Innovations in Measuring Cellular Mechanics

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Abstract

This article describes several novel mechanical methods for elucidating cellular responses to different types of mechanical loading (adhesive, pulling, pushing, shearing, and stretching forces). Understanding how cells deform and transmit stresses into the cell is important for gene expression, cytoskeletal remodeling, and focal adhesion reorganization and crucial for a variety of higher fundamental cell functions including cell division, motility, and differentiation. Introducing these unique methods of measuring and understanding cellular mechanics, therefore, provides a valuable platform for cell biology research.

Keywords

Magnetic tweezer • Magnetic twisting cytometry • Traction force microscopy • Cell poking • Plate rheometer • Nano-scale tracking

14.1 Introduction

14.1.1 Muscle Physiology, Biomechanics, and Biochemistry

Biomechanical studies of single cells and cell populations have been of interest for a while, and in recent years applied methods have experienced rapid development (Goldmann 2000, 2002; Mierke et al. 2008, 2010; Möhl et al. 2009; Lange

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et al. 2013). Measuring cell mechanical properties in the field of muscle physiology, which has a century-old tradition, has contributed significantly to the current understanding of the mechanisms of muscle contraction (Huxley 1957; Ra et al. 1999). The dynamics and kinetics of biochemical reactions between the most important muscle contractile proteins, actin and myosin, were determined with high accuracy and temporal resolution based on relatively simple measurements of force and length changes of the skeletal muscle as determined by Hill (1965). The regular, almost crystalline, arrangement in the muscle contractile apparatus allowed the use of simple mathematical models to interpret the measurements obtained macroscopically in relation to molecular processes (Huxley 1957; Hill 1965; Kawai and Brandt 1980).

In nonmuscle cells, however, the conditions are more complex. Their diverse mechanical functions including migration, division, phagocytosis are determined by myosin motors, but the numerous regulatory molecules and structure-forming proteins of the cytoskeleton are also significantly involved (Hartmann and Spudich 2012). This complexity is often exasperated by the time-varying irregular network-like structures of the cytoskeleton. In nonmuscle cells, the assignment of mechanical measurements and biochemical processes are not clear, particularly the absence of macroscopic tissue that embeds cells. Nonetheless, tests can be carried out at the cellular level. In the following paragraphs, some important methods for measuring mechanical properties at the cellular level and some common conceptual models for their interpretation are listed.

14.1.2 Methods for Measuring Cell Mechanical Properties

James Crick and Arthur Hughes developed in the 1950s a method for quantitative measurements of rheological (mechanical) properties of individual cells under culture conditions (Crick and Hughes 1950). The method was based on microscopically small (about 5 μm) acicular ferromagnetic particles, which had been endocytosed or phagocytosed by cells. These particles were magnetized along their major axis and then subjected to a magnetic field in the plane perpendicular to the major axis, which results in a rotation in the direction of the magnetic field. The rotation angle and the rotation speed of the particles were recorded by a camera, and their movement gives information about the mechanical properties of the cell.

In the 1970s, Brain and Cohen replaced the complex evaluation of particle rotation by filming magnetometric measurements (Cohen et al. 1979), which was also used later by Valberg et al. (Valberg and Albertini 1985). Because of the limited sensitivity of the magnetometer, several thousand particles had to be added to the cell culture, and the behavior of individual cells could not be observed. However, the method was suitable for measurements in living animals, for instance, in determining the mechanical properties of alveolar macrophages. The limitation of the method for examining only endocytotic or phagocytotic cells was overcome in the 1990s by Wang et al. (1993), who used magnetic particles coated with

peptides, proteins, or antibodies that bind to specific receptors on the cell surface. The restriction of measuring only the behavior of cell populations was resolved in 2001 by Fabry et al. (2001a, b) using computerized video analysis of the microscopic particle motion on individual cells.

