obtained on the same cell and for the same experiment by the application of different models, which are suitable for the evaluation of elasticity. Some models have been known for a long time; the most commonly used ones are Hertz-Sneddon (22, 23), Johnson-Kendall-Robins (24), and Derjaguin-Muller-Toporov (25) models. Each of the mentioned models has some shortcomings; therefore, to fix them, finite element models are applied.

The calculated elastic properties of cells depend not only on the applied model, but also on the conditions of measurement, such as cell fixation, ambient environment (solution or air), temperature, measuring time, scanning speed, probe geometry, applied force, mode of measurement, quality of a tip, and sample preparation procedures (26). Therefore, AFM results are usually evaluated by comparing healthy cells with cancer cells or cancer cells before and after treatment with drugs.

In this review, the AFM-based methods suitable for the determination of elastic properties of cells and for the imaging of cells are described. Some methods suitable for cell preparation are presented. The estimation of cell elasticity by the evaluation of force-distance curves is discussed, and the values of elasticity are presented. Moreover, a practical part of measurements applied to different kinds of cells is presented.

Influence of Cell Preparation on Measurement Results

The most challenging cell preparation is cell fixation, which is required to protect cells from detachment and to eliminate their movements. Therefore, measurement results strongly depend on cell fixation methods. Cell rigidity of fixed cells compared with unfixed cell increases due to chemical fixation, and for this reason, image resolution becomes much higher. The fixation of round cells (yeasts and some bacteria) by capturing them in the pores of the membrane or the filter causes the deformation of cells, and therefore, holes of a different diameter in the membrane are needed for cells in various dimensions. However, this method allows investigating living cells without any additional procedures such as drying, coating, or chemical treatment. Otherwise, during force measurements, the immobilization method is not critical, since results are usually presented in a comparative way.

When cells are immobilized by electrostatic interactions (Fig. 1) and measurements are performed in phosphate buffer saline (PBS) or 3-(N-morpholino) propane sulfonic acid (MOPS), they are often detached from the support. To solve this problem, Meyer et al. (27) proposed to use a layer of polyphenolic adhesive proteins formed on the surface for the attachment of cells (Fig. 2). Some other methods can also be applied for the immobilization of cells, e.g.: (i) physical confinement by capture in holes (28, 29) (Fig. 3), (ii) attractive electrostatic interactions (30) (Fig. 1), (iii) covalent binding to amine-functionalized surfaces after activation of carboxyl groups on the cell surface by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysuccinimide (EDC-NHS) (27) (Fig. 4), (iv) covalent binding to carboxyl-functionalized surfaces by EDC-NHS (27) (Fig. 5), (v) covalent binding to amine-functionalized surfaces by glutaraldehyde (31) (Fig. 6), and (vi) covalent binding to self-assembled monolayers (32) (Fig. 7).

Mechanical Immobilization of Cells. Cells can be fixed mechanically in porous membranes, e.g., in our group, the cells were trapped within the micrometer-diameter holes of polymeric membranes (Fig. 3) (33). Wang et al. invented a vacuum-based cell-holding device (34). Evenly spaced holes

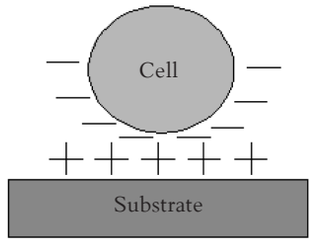

Fig. 1. Schematic representation of electrostatic interaction between the cell and the surface

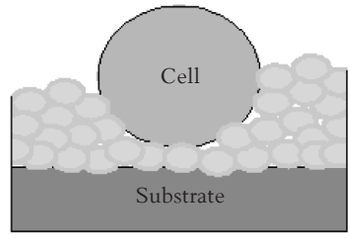

Fig. 2. Schematic representation of cell immobilization attaching them to a polyphenolic adhesive protein

