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Minireview

Mechanical manipulation of animal cells: cell indentation*

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Abstract

Determining the mechanical properties and behavior of cells has been studied through a variety of methods including micropipette aspiration, atomic force microscopy (AFM), magnetometry, rheology, and cell indentation. Using the cell poker technique, the force required to indent the cell surface by a glass stylus or the relaxation time of the cell membrane can be determined. This method provides information about both the mechanical properties of adherent cells and the internal cytoskeleton. For example, using the cell poking technique showed that F9 mouse embryonic carcinoma cells were ∼20% more resistant to indentation by the cell poker compared to F9 vinculin-deficient (5.51) cells, which were derived by chemical mutagenization of F9 cells. This was confirmed in viscoelastic measurements using AFM, magnetometry, and rheology.

Introduction

An important question in biological science and in many other fields is how groups of animal cells and molecules associate to form three-dimensional tissues that exhibit specialized form, shape, and function (Ingber *et al.* 1994). These interactions between the cells and molecules are controlled by a complex system of chemical and physical determinants at the molecular level; actin-containing contractile microfilaments are responsible for force transduction in cells and thus play a central role in determining cell shape (Ingber *et al.* 1995). In addition, extracellular matrix (ECM) molecules can play a significant role in these tissue formations: the ECM appears to be structurally interconnected with microfilaments via a continuous series of noncovalent binding interactions, involving actin-associated proteins like vinculin, talin, α-actinin, filamin, and transmembrane integrin receptors (Goldmann *et al.* 1996, Giancotti & Ruoslahti 1999). Therefore, changes in cell shape must be seen as the manifestation of an underlying physical force distribution between molecules, both intracellular and extracellular, as demonstrated in studies by Wang & Ingber (1995) and Eichinger *et al.* (1996).

In the force distribution, mechanical perturbation of cell surfaces or cortical cytoskeleton may have direct effects on the tension of capillary endothelial cells; recent studies suggest that extracellular matrix-dependent changes in cell shape may affect cell growth as well (Dike *et al.* 1999). Another possibility is that tension-dependent changes in cell shape and subsequent cytoskeletal reorganization might alter the structural system for signal transduction within capillary cells (Singhvi *et al.* 1994). For example, extracellular alterations of physical force distributions may be translated directly into changes of mechanical forces of actin filaments that physically link the plasma membrane through structural reorganization of the cytoskeleton. Ingber (1993) explains this as a 'mechano-chemical relation': the dependence of cytoskeletal polymerization upon tension and compression has a thermodynamic basis and viscoelastic implication that forms a continuum within living cells. Observations of changes in torsional strain (Wang &

*This article is dedicated to Professor Erich Sackmann on his 65th birthday for his achievements in the field of biophysics and biomechanics.

Ingber 1994) confirm that cell shape changes in a coordinated fashion as the cell progresses from a round to a spread form. This suggests that biophysical interactions may play a central role in these processes by altering cell shape through external force stumuli. To understand the role of external forces with regard to cell shape change and viscoelasticity, it is necessary to elucidate the mechanical/physical basis of the connection between the plasma membrane and cytoskeleton though the most likely pathway, viz., the focal adhesion complex (FAC).

Although there has been intensive investigation into how the cytoskeleton responds to chemical stimuli, the mechanism by which external forces are transmitted across the cell surface and transduced into a cytoskeletal response is only poorly understood. The mechanical properties of cells have been measured by micropipette aspiration, micromanipulation, magnetometry, atomic force microscopy, and cell-poking elastometry. Evans and coworkers (Evans 1980, 1983, Discher *et al.* 1994) have used the micropipette aspiration technique for the past two decades on red blood cells to determine how the shear rigidity is associated with membrane-linked proteins like spectrin, band 3, and protein 4.1; and recent findings further have shown that the underlying cytoskeleton contributes significantly to cell elasticity. The atomic force microscope (AFM) has also proven to be an effective tool for investigating cell elasticity by using its ability to detect dynamic changes in the viscoelastic properties of the cell with a high spatial and temporal resolution (Radmacher *et al.* 1992). The AFM has been used to examine the surface morphology and mechanical properties of MDCK, human platelets, and F9 mouse embryonic carcinoma cells (Hoh & Schoenenberger 1994, Radmacher *et al.* 1996, Goldmann *et al.* 1998a, b). Another technique, developed by Wang and colleagues, is a magnetic twisting device, which controls mechanical stresses applied directly to cell surface receptors and hence the cytoskeleton. This is accomplished by using magnetic microbeads that are coated with specific integrin ligands (Wang *et al.* 1993). The cellular response to applied stress can be measured simultaneously by quantitating changes in the rotation (angular strain) of the surface-bound magnetic beads using a sensitive in-line magnetometer. These researchers found that the stiffness (ratio of stress to strain) of the cytoskeleton increases in direct proportion to the applied stress. A group led by Dr Elliot Elson has developed the cell-poking device in order to measure the force required to indent rigid surfaces, i.e., 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. Changes in cell shape and cytoskeletal organization associated with physiological processes should be quantitatively detectable with the cell poker. These researchers laid down 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).