Over the past 25 years, many other methods for measuring the mechanical properties of cells in culture have been developed. These include microindentation (cell poking) (Goldmann 2000; Zahalak et al. 1990), aspiration of cells into micropipettes (Evans and Yeung 1989; Merkel 2001), measurements of diffusive motion of intracellular particles or granuoles (Caspi et al. 2002), atomic force microscopy (Moy et al. 1994; Goldmann et al. 1998; Alcaraz et al. 2003), disk-microrheometry (Müller et al. 1991; Goldmann and Ezzell 1996; Thoumine and Ott 1997), and diffusion measurements of receptor-bound particles (beads) in optical traps (optical tweezer) (Choquet et al. 1997) or in magnetic field gradients (Alenghat et al. 2000). All these methods have their own advantages and limitations for specific applications. A selection of the methods is described below in detail: (i) nano-scale particle tracking, (ii) magnetic tweezer, (iii) rotation disc rheometer, (iv) magnetic twisting cytometry, (v) cell poking, and (vi) traction microscopy for the application in general biology research.

14.2 Methods

14.2.1 Nanoscale Particle Tracking

To describe active dynamic, mechanical operations such as cytoskeletal remodeling processes in cell migration, cell proliferation, adhesion, or contraction quantitatively, the method of nanoparticle tracking is ideally suited (An et al. 2004; Raupach et al. 2007; Metzner et al. 2007, 2010; Alonso and Goldmann 2012). The method is based on the assumption that a bead (<1 μm diameter) is firmly bound to the cytoskeleton via integrins and focal adhesions and moves when the actin cytoskeleton is spontaneously restructured, i.e., without the influence of (e.g., external magnetic) forces. The bead movement, therefore, reflects the extent and speed of cytoskeletal reorganization. The impromptu bead motion can be determined by subpixel arithmetic with nanometer accuracy (Fig. 14.1).

14.2.2 Magnetic Tweezer

When focal adhesion contacts of adherent cells are stimulated by external forces, they respond by reconstructing and reinforcing the contacts (focal adhesion strengthening) (Goldmann 2002; Choquet et al. 1997; Giannone et al. 2003; Deng et al. 2004). A controlled generation of such forces allows studying dynamic processes in great detail. For this purpose, a magnetic tweezer was developed with which forces of up to 10 nN on superparamagnetic beads of 4.5 μm diameter can be applied. The design of the magnetic tweezer was based on a prototype developed by

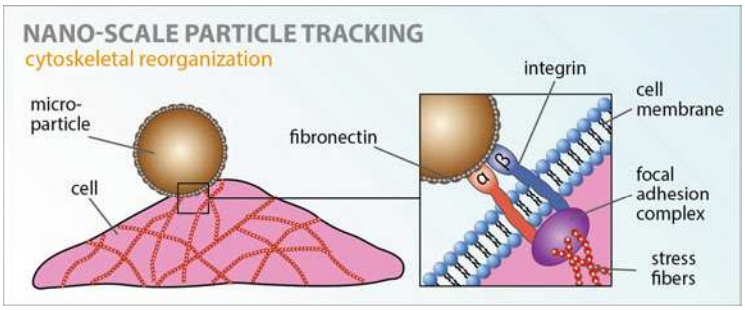

Fig. 14.1 Nano-scale particle tracking. Schematic representation of a < 1 μm RGD-coated polystyrene bead attached to integrins that span the cell membrane (*left*). Integrins are connected *via* focal adhesions to the actin cytoskeleton (inset, *right*)