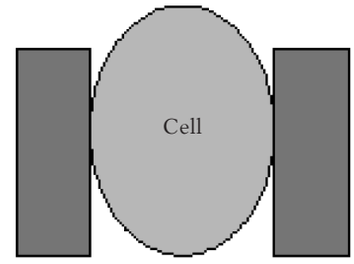

Fig. 3. Schematic representation of mechanical immobilization of a cell

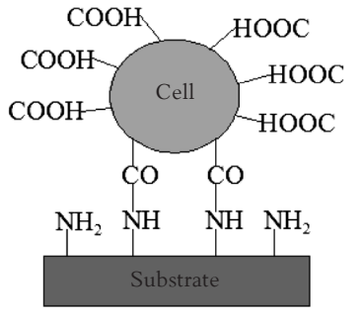

Fig. 4. Schematic representation of cell immobilization by covalent binding to amine-functionalized surfaces by EDC-NHS

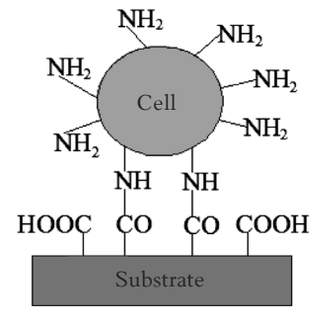

Fig. 5. Schematic representation of cell immobilization by covalent binding to carboxyl-functionalized surfaces by EDC-NHS

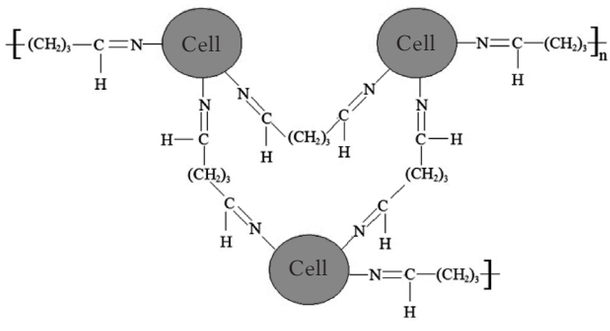

Fig. 6. Schematic representation of covalent binding of cells to amine-functionalized surfaces by glutaraldehyde

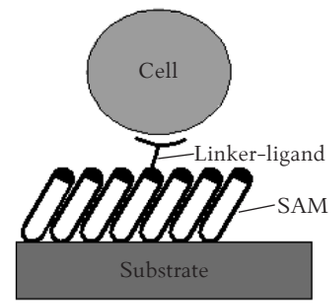

Fig. 7. Schematic representation of cell immobilization on a self-assembled monolayer (SAM)

(~400 μm in diameter) are connected to a vacuum source. A negative sucking pressure of 7–24 kPa (2–7 inHg) enables each through-hole to trap a single cell without damaging. To increase the efficiency of immobilization during entrapment, some processing conditions such as vacuum, pressure, and cell concentration are controlled. However, physical entrapment in membranes can cause severe structural and mechanical deformations of the cell membrane (28). In order to overcome the limitations and difficulties of imaging and manipulating the protein molecules by AFM, Fung et al. (35) proposed to use a biocompatible and flexible polymer micromesh (parylene membrane) with openings of 100 μm in diameter specifically designed to immobilize mechanically living cells and protein structures. Human epithelial cells immobilized by this approach were successfully imaged using the AFM tapping mode.

Electrostatic Immobilization of Cells. For the immobilization of cells using electrostatic interactions, the surface has to be coated with positively charged substances, such as polyethyleneimine (PEI) (30), poly-L-lysine (PLL), or gelatin. For example, *Staphylococcus sciuri* suspended in distilled water or PBS was immobilized by electrostatic interactions to the N-(2-aminoethyl)-3-aminopropyltrimethoxy-silane (EDS), 3-aminopropyltriethoxysilane (APTES), polyethyleneimine, poly-L-lysine, and gelatin-coated slides (27).

Chemical Fixation of Cells. The fixation of cells is a process when cells are treated with different agents that cross-link proteins present on the cell surface, thereby "freezing" the morphology of the cell and fixing cells to the substrate and/or attaching cells to each other. The chemical fixation of cells simplifies the measurement process and improves image resolution. Glutaraldehyde, formaldehyde, methanol, ethanol/acetic acid, paraformaldehyde, and methanol/acetone are most frequently used for the chemical fixation of cells. Chemical fixation dehydrates the cell and increases membrane stiffness; therefore, small components, such as cytoskeletal fibers, are not revealed in a topographical image (6).

