Biological Machines, Cell Mechanics and Nanotechnology

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### Remaining course overview

<table>
<thead>
<tr>
<th>Date</th>
<th>Topic</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/13</td>
<td>Kinesins, their mechanical properties and MEMS</td>
<td>王歐力</td>
</tr>
<tr>
<td>4/20</td>
<td>Myosins, Dynein and the problems of trafficking</td>
<td>王歐力</td>
</tr>
<tr>
<td>4/27</td>
<td><strong>Midterm Exam</strong> =&gt; only Dr. Perng Ming-Der's Part</td>
<td>彭明德</td>
</tr>
<tr>
<td>5/04</td>
<td>Biological and non-biological nanomachines</td>
<td>王歐力</td>
</tr>
<tr>
<td>5/11</td>
<td>Cell mechanics I</td>
<td>王歐力</td>
</tr>
<tr>
<td>5/18</td>
<td>Diffusion, friction and entropic forces acting on molecular motors II</td>
<td>吳見明</td>
</tr>
<tr>
<td>5/25</td>
<td>Diffusion, friction and entropic forces acting on molecular motors II</td>
<td>吳見明</td>
</tr>
<tr>
<td>6/01</td>
<td>Cell mechanics II</td>
<td>王歐力</td>
</tr>
<tr>
<td>6/08</td>
<td>Journal club 1: 張妍, 謝榕, 黃玳軒, 李詠哲</td>
<td>王歐力</td>
</tr>
<tr>
<td>6/15</td>
<td>Journal club 2: 蘇子翔, 謝錚澤, 林淑娟, 陳莉菁</td>
<td>王歐力</td>
</tr>
</tbody>
</table>

#### Evaluation:
- Presence 25%
- Class Performance 40%
- Journal Club 35%

#### Journal Club:
- Pick an article from a journal with IF >5 about **molecular motors** or **cell mechanics**
- Presentation time 20 min. + 10 min discussion (total 2 hours for 4 students)
Computer model of cellular tensegrity

Tensegrity is the structural interplay between compression elements (microtubules) and tension elements (actin filaments)

Computer model shows how hierarchical tensegrity structures, such as a cell with a nucleus, behave when pulled, sheared and stretched
Axonal tensegrity: Mechanical properties of neurons

Abrupt axon retraction observed after nocodazole treatment (MT depolymerization):

=> Mechanical balance of an axon is provided by creating a tension of actin along the cortex (as well as substrate adhesion) and antagonistic compression forces provided by microtubules.
Difference between shear stress and compression

**Compressed**
- Network area changed but **no changes in internal angles**

**Undeformed**
- Internal network angles changes but **area unchanged**

**Sheared**
- Similar after applying a two-dimensional stress

Effect of **thermal fluctuations**
- Zero-temperature network
- Network becomes more erratic

David Boal, Mechanics of the Cell, 1st Ed.
Cell compression induces reversible spindle widening and elongation

Mild compression with agarose coated metal rod

Spindle elongation is not affected by actions of actin network, but by MT polymerization

The drug STLC inhibits kinesin 5

=> Also here no effect on spindle elongation

Conclusion:
• Neither the action of the actin network nor that of kinesin 5 affects the spindle elongation upon compression.
• It is assumed that a mechanochemical switch at the poles regulates the depolymerization rate of kinetochore MTs.

Importance of cytoskeleton and cytomechanics in environmental cell responses

Filopodia (made of thick actin bundles) of white blood cells catching bacteria for lysosomal digestion
Actin Microtubules Cytoskeletal response to cell spreading

Stress fiber development upon spreading of a fibroblast on glass
Cellular response to substrate stiffness

**Soft Substrate**

- Polyacrilamide: 0.5 kPa

  NIH3T3 Fibroblast
  
  Model

  Soft substrate with **small spring constant** (k):
  
  Cell can easily pull on the gel (no need for stress fibers)

  C

  no actin stress fibers

**Stiff Substrate**

- Polyacrilamide: 12 kPa

  NIH3T3 Fibroblast

  Model

  Stiff substrate with **larger spring constant** (k):
  
  Cell need greater force to displace the polyacrilamide gel (cell need power of stress fibers)

  D

  no actin stress fibers
Prestress visualized in a computer model

- A rounded cell on a soft substrate exhibits a **uniform and constant prestress** from the edge (cell border) to the nucleus (cell center)
- Prestress is **generated by actin-myosin contraction** and transmitted to the substrate
- This computed strain distribution is consistent with the tensegrity model

Discher et al., Science, 2005
Rearrangement of stress fibers after cyclic cell stretching

How do cells handle mechanical forces generated in organs as the heart or the blood pressure in vessels?