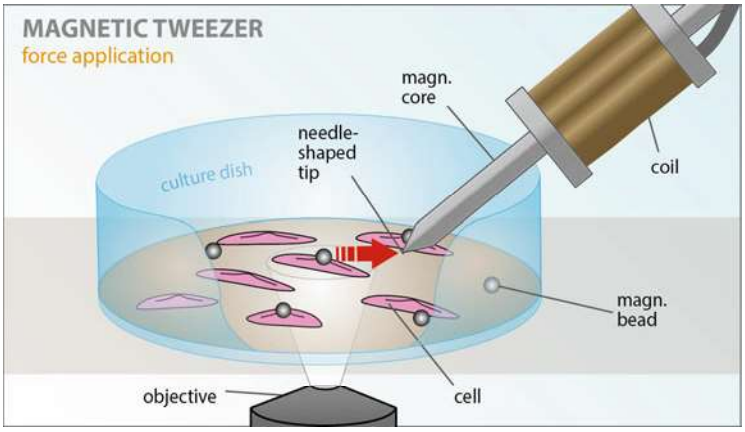

Fig. 14.2 Schematic image of the magnetic tweezer experimental setup. A high magnetic field gradient is generated by a needle-shaped high-permeability core of a solenoid attached to a micromanipulator. The gradient force generated by the magnetic tweezer acts on superparamagnetic beads coated with extracellular matrix proteins. Beads coated with extracellular matrix proteins are bound to the cell surface *via* integrin receptors, which connect the extracellular space with the intracellular cytoskeleton. The bead displacement is the basis for the calculation of cellular stiffness (Bonakdar et al. 2012, 2014, 2015)

Alenghat et al. (2000), which allowed force generation up to about only 1 nN. The latest generation of magnetic tweezers exerts forces up to 100 nN (Kollmannsberger and Fabry 2011).

Figure 14.2 shows the schematic image of a magnetic tweezer setup. The tweezer is composed of a soft, superparamagnetic iron alloy (HyMU80) core, which is tapered at one end, and a surrounding magnetic induction coil (copper wire of 0.5 mm diameter and 250 turns), which magnetizes the core with a maximum current of 3 A and a power up to 10 W. The current for the coil is generated by a microprocessor-controlled current source, which is connected to the

PC via a DA converter board. Near the core tip, the magnetic field shows a strong inhomogeneity, i.e., a high local field gradient. A superparamagnetic bead in close vicinity is magnetized and pulled in the direction of the strongest field gradient. By coating the beads with ligands, e.g., proteins of the extracellular matrix, the beads can adhere to specific receptors of the cell and transfer controlled forces. The lateral bead movement toward the needle tip allows the calculation of the mechanical properties of the cell (Alenghat et al. 2000). Because of higher forces per bead in the nano-Newton range compared to other methods (laser tweezer in pN range), the magnetic tweezer is a versatile tool that is suitable for the investigation of focal adhesion reinforcement and many other cellular processes (Kollmannsberger and Fabry 2007).

14.2.3 Rotation Disk Rheometer

Important advances have been made in recent years regarding the investigation of passive rheological properties of living cells occurring within short timescales. Rheological experiments that probe at the micron level have shown broad power-law frequency responses. In Fig. 14.3, we briefly describe the apparatus and details of the data analysis used by Müller et al. (1991). The apparatus consists of a cylindrical glass cuvette containing the cells, a metal disk on top of the cells, and two pairs of surrounding perpendicularly oriented magnetic coils. One of these serves to fix the orientation of the disk and the other (the deflection coil) to apply an

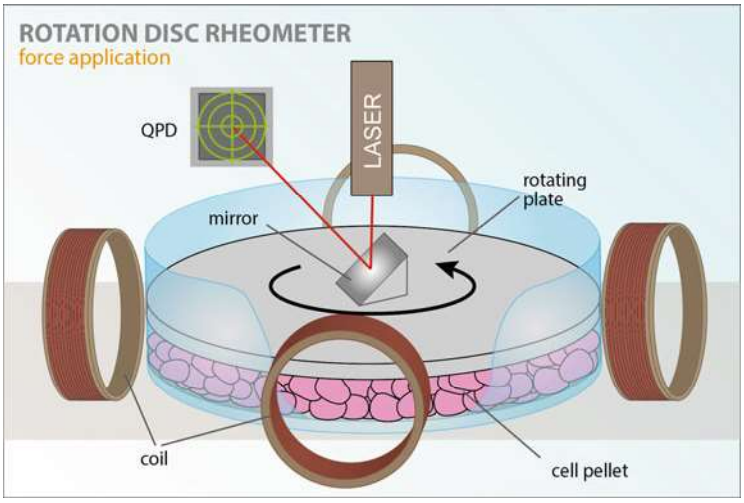

Fig. 14.3 A schematic representation of the rotation disc rheometer setup designed to perform cell rheology measurements. Two pairs of coils serve to orient the mirror on top of the metal plate and to oscillate the disc by a small amplitude. A laser beam hits the mirror and is reflected onto a quadrant photodiode. The rotation of the disc leads to a lateral deflection of the laser spot on the detector

