Introduction to Cell Adhesion and Focal Adhesions

Focal adhesions (FAs) are specialized, dynamic structures that enable cells to **anchor** to the extracellular matrix (ECM) and **communicate biochemical and mechanical signals** across the cell membrane.

They play essential roles in cell migration, differentiation, and mechanotransduction—the process by which cells sense and respond to mechanical signals. These adhesions are a major research area in biophysics due to their role in mechanobiology and cellular force transmission (Geiger et al., 2009).

"The term **sensing** is used metaphorically and refers to those **environmental features that can exert measurable effects on cell dynamics, function and fate** following specific modulation"

REVIEWS OF MODERN PHYSICS, VOLUME 85, JULY–SEPTEMBER 2013

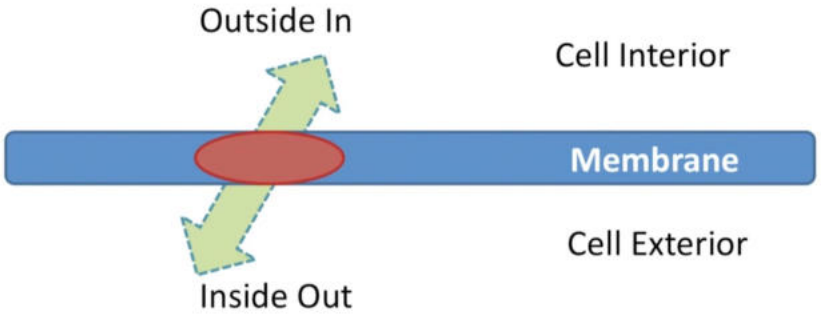
Physics of adherent cells

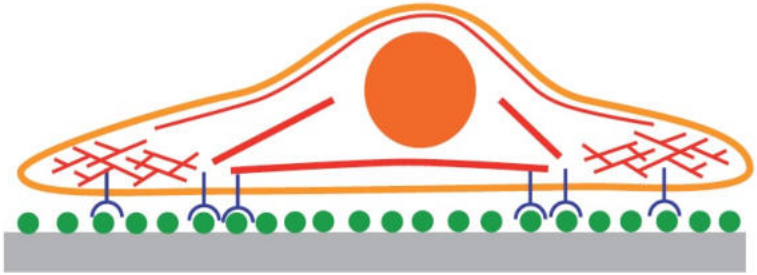
Ulrich S. Schwarz*
BioQuant and Institute for Theoretical Physics, Heidelberg University, Philosophenweg 19, 69120 Heidelberg, Germany

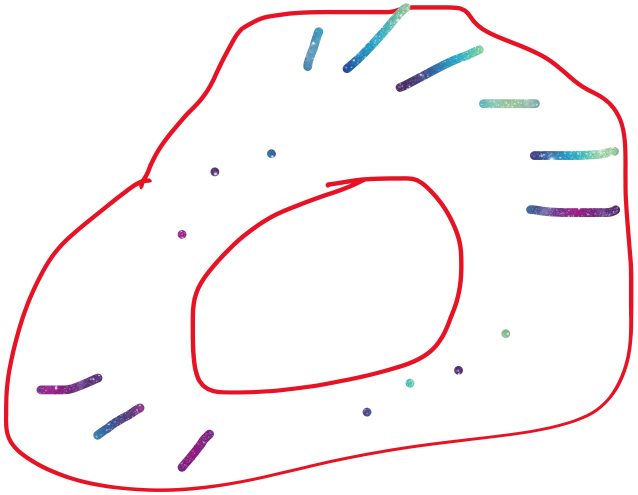
Samuel A. Safran†
Department of Materials and Interfaces, Weizmann Institute, Rehovot 76100, Israel

