Micromechanics and Microviscoelasticity of Cells .

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Abstract: Magnetic tweezer microrheometry combined with deformation field mapping enables local measurements of viscoelastic moduli of cell envelopes or cytoplasms. Cell envelopes behave as viscoelastic shells composed of slightly crosslinked actin gels while cytoplasms form highly heterogeneous viscoplastic fluids. Creep function analyses enable mapping of viscosites and measurement of local transport forces.

Mechanical forces control life processes on all length scales. On the molecular level forces can act catalytically on chemical reaction and molecular motors serve the directed material transport along macromolecular tracks or drive the replication and transcription of DNA. On the cellular level hydrodynamic shear forces control the blood pressure through force-dependent synthesis of the signal molecule nitroxid (NO) by endothelial cells lining the inner wall of blood vessels which mediate the smooth muscle contraction. Cell adhesion is controlled by interplay of specific (lock-and-key) forces a manifold of interfacial forces and, most importantly, by elastic stresses in the cell membranes. For that reason close relations exist between adhesion strength and elastic properties of cells On the macroscopic scale mechanical forces control the structure and mechanical strength of materials during growth . A particularly beautiful example is the regulation of the mechanical strength of trees by variation of the pitch of helically arranged cellulose fibrilles .For many such tissue growth processes by self assembly of materials, cells play a twofold role by acting as mechanical sensors and as machines for the synthesis of new materials such as collagen, hyaluronic acid and cellulose.

The biological function of forces is mediated by the activation of intracellular signal cascades resulting in the generation or libration of second messengers (such as cyclic AMP) or in the increase of intracellular Ca levels which can stimulate the reorganization of intracellular macromolecular scaffold ,such as the actin cortex , or activate the genetic expression. To gain insight into correlations between mechanical forces and biological functions it is essential to understand the transmission of forces within the composite biological materials .which is determined by the heterogeneous viscoelastic properties.

The main thrust of my lecture is to show that despite of the complex design of cells local viscoelastic moduli can be measured by application of the magnetic tweezer microrheometry and dynamic strain field analysis by colloidal force probes (cf Fig 1). Insight into the correlations between the viscoelastic impedance and structural or dynamic molecular properties of the materials is gained by parallel investigation of natural systems and realistic *in vitro* mechanical models of cells, such as giant vesicles with self-assembled reconstituted actin corteces

In the first part of the lecture we show that creep experiments combined with strain-field analysis [1] enable measurement of absolute Young moduli of the cell envelope. Comparison with viscoelastic impedances data of vitro actin networks show that the deformability of the cell envelope s determined by the membrane associated actin cortex .

The second part describes the application of the technique to evaluate in real time the rapid reorganization of the actin cortex of endothelial cells (embedded in confluent monolayers) by activation with inflammational agents;

such as histamine involved in allergic reactions[2]. We show that the actin cortex is stiffened by a factor of ~100 within ~ 1 sec by formation of stress fibers;a process associated with a sol-gel transition of the actin network which is triggered by activation of myosin and Ca-induced actin polymerization.We provide evidence that the centripetal contraction of cells (leading to the formation of gaps within the endothelium) is driven by the tension generated in the composite shell by the actin reorganization .The important role of integrin receptors as force transducers is discussed .

The viscoelasticity of the cytoplasm is studied by analysing the quasi-random transport of magnetic tweezers internalized by the cells and by local creep experiments. The motion consisists of intermittent transport along microtubuli and local random walks driven by intracellular dynamics; such as flagella-like motions of microtubule. The cytoplasm consists of soft streets separated by forbidden zones and these organized structures prevail for hours

The intracellular transport forces are measured by analysing the change in velocities induced by external force pulses and we show that forces between 8 and 30 pN are found. .Viscoelastic creep experiments demonstrate that the intracellular viscosity varies by orders of magnitude (10 -500 Pasec) .The intracellular space behave as viscoplastic rather than viscoelastic body. Similar to fracture processes in metals , the creep responses are described by force dependent local mobilities and random transitions within a multi-well potential. An important consequence of the viscoplasticity is that weak forces can mediate transport of intracellular compartments through stiff regions by statistical breakage of bonds.

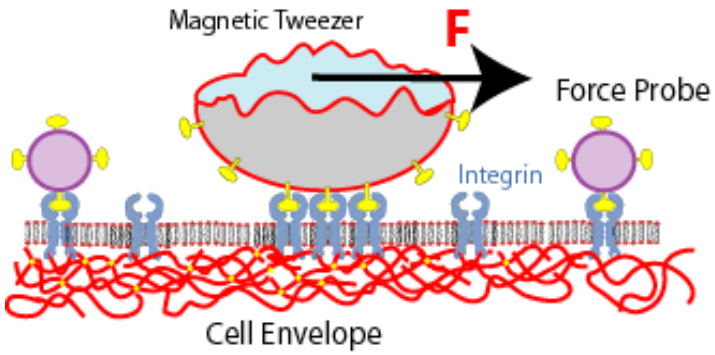

Fig 1. Viscoelastic microscopy with magnetic tweezers combined with strain field analysis based on induced creep of nonmagnetic force probes

[1] E. Sackmann and R. F. Bruinsma , Cell Adhesion as Wetting Transition?
ChemPhysChem 3 , 262-269,2002

[2] A.R. Bausch, U. Hellerer, M. Essler, M. Aepfelbacher and E. Sackmann , Rapid Stiffening of Integrin Receptor-Actin Linkages in Endothelial Cells Stimulated with Thrombin: A magnetic Bead Microrheology Study .Biophys. J. 80, 2649-2657, 2001

Viscoelastic microscopy of cells.

Superparamagnetic beads are biofunctionalized by surface grafting of ligands (such as fibronectin or bacterial proteins) for binding to specific cell surface receptors (such as receptors of the integrin family) or of motor proteins mediating intracellular transport for measurement of intracellular viscoelastic moduli or transport forces