oscillatory shear force to the cell pellet. The "in-phase" and "out-phase" components of the resulting rotational amplitude $\alpha(t)$ of the disk are analyzed as follows: The beam of a He–Ne laser in a direction vertical to the rotational axis of the disk is horizontally deflected by the mirror mounted on the disk, and its horizontal orientation is recorded by a gradient photodiode. The complex shear modulus $G^*(\varpi\Psi\psi\rho) = G'(\varpi\Psi\psi\rho) + G''(\varpi\Psi\psi\rho)$, where $G'(\varpi\Psi\psi\rho)$ is the frequency-dependent storage modulus and $G''(\varpi\Psi\psi\rho)$ is the loss modulus, was determined for a pellet of $\sim 5.5 \times 10^7$ F9 wild-type and vinculin-deficient cells (Goldmann and Ezzell 1996). For further information regarding disk rheometers, refer to (Fernandez et al. 2007).

14.2.4 Magnetic Twisting Cytometry (MTC) with Optical Detection

14.2.4.1 Principle

The basic idea of the MTC is to manipulate living, adherent cells mechanically in a microrheometer to measure their mechanical properties. Similar to the conventional rheometer, in which the material to be examined is sheared between two plates, the cells are sheared between a rigid plate (disk) and magnetically rotated ("twisted") ferromagnetic beads adhered to the cells. From the relationship between the generated magnetic shear forces and the measured deformation of the cells, the constitutive mechanical properties of the living cells can be derived. The advantage of this method is the possibility (i) of coating the beads with different ligands and thus coupling them to specific receptors, (ii) of tracking several (up to 100) cells simultaneously per field of view, and (iii) of measuring the mechanical behavior of cells in a frequency-dependent manner over a wide frequency range (0.01–1000 Hz).

Commercially available ferromagnetic (chromium dioxide) beads coated with polystyrene (Spherotech, Libertyville, IL) or homemade ferromagnetic beads made from magnetite can be used. Spherotech beads can easily be coated with secondary antibodies (due to their factory-functionalized surface, e.g., carboxylated or coated with antibodies) or with proteins (fluorescent or nonfluorescent) to bind cell surface receptors. It should be noted that beads on the basis of low-ferromagnetic fraction (<40 vol.%) are only suitable for the production of mechanical shear forces of up to 50 Pa. Magnetite beads of defined sizes (1–6 μm diameter) can be obtained to produce shear forces up to 150 Pa.

After the beads are bound to the cells, they are magnetized by a short (10 μs) and strong (>150 mT) pulse from a permanent magnet in the horizontal direction. Following this procedure, the beads can be rotated in a weaker vertically aligned, uniform magnetic field (up to 8 mT), which is similar to the situation of a compass needle responding to the Earth's magnetic field. The torque T acting on each magnetic bead depends on the magnetization constant c of the beads and the rotation angle θ between the magnetic moment M and the magnetic field H according to the relation $T = c \times H \times \cos\theta$ (Wang et al. 1993; Fabry et al. 2001a). The magnetization constant c is calibrated by measuring the rotational velocity of the beads in a viscous standard. For Spherotech beads, c is about