Materials and methods

Cell indentation

The principle of the cell poking elastometer and its schematic representation are shown in Figure 1A, B. This is a purpose-built apparatus, developed by Dr Markus Ziegler, Technical University of Munich and based on the design by Duszyk *et al.* (1989), and described in Goldmann *et al.* (1998b). In brief, this instrument is mounted on an inverted microscope that allows accurate three-dimensional lateral positioning (within 1 nm precision) of the glass stylus. The deformation of the cell membrane (a human erythrocyte) attached to a bovine serum albumin (BSA)-coated glass coverslip by the glass stylus is measured by reflection interference contrast microscopy (RICM) and bright field optics. After instantaneous computer-controlled retraction of the glass poker up to 5 μm, ten individual video frames were captured at 25 Hz (Figures 1B, 1C), and the membrane deformation was analyzed according to Equation (1). The cell is suspended in 123 mM NaCl, 25 mM glucose, 11 mM sodiumcitrate, 0.1 mM adenine, 0.1 mM inosin, and 0.1 vol% vitamin and antibiotic solution, pH 7.4.

Theoretical considerations

To describe the elastic response the following equation (1) is used:

$$\ln\ \Delta x(t_i) = -k * t_i, \tag{1}$$

where $\Delta x(t_i)$ = time-dependent 'lateral deformation' relaxation (nm); k = rate of relaxation (s). From the plot $\Delta x(t_i)$ against time t_i the decay of the indentation is observed, with $k = 6.7\ \mathrm{s}^{-1}$ being a measure of the

