Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Micron 38 (2007) 824–833
micron
www.elsevier.com/locate/micron

Atomic force microscopy probing of cell elasticity

Tatyana G. Kuznetsova [a], Maria N. Starodubtseva [a,*], Nicolai I. Yegorenkov [b], Sergey A. Chizhik [c], Renat I. Zhdanov [d]

[a] Gomel State Medical University, 5, Lange str., Gomel 246000, Belarus
[b] Gomel State Technical University, 48, ave. Oktyabrya, Gomel 246746, Belarus
[c] A.V. Lykov Heat and Mass Transfer Institute of NASB, 15, Brouki str., Minsk 220072, Belarus
[d] Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, 8, Baltiiskaya St., Moscow 125315, Russian Federation

Abstract

Atomic force microscopy (AFM) has recently provided the great progress in the study of micro- and nanostructures including living cells and cell organelles. Modern AFM techniques allow solving a number of problems of cell biomechanics due to simultaneous evaluation of the local mechanical properties and the topography of the living cells at a high spatial resolution and force sensitivity. Particularly, force spectroscopy is used for mapping mechanical properties of a single cell that provides information on cellular structures including cytoskeleton structure.

This entry is aimed to review the recent AFM applications for the study of dynamics and mechanical properties of intact cells associated with different cell events such as locomotion, differentiation and aging, physiological activation and electromotility, as well as cell pathology. Local mechanical characteristics of different cell types including muscle cells, endothelial and epithelial cells, neurons and glial cells, fibroblasts and osteoblasts, blood cells and sensory cells are analyzed in this paper.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Atomic force microscopy; Cell elasticity; Young's modulus; Cytoskeleton dynamics

1. Introduction

It is now well accepted that the cell functions are essentially determined by its structure. At different hierarchy levels the structural organization of cells is characterized by certain mechanical properties. It is obvious that cell structure should be different both in a variety of physiological processes (such as cell differentiation, growth, adhesion, etc.) and under pathogenesis (oxidative stress, attack of viruses, parasites, etc.). For a long time there have been two approaches for the study of cells' mechanical properties: (i) the cell mechanical properties were integrally studied, when the cell was considered as a single whole and (ii) mechanical properties of the cell structural components were studied in details using isolated lipid bilayers, biomembrane and cytosolic proteins. Only recently it has become possible to probe micro- and nanomechanical properties of cell structures and to study the spatial distribution of mechanical properties of cellular structures within a single cell by the atomic force microscopy (AFM) technique (Hansma, 2001).

AFM is a novel method for high-resolution imaging of any surface including those of living and fixed cells (Bischoff and Hein, 2003, 2004, 2005). This powerful technique is also used for characterization of the mechanical, electrical and magnetic characteristics of samples to be studied both qualitatively and quantitatively. AFM operation is based on the detection of repulsive and/or attractive surface forces. The interaction between the sample surface and a tip (probe) located very close to it corresponds to the force between the atoms of the sample and those of the tip that scans its surface. Image contrast is generated by monitoring the forces of interaction between the tip and the surface. The tip is fabricated under a flexible cantilever responsible for the signal transduction. The interaction between the sample and the tip causes bending or twisting of the cantilever in a manner proportional to the interaction force. A small laser, which is focused on the cantilever, detects any bending or twisting of the cantilever. The reflection of the laser beam is focused on a photodiode detector. The interaction of the sample with the tip is measured by the variation in the reflected beam's point of incidence on the photodiode. Deflection of the cantilever by interaction with features on the sample surface is monitored during scanning and is translated into a three-dimensional image of the surface (Mozafari et al., 2005).

* Corresponding author at: Gomel State University, 5, Lange str., Gomel 246000, Belarus. Tel.: +375 232 745274; fax: +375 232 749831.
E-mail address: marysta@mail.ru (M.N. Starodubtseva).

0968-4328/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micron.2007.06.011

AFM can be operated in a number of different imaging modes depending on the nature of the interaction between the tip and sample surface. The micromechanical properties of cell surface and subsurface layers can be detected either by contact mode AFM techniques (force modulation, lateral force microscopy and force-curve analysis) or by phase imaging in the tapping mode AFM (intermittent, semi contact) (Fig. 1).

Probing of cell surface by AFM techniques can reveal heterogeneities of mechanical properties of the surface at nanolevel, and subsurface layers of cells. The resolution of AFM in air at vertical direction is ~0.1–0.5 nm, and at horizontal direction is ~1–5 nm, depending on sample rigidity. The horizontal resolution can be solved for living cells in aqueous medium even at several tens of nanometers range due to the softness of the cell membrane. The thickness of cellular membranes is known to be around 5–10 nm. When analyzing their heterogeneities by AFM techniques we are able to create the picture of the cellular structure of certain regions within a single cell. The sensitivity and resolution of AFM method depend also on tip and cantilever characteristics (e.g., radius, shape, material) (Alessandrini and Facci, 2005).

The basic AFM technique for quantitative study of mechanical characteristics of cells and tissues is the force spectroscopy (namely, force-curve analysis). By recording the force value and vertical deflection of the cantilever, the probe approaches the surface under the study at the fixed point and usually performs force-curve analysis. The force value versus distance between the probe and the surface can be plotted in this case. The force curve contains information about long- and short-range interactions and represents a basis for estimation of sample Young's modulus. Today, there is a serious problem in estimation of absolute value of cellular Young's modulus using AFM force-curve analysis due to the problem of what appropriate mechanical model to choose.

Up to now the Hertz model has been used in the majority of articles devoted to the evaluation of Young's modulus of cells. The Hertz model describes the simple case of elastic deformation of two perfectly homogeneous smooth bodies touching under load (Hertz, 1881; Johnson, 1985). Two important assumptions of the Hertz model are the followings: (i) the indenter must have a parabolic shape and (ii) the indented sample is assumed to be extremely thick in comparison to the indentation depth. The first assumption remains a valid one for the case when a spherical tip radius is much bigger than the indentation depth ($h < 0.3R$) (Mahaffy et al., 2000).

If the tip of an AFM nanoscope is approximated by a sphere with the radius R, then the force on cantilever $F(h)$ is given by:

$$F(h) = \frac{4\sqrt{R}}{3} E^{*} h^{3/2}$$

where h is the depth of the indentation, E^{*} the effective modulus of a system tip-sample, which is calculated from

