Methods of measuring
Cell Elasticity

Courtesy of Katja Taute, modified by Björn Stuhrmann

Motivation

• Apply laws of physics (derived from inanimate matter) to living objects → learn about amazing material properties of cells

• Mechanical properties of cells reveal structural characteristics

• How are cell function and mechanical properties correlated?

• Changes in cell elasticity often monitor physiological status of cell, e.g. cancer cells are less elastic than healthy cells.
Basics: What is Elasticity?

In the easiest case (homogeneous isotropic solid):

<table>
<thead>
<tr>
<th>Compression/Stretching</th>
<th>Shear</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hooke’s law:</strong> $F \sim \Delta x$</td>
<td><strong>$F_T \sim \Delta x_T$</strong></td>
</tr>
<tr>
<td>Stress: $\sigma = F/A$</td>
<td>Shear stress: $\sigma_S = F_T/A$</td>
</tr>
<tr>
<td>Strain: $\varepsilon = \Delta x/x$</td>
<td>Shear strain: $\varepsilon_S = \Delta x_T/x$</td>
</tr>
<tr>
<td>$\sigma = E \varepsilon$</td>
<td>$\sigma_S = G \varepsilon_S$</td>
</tr>
<tr>
<td>$E$: Elasticity or Young’s Modulus</td>
<td>$G = E/(2 + 2\mu)$, $\mu$: Poisson number</td>
</tr>
<tr>
<td></td>
<td>$G$: Shear modulus</td>
</tr>
</tbody>
</table>

Does this apply to cells?

- **Cells are neither homogeneous nor isotropic**
  - Cells have internal structure
  - Cell components have different characteristics
- **Cells are not purely elastic but show also viscous behavior**
  - Cell material rather resembles a polymeric liquid or gel than a solid
  - Mechanical energy can be dissipated into heat
- **Cells are active**
  - Cell can respond to external forces e.g. by strengthening the cytoskeleton

⇒ Some difficulties extracting $E$ for a cell
How to deal with the difficulties

• **Inhomogeneity**
  → Use local measurements or averages over whole cell

• **E not practicable**
  → Incorporate viscosity in the model
    • Use complex elasticity modulus $E^*(\omega)$ or shear modulus $G^*(\omega)$
    • Storage modulus $G'(\omega) = \text{Re}(G^*)$ accounts for elasticity
    • Loss modulus $G''(\omega) = \text{Im}(G^*)$ accounts for viscosity
    • Be aware that these quantities are frequency-dependent

• **Living subject of study**
  → Allows for the study of biological response!

Techniques

• **Basic mechanical methods**
  – Micropipette Aspiration
  – Cell Poking
  – Silicon Micromachines
  – Atomic Force Microscopy
  – Biointerface Probe
  – Tensile Tester
  – Microplates

• **Magnetic methods**
  – Magnetic Twisting
  – Attached Magnetic Beads
  – Embedded Magnetic Beads

• **Optical methods**
  – Optical Tweezers
  – Optical Stretcher

• **Other**
  – Acoustic Microscopy
  – Laser Tracking
    Microrheology
  – Hydrodynamic Flow
Micropipette Aspiration

**Principle**
- suction pressure is created in micropipette
- leading edge of aspirated cell is tracked

**Details**
- tracking accuracy: ±25nm
- suction pressure range: 0.1-1000Pa
- force range 10pN-10mN
- pressure vs edge position yields E


Cell Poker (1)

**Principle:**
- tip mounted on flexible wire
- position of both tip and wire arm are measured to determine deflection

**Details**
- tip: glass stylus d=2µm
- position precision: 0.1µm
- $k_{wire}=38pN/nm$
- force resolution ~4nN
- force range: <30nN

Cell Poker (2)

**Principle**
- indentation by poker
- immediate retraction
- monitor cell relaxation by reflection interference contrast microscopy (RICM)

**Details**
- position precision: ~1nm
- analysis: \( \ln(x(t)) \sim -k t \)
- \( k \) characterizes resistance to indentations

Goldmann et al. (1998) FEBS Lett. 424:139-142

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Silicon Micromachines

**Principle**
- silicon poker fixed to beam of known elasticity
- poker and sensor position are monitored
- Also stretching experiments by glueing tip to cell