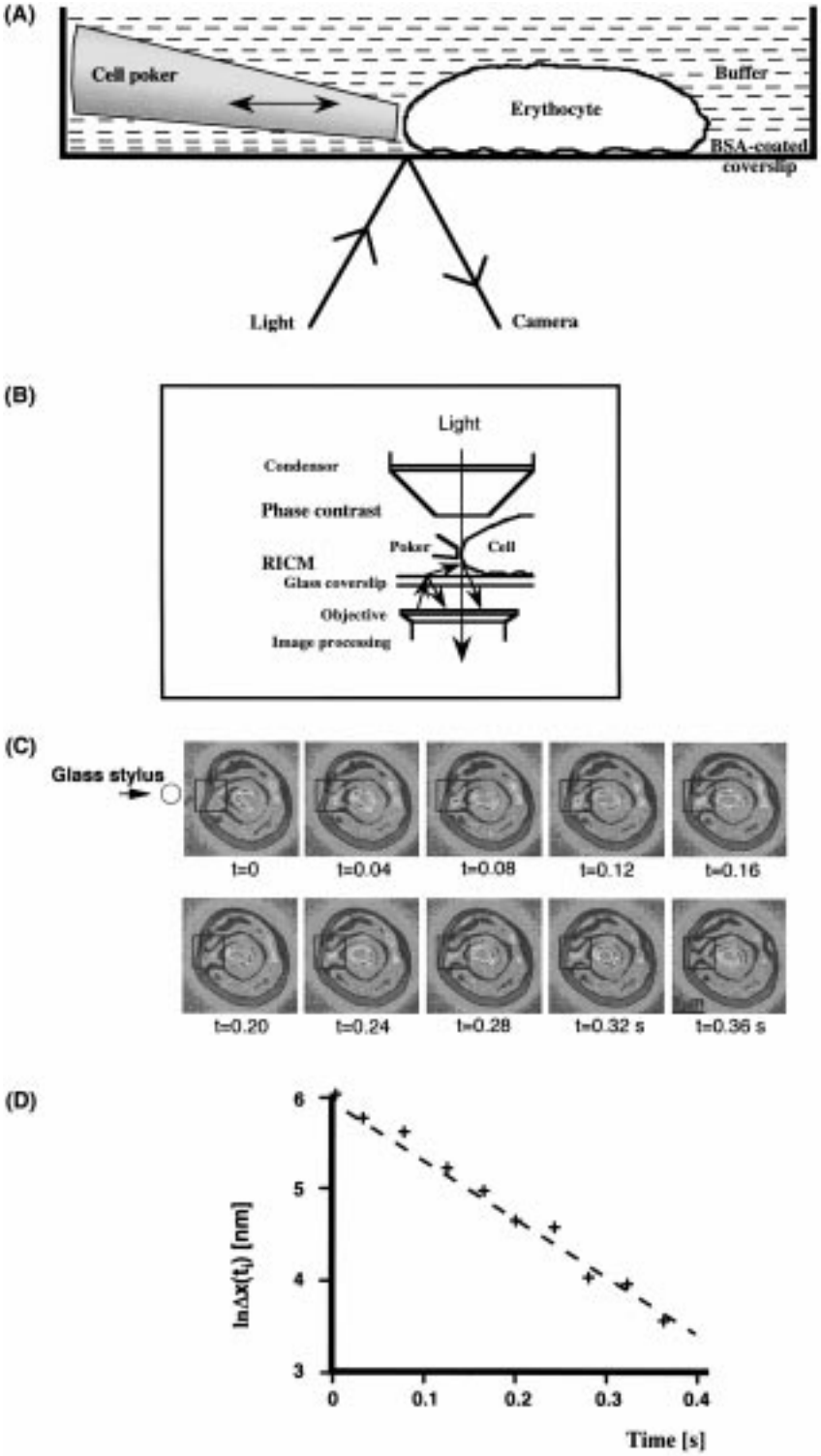

Fig. 1. Schematic representation of the cell-poking device (A) and detection system (B). The coverslip and glass stylus are coated with 2.5 mg and 25 mg BSA ml^{-1}, respectively, to immobilize the erythrocyte and to prevent its attachment to the glass stylus. Images taken after poking of an erythrocyte and immediate retraction of the glass stylus at $t = 0$ (s). The retraction of the cell membrane after deformation is recorded in ten images (C). The rate of relaxation is determined from the analysis of these ten video frames using an elastic response theory (D).

cell elasticity (Figure 1D). The theoretical basis for using this analysis form is given in detail by Strey *et al.* (1996).

Results and discussion

We have used this device to study the viscoelasticity of a wild-type F9 mouse embryonic carcinoma and an F9 vinculin-deficient (5.51) cell line, which was produced by chemical mutagenesis and is defective in both cell–matrix and cell–cell adhesivity (Goldmann *et al.* 1998b). We measured the effects of the deficiency of vinculin on the elastic properties of these cells. Wild-type cells were ∼20% more resistant to indentation by the cell poker (=glass stylus) than 5.51 cells. To further verify this finding, we used AFM to map the elasticity of the F9 cell lines by 128 × 128 force scans. These AFM findings, which correlate with cell-poking elastometric measurements as well with previous cell magnetometric and rheologic measurements (Goldmann & Ezzell 1996, Ezzell *et al.* 1997), indicate that sensing of physical forces within a cell's environment seems to be mediated by membrane-associated proteins known as focal adhesion proteins (FACs), which are an integral part of the cytoskeletal network in promoting cell adhesion and spreading by stabilizing focal adhesions. These results demonstrate the importance and feasibility of using biophysical techniques to examine the function of proteins linking actin to integrins and the plasma membrane and further the mechanical and viscoelastic properties of the cell.

Conclusion

This minireview has briefly shown the potential of the biophysical technique of cell indentation or poking, which is presently used to manipulate and probe cell structure and mechanics. This and other methods have led to further insights in how cellular viscoelasticity is regulated based on its architecture where mechanical, biological, and biochemical events are tightly coupled. Better understanding of how cells control these functions will require further optimization of the present techniques and will incorporate methods for controlling and quantitating changes in the cell. For more detailed reading see Goldmann *et al.* (2000).

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