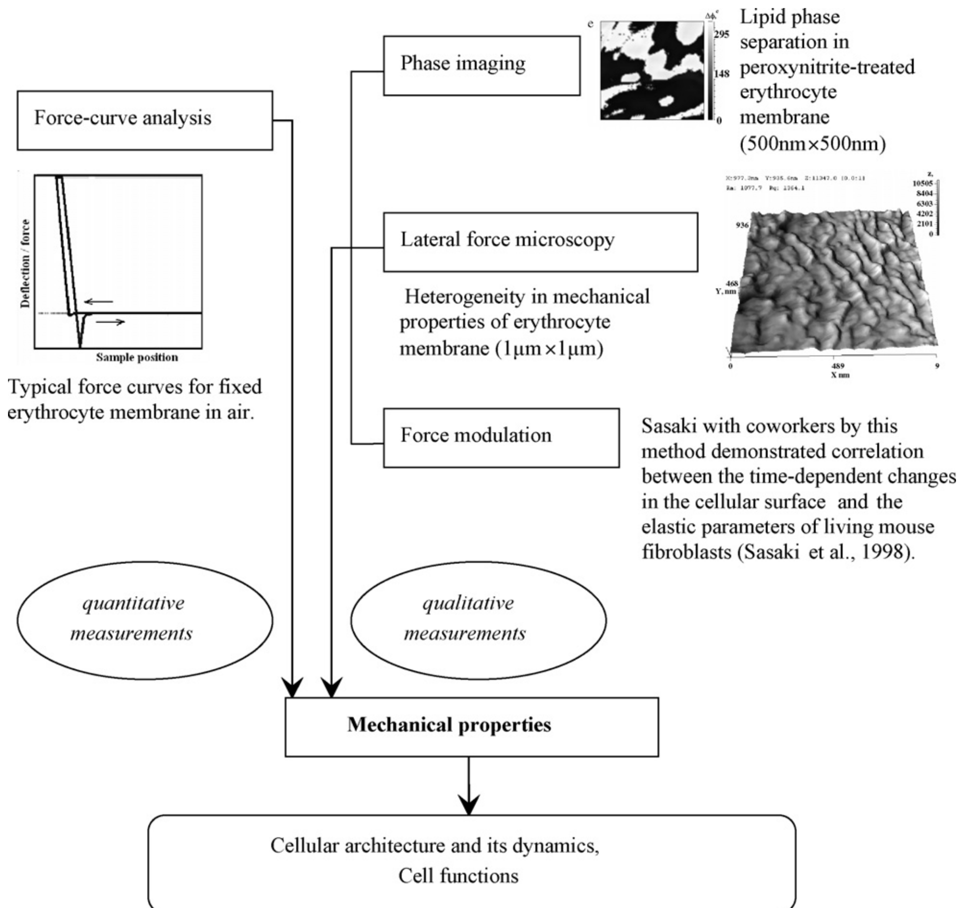

Fig. 1. Scheme of atomic force microscopy (AFM) probing the cells.

the equation:

$$\frac{1}{E^*} = \frac{1-\upsilon_{\text{tip}}^2}{E_{\text{tip}}} + \frac{1-\upsilon_{\text{sample}}^2}{E_{\text{sample}}}$$

in which E_{tip}, υ_{tip} and E_{sample}, υ_{sample} are the Young's modules and the Poisson ratios for the materials of tip and the sample, respectively. If the material of the tip is considerably harder than the sample the following equation is used (Vinckier and Semenza, 1998):

$$E^* \approx \frac{E_{\text{sample}}}{1-\upsilon_{\text{sample}}^2}$$

The Sneddon's variation of the Hertz model is used for the case of cone tip of AFM cantilever (Laurent et al., 2005):

$$F(h) = \frac{2}{\pi} \tan\alpha \frac{E_{\text{sample}}}{1-\upsilon_{\text{sample}}^2} h^2$$

where α is the half-opening angle of the AFM tip.

Though the indentation depth in case of AFM probing of the cell is in the range of hundreds of nanometers, which is higher than an appropriate depth for the Hertz model, it was shown in many studies that the Hertz model describes sufficiently the experimental data. The original Hertz theory did not allow adhesion of the indenter to material. Johnson, Kendal and Roberts modified the theory for that case (Johnson et al., 1971). The Hertz model was mainly used for an estimation of static Young's modulus of cells, but dynamic Young's modulus was sometimes used for the characterization of cell elastic properties (Mahaffy et al., 2004). Because cell surface is heterogeneous (that is a network of cell membrane and sub-membrane structures), the cellular Young's modulus evaluation using the Hertz model assumes an error.

The second model used for studying cell elastic properties basing on force spectroscopy data is a model based on the theory of elastic shells (ES) (A-Hassan et al., 1998; Scheffer et al., 2001; Timoshenko and Woinowsky-Krieger, 1970). This theory considers cells as the shells filled with liquid. In such an approach, effective Young's modulus can be evaluated from the relationship between effective Young's modulus, shell thickness and bending modulus. The serious problem of such evaluation procedure is the determination of the boundary conditions for the calculation of any constants involved in main relation and the definition of the tip-sample contact radius.

Among other theoretical models used in AFM-based evaluation of cell mechanical characteristics we should mention the finite element model, which is the most popular model for analysis of elasticity problems in engineering (Ohashi et al., 2002).

The values of elasticity parameters calculated using various models differ each other. For example, Ohashi's group studying mechanical properties of endothelial cells exposed to shear stress (Ohashi et al., 2002) calculated different values of Young's modulus using various models. The modulus values calculated using a finite element model appeared to be significantly higher: from 12.2 ± 4.2 to 18.7 ± 5.7 kPa with exposure to shear stress. The modulus value calculated using the Hertz model reflects the same tendency, but has different means (0.87 ± 0.23 and 1.75 ± 0.43 kPa for control and sheared endothelial cells, respectively).

The AFM experimental approach named as force integration to equal limits (FIEL) mapping, for producing quantitative maps of relative cell elasticity was developed in 1998 (A-Hassan et al., 1998). FIEL theory assumes a simple relationship between values of the works done by the AFM cantilever during an indentation and the elastic constants at different surface positions.

The collection of force curves over a certain area allows creation of the elasticity map of the cell surface. The surface elasticity map can be also obtained using either force-modulation (static mode) or phase imaging (tapping mode) techniques. The characteristics of cantilever oscillation (amplitude and phase shift) carry on information about local elastic and friction properties of the sample in both cases. The image of the changes in oscillation characteristics represents a map of relative mechanical properties of cell surface.

Indirect information on elastic properties of cell surface can be provided by recording the lateral force map of surface to be studied. The AFM cantilever lateral deflections (torsion) arise because of either the changes in the surface slop or heterogeneity of surface frictional properties. The lateral force map is simultaneously analyzed with sample surface topography to elaborate the specificity of elastic property map.

This paper is aimed to analyze the progress in the usage of AFM probing elasticity of mammalian cells for the study of their temporal and spatial structural dynamics under physiological and pathological processes. To analyze the mechanical properties of different cells comparatively, we only chose the articles in which Young's modulus were used for the characterization of cell mechanical properties.

2. Young's modulus and its AFM measurements in living cells

Table 1 summarizes the results of Young's modulus measurement for different types of living mammalian cells. It shows that the elastic modulus value of living cells varies in wide range. It is evident that both real variability of the parameter and imperfection of AFM methods of measurement and numerical estimation of cell elasticity are present. The analysis of almost a decade progress in this area allows generalizing some methodological factors having a significant influence on Young's modulus value. The technical and theoretical problems of those studies were discussed in the Section 1. Here, we discuss the problems connected with cell specificity. Literature survey demonstrated that under a change of the external conditions, the elasticity of cell membranes changes much stronger than the morphology of cell.