Cells can also be explored in a dried state; for this purpose, some additional procedures are needed, and the cell immobilization method is important to get reliable measurement results. Using AFM, Starodubtseva et al. (36) investigated erythrocytes, thymocytes, human embryonic fibroblast cells, and human lung adenocarcinoma cell line A549 samples. Three-dimensional AFM images were obtained by measuring erythrocytes prepared in one of the 2 ways: (i) by spreading the whole blood drop on the glass slide and (ii) by drying at room temperature (rat erythrocyte) or by the fixation of the erythrocyte shape with glutaraldehyde in a cellular suspension, placing cells on the glass slide and drying at room temperature. The image showed that glutaraldehyde fixed an intravital shape of cells. Moreover, they found that the parameters (averaged lateral forces and roughness of lateral force map) of lateral force microscopy of single cells (erythrocytes and thymocytes) were different for unfixed cells and for those fixed with glutaraldehyde.

It was demonstrated that chemically pretreated yeast cells became much stiffer compared with the intact ones. In addition, morphology studies of the cell wall revealed that the chemical treatment of cells enhanced the roughness of the yeast cell surface (33).

For *E. coli* K12 strains, a 4-fold increase in cell stiffness due to fixation was determined (37). An enzymatic digestion or chemical treatment of components involved in the formation of the cytoskeleton leads to a softening of the cell, and it should reveal information on cytoskeleton contribution to the observed stiffness of the cell.

Immobilization of Cells on Glass a Cover Slip. In order to immobilize cells on a transparent surface, cells can be grown on a glass cover slip, a plastic substrate, or mica. The glass cover slip can be coated with polyionic polymers like poly-L-lysine or poly-L-ornithine (16), which support the attachment of cells to the surface. The flat pieces of fresh tissues can be "glued" directly to magnetic disks used for the fixation of AFM samples.

El-Said et al. studied healthy and cancerous cells on the following surfaces or substrates: (i) a polystyrene-based Petri dish, (ii) aluminium foil, (iii) a first anodized alumina substrate, (vi) a second anodized uncoated alumina substrate, and (v) a polypyrrole (PPy) nanowire/nanoporous alumina substrate (38). They found the best cell adhesion and proliferation on PPy-coated alumina surfaces, where cell migration was easier and cell motility was higher than on other studied surfaces (38).

Principle of Atomic Force Microscopy Operation

AFM can be applied for the investigation of surface morphology of cells at nanometric resolution under physiological conditions.

A very important issue in cell investigation is to maintain them in a living stage in order to investigate cells for an extended period of time (6). AFM is suitable for the nanoscaled resolution imaging of living cells, and it has been successfully employed for the imaging of a wide variety of primary cells and cell lines (39–43). Currently, AFM is a label-free technique that can image the surface of living microbial cells at high resolution and practically "in real time," thereby providing information that is complementary to that obtained by electron microscopy (8). However, neither transmission electron microscopy nor scanning electron microscopy (SEM) is suitable for the visualization of live cells because cells are usually negatively affected by metallization, which is absolutely necessary in order to get conducting samples required for SEM imaging. Moreover, SEM or other most frequently used visualization methods do not allow providing any information about cell stiffness and other physicochemical surface characteristics, which becomes possible if AFM is applied in such investigations.

The principle of the AFM system is shown schematically in Fig. 8. The probe is the tip fixed on a flexible end of the cantilever with the diameter ranging between 1 and 50 nm. One of the most important AFM operation modes is the "contact mode," which maintains a constant interaction force between the probe and the surface. The AFM probe moves in the x and y directions in the horizontal plane by the piezo scanner and scans the sample line by line. The cantilever deflects depending on applied and/or acting forces. This deflection is determined by the optical system. The electronics, which usually operate in a "closed loop mode," control the probe movement in the z axis, according to the signal received from the detector. This feedback circuit keeps a constant distance between the probe and the surface. The computer processes the "error signal," i.e., the difference between the set force and the detector signal, and converts this computation into the image of topographical or other characteristics.