**Details**
- sensor is grown in SCREAM process
- \( k_{beam} = 3.4 mN/\mu m \)
- position accuracy: ~0.14\( \mu m \)
- force resolution ~0.5nN
- nN and \( \mu m \) range

Atomic Force Microscopy (AFM)

**Principle**
- Record player principle: cantilever scans sample
- Cantilever deflection is recorded by laser reflection
- Force-distance-maps can be produced for each x-y-position

**Details**
- $k_{\text{cantilever}} \approx 10^{-5} \text{pN/nm}$
- Deflection of laser beam is recorded with 2-segment photo diode
- Cantilever tip can be modified with beads for more convenient geometry and nondestructive imaging (Mahaffy et al. (2000). Phys. Rev. Lett. 85:880-883)

Al-Hassan et al. (1998) Biophys. J. 74:1564-1578

Biointerface Probe

**Principle**
- bead probe glued to red blood cell (RBC) held by micropipette
- RBC acts as variable force transducer

**Details**
- latex bead $d=2-3\mu m$
- probe position precision $\approx 5\text{nm}$
- suction pressure $\approx$ RBC surface tension $\approx$ RBC stiffness
- $k=1\text{fN/nm}-10\text{pN/nm}$
- force range $10^{-2}-10^3\text{pN}$

**Tensile Tester**

**Principle:**
- cell is stretched between two micropipettes
- distance between pipette tips measures elongation

**Details**
- displacement resolution: 0.24µm
- forces are measured with semiconductor strain gauge, accuracy ±50nN

Miyazaki et al. (2000) J. Biomech. 33:97-104

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**Microplates**

**Principle**
- cell is squeezed/stretched between a solid and a flexible microplate
- cell deformation is observed

**Details**
- solid microplate is moved with piezo
- force range: 10-100nN
- $k_{\text{microplate}}=1-10nN/\mu m$
- position accuracy: 0.4µm
- Analysis: $\sigma(t)=\varepsilon_0(k_0+k_1e^{-t/\tau})$

**Magnetic Twisting**

**Principle**
- surface-bound ferromagnetic beads are magnetized by strong field pulse
- then twisted by small perpendicular field
- twisting angle reflects shear response

**Details**
- field pulse: 0.1T for 10µs
- twisting field: <25·10⁻⁷T
- twisting angle is inferred from magnetometer measurements

Wang et al. (1993) Science 260:1124-1127

**Attached Magnetic Beads**

**Principle**
- force pulses are applied to paramagnetic beads bound to cell surface
- creep response and relaxation are recorded

**Details**
- bead d=4.5µm bound to integrin receptors
- force range: up to 10nN
- position accuracy: ~10nm
- time resolution: 0.04s

Bausch et al. (1998) Biophys. J. 75:2038-2049
**Embedded Magnetic Beads**

**Principle**
- field pulses are applied to ferromagnetic beads in cells (macrophages)
- creep response and recovery curves determine viscoelasticity

**Details**
- bead size: $d=1.3 \mu m$
- force range: $300-700pN$
- time resolution: $0.04s$


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**Optical Tweezers**

**Principle**
- beads glued to cell are held in optical traps
- one is moved and the response of the second is measured

**Details**
- $k_{trap}=0.08pN/nm$
- forces $<25pN$
- bead $d=1\mu m$
- bead 1 is moved using acousto-optical modulator
- bead 2 response is monitored with quadrant detector

Sleep et al. (1999) Biophys. J. 77:3085-3095
Optical Stretcher

**Principle**
- No absorption → laser beam passes through cell
- Momentum conservation → surface stress
- Cell deformation is measured in terms of aspect ratios
- No physical contact required!