The first factor is a question of AFM sample handling. The appropriate object for an illustration of the progress in this acute area is erythrocytes. AFM method has been used to study both the living (Nowakowski et al., 2001; Kamruzzahan et al., 2004) and fixed erythrocytes either in air (Gould et al., 1990) or in buffer (Butt et al., 1990). The living cells are rather soft and delicate for

Table 1
Young's modulus of mammalian cells

Cell type	*E* (kPa)	Commentary	References
Endothelial cells			
HUVEC	10–11		Sato et al. (2004)
–	1.3–7.2	Spatial heterogeneity	Mathur et al. (2000)
–	0.9–1.7; 12.0–18	Shear stress, different models	Ohashi et al. (2002)
BPAEC	0.2–2.0	Spatial heterogeneity	Pesen and Hoh (2005)
Leukocytes			
Leukemia myeloid cells (HL60)	0.2–1.4		Rosenbluth et al. (2006)
Leukemia lymphoid (Jurkat) cells	0.02–0.08		
Neutrophils	0.2–0.07		
Corti organ's cells			
Outer hair cells	300–400	Cortical lattice	Tolomeo et al. (1996)
Guinea pig's outer hair cells	2–4	Different levels of cochlea	Sugawara et al. (2002)
Mouse outer hair cells	2–4		Murakoshi et al. (2006)
Guinea pig's inner hair cells	0.1–0.5		Sugawara et al. (2002)
Hensen's cells	0.3–1.1		–
Osteoblasts	0.3–20.0	Changes at adhesion	Simon et al. (2003)
Astrocytes	2–20	Spatial heterogeneity	Yamane et al. (2000)
Fibroblasts	4–5		Bushell et al. (1999)
Migrating 3T3 cells	3–12	Spatial heterogeneity	Rotsch et al. (1999)
–	0.6–1.6	Changes at adhesion	Mahaffy et al. (2004)
L 929	4–5		Wu et al. (1998)
Epidermal keratocytes	10–55	Spatial heterogeneity	Laurent et al. (2005)
Platelets	1–50	Spatial heterogeneity at activation	Radmacher et al. (1996)
Skeletal muscle cells			
Murine C_2C_{12} myoblasts	11–45	Change at differentiation	Collinsworth et al. (2002)
Murine C_2C_{12} myotubes	8–14	Changes at differentiation, Treating with L-arginine	Zhang et al. (2004)
–	10–17		–
–	28–21		Mathur et al. (2001)
Myofibrils	40–45		Yoshikawa et al. (1999)
Cardiocytes	90–110		Mathur et al. (2001)
Rat	32–42	Aging changes	Lieber et al. (2004)
Chicken	5–200	Spatial heterogeneity	Hofmann et al. (1997)
Erythrocytes	14–18		Mozhanova et al. (2003)
	19–33	Normal	Dulinska et al. (2006)
	22–64	Hereditary spherocytosis, thalassemia, G6PD deficiency	–
	16–64		–
	70–110		–

their AFM probing under physiological conditions. Their drying, freezing and fixing with chemical agents improve the AFM images and AFM indentation results. However, these procedures change cell structure, viability and elasticity. The Young's modulus values for erythrocytes treated with 5% formalin solution are increased 10-fold (119.5 ± 15 kPa) compare to viable (native) erythrocytes (16.05 ± 2.3 kPa) (Mozhanova et al., 2003). Transverse stiffness of cardiomyocytes is also increased by a factor of 16 after fixing with formalin (Shroff et al., 1995). Takeuchi et al. (1998) comparing a variety of methods for preparing erythrocyte ghost for AFM studies showed that air drying is not suitable even after fixation in glutaraldehyde. On the other hand, fixation enhances the images of cell structures like the cytoskeleton (Shroff et al., 1995, Hofmann et al., 1997). Moreover, the highest resolution for cells, 10 nm, may be achieved only in air, which presumes cell fixation before AFM probing. The high mobility of the erythrocyte shape in buffer solutions leads to smearing of the AFM image and the maximal space resolution of living erythrocytes can be only about 200 nm (Mozhanova et al., 2003).

Standard AFM technique for cell elasticity measurement is based on indentation of the cells firmly attached to the substrate. For reliable results of indentation, the firm substrate/cell contact is required and that is a problem for nonadhered cells in solution. A good approach for the immobilization of native erythrocytes in liquid is attachment to glass surface previously modified with poly-L-lysine solution. Poly-L-lysine provides the accurate localization of red blood cells on the glass surface due to electrostatic interaction between the negatively charged cell surface and the positively charged poly-L-lysine layer. However, poly-L-lysine can induce membrane rearrangement with the formation of specific membrane deformation pattern within contact area (Dulinska et al., 2006). Recently, a new method of indentation of leukemia cells placed at special

microwells was reported (Rosenbluth et al., 2006). This method provides the mechanical immobilization of cells, but also has an influence on the estimated Young's modulus value. In this case the cell deformation is well described by elastic model based on Hertzian mechanics.

The second factor is related to the heterogeneity of mechanical properties of cells. There are significant variations of the values of elastic modulus at different cell regions. Mathur et al. (2000) showed that the elastic modulus value of human umbilical vein endothelial cells was 7.22 $\pm$ 0.46 kPa over the nucleus; 2.97 $\pm$ 0.79 kPa over the cell body in proximity to the nucleus, and 1.27 $\pm$ 0.36 kPa on cell body near the edge. Costa and Yin (1999) reported that the cell body of bovine pulmonary artery endothelial cells was two- to three-fold softer than the cell periphery. The corresponding study on cardiomyocytes also revealed that cells are softer at the nuclear region, and become stiffer toward the periphery (Shroff et al., 1995). Mapping of the Young's modulus across the living chicken cardiocytes, Hofmann et al. (1997) stated that the stress fibers were characterized by the presence of areas with a stiffness of 100–200 kPa embedded in softer parts of the cell, with elastic modulus values between 5 and 30 kPa. The elasticity map images of living astrocytes (glial cells of nerve tissue) showed that cell membrane above the nucleus was softer (2–3 kPa) than the surroundings, and that the cell membrane above ridge-like structures reflecting F-actin was stiffer (10–20 kPa) than the surroundings. In the elasticity map images of fixed astrocytes, on the other hand, the elasticity value of cells was found to be relatively uniform (200–700 kPa) irrespective of the inner structures of cells (Yamane et al., 2000). On the other hand, the variations of elastic modulus value within single cell are often objects of the study themselves.

The third factor influencing elastic modulus measurement is connected with cell thickness. Substrate contributions can be neglected if AFM tip never indented more than 10% of the cell thickness (Mathur et al., 2001). If the cell compartment under the study is very thin ($<$1000 nm), e.g., in the case of lamellipodium, it is necessary to impart special challenges for accurate measurements of its viscoelastic behavior. Mahaffy et al. (2004) reported the method for AFM-based microrheology that allowed to estimate the viscoelastic constants of thin parts of cell ($<$1000 nm) as well as those of thick areas, applying two different models—a model for well-adhered regions and a model for nonadhered regions.

3. Cell biology and cell elasticity

Although Young's modulus obtained by AFM techniques must be carefully assessed as the absolute values, it is very useful as relative parameter in certain experiments. Therefore, Young's modulus can be successfully used in the study of a variety of cell functions, some examples of which are given below.