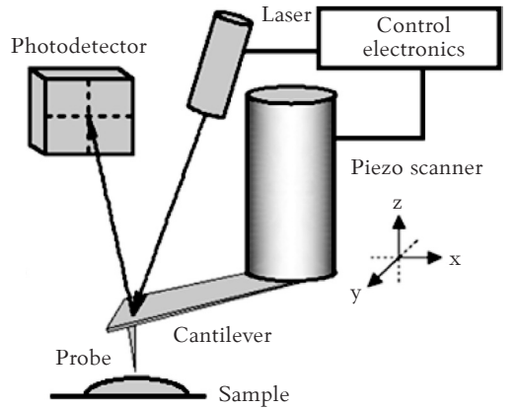

Fig. 8. Schematic representation of the most important acting parts of the atomic force microscope

Imaging of Living Cells by Contact Mode

Resolution in the imaging of biological samples is a very important issue; significant AFM resolution has been achieved in virus research, DNA and chromosome studies, bacterium and mammalian cell imaging, as well as in protein and peptide analysis (44). Moreover, cell differentiation (45), cell division (46), shear stress (47,48), cultivation time (49), and cell spreading (50) have been studied using AFM. Image resolution depends on tip geometry, cell stiffness, adhesion forces, the external force applied to the AFM probe, scanning speed (6), control over tip-cell interaction, and tip contamination (51). The highest (subnanometer) resolution of images has been achieved in the contact mode, e.g., in the visualization of membrane proteins (51). AFM resolution can also be improved by the chemical fixation of a cell because cells become stiffer. Deflection images are usually of better resolution (8); such an image can be measured in the same time as topography. However, the quality of living cell images is deteriorated within scanning time due to the contamination of the AFM probe.

An important parameter of AFM imaging is scanning force, which is applied on the cantilever. Applied low scanning force (in the range of pN) is required to minimally deform the cell surface, but low force results in lower contact stiffness between the tip and the surface. It decreases image resolution because small details become "invisible." Soft cantilevers should be used for living cell imaging at low forces. However, to date, even the softest cantilever is 10 times stiffer than a mammalian cell surface (52). Therefore, scanning forces have to be minimized down to $\leqslant$50 pN in order to prevent tip penetration into the cell membrane and dislodging or dissecting of fine cellular surface structures, such as microvilli and filopodia (6). On the other hand, high scanning forces induce cell retraction out of the substrate (6). High viscoelasticity of the plasma membrane can be exploited to image the underlying cortical cytoskeleton in living cells with high resolution (52) when scanning force is increased (6). With increasing scanning force, the AFM tip will deform the compliant membrane and force it against the much stiffer underlying cortical actin cytoskeleton (6). In order to solve this problem, the control of forces applied between the tip and the surface has to be very accurate.

Another important issue is low scanning speed. The reduction of scanning velocity down to 5 μm/s improves sample tracking by the tip and, consequently, provides much higher resolution of living cell images (6). However, biological samples can vary over time, e.g., living cells constantly change their shape, detach from or adhere to the substrate, or interact with neighboring cells (53). Moreover, the AFM tip during long scanning periods may become contaminated with phospholipids and/or proteins. Therefore, there are many attempts to create fast scanning AFM systems.

A disruptive effect of the scanning cantilever tip on cell morphology in the AFM contact mode has been investigated by You et al. (53). The experimental results showed that the disruptive effect often took place even under normal imaging conditions. Cells being scanned suffered injuries to various degrees, depending on the cell type and viability, and injured cells might undergo remarkable changes in morphology (53). Some researchers indicated that the improvement of cell adhesion is a way to promote cell resistance to a disruptive effect of the cantilever.

It could be concluded that the contact mode is the simplest mode of the AFM operation, which allows much faster scanning than in the tapping mode and force control, the best image resolution compared with other modes, and a good signal-to-noise ratio even in a noisy environment (18). However, there are major disadvantages to this mode: cells can be retracted from the surface and the tip can become contaminated, then providing false information about cell surface morphology and/or other cell surface-characterizing parameters.