**Details**
- surface force scales with \( n_{\text{cell}} \) and laser intensity
- force range: \( \sim 10^2 \text{pN} \)

Guck et al. (2005) Biophys J. 88:3689-3698

Acoustic Microscopy

**Principle**
- acoustic lens scans sample
- phase and amplitude of reflected wave encode sound velocity and attenuation
- sound velocity is a measure of elasticity

**Details**
- VHF ultrasound: \( \sim 1\text{Ghz} \)
- scan: 512×256 pixels
- spatial resolution: 3µm²

Kundu et al. (2000) Biophys. J. 78:2270-2279
Laser Tracking Microrheology (LTM)

**Principle**
- Some cells contain granules (lipid droplets), $d=0.3\mu$m
- Their Brownian motion in cytoplasm is observed by light scattering
- Mean square displacement is a measure for viscoelasticity of the surrounding medium

**Details**
- Laser: $P=0.13\text{mW}$, $\lambda=670\text{nm}$
- Displacement calibration with polystyrene beads (similar optical properties)
- Displacement resolution: $<1\text{nm}$
- MSD: $<R^2(\tau)>=(r(t+\tau)-r(t))^2$

Yamada et al. (2000) Biophys. J. 78:1736-1747

Hydrodynamic Flow

**Principle**
- Hydrodynamic flow deforms the cell
- Cell profile is determined by membrane bending modulus

**Details**
- Shape is analyzed using reflection interference contrast microscopy (RICM)
- $\lambda$ yields bending modulus

Brief Review of Techniques

• Basic mechanical methods
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  – Hydrodynamic Flow

Summary

• Different techniques measure different aspects
• Quantity characterizing elasticity needs to be chosen with care
• Results obtained with different techniques may be difficult to compare
• Choice of technique must depend on the specific aim of the experiment
Experimental Results

Techniques for Attached Cells

Adherent cells

Beads as handles
Techniques for Attached Cells

Magnetic bead twisting
(Fabry et al., PRL 2001; PRE 2003)

Optical Tweezers
(Balland et al., Eur. Biophys. J., 2005)

Rheological Results: Attached Cells

Rheological Results

\[
\log(G') = x \cdot \log(\omega) + c
\]

All experiments with all kinds of different cells can be described by a Master Curve; only difference is \(x\)!

Fabry et al., *PRL* 2001; *PRE* 2003

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Rheological Results

Can be described by theory of “soft glassy materials” (Sollich, 1997, 1998) (Foams, pastes, colloids, slurries, ...)
- very soft (Pa – kPa)
- \(G'\) and \(G''\) follow the same power law (no single relaxation time)
- \(x\) is an effective noise temperature
- microscopic origin???
Deforming Cells in Suspension?

The Optical Stretcher
The Optical Stretcher

Optical Surface Forces

Momentum of a light ray

\[ p = \frac{nE}{c} \]

Conservation of momentum at surface

\[ \Delta p = \frac{E}{c} (n_1 + Rn_1 - (1 - R)n_2) < 0 \]

\[ n = \frac{n_2}{n_1} > 1 \]

Whenever light enters or exits a dielectric medium it exerts a force AWAY from the denser medium and NORMAL to the surface.

Surface Deformation


Surface Forces in Gaussian Beams
Surface Forces in Gaussian Beams

Optical Stress in DBLT
Microrheology on Fibroblasts

Step-stress experiment

Deformation ($\gamma_{\text{max}} = 7.5\%$)

Viscoelastic Characterization

$$\sum_{n=0}^{\infty} a_n \dot{\gamma}(t) = \sum_{j=0}^{\infty} b_j \dot{\gamma}(t)$$

$$a_0 \dot{\gamma}(t) + a_1 \dot{\gamma}(t) = \sigma(t) + b_0 \sigma(t)$$

Viscoelastic Models

a) Maxwell element
b) Voigt element
c) Voigt element w/ dashpot

Viscoelastic Characterization

\[ \frac{1}{D(t)} = \frac{\sigma(t)}{\gamma(t)} \]
\[ L \{ \sigma(t) \} = \frac{1}{p^2} \]
\[ E(t) = 2(1 + \mu)G(t) \]
\[ G^*(\omega) = i\omega \int_0^\infty dt e^{-\omega t} G(t) \]
\[ \gamma(t) \rightarrow D(t) \rightarrow E(t) \rightarrow G(t) \rightarrow G^*(\omega) \]

Frequency dependent shear modulus:

\[ G^*(\omega) = G'(\omega) + iG''(\omega) \]