3.1. Functional mechanics of endothelial cells

Vascular endothelial cells represent an interesting system for studying cell mechanics and cytoskeleton itself. These cells are found in mechanically active environment, and they are required to withstand shear stress, blood pressure, and any changes in pressure due to breathing cycles. The earliest AFM works on living aortic endothelial cells were devoted to studying the effects of shear stress on cellular organization and other factors which may influence the mechanical response of cells to flow (Barbee et al., 1994; Barbee, 1995; Sato et al., 2000; Ohashi et al., 2002). Ohashi et al. (2002) reported that the local elastic parameters of aortic endothelial cells significantly increased (from 0.87 $\pm$ 0.23 to 1.75 $\pm$ 0.43 kPa) with exposure to shear stress. The average elastic modulus values of bovine pulmonary artery endothelial cells (BPAECs) reported by Pesen and Hoh (2005) were in the similar range of 0.2–2 kPa. Miyazaki and Hayashi (1999) demonstrated the difference in the mechanical properties of rabbit endothelium. So, cells were stiffer in the medial wall of aortic bifurcation than in the lateral wall.

Using AFM and human umbilical vein endothelial cells (HUVEC), Mathur et al. (2000) showed that the cell responds globally to the localized applied force over the cell edge and the nucleus. They concluded that the nuclear region of the cell appears to be stiffer than the rest of the cell body and although the nucleus appears to be offset from the basal surface, the focal adhesion movement upon the apical cell surface perturbation indicates a link between the nucleus and the focal adhesions via the cytoskeleton (Mathur et al., 2000).

3.2. Platelet activation

Activation of platelets, leading to a drastic change in the cytoskeletal structure and marked change in cell shape, can be induced by a contact with wettable surfaces or with tip during AFM probing. Fritz et al. (1994, 1993) demonstrated that platelets, which have bound to the surface but not yet activated, can be scanned by AFM at low forces without any noticeable time-dependent changes in shape. However, scanning at higher forces promotes their activation. Topographic and elasticity maps of viable-activated cells were created and analyzed using force mapping techniques (Radmacher et al., 1996). The authors reported that the elastic modulus values of activated platelets were in a range of 1–50 kPa.

3.3. Cell locomotion

Cell motility is ultimately a mechanical phenomenon, but very little is known about the mechanical and physical properties of moving cells, including these properties in specific cell regions at specific stages of the cell migration process. So, nanoindentation can be very useful in this case.

A few AFM studies of time-dependent structural changes, cell migration and the associated changes in mechanical properties have been reported for fibroblasts (Sasaki et al., 1998; Nagayama et al., 2001; Haga et al., 2000). The usage of the force modulation mode allowed demonstrating the existence of a correlation between the time-dependent changes of cell surface and the elastic parameters of viable mouse fibroblasts in culture medium (Sasaki et al., 1998). It is interesting that some cells continued to

shrink and change their softness for several hours. The results indicated that the AFM force-mapping technique does not appreciably perturb cell mechanical dynamics.

A clear relationship between the local stiffness distribution on cell and cell migration was also found. The stiffness distribution of cell surface was quite constant for stationary cells, but if cells started to move, the stiffness in their nuclear regions was drastically decreased (Nagayama et al., 2001).

The mechanical properties of the leading edge of migrating cells, i.e., the lamellipodium, are important for a deeper understanding of cell locomotion mechanism. Haga et al. (2000) found that the stable part of the fibroblast cell body was stiffer and did not demonstrate morphological changes for over 1 h. The authors proposed that it is connected with excess condensation of the actin network, hardening cell cortex, and lowering cytoskeletal activity. The nuclear region of cell body was slightly less viscous than the peripheral region.

Dimensional and mechanical dynamics of active and stable edges in motile 3T3 fibroblasts in culture were also investigated (Rotsch et al., 1999). The cortical stiffness calculated for the stable edges was 12 kPa. Contrary to that, the leading edge stiffness had an upper limit of 3–5 kPa.

Recently, the fish epidermal keratocytes with their stable shape and steady motion were studied (Laurent et al., 2005). The authors demonstrated that though vertical lamellipodia thickness remained nearly constant, the rigidity value was highest at the front protruding edge of the lamellipodia, and gradually decreased along with a distance from the edge. The values of the elastic modulus exhibited a tendency to decrease from 55 kPa at the front of the lamellipodium to 10 kPa at its rear. Those differences in rigidity may indeed reflect the differences in the structure of the leading edge between different types of fibroblasts.

3.4. Cell adhesion

AFM method is also effective for the study of cell mechanical properties during cell adhesion. There are some AFM studies which characterized osteoblast elastic properties during adhesion (Domke et al., 2000; Takai et al., 2005, Simon et al., 2003). Liang et al. (2004) employed AFM and model cells for the elucidation of micromechanical properties of biomembranes (Fig. 2). Domke et al. (2000) used the AFM method for testing the biocompatibility of implanted materials by the study of the elastic properties of osteoblast cells adhered to the different surfaces. Takai et al. (2005) determined, e.g., the apparent elastic modulus of osteoblast-like MC3T3-E1 cells adhered to different surfaces. They stated that the elastic modulus values of osteoblasts adhered to extracellular matrix proteins that bind cells using integrins were higher compared to cells on glass and poly-L-lysine adhering the cells through nonspecific binding. It was suggested that the enhanced modulus value of osteoblasts adhered to extracellular matrix proteins was due to remodeling of the actin cytoskeleton, and modulation of cell stiffness upon adhesion to various substrates may influence mechanosignal transduction in osteoblasts. The elastic properties of osteoblasts cultured on two types of surface to induce weak and strong cellular adhesions were also studied by AFM technique (Simon et al., 2003). The values of elastic modulus were between 0.3 and 200 kPa depending on the level of cell adhesion and the approaches used to measure this elastic modulus value. The authors concluded that a comparison between the elasticity of viable cells may be used as demonstration of cytoskeletal reorganization and the state of cell adhesion.

Studying the elastic properties of fibroblast lamellipodium, Mahaffy et al. (2004) applied two different models: a model for well-adhered regions and a model for nonadhered regions. They showed that very thin regions relatively near the edge of NIH 3T3 fibroblasts were strongly adherent with an elastic strength value of 1.6 ± 0.2 kPa, and the regions quite far from the edge of these cells adhered worse, and therefore Young's modulus value was less (0.6 ± 0.1 kPa).

3.5. Physiology of sensory cells

The mechanical property measurement can also be useful for the study of sensory cells physiology including sensory hearing

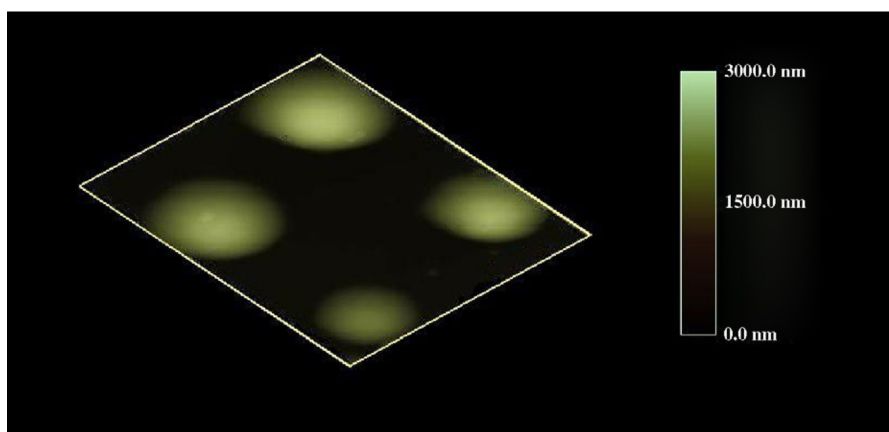

Fig. 2. A representative AFM height image of fixed model cell membranes (EggPC:DCP:Chol, 7:2:1 molar ratio). Imaging was done at a scan rate of 0.3 Hz. Courtesy of Dr. M.R. Mozafari.