Imaging of Living Cells by Tapping Mode

Cells can be imaged by the tapping motion of the AFM probe, which is usually performed at high frequencies. At a high tapping frequency, the cell surface behaves like a solid material. In this imaging mode, the cantilever is oscillated at its resonant frequency, and only an intermittent contact with the cell surface is observed; as a result, destructive shear forces are minimized (54). Therefore, the high-resolution imaging of subcellular structures is possible using tapping-mode AFM. The resonant frequency of the cantilever in liquid is relatively low, typically

in the range of 8 to 35 kHz, resulting from substantial liquid damping (54). On the other hand, the operation of tapping mode AFM in liquid appears to be more complicated than in the air (55).

For the imaging of soft samples, it is very important that the tip does not deform or scratch the surface, and the tip is not contaminated. The tapping mode allows collecting the various kinds of information related to the properties of the surface, including viscoelasticity, mechanical properties, and local changes in physical or mechanical properties of the material. However, tapping force cannot be controlled precisely, and therefore, scanning velocity is lower than in the contact mode.

Measurement of Elastic Properties of Cells

Stiffness of the cell, measured by indentation, can be caused by the cell wall itself or by underlying structures as the cytoskeleton or by a pressure difference between the cell interior and the exterior (56). Cancer cells have an elastic modulus between 0.1 and 18 kPa (Table), depending on the cell type, preparation, and the fixation method. Wu et al. (57) tested the influence of some toxins, which affect the cytoskeleton, and some fixing agents reacting with mouse fibroblast (L929) cells. The potential of AFM for the stable imaging and acquisition of force curves observed on the surface of living cells for extended periods facilitates the study of dynamic processes induced by external stimuli. AFM was applied in the assessment of changes in cell stiffness upon an increasing Ca^{2+} concentration (58, 59) and cellular contractility (60). The intrinsic variability of biological forms is considerably higher than that typically described by basic physical models. Furthermore, even within one cell, there are many different areas of rigidity, which can be different by few orders of magnitude (61, 62). For example, the difference in rigidity at the cytoplasmic area and the cell edge can vary by 2 orders of magnitude or even more (62). The results of Berdyyeva et al. (62) show that a similar change in rigidity (about 10 times) can be observed on epithelial cells while aging in vitro. The next factor increasing uncertainty can be cell age and its stage in mitosis or meiosis. It was demonstrated (63) on normal rat kidney fibroblast cells that the Young's modulus of the middle (between the nuclei and the membrane) of a dividing cell changed from 1 kPa (during interphase) to 10 kPa (during cytokinesis).

Young's modulus can depend on the depth of probe penetration and on the calculation model (18). There is a considerable variation of rigidity within the cells of different types. Epithelial cells seem to be the softest (18). The edge of a young epithelial cell can have a Young's modulus of 0.2 kPa (62). Platelets are the toughest with Young's modulus as high as hundreds of kPa. It has been reported that cells in vitro have the values of Young's modulus in the range of 1–100 kPa (18, 64, 65), which encompasses the different types of investigated cells, including vascular smooth muscle cells, fibroblasts, bladder cells, red blood cells, platelets, and epithelial cells. Moreover, it was determined that the addition of chitosan led to an increase in the stiffness of cancer cells, whilst nonmalignant cells were not influenced by the addition of chitosan (66).

A direct comparison of several cancerous cell lines (prostate: PC-3, LNCaP, and Du145; breast: MCF7 and T47D) with a normal counterpart showed a significantly lower Young's modulus value for the cancerous ones (17). Studies on the mechanical properties of single cells originating from various cancer types (bladder, melanoma, prostate, breast, and colon) are characterized by a lower Young's modulus, denoting the higher deformability of cancer cells (13).

A comparison of elastic properties of normal human epithelial cell lines (Hu609 and HCV29) and 3 cancerous ones (Hu456, T24, BC3726) showed that healthy cells had a Young's modulus value higher by about 1 order of magnitude than cancer cells (67). Malignant (MCF-7) breast cells had an apparent Young's modulus value significantly lower (1.4–1.8 times) than that of nonmalignant (MCF-10A) cells at physiological temperature (37°C), and their apparent Young's modulus increased with the loading rate (68).

Some values of Young's modulus of cancer cells are presented in Table.