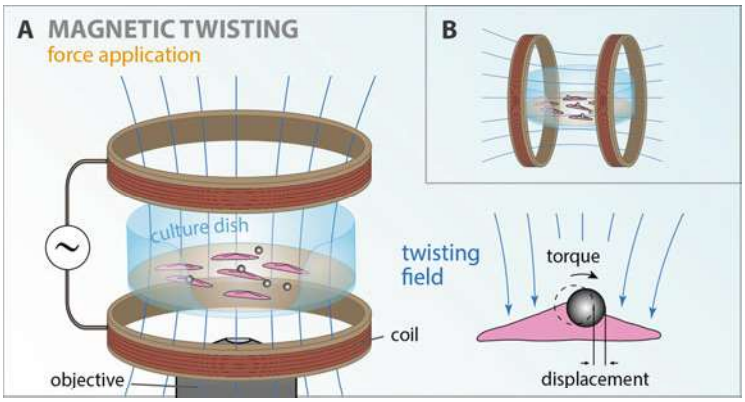

Fig. 14.4 Magnetic twisting device. Prior to experimentation, cells are incubated for 20 min with magnetite beads that are coated with, e.g., a synthetic peptide (RGD) that in turn connects the beads with the cellular actin cytoskeleton *via* integrin receptors. A brief (10 μs) but strong (>150 mT) magnetic pulse in the horizontal direction (parallel to the culture surface), using one pair of magnetic coils, is applied (*top, right*, **B**). Then after several seconds, a much weaker magnetic twisting field (up to 8 mT) in the vertical direction is applied (*left*, **A**). The vertical magnetic field produces a torque of the beads, which depends on the mechanical properties of the underlying cells in response to the deflection of the beads. The bead rotation (displacement) can be measured microscopically with high accuracy (*bottom, right*) (Goldmann et al. 2000)

5 Pa/mT and for magnetite beads 20 Pa/mT. With the usually small rotation angles of less than 5°, cos θ is negligible. The time characteristic of the homogeneous magnetic field is varied sinusoidally, which causes a rotation and movement of the beads with the same frequency along the direction of magnetization (Fig. 14.4).

The amplitude of the bead movement is typically around 100 nm but may vary depending on the cell type and other factors such as coating by up to two orders of magnitude. The bead movement can be directly visualized by a microscope with a CCD camera, and bead positions can be highly resolved with subpixel arithmetic. The round shape of the beads allows analysis via a simple center-of-mass algorithm. About 100 cells can be measured simultaneously, which ensures a high statistical confidence of the results. The image acquisition of the CCD camera is synchronized with the generation of the magnetic field. This is achieved by a phase-synchronous control of the current source and by means of a real-time trigger microcontroller of the camera (Fabry et al. 2001a).

14.2.4.2 Calculating Cell Mechanical Properties

Forced bead movement in the magnetic field gives rise to a deformation of the cell and of bead-associated intracellular structures. Within the cell, mechanical shear forces are generated opposite to the bead movement. Consequently, the mechanical properties can be derived from the relationship between the magnetically generated mechanical torque and bead movement. Due to periodic excitation, Fourier analysis can be used to calculate the mechanical properties, greatly simplifying the calculation and making it substantially insensitive to noise effects (Fabry et al. 2001a).

14.2.4.3 Measuring Temporal Changes

Cell mechanical parameters measured at constant twist rates allow the detection of changes over time for each cycle of twist. The possibility of time-resolved measurements using MTC allows measuring cell-specific responses to pharmacological stimulations in the same cell under the same conditions (before/after). Since a considerably high number of cells are measured in each experiment (~100), this gives a high confidence level and allows the detection of small mechanical changes (<5 %). Many substances that activate, e.g., motor proteins, or trigger polymerization/depolymerization of the actin cytoskeleton can lead to pronounced changes in cell mechanics within seconds. Time-resolved measurements are therefore essential to quantify dynamic (kinetic) reaction processes. For instance, time-resolved measurements could provide evidence that smooth muscle cells of the respiratory tract under culture conditions have a significant muscle tone (Fabry et al. 2001b) and that individual muscle cells show considerable heterogeneity regarding contractile behavior (Fabry et al. 2001b) or that mechanical stimulation of muscle cells leads to actin polymerization (Deng et al. 2004).