cells, capable to alter their length in response to changes in membrane potential and subject the basilar membrane of inner ear to force, resulting in cochlear amplification. The force produced by piliform (hear hair) cells' electromotility is thought to depend not only on the conformational change of the protein motors, but also on the mechanical properties of the cell lateral wall (Murakoshi et al., 2006). Sugawara et al. (2002) revealed that elastic modulus values in the apical region of the outer piliform cells were three times higher than those in the basal and middle regions. Other reports (Wada et al., 2003) also state that the stiffness of the apical region of outer piliform cells is greater than that in other regions. According to Wada's results, a difference between the intervals of the actin circumferential filaments in the apical region and those in other regions is one factor that causes the high stiffness in this part of cell lateral wall. It was found that Young's modulus value decreases with an increase in the piliform cell length. Young's modulus values in the middle region of a long outer piliform cells obtained from the apical turn of the cochlea and that of a short outer piliform cells obtained from the basal turn or the second turn were 2.0 ± 0.81 kPa and 3.7 ± 0.96 kPa, respectively (Sugawara et al., 2002).

The calculated Young's modulus values of the guinea pig cells were 0.29 ± 0.20 kPa for inner hair cells and 0.69 ± 0.45 kPa for Hensen's cells (Sugawara et al., 2004). It is interesting that the species differences in the estimated values of elastic modulus of each type of hair cells is insignificant. So, the Young's modulus value of the mouse outer hair cells in the apical turn of the cochlea (2.1 ± 0.5 kPa) was similar to that of the guinea pig cochlea (Murakoshi et al., 2006).

3.6. *Role of cytoskeleton in elastic property formation*

The unique advantage of AFM method is the opportunity to perform measurement of the mechanical properties of cells and visualization of important cellular structures like cytoskeleton simultaneously (Fig. 3). It allows understanding the mechanisms of cell functions thoroughly. To provide the fast progress in studying cellular mechanisms with cytoskeleton involvement, AFM analysis is often combined with confocal fluorescent microscopy or usage of a drug destructing cytoskeleton components. The data collected show clearly that elastic response of cell is due to the actin network to a high degree. Using all these methods Pesen and Hoh (2005) characterized the micromechanical architecture of the cortex in bovine pulmonary artery endothelial cells. The authors showed that the cortex in these cells is organized as a polygonal mesh at two levels: a coarse mesh with dimensions of several micrometers and a fine overlapping mesh with dimensions of hundreds of nanometers. These meshes appear to be intertwined and are in part composed of actin and vimentin. The analysis of fluorescent images and elasticity maps revealed that in the case of activated platelets (Radmacher et al., 1996) and cardiomyocytes (Shroff et al., 1995) the variation of elastic properties across the cell was correlated with the cytoskeletal heterogeneity as well as the significant increase in the elastic modulus value of endothelial cells with exposure to shear stress occurred due to the remodeling of cytoskeletal structure (Ohashi et al., 2002).

The role of cytoskeletal elements in formation of the mechanical properties of cells was found more precisely using a variety of medicines affecting the cytoskeleton. According to Rotsch et al. (1997), who studied viable cultured rat liver macrophages, the chemical disassembly of the actin network by applying cytochalasin B decreases the cell's average elastic modulus seven-fold within less than 40 min. Treating cells with latrunculin A results in a two-fold decrease in the elastic modulus of the perinuclear region after 40 min, whereas other parts of cell are not affected. The latruculin-induced disruption of the cytoskeleton network was also observed by AFM images of skin fibroblasts (Braet et al., 2001) and two different fibroblast cell lines (Rotsch and Radmacher, 2000). A decrease of the elastic modulus for viable chicken cardiocytes after their treatment with cytochalasin B as well as for L929 cells affected with cytochalasin D was demonstrated by two groups

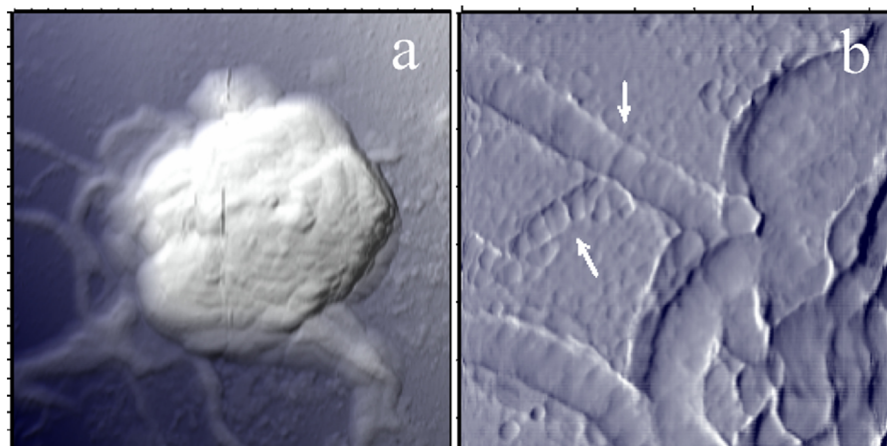

Fig. 3. The unique structure of lamellipodium cytoskeleton determines its local mechanical properties: (a) topography of small lymphocyte ($5\ \mu m \times 5\ \mu m$) and (b) lateral force map of its lamellipodia ($1.5\ \mu m \times 1.5\ \mu m$). Arrows show the structures arranged transverse to lamellipodium growth direction. The lymphocytes were isolated from peripheral venous blood obtained from healthy volunteers by centrifugation over a Ficoll gradient. The cells were treated with $0.5\ \mu M$ peroxynitrite in suspension, placed on glass plate for 20 min adhesion, fixed with 1% glutaraldehyde, dried on an air. The topography and lateral force map of lymphocyte surface were produced by AFM "NT-206" (Belarus).

(Hofmann et al., 1997; Wu et al., 1998). On the contrary, the treatment of fibroblasts with nocodazole or colcemid induces a marked increase in their elasticity (Wu et al., 1998). Using such cytoskeleton medicines as cytochalasins B and D, latrunculin A and Jasplakinolide, Rotsch and Radmacher (2000) showed that disaggregation of actin filaments results in a decrease in the elastic modulus of fibroblasts, while reorganization of microtubules did not affect cell elasticity.

The rigidity profile of migrating epidermal keratocytes closely resembles the actin density profile, suggesting that the dynamics of rigidity is due to actin depolymerization (Laurent et al., 2005). The authors suggest that a decrease of rigidity may play a role in facilitating the contraction of the actin-myosin network at the lamellipodium/cell body transition zone. Haga et al. (2000) also propose that higher rigidity of cell body compared to lamellipodia of migrating fibroblast is connected to an excess condensation of the actin network, hardening the cell cortex, and lowering the cytoskeletal activity.