Force Spectroscopy

To measure the elastic properties of cells, the force spectroscopy mode is very useful. From force-displacement curves, it is possible to draw information about a viscoelastic behavior of cells (69). In this mode, deflection-distance curves are measured (Fig. 9). Further, they are recalculated to force-distance curves by applying the Hooke's law:

$$F=k\Delta d, \qquad (1)$$

where k is an AFM cantilever stiffness constant and Δd is cantilever deflection.

The elastic properties of cells are calculated from the force-distance curves that are usually measured on stiff and compliant surfaces (Fig. 3). This is represented by a straight-sloped line, and it is usually applied as a reference line required for force calibration. For compliant samples like cells, cantilever deflection is much smaller, and the resulting force curve has a nonlinear character (70).

Mechanical properties are mapped (8) by recording force-volume images usually consisting of arrays of 32×32 force curves using the maximum applied

Table. Young's Modulus of Cells

Cell	Young's Modulus, kPa	Ref.
Prostate cancer cells, mean (SD)		
Lymph node metastatic prostate carcinoma LNCaP	0.45 (0.21)	
Brain metastatic prostate carcinoma Du145	1.36 (0.42)	(10)
Prostatic adenocarcinoma PC-3	1.95 (0.47)	
Nontumorigenic prostate cells PZHPV-7	3.09 (0.84)	
Breast cancer cells, mean (SD)		
Breast cancer from pleural effusion T47D	1.20 (0.28)	(10)
Breast adenocarcinoma MCF7	1.24 (0.46)	
Normal mammary cell 184A	2.26 (0.56)	
Normal human fibroblast cells HS68	1.86 (1.13)	
Normal human breast epithelial cells (MCF10AMC10A)	1.13 (0.84)	(18)
Metastatic human breast cancer cells (MDA-MB-231)	0.51 (0.35)	
Human bladder cancer cell lines, mean (SD)		
Transitional cell cancer of urine bladder T24	0.77 (0.25)	(58)
Transitional cell cancer of bladder Hu456	0.80 (0.23)	
HCV29 transfected with v-ras oncogene BC3726	0.17 (0.08)	
Nonmalignant epithelial cells, mean (SD)		
Human bladder cells HCV29	3.19 (0.27)	(58)
Nonmalignant ureter cells Hu609	3.29 (0.35)	
Normal human esophageal cells (EPC2), mean	4.7	
Nondysplastic metaplasia (CP-A), mean	3.1	(70)
High-grade dysplasia (CP-D), mean	2.6	
Hepatocellular carcinoma cells (HCC), mean		
Calculated using hyperelastic optimized parameters		(69)
Conical tip	8.52	
Sphere tip	7.44	

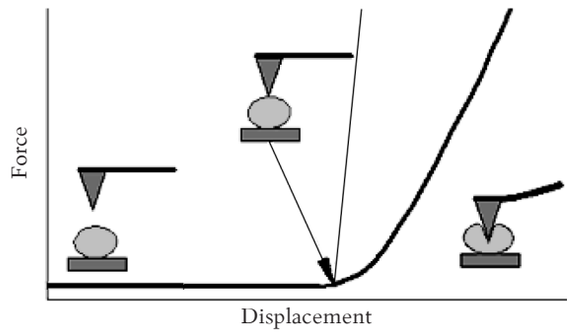

Fig. 9. Force-displacement curves for hard and soft (thick line) samples

The arrow shows the point of contact. For more details see also (22).

force of 1.2 nN. Young's modulus of the cell under different experimental conditions can be calculated by performing the curve fitting of force-indentation curves using the Hertz-Sneddon model. The quantity, which determines cell elastic properties, is Young's modulus. Several theories describe elastic deformation of a sample. These theories have been developed by Hertz (22), Johnson-Kendall-Roberts (JKR) (24), and Derjaguin-Muller-Toporov (DMT) (25). The Sneddon model of indentation (23) is applied in order to gain a perfectly elastic indentation of a half-space material, in which case force measurements are performed with an axisymmetric indenter and elimination of adhesion forces. The JKR model can be applied to evaluate the mechanical contacts of soft and elastic spherical bodies interacting by short-range adhesion forces. All these equations are valid for spherical tips. The Hertz model is appropriate for the determination of cell elasticity; however, adhesion force is neglected. This model is well adopted to describe sphere-shaped and flat surfaces; however, the surface of some cells is rough; therefore, in such cases, we face some disadvantages of this model. To calculate Young's modulus, the theoretical force model has to be fitted to measured force-distance curves. Usually, the Hertz-Sneddon (22, 23) contact model is used for cells:

$$F = \frac{4}{3}\frac{E\sqrt{\delta^3 \cdot R}}{1-\nu^2}, \qquad (2)$$

where F indicates applied force; E, Young's modulus of the cell; δ, depth of indentation; ν, Poisson ratio; R, spherical radius of the tip end.

The end of the AFM tip can be of different forms: pyramidal, conical, or parabolic. Therefore, contact behavior of conical indenters is described differently:

$$F = \frac{2}{\pi}\cdot\frac{E\cdot tan(\alpha)}{1-\nu^2} \text{ or } F = \frac{2}{\pi}\cdot\frac{E}{1-\nu^2}\cdot\frac{1}{tan(\alpha)} \qquad (3)$$

The above presented descriptions assume deformations arising from perfectly flat elastic substrates (71). However, living cells do not completely meet the assumptions of the Hertz model mainly due to their inhomogeneity (56). Another problem is that the Poisson ratio of the cell is not exactly known, and it usually varies over the cell surface in the range of 0.3–0.5 (56).

In an approach applied by Sirghi et al. (72), the elastic force corresponding to the deformation of the cell membrane is neglected, but the effect of short-range adhesive forces of the cell membrane to the indenter surface is retained. The indenter-cell contact area and associated indenter-cell adhesion energy suffer a continuous variation during cell indentation. Then, the cell indentation force is considered to be determined mainly by elastic force of the cell cytoskeleton and by the indenter-cell membrane adhesive force. Therefore, this approach may result in an erroneous determination of cell elasticity. The authors (72) gave an expression of force P, which is the sum of elastic and adhesion forces:

$$P = \frac{4\,E^{*}\cdot tan(\alpha)}{\pi\sqrt{\pi}} \cdot h^{2} - \frac{32\,\gamma_{a}\cdot \tan(\alpha)}{\pi^{2}\cos(\alpha)} \cdot h, \qquad (4)$$

where $E^{*} = E/(1-\nu^{2})$; α, cone angle of the AFM cantilever pyramidal indenter; h, total inward displacement of the AFM tip; γ_{a}, work of adhesive forces at the tip-sample interface.

Kim et al. presented significantly different results obtained during the measurements of hepatocellular carcinoma cells (HCC) with spherical and conical tips. They found the Young's modulus to be 15.0±3.2 kPa and 52.7±5.3 kPa, respectively (73). The authors concluded that the Hertz-Sneddon model could not explain these results, and the created finite element models resulted in 8.52 kPa and 7.44 kPa for the conical tip and the sphere-shaped tip, respectively.

Concluding Remarks

Recently, atomic force microscopy has become a powerful tool for the investigation of living and immobilized cells. Atomic force microscopy provides a unique characterization of living and fixed cells, which cannot be obtained by any other method. Atomic force microscopy-based measurements provide information on cell surface morphology, presence and distribution of surface proteins and receptors, and viscoelastic properties of the cell membrane. The results of atomic force microscopy depend on a number of experimental conditions. In some measurements of atomic force microscopy, cells must be specially prepared and should be immobilized on the substrate or entrapped in porous membranes. Various immobilization methods are used for different kinds of cells. Fixed cells have a higher Young's modulus compared with unfixed cells. Atomic force microscopy is a powerful tool for the investigation and determination of cancer cells. Atomic force microscopy-based elasticity studies of cancer cells show that they have a lower Young's modulus compared with healthy cells.

Acknowledgments

This research was funded by the European Social Fund under the Global Grant measure, "Enzymes functionalized by polymers and biorecognition unit for selective treatment of target cells" (NanoZim's, project No. VP1-3.1-ŠMM-07-K-02-042). The authors are thankful to the Research Council of Lithuania.

Statement of Conflicts of Interest

The authors state no conflict of interest.

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Received 17 December 2012, accepted 30 April 2013