F. Wottawah et al., Acta Biomater. (2005)
Microscopic Origin

**non-crosslinked / entangled**

\[ G' \approx 5 \text{ Pa} \]


**transiently crosslinked**

\[ G_{NH3}^{t} = 100 \pm 10 \text{ Pa} \]


**fully crosslinked**

\[ G' \approx 680 \text{ Pa} \]

F. Wottawah et al., Acta Biomater. (2005)

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**Microscopic Origin**

**non-crosslinked / entangled**

Reptation time

\[ \tau_r \approx 400 \text{ s} \]

Wachowiak et al., Biophys J. (1994)


Mullins et al. PNAS (1998)

**transiently crosslinked**

Relaxation time

\[ \tau_t = 2.8 \pm 0.5 \text{ s} \]

**fully crosslinked**

Wachowiak et al., Biophys J. (1994)


<table>
<thead>
<tr>
<th>Actin Crosslinker</th>
<th>k (Dissociation Rate Constant)</th>
<th>1/k (secs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-actinin</td>
<td>2.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Filamin/ARF</td>
<td>0.6</td>
<td>1.667</td>
</tr>
<tr>
<td>Arp 2/3</td>
<td>0.5-3</td>
<td>0.2-2</td>
</tr>
</tbody>
</table>

Microscopic Origin

Transiently crosslinked: beyond $1/k$, the cell behaves like a fluid

Explanation of the Difference?

Attached

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>G' (Pa)</th>
<th>G'' (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>1</td>
<td>1000</td>
<td>10000</td>
</tr>
</tbody>
</table>

Suspended

<table>
<thead>
<tr>
<th>Frequency (rad/s)</th>
<th>G' (Pa)</th>
<th>G'' (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>0.15</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>0.2</td>
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<td>10000</td>
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<tr>
<td>0.3</td>
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<td>100000</td>
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<tr>
<td>0.5</td>
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</tr>
<tr>
<td>0.7</td>
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<td>10000000</td>
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<tr>
<td>1</td>
<td>1000000</td>
<td>10000000</td>
</tr>
<tr>
<td>1.5</td>
<td>10000000</td>
<td>10000000</td>
</tr>
<tr>
<td>2</td>
<td>10000000</td>
<td>10000000</td>
</tr>
</tbody>
</table>
Cytoskeleton in Adherent Cells

Handles attach to Stress Fibers

Methods probe dynamics of the contractile actin-myosin stress fibers (very local measurement). Response on many time-scales!
Cytoskeleton in Suspension

No stress fibers present, only isotropic network. This allows the application of polymer theories and microscopic interpretation of results! Optical Stretcher measures global properties.

The Effect of Blebbistatin

Blebbistatin inhibits the activity of myosin. 

--> other relaxation times appear!!

Cell Mechanics as a Cell Marker

The Cytoskeleton

Far from being static and passive, it is a very dynamic system that fulfills many important cell functions.

Courtesy of Dr. Stefan Grill, MPI f. MCB Dresden
The Cytoskeleton

Elasticity Function

Comparison of Different Cell Types

<table>
<thead>
<tr>
<th></th>
<th>Normal Cell</th>
<th>Cancer Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G$ [Pa]</td>
<td>10$^9 \pm 10$</td>
<td>8$^9 \pm 5$</td>
</tr>
<tr>
<td>$\eta$ [Pa s]</td>
<td>426 $\pm$ 97</td>
<td>232 $\pm$ 44</td>
</tr>
<tr>
<td>$\tau$ [s]</td>
<td>2.2 $\pm$ 1.1</td>
<td>1.9 $\pm$ 0.6</td>
</tr>
</tbody>
</table>

Deformability of Cells

Only 30 cells each measured.

Deformability of cells is a tightly regulated cell marker

S. Schinkinger et al., *J. Biomed. Opt.* (submitted)
Cytoskeleton in Cancer Cells

Structure of cytoskeleton in cancer cells is different

Amount of F-Actin

Actin amount is reduced by 30-35%

J. Guck et al., Biophys. J. 88(5) (2005)
Cell deformability provides built-in, strong amplification of molecular changes in single living cells.