3.7. Cell differentiation and aging

The changes in mechanical properties and cytoskeleton reorganization seem to be correlated with cell cycle stages (Sato et al., 2004; Collinsworth et al., 2002; Zhang et al., 2004; Lieber et al., 2004; Berdyyeva et al., 2005). Therefore, these results form the basis for understanding the mechanisms of cell differentiation and organism ageing.

AFM studies of human umbilical vein endothelial cells reveal that cell elasticity depends on the culture period (Sato et al., 2004). The elasticity of cells cultured on type IV collagen for longer than 4 days leads to average elasticity values higher than 10 kPa. The data obtained for human epithelial cells (Berdyyeva et al., 2005) also prove an increase in cell rigidity during ageing in vitro.

AFM-nanoindentation has been recently used to analyze the ageing changes of cardiac myocytes of young and old male Fischer 344 × Brown Norway F1 hybrid rats (Lieber et al., 2004). The significant increase of the apparent elastic modulus of cardiac myocytes with advanced age was found. The elastic modulus values are changed from 35.1 ± 0.7 kPa for young rat cells to 42.5 ± 1.0 kPa for old rat cells. Results of the study support the authors' concept that the mechanism mediating LV diastolic dysfunction in ageing hearts resides, in part, at the level of the myocyte.

The differentiation of skeletal muscle is a complex process, which helps to understand the functional properties and mechanisms of the muscle tissue regeneration. It includes subsequent fusion of myoblasts to form multinucleated myotubes or myofibers, and expression of differentiation-specific proteins. Cell transformation is accomplished by the changes of cytoskeletal structures and probably lead to the changes in the mechanical properties of differentiated and undifferentiated skeletal muscle cells.

Collinsworth et al. (2002) used AFM to elucidate the nature of mammalian myocyte mechanical properties throughout their development and to test the hypothesis that the transverse elastic and viscous properties of skeletal muscle cells change throughout the time course of differentiation of myoblast to myofiber. They demonstrated the dramatic change in the passive mechanical behavior of mouse skeletal myocytes which appeared in a significant increase of the apparent elastic modulus with differentiation from 11.5 ± 1.3 kPa for undifferentiated myoblasts to 45.3 ± 4.0 kPa after 8 days of differentiation. Results of examining the actin, myosin, and tubulin demonstrated that major contributors to changes in the transverse elastic modulus during differentiation are actin and myosin.

The elastic modulus of the multinucleated myocytes and its varieties during cell differentiation were also estimated (Zhang et al., 2004). As it turned out at the second day of cell differentiation, elastic modulus for statically stretched cells was 8.3 ± 1.6 kPa and increased until fourth day up to 14.3 ± 2.4 kPa.

3.8. Pathology

AFM investigations can be useful for studying cell pathology. Any factors having an influence on cell structures can cause the alterations in mechanical properties of cell (Garcia et al., 1997). The determination of the local elastic properties of cells in their culture conditions has opened the possibility for the measurement of the influence of different factors on the mechanical properties of the living cells. That is why the AFM estimation of cell mechanical properties seems to be a perspective method of diagnostics of different pathologies.

Dulinska et al. (2006) studied the elastic properties of erythrocytes from patients with different types of anemias using force spectroscopy and compared the results with those obtained in normal cells. Additional comparison was performed for anisocytic erythrocytes since the authors considered that alteration of the erythrocytes shape could be reflected by changes of Young's modulus. According to Dulinska et al. (2006) Young's modulus of pathological erythrocytes was two to three times higher than in normal cells. The values of elastic modulus were: 26 ± 7, 43 ± 21, 40 ± 24 and 90 ± 20 kPa for control, hereditary spherocytosis, thalassemia, and G6PD deficiency, respectively. The maximal change was observed for erythrocytes with G6PD deficiency, where the calculated Young's modulus was more than three times larger than in normal cells. The authors attribute the increase of the Young's modulus to the change in cytoskeleton structure whether due to the changes of spectrin structure, the molecular anomaly in hemoglobin structure or impaired ATP metabolism. They also showed the change in distribution of Young's modulus in erythrocytes. It became broader in case of pathologically altered erythrocytes and even became bimodal at anisocytosis.

In our laboratory the comparative AFM study of elastic properties of normal and peroxynitrite-treated erythrocyte was carried out. Peroxynitrite as a reactive nitrogen species reacts with both protein and lipid components of membrane and membrane skeleton. According to our data peroxynitrite (>1 mM) causes about two- to three-fold increase in Young's modulus of erythrocyte and also results in the broadening of the Young's modulus distribution peak. We also attribute these changes of erythrocyte mechanical properties distribution to

cytoskeletal rearrangements. We visualized those cytoskeleton rearrangements with lateral force map of erythrocyte surface.

Influence of hydrocortisone on the mechanical and morphological properties of confluent cell layers of brain microvascular endothelial cells was examined by Schrot et al. (2005). They showed that hydrocortisone induced a reduction of the intercellular contact surface and changes in cell elasticity.

Because epithelial cells most often are subjected to carcinogenic transformation, comparative studies of normal and cancerous human epithelial cells were also carried out (Lekka et al., 1999). According to this work the normal cells had a Young's modulus of about one order of magnitude higher than cancerous ones. The authors suggested that such change in elastic properties might be attributed to a difference in the organization of cell cytoskeletons. This change is associated with the increased crosslinking of extracellular matrix proteins. In a later work the same authors (Lekka et al., 2001) showed the strong correlation between the decrease of the energy production and the increase in Young's modulus values obtained for the cancer cells treated with chitosan.

4. Conclusions

AFM probing of whole cells is an effective tool for studying membrane and sub-membrane cell structures. Because the heterogeneity of cell mechanical properties is mainly defined by membrane cytoskeleton, AFM probing of the cell elasticity can be effectively used in investigation of cytoskeleton characteristics and dynamics, for example, for studying migrating cells. Although for these purposes the force spectroscopy methods are commonly used, the lateral force microscopy, phase imaging and force modulation methods can be useful in the evaluation of spatial distribution of mechanical and structural characteristics of cytoskeleton. Another perspective opportunity of AFM probing cell elasticity is investigation of the lipid phase characteristics and dynamics on living and fixed cells using phase imaging in intermittent mode. Estimation of structural state (cytoskeleton or membrane lipid phase) of living cells with AFM can be of great importance in diagnostics of different human diseases.

In this article, we were focused at AFM applications to mainly characterize the mechanical properties of intact cells both quantitatively and qualitatively. We realize that only a part of the work done in the field could be considered in a single article, other contributions will be covered in future papers.

References

A-Hassan, E., Heinz, W.F., Antonik, M.D., D'Costa, N.P., Nageswaran, S., Schoenenberger, C.-A., Hoh, J.N., 1998. Relative microelastic mapping of living cells by atomic force microscopy. Biophys. J. 74, 1564–1578.

Alessandrini, A., Facci, P., 2005. AFM: a versatile tool in biophysics. Meas. Sci. Technol. 16, R65–R92.

Barbee, K.A., 1995. Changes in surface topography in endothelial monolayers with time at confluence: influence on subcellular shear stress distribution due to flow. Biochem. Cell Biol. 73, 501–505.

Barbee, K.A., Davies, P.F., Lal, R., 1994. Shear stress-induced reorganisation of the surface topography of living endothelial cells imaged by atomic force miroscopy. Circ. Res. 74, 163–171.