Unstretched human aortic endothelial cell: **random distributed stress fibers**

After 3 hours of stretching: **stress fibers are oriented into direction of stretching**

Very dynamic features of stress fibers are critical for **force sensing** and **force transduction**
Cellular response to substrate composition

Cultured fibroblast align on a furrowed surface in the direction of the grooves

Preference of the substrate coating is obvious since growing does not occur across the furrows

Groove dimensions:
2 µm deep
3 µm wide
3 µm spaced apart

Bray, Cell Movements, 2nd Ed.
Cellular response to “cell traffic”: contact inhibition

When one cell collides with another a phenomenon named **contact inhibition** occurs:

- At the region of contact (cell’s ruffles) a **stationary (quiet) zone** is formed in which cells seemed to form **contact by filopodia**
- Ruffling now occurs in the **opposite direction**
- Cells are moving away from each other
Cellular response to an electric field

Before the field, the epithelial cell rounded

After 1 hour exposure to an electric field of 150 mV/mm cell becomes elongated (90° to the field) and starts to move to the minus-pole

Switching the polarity of the field results in a movement to the preferred minus-pole (the cathode)
Internal cellular hydrostatic pressure as a cytomechanical factor

- Cell contains **bulk water** (free water) and **bound water** (bound by proteins)
- Under hyperosmotic conditions, only the bulk water will be lost
- On the other hand, the **high ionic content in the cell** might lead to a constant flow of water inside the cell
- To avoid this, the cell develops and maintains a constant hydrostatic pressure to stop water flowing inside
- Some plant cells and bacteria can develop internal pressures up to $10^6\text{Pa}$
- Relaxation of cortical tension might result in redirecting of internal pressure that may drive cell membrane extension
- Water ingress might also **swell the cytoskeleton** leading to increased osmotic forces
- How much does hydrostatic pressure contribute to cell mechanics?

Model of cortical relaxation (based on osmotic forces) after adding an actin depolymerizing factor (gelsolin)
Biomechanics and biophysics of cancer cells

**Deformability** of breast cancer cell is increased (based on f-actin reduction) that also increases metastatic potential.

Microfluidic optical stretcher: trapping and stretching cells with two laser beams.

TPA is a type of phorbol ester to treat leukemia or lymphoma cancer.

Guck et al., Biophys. J, 2005
The substance SPC decreases the IF network which in turn increases metastatic potential.

Invasion of Panc-1 epithelial tumor cells in the human pancreas by the bioactive lipid SPC

**Structure**
- Dramatic reorganization of the intermediate filament (keratin) network in the perinuclear region

**Property**
- More than three-fold reduction in Panc-1 cell elastic modulus and increase in hysteretic energy dissipation during cell deformation

**Disease**
- Greater motility of tumor cells through size-limiting pores and metastatic invasion?

(High SPC levels found in blood in patients with pancreatic tumors)

Effects of chemotherapy on elastic properties of cancer cells

• Chemotherapy to treat leukemia leads to cell stiffening that might explain observed vascular compilations (atherosclerosis etc.)
• Parallel treatment with cytochalasin D to weaken the actin-network helped to make the dead cells softer for better dead-cell recycling (not shown)

Yellow bars: blood cells before chemotherapy
Red bars: dead cells after chemotherapy (drug: daunorubicin)
Besides the Cytoskeleton the ECM is important for Cell Mechanics

3 principles act to form a tissue from single cells:
1) **Cytoskeleton** not only acts to stabilize single cells but also helps to connect a cell to a neighbor cell
2) Specialized (polymeric) proteins stabilize cell-cell contacts (cell adhesion molecules, **CAM**)
3) An matrix outside the cell (extracellular matrix, **ECM**) acts as a fibrous filling material and to glue cells to each other
Integrating single cells into stable tissues