14.2.4.4 Measuring the Frequency Response

The constitutive mechanical properties of engineering materials are usually determined from the step or frequency responses of the material deformation. A similar approach has also been used in traditional muscle physiology. The frequency dependence of different types of cells using various agonists and different bead (coating) attachments over a large frequency range has been measured by MTC (Fabry et al. 2001a, 2003; Puig-de-Morales et al. 2004). The main aim of these studies was to make predictions about which molecular processes dominate in cell mechanics; i.e., dominant molecular processes should have characteristic relaxation times, which are reflected in a characteristic frequency response. This hypothesis could not be proven. Instead, a surprising power-law behavior was determined for cellular mechanical properties, for which no explanation in the literature of cell biology existed (Fabry et al. 2001a, 2003; Puig-de-Morales et al. 2004). The power-law behavior of cell mechanical properties can be described empirically by the so-called structural damping equation. This has been used for a systematic investigation of cell mechanics, in which cells were stimulated with contractile, relaxant, or cytoskeleton-changing agonists (histamine, bradykinin, ML-7, ML-9, BDM, wortmannin, N-formyl-methionyl-leucylphenylalanine (FMLP), N6, 2′-O-dibutyryladenosine 3′, 5′-cyclic monophosphate (DBcAMP), latrunculin A, jasplakinolide, and cytochalasin D) (Fabry et al. 2003; Puig-de-Morales et al. 2004). All mechanical cell responses were mainly determined by one parameter, while the parameters in the power-law remained largely unchanged (Fabry et al. 2003; Puig-de-Morales et al. 2004). This observation was confirmed in different cell types (macrophages, leukocytes, epithelial cells, endothelial cells, and fibroblasts) and with different ligations to the beads (e.g., AcLDL, urokinase, and different activating and nonactivating antibodies against various β-integrin subunits) (Fabry et al. 2003; Puig-de-Morales et al. 2004).

14.2.5 Cell Poking

The cell poking device was developed by Dr. Elliot Elson's group to measure the forces required to indent rigid surfaces, for instance, the cell membranes of circulating blood cells and adherent cells (Daily et al. 1984, Duszyk et al. 1989). Their aim was to record the dynamic response of the cell membrane to external signals in order to quantitatively investigate changes in cell shape and cytoskeletal organization associated with physiological processes. These researchers provided the theoretical basis for measuring elastic and viscous material properties resulting from tension generation at the lipid/protein interface, i.e., at the cell membrane-cytoskeleton connection (Zahalak et al. 1990). We have used this biophysical technique of cell indentation or poking to manipulate and probe cell structure and mechanics. The principle is shown in Fig. 14.5. A cell adhered to the bottom of a culture dish is indented by a glass stylus. In order to prevent attachment to the cell, the glass stylus is coated with BSA. With a high-speed video camera, the relaxation of the cell membrane after poking and retraction of the glass stylus is recorded and the relaxation rate determined from video analysis using an elastic response theory. This method has led to further insight into how cellular viscoelasticity is regulated based on the cell architecture, where mechanical, biological, and biochemical processes are tightly coupled. Better understanding of how cells control these functions will require further optimization of the present techniques and inclusion of methods for controlling and quantitating changes in the cell (Goldmann 2000).

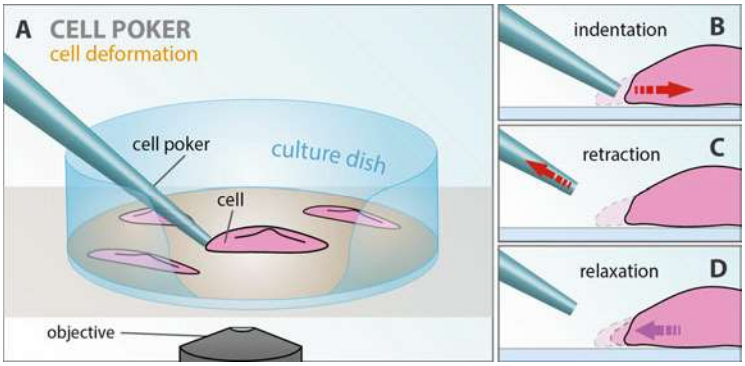

Fig. 14.5 Schematic representation of the cell poking device and detection by microscopy (**A**). The adherent cell is indented by a glass stylus that is coated with BSA to prevent its attachment to the cell (**B**). Images of the cell edge are taken after poking and immediate retraction of the glass stylus (**C**). The relaxation of the cell membrane after deformation is recorded by a high-speed video camera (**D**). The rate of relaxation is determined from the analysis of video frames using an elastic response theory (Goldmann 2000)