Berdyyeva, T.K., Woodworth, C.D., Sokolov, I., 2005. Human epithelial cells increase their rigidity with ageing in vitro: direct measurements. Phys. Med. Biol. 50 (1), 81–92.

Bischoff, G., Hein, H.-J. (Eds.), 2003. Micro- and Nanostructures of Biological Systems, vol. 1. Shaker Verlag, Aachen.

Bischoff, G., Hein, H.-J. (Eds.), 2003. Micro- and Nanostructures of Biological Systems, vol. 2. Shaker Verlag, Aachen.

Bischoff, G., Hein, H.-J. (Eds.), 2003. Micro- and Nanostructures of Biological Systems, vol. 3. Shaker Verlag, Aachen.

Braet, F., de Zanger, R., Seynaeve, C., Baekeland, M., Wisse, E., 2001. A comparative atomic force microscopy study on living skin fibroblasts and liver endothelial cells. J. Electron Microsc. (Tokyo) 50 (4), 283–290.

Bushell, G.R., Cahill, C., Clarke, F.M., Gibson, C.T., Myhra, S., Watson, G.S., 1999. Imaging and force–distance analysis of human fibroblasts in vitro by atomic force microscopy. Cytometry 36 (3), 254–264.

Butt, H.-J., Wolff, E.K., Gould, S.A.C., Northern, B.D., Peterson, C.M., Hansma, P.K., 1990. Imaging cells with the atomic force microscope. J. Struct. Biol. 105, 54–61.

Collinsworth, A.M., Zhang, S., Kraus, W.E., Truskey, G.A., 2002. Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation. Am. J. Physiol. Cell Physiol. 283, C1219–C1227.

Costa, K.D., Yin, F.C.P., 1999. Analysis of indentation: implications for measuring mechanical properties with atomic force microscopy. J. Biomech. Eng. 121, 462–471.

Domke, J., Dannohl, S., Parak, W.J., Muller, O., Aicher, W.K., Radmacher, M., 2000. Substrate dependent differences in morphology and elasticity of living osteoblasts investigated by atomic force microscopy. Colloids Surf. B: Biointerfaces 19 (4), 367–379.

Dulinska, I., Targosz, M., Strojny, W., Lekka, M., Czuba, P., Balwierz, W., Szymonski, M., 2006. Stiffness of normal and pathological erythrocytes studied by means of atomic force microscopy. J. Biochem. Biophys. Meth. 66, 1–11.

Fritz, M., Radmacher, M., Gaub, H.E., 1994. Granula motion and membrane spreading during activation of human platelets imaged by atomic force microscopy. Biophys. J. 66 (5), 1328–1334.

Fritz, M., Radmacher, M., Gaub, H.E., 1993. *In vitro* activation of human platelets triggered and probed by atomic force microscopy. Exp. Cell Res. 205, 187–190.

Garcia, C.R.S., Takeuschi, M., Yoshioka, K., Miyamoto, H., 1997. Imaging *Plasmodium falciparum*-infected ghosts and parasite by atomic force microscopy. J. Struct. Biol. 119, 92–98.

Gould, S.A.C., Drake, B., Prater, C.B., Weisenhorn, A.L., Manne, S., Hansma, H.G., Hansma, P.K., Massie, J., Longmire, M., Elings, V., Northern, B.D., MuKergee, B., Peterson, C.M., Stoeckenius, W., Albrecht, T.R., Quate, C.F., 1990. From atoms to integrated circuit chips, blood cells, and bacteria with the atomic force microscope. J. Vac. Sci. Technol. A8, 369–373.

Haga, H., Nagayama, M., Kawabata, K., Ito, E., Ushiki, T., Sambongi, T., 2000. Time-lapse viscoelastic imaging of living fibroblasts using force modulation mode in AFM. J. Electron Microsc. (Tokyo) 49 (3), 473–481.

Hansma, H.G., 2001. Surface biology of DNA by atomic force microscopy. Annu. Rev. Phys. Chem. 52, 71–92.

Hertz, H., 1881. Ueber den kontakt elastischer koerper. J. fuer die Reine Angewandte Mathematik 92, 156.

Hofmann, U.G., Rotsch, C., Parak, W.J., Radmacher, M., 1997. Investigating the cytoskeleton of chicken cardiocytes with the atomic force microscope. J. Struct. Biol. 119, 84–91.

Johnson, K.L., 1985. Contact Mechanics. Cambridge University Press, Cambridge.

Johnson, K.L., Kendall, K., Roberts, A.D., 1971. Surface energy and the contact of elastic solids. Proc. R. Soc. Lond. Ser. A 324, 301–321.

Kamruzzahan, A.S., Kienberger, F., Stron, C.M., Berg, J., Huss, R., Ebner, A., Zhu, R., Rankl, C., Gruber, H.J., Hinterdorfer, P., 2004. Imaging morphological details and pathological differences of red blood cells using tapping-mode AFM. Biol. Chem. 385 (10), 955–960.

Laurent, V.M., Kasas, S., Yersin, A., Schäffer, T.E., Catsicas, S., Dietler, G., Verkhovsky, A.B., Jean-Jacques Meister, J.-J., 2005. Gradient of rigidity in the lamellipodia of migrating cells revealed by atomic force microscopy. Biophys. J. 89, 667–675.

Lekka, M., Laidler, P., Gil, D., Lekki, J., Stachura, Z., Hrynkiewicz, A.Z., 1999. Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy. Eur. Biophys. J. 28 (4), 312–316.

Lekka, M., Laidler, Ignacak, J., Labedz, M., Lekki, J., Struszczyk, H., Stachura, Z., Hrynkiewicz, A.Z., 2001. The effect of chitosan on stiffness and glycolytic activity of human bladder cells. Biochim. Biophys. Acta 1540 (2), 127–136.

Liang, X., Mao, G., Simon Ng, K.Y., 2004. Probing small unilamellar EggPC vesicles on mica surface by atomic force microscopy. Colloids Surf. B: Biointerfaces 34, 41–51.

Lieber, S.C., Aubry, N., Pain, J., Diaz, G., Kim, S.-J., Vatner, S.F., 2004. Aging increases stiffness of cardiac myocytes measured by atomic force microscopy nanoindentation. Am. J. Physiol. Heart Circ. Physiol. 287, H645–H651.

Mahaffy, R.E., Park, S., Gerde, E., Käs, J., Shih, S.K., 2004. Quantitative analysis of the viscoelastic properties of thin regions of fibroblasts using atomic force microscopy. Biophys. J. 86, 1777–1793.

Mahaffy, R.E., Shih, C.K., MacKintosh, F.C., Käs, J., 2000. Scanning probe-based frequency-dependent microrheology of polymer gels and biological cells. Phys. Rev. Lett. 85, 880–883.

Mathur, A.B., Truskey, G.A., Reichert, W.M., 2000. Atomic force and total internal reflection fluorescence microscopy for the study of force transmission in endothelial cells. Biophys. J. 78, 1725–1735.

Mathur, A.B., Collinsworth, A.M., Reichert, W.M., Kraus, W.E., Truskey, G.A., 2001. Endothelial, cardiac muscle and skeletal muscle exhibit different viscous and elastic properties as determined by atomic force microscopy. J. Biomech. 34, 1545–1553.