- **Intracellular anchor proteins** connect the cytoskeleton to **transmembrane adhesion proteins (CAMs)**
- **Transmembrane adhesion proteins** are embedded in the **extracellular matrix (ECM)**

Cytoskeletal filaments are connected to the anchoring junctions
Integrating single cells into stable tissues

A variety of cell-cell contacts functions differently in the tissue:

- **Tight junctions**: make part of the membrane almost impermeable (diffusion barrier)
- **Gap junctions** form ion-channels for electrical communication between cells
- **Adherens junctions** are guided by an elastic actin-myosin cable to shape the cell

- **Desmosomes** are protein complexes to interconnect the internal intermediate filament network and to form contacts to neighboring cells
- **Hemidesmosomes** connect cells to the extracellular matrix (connective tissue)
- The cell is separated from the ECM by an tight and impermeable network-layer (“carpet”) named **basal lamina**
Integrating single cells into stable tissues

• While **tight junctions** make the cell almost impermeable, **gap junctions** allow small metabolites and messaging molecules to pass.

• The actin or intermediate filaments network first connects to **specific adapter proteins** which in turn connect to the large CAM macromolecules.
Cell adhesion molecules (CAM) form homophilic (self) cross-bridges

Cell-cell contact is generated in two steps:
- CAMs first form dimers by lateral interaction and clustering in the cell membrane
- Strong cell-cell adhesion is generated by cross-bridging CAMs to opposite CAMs (homophilic (self) cross-bridging depends on and is regulated by Ca^{2+})
Dissecting an adherens junction

• An adherens junction is a complex apparatus consisting of many components for successful connecting cells to each other and the cytoskeleton to the junction.
• Some adapter proteins do directly participate in intracellular signaling pathways (for example, β-catenin).
Desmosomes are button-like structures connecting two cells

Thick intermediate filament bundles connected to electron dense structures can be seen in EM of two keratinocytes (skin cells) firmly connected to each other.

In the skin disease *pemphigus vulgaris* the protein *desmoglein* is non-functional, resulting in severe skin blistering.
Gap junctions are 2-3 nm wide channels

**Gap junctions** form a channel system for the exchange of small metabolites (as ions, sugars, vitamins, ATP etc.) between two cells.

The gap junction channel is formed by the hexagonal protein **connexin**.

Atomic structure of gap junctions

C = cytosol, M = membrane bilayer, E = extracellular gap.
Cell junctions are crucial for tension and mechanical stability of tissues

Since **junctions** integrate a cell’s cytoskeleton and at the same time strongly connect to neighboring cells, shape, rigidity and cell strength are largely increased.

<table>
<thead>
<tr>
<th>Functions of cell junctions</th>
</tr>
</thead>
<tbody>
<tr>
<td>JUNCTION</td>
</tr>
<tr>
<td><strong>Anchoring junctions</strong></td>
</tr>
<tr>
<td>1. Adherens junctions</td>
</tr>
<tr>
<td>2. Desmosomes</td>
</tr>
<tr>
<td>3. Hemidesmosomes</td>
</tr>
<tr>
<td><strong>Tight junctions</strong></td>
</tr>
<tr>
<td><strong>Gap junctions</strong></td>
</tr>
</tbody>
</table>
ECM (extracellular matrix)

- Extracellular matrix (ECM) is the (connective) tissue below an epithelium
- ECM contains **many highly elastic fibers** but also the cells that secrete these fibers
- These fibers and cells are embedded in a gel (**hyaluronan** and **proteoglycans**)
ECM contains stiff/non-elastic and highly elastic fibers

Highly elastic aorta need to resist strong and alternating blood pressure

- Elastin molecules are highly cross-liked by covalent bonds
- An elastin assembly can stretch and relax like a rubber-band

Elastic fiber (elastin) in the outer layer of the aorta
Hyaluronan resists compression and gives cartilage its gel-like properties

- Major component of cartilage is the **aggrecan aggregate**: huge molecule (MW 2 x 10^8) with a size of a bacterium
- Up to 100 **aggrecan** molecules are connected to a **hyaluronan** backbone
The ECM is composed of a variety of **proteins** and **GAGs** = glycosaminoglycan (or combinations of them)

- fibronectin
- fibrillar collagen
- laminin
- tenascin
- type IV collagen
- hyaluronan
- decorin
- perlecan
- aggrecan

**GAG chain is a repeating disaccharide sequence**
Collagens are rather inelastic fibers found in skin and bone.

Collagens are complex molecules embedded in the ECM.