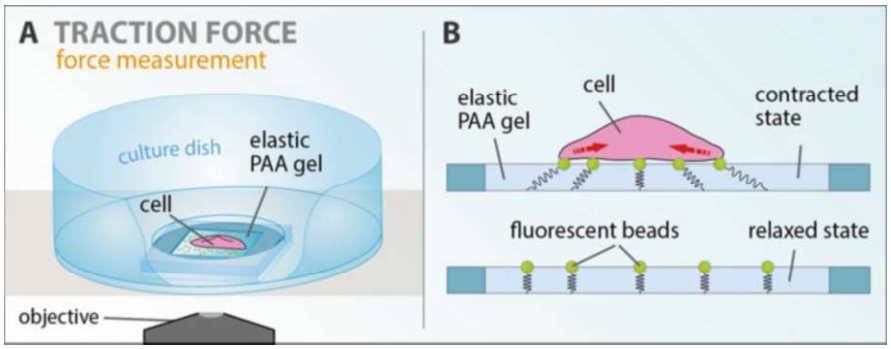

Fig. 14.6 Basic principle of traction force microscopy (**A**). Cells are seeded on an elastic polyacridamide (PAA) gel containing 200 nm diameter fluorescent beads embedded on the surface, which serve as markers to visualize any gel deformation due to cell contraction (**B**). The traction forces are calculated from the gel deformation; i.e., relaxed versus contracted state (as shear stress in Pa) caused by the cells (Bonakdar et al. 2014, 2015)

14.2.6 Fourier Transform Traction Force Microscopy

Adherent cells transfer forces to the extracellular matrix. When cells are cultured on a polyacrylamide elastic substrate coated with extracellular proteins in which fluorescent markers (e.g., 200 nm FluoSpheres) are embedded (Fig. 14.6), such forces can be optically determined (Deng et al. 2004; Butler et al. 2002; Stamenovic et al. 2002, 2004). Depending on the elastic modulus of the substrate and the contractile state of the cells, the fluorescent-labeled beads will be displaced from their resting position. The surface of the polyacrylamide gel can be activated with sulfosuccinimidyl 6 (4′-azido-2′-nitrophenyl-amino) hexanoate (sulfo-SANPAH) and then covalently coated with any adhesive matrix molecule. The elastic properties of the polyacrylamide substrate can be adjusted over a wide range (100 Pa to >50 kPa) by precisely varying the acrylamide/bis-acrylamide cross-linker concentration. This permits adaptation to different cell types and also induces different responses of the cells to substrate properties.

14.3 Method Application to Smooth Muscle Cells

To determine the contractile response of smooth muscle cells under culture conditions, the methods of traction force microscopy and optical magnetic twisting cytometry (MTC) can be applied. Traction force microscopy allows the direct determination of the contractile moment of individual cells, while MTC gives information about cell elasticity (G'). Classic muscle physiology predicts a linear relationship between contractile strength and elasticity of a muscle; for instance, when a muscle is activated, each individual actomyosin binding contributes to both the strength and the elasticity of the entire muscle. A linear relationship even at the

cellular level has been reported (Butler et al. 2002; Stamenovic et al. 2004). Therefore, it seems reasonable to equate the cytomagnetometrically measured temporal changes of G' with a proportional change in force development. The advantage of this approach is the high statistical quality of cytomagnetometric measurements (up to 100 individual cells per measurement) and the high temporal resolution compared with traction force microscopic measurements. Interestingly, the linear relationship between cell contraction stress P and cell elasticity G' is lost when the mechanical properties are determined with higher frequencies of >1 Hz. Therefore, the deviation from the linear relationship between G′ and contractile force is related to the frequency dependence of G′. This relationship could possibly be a direct function of the power-law exponent of the acting cytoskeletal contractile force (prestress) (Stamenovic et al. 2004; Gardel et al. 2004).