DOI: 10.1103/RevModPhys.85.1327

Focal adhesions mediate bi-directional signaling

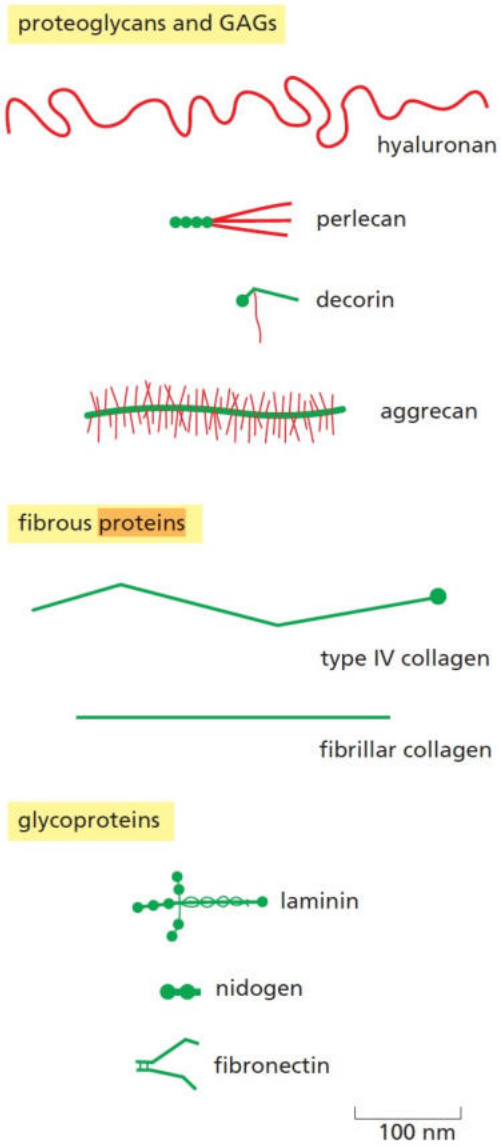






FA are made by more than 100 different proteins

Areas are spreaded : (0.5-1.0) μm^2
(3-10) μm^2

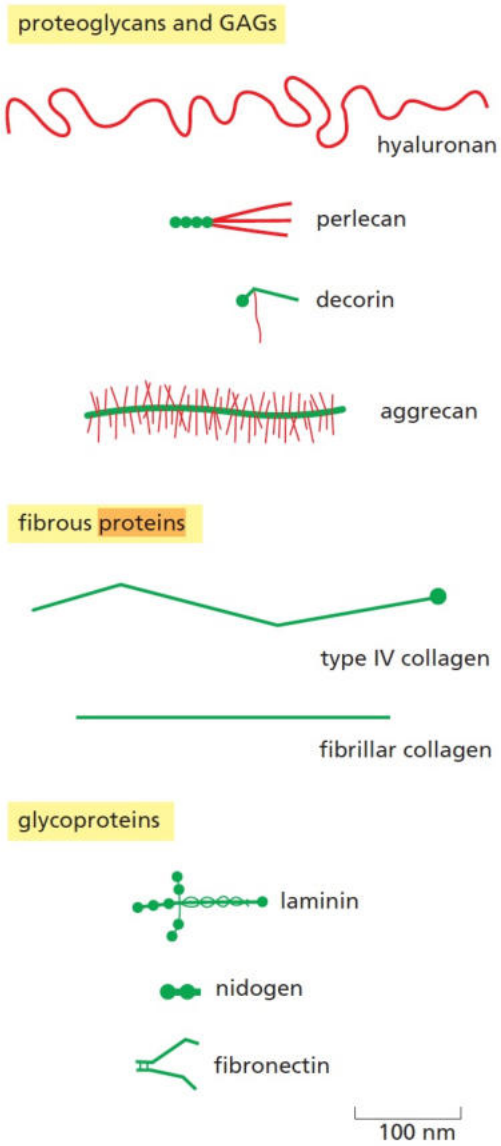


Extracellular Matrix

ECM macromolecular composition:

(1) **glycosaminoglycans (GAGs)**, which are large and highly charged polysaccharides that are usually covalently linked to protein in the form of proteoglycans;
(2) **fibrous proteins**, which are primarily members of the **collagen** family;
(3) a large class of **noncollagen glycoproteins**, which carry conventional asparagine-linked oligosaccharides.

Mammals have almost 300 matrix proteins: 36 proteoglycans, about 40 collagens, and over 200 glycoproteins, which usually contain multiple subdomains and self-associate to form multimers.
Add to this the large number of matrix-associated proteins and enzymes that can modify matrix behavior by cross-linking, degradation, or other mechanisms:
the matrix is an almost infinitely variable material. Each tissue contains its own unique blend of matrix components, resulting in an ECM that is specialized for the needs of that tissue.



Extracellular Matrix

The **proteoglycan** molecules in connective tissue typically form a **highly hydrated, gel-like** “ground