A single collagen fiber in the gel-forming matrix of cartilage
=> inflexible but resilient

Collagens provide great tensile strength
(high mechanical load capacity)
Collagen fibers can be arranged into a network by type IX collagen.
Basal lamina acts as a permeability barrier and integrates cells into tissues

Epithelial cells sitting on top of the basal lamina

Mat-type basal lamina acting as a **tight barrier** for small molecules

Collagen in the ECM below the basal lamina
Basal lamina acts as a permeability barrier and integrates cells into tissues

- **Type IV collagen** is a sheet-forming protein making the basal lamina a tight barrier for selecting molecules (filter function/blood brain barrier)
- **Laminin** integrates the major components of the basal lamina
Type IV collagen can form a complex network by forming **dimer** (tail-tail) and **tetramer** (head-head).
Laminin is a multiadhesive matrix protein found in all basal lamina.

- Structure of laminin: it is a complex molecule built from three chains containing several globular domains and coiled-coils.
- It can bind collagen, integrins, lipids, carbohydrates, and even neurites.
Cell contact with the ECM is important for growth, proliferation and survival

If a cell cannot nicely spread on a substrate it will eventually die

The extent of cell spreading is more important than the amount of molecules the cell interacts with
The basal lamina is important for cell survival

Embryonic development of bodies cavities:
- **Endoderm cells** send out signals that make **ectoderm cells** die (forming a cavity)
- However, those with direct contact to the **basal lamina** survive
Literature

**Molecular Cell Biology 6th Edition**
by Harvey Lodish etc.

**Cell Biology 2nd Edition**
by Thomas D. Pollard etc.

**Molecular Biology of the Cell, 5th Edition**
by Bruce Alberts etc.

Aug 2007

Apr 2007

Nov 2007
Free online books

88 items in Eurekah Bioscience Collection.
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50 items in Cancer Medicine. 6th ed.

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Cooper, Geoffrey M.

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The C. elegans Research Community, editors
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Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Raff, Martin; Roberts, Keith; Walter, Peter
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Cytoskeleton Methods and Protocols by Ray H. Gavin (Editor)

Cytoskeletal Mechanics: Models and Measurements by Mohammad R. K. Mofrad and Roger Kamm

Mar 2000  Jan 2001  Sep 2006
Focused/specialized literature

G Proteins, Cytoskeleton and Cancer
by Hiroshi, Ed. Maruta

Aspects of the Cytoskeleton
by Seema Khurana

Molecular Motors
by Manfred Schliwa

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**Mechanics of Motor Proteins and the Cytoskeleton** by Jonathon Howard

Feb 2001

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**Guidebook to the Cytoskeletal and Motor Proteins** by Thomas Kreis and Ronald Vale

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by Vincenzo Balzani etc.

Our Molecular Nature: The Body's Motors, Machines and Messages
by David S. Goodsell

Molecular Machines
by T. Ross Kelly

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by Jesus Paramio

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by M. Bishr Omary and Pierre A. Coulombe

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Dec 2004
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**Molecular Interactions of Actin**
by [D.D. Thomas](https://example.com) and [C.G. dos Remedios](https://example.com)

**Actin-Binding Proteins and Disease** by [Cris dos Remedios](https://example.com) and [Deepak Chhabra](https://example.com)

**Actin-Monomer-Binding**
by [Pekka Lappalainen](https://example.com)

- Mar 2002
- Nov 2007
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**Myosins**
by Lynne M. Coluccio

**The Role of Microtubules in Cell Biology, Neurobiology, and Oncology**
by Antonio Tito Fojo

**Microtubule Protocols**
by Jun Zhou

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