To quantify cytoskeletal remodeling processes in smooth muscle cells, the nanoscale particle tracking method can also be applied (An et al. 2004). From the bead movement (expressed by the mean square displacement) within a defined time interval, the effective diffusion coefficient that increases with increasing speed of cytoskeletal remodeling can be determined. Cytoskeletal remodeling processes can be directly manipulated pharmacologically by using, for instance, cytochalasin D or latrunculin A (leading to actin depolymerization) or jasplakinolide (stabilizing the actin cytoskeleton). Cytoskeletal conversion processes can also be affected indirectly, for example, by stimulating cells with contractile (histamine or bradykinin) and relaxant (isoproteronol or DBcAMP) substances.

14.3.1 Relationship Between Contraction and Cytoskeletal Remodeling in Airway Smooth Muscle Cells

In human respiratory muscle cells, depending on the dynamics of the cytoskeleton remodeling induced pharmacologically over a wide range, the beads showed a more or less pronounced superdiffusive motion (An et al. 2004). Likely causes for this are active processes that keep the cytoskeletal matrix in a thermodynamic imbalance. The exact molecular details of the superdiffusive behavior are still unclear. It was shown, however, that metabolic, ATP-consuming processes influence spontaneous bead motion strongly; for instance, when the ATP turnover of cells was reduced with 2 mM deoxyglucose and 2 mM NaN_3 to about 5 % of baseline activity, the transition from subdiffusive to superdiffusive behavior was observed only at Δt of ~ 5 s (An et al. 2004).

Cytoskeletal remodeling processes can also be made directly visible, either by rhodamine-phalloidin staining of fixed cells (An et al. 2004; Deng et al. 2004) or with transfection of GFP-actin (Hu et al. 2003). Numerous experiments not only confirm the already suspected correlation between contraction and cytoskeletal remodeling in airway smooth muscle cells but also suggest a previously undescribed close temporal coupling of these two processes. Data showed under existing baseline conditions that the cytoskeleton is affected by the baseline tone of cells; i.e., the baseline cytoskeleton is too weak to transmit additional forces to the

extracellular matrix resulting from myosin activation. For this, it needs a reinforcement of the existing cytoskeletal and adhesive structures. It is particularly surprising that the reinforcement and restructuring of the cytoskeleton can occur with the same speed as the contractile activation of muscle cells, namely, within a few seconds. Such a coupling between contraction and cytoskeletal remodeling has been described only for longer time intervals (Balaban et al. 2001; Gunst and Fredberg 2003).

The relationship between contractile forces and cell elasticity under steady state is well documented in smooth muscle cells. However, doubts about this relationship were reported (An et al. 2002). For example, myosin inhibitors such as 2,3-butanedione monoximes (BDM), wortmannin, or ML-7 are not able to completely block the increase in elasticity of cells after stimulation (Smith et al. 2007). A possible reason for this could be a myosin-independent actin polymerization after contractile stimulation (An et al. 2002; Mehta et al. 1998). Such a decoupling between actin polymerization and prestress, however, contradicts the well-established view of the cell as being a "stress-supported structure" (Wang et al. 2002). For example, a tight, timely coupling between the pharmacologically (BDM) modified prestress of fibroblasts and the size of focal adhesion contacts was observed (Balaban et al. 2001). An alternative, more trivial explanation for the increase in elasticity after contractile stimulation (despite myosin inhibition) could be an incomplete effect of the inhibitors. For a definite decision between these alternative hypotheses, the time course of the contractile force of the cell elasticity and the dynamic cytoskeletal reorganization together (preferably simultaneously) must be investigated.

Future experiments using airway smooth muscle cells should focus on the time course of contractile forces after stimulation at a time resolution of 1 s using traction force microscopy, on the elasticity using magnetic twisting cytometry, and on the dynamics of cytoskeletal reorganization using nanoscale particle tracking as well as live cell microscopy with fluorescently tagged actin, vinculin, and paxillin. Cells should be treated with myosin inhibitors such as wortmannin, BDM, or ML-7, or with actin-active substances such as cytochalasin D, latrunculin-A, or jasplakinolide. The chronological sequence of strength, elasticity, and cytoskeletal reorganization should be determined during pretreatment with these substances. These studies should be designed in a way that can provide clear answers to key open questions about smooth muscle contraction processes. In addition, future studies should provide answers to general questions about cell mechanics and mechanotransduction.

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