Miyazaki, H., Hayashi, K., 1999. Atomic force microscopic measurement of the mechanical properties of intact endothelial cells in fresh arteries. Med. Biol. Eng. Comput. 37 (4), 530–536.

Mozafari, M.R., Reed, C.J., Rostron, C., Hasirci, V., 2005. A review of scanning probe microscopy investigations of liposome–DNA complexes. J. Liposome Res. 15, 93–107.

Mozhanova, A.A., Nurgazizov, N.I., Bukharaev, A.A., 2003. Local elastic properties of biological materials studied by SFM. SPM-2003. In: Proceedings Nizhni Novgorod, March 2–5, pp. 266–267.

Murakoshi, M., Yoshida, N., Iida, K., Kumano, S., Kobayashi, T., Wada, H., 2006. Local mechanical properties of mouse outer hair cells: atomic force microscopic study. Auris Nasus Larynx 33 (2), 149–157.

Nagayama, M., Haga, H., Kawabata, K., 2001. Drastic change of local stiffness distribution correlating to cell migration in living fibroblasts. Cell Motil. Cytoskel. 50 (4), 173–179.

Nowakowski, R., Luckham, P., Winlove, P., 2001. Imaging erythrocytes under physiological conditions by atomic force microscopy. Biochim. Biophys. Acta 1514, 170–176.

Ohashi, T., Ishii, Y., Ishikawa, Y., Matsumoto, T., Sato, M., 2002. Experimental and numerical analyses of local mechanical properties measured by atomic force microscopy for sheared endothelial cells. BioMed. Mater. Eng. 12, 319–327.

Pesen, D., Hoh, J.H., 2005. Micromechanical architecture of the endothelial cell cortex. Biophys. J. 88, 670–679.

Radmacher, M., Fritz, M., Kacher, C.M., Cleveland, J.P., Hansma, P.K., 1996. Measuring the viscoelastic properties of human platelets with the atomic force microscope. Biophys. J. 70 (1), 556–567.

Rosenbluth, M.J., Lam, W.A., Fletcher, D.A., 2006. Force microscopy of nonadherent cells: a comparison of leukemia cell deformability. Biophys. J. 90, 2994–3003.

Rotsch, C., Braet, F., Wisse, E., Radmacher, M., 1997. AFM imaging and elasticity measurements on living rat liver macrophages. Cell Biol. Int. 21 (11), 685–696.

Rotsch, C., Jacobson, K., Radmacher, R., 1999. Dimensional and mechanical dynamics of active and stable edges in motile fibroblasts investigated by using atomic force microscopy. Proc. Natl. Acad. Sci. U.S.A. 96, 921–926.

Rotsch, C., Radmacher, R., 2000. Drug-induced changes of cytoskeletal structure and mechanics in fibroblasts: an atomic force microscopy study. Biophys. J. 78 (1), 520–535.

Sasaki, S., Morimoto, M., Haga, H., Kawabata, K., Ito, E., Ushiki, T., Abe, K., Sambongi, T., 1998. Elastic properties of living fibroblasts as imaged using force modulation mode in atomic force microscopy. Arch. Histol. Cytol. 61 (1), 57–63.

Sato, H., Nagayama, K., Kataoka, N., Sasaki, M., Hane, K., 2000. Local mechanical properties measured by atomic force microscopy for cultured bovine endothelial cells exposed to shear stress. J. Biomech. 33 (1), 127–135.

Sato, H., Kataoka, N., Kajiya, F., Katano, M., Takigawa, T., Masuda, T., 2004. Kinetic study on the elastic change of vascular endothelial cells on collagen matrices by atomic force microscopy. Colloids Surf. B: Biointerfaces 34 (2), 141–146.

Scheffer, L., Bitler, A., Ben-Jacob, E., Korenstein, R., 2001. Atomic force pulling: probing the local elasticity of the cell membrane. Eur. Biophys. J. 30, 83–90.

Schrot, S., Weidenfeller, C., Schäffer, T.E., Robenek, H., Galla, H.J., 2005. Influence of hydrocortisone on the mechanical properties of the cerebral endothelium in vitro. Biophys. J. 89, 3904–3910.

Shroff, S.G., Saner, D.R., Lal, R., 1995. Dynamic micromechanical properties of cultured rat atrial myocytes measured by atomic force microscopy. Am. J. Physiol. Cell Physiol. 269, C286–C292.

Simon, A., Cohen-Bouhacina, T., Porte, V.C., Aime, J.P., Amedee, J., Bareille, R., Baquey, C., 2003. Characterization of dynamic cellular adhesion of osteoblasts using atomic force microscopy. Cytometry A 54 (1), 36–47.

Sugawara, M., Ishida, Y., Wada, H., 2002. Local mechanical properties of guinea pig outer hair cells measured by atomic force microscopy. Hear. Res. 174 (1–2), 222–229.

Sugawara, M., Ishida, Y., Wada, H., 2004. Mechanical properties of sensory and supporting cells in the organ of Corti of the guinea pig cochlea—study by atomic force microscopy. Hear. Res. 192 (1–2), 57–64.

Takai, E., Costa, K.D., Shaheen, A., Hung, C.T., Guo, X.E., 2005. Osteoblast elastic modulus measured by atomic force microscopy is substrate dependent. Annals Biomed. Eng. 33 (7), 963–971.

Takeuchi, M., Miyamoto, H., Sako, Y., Komizu, H., Kuzumi, A., 1998. Structure of the erythrocyte membrane skeleton as observed by atomic force microscopy. Biophys. J. 74, 2171–2183.

Timoshenko, S.P., Woinowsky-Krieger, S., 1970. Theory of Plates and Shells. McGraw-Hill, New York.

Tolomeo, J.F., Steele, C.R., Holley, M.C., 1996. Mechanical properties of the lateral cortex of mammalian auditory outer hair cells. Biophys. J. 71 (1), 421–429.

Vinckier, A., Semenza, G., 1998. Measuring elasticity of biological materials by atomic force microscopy. FEBS Lett. 430, 12–16.

Wada, H., Usukura, H., Katori, Y., Kakehata, S., Ikeda, K., Kobayashi, T., 2003. Relationship between the local stiffness of the outer hair cell along the cell axis and its ultrastructure observed by atomic force microscopy. Hear. Res. 177 (1–2), 61–70.

Wu, H.W., Kuhn, T., Moy, V.N., 1998. Mechanical properties of L929 fibroblasts measured by atomic force microscopy: effects of anticytoskeletal drugs and membrane crosslinking. Scanning 20 (5), 389–397.

Yamane, Y., Shiga, H., Haga, H., Kawabata, K., Abe, K., Ito, E., 2000. Quantitative analyses of topography and elasticity of living and fixed astrocytes. J. Electron Microsc. (Tokyo) 49 (3), 463–471.

Yoshikawa, Y., Yasuike, T., Yagi, A., Yamada, T., 1999. Transverse elasticity of myofibrils of rabbit skeletal muscle studied by atomic force microscopy. Biochem. Biophys. Res. Comm. 256, 13–19.

Zhang, S., Kraus, W.E., Truskey, G.A., 2004. Stretch-induced nitric oxide modulates mechanical properties of skeletal muscle cells. Am. J. Physiol. Cell Physiol. 287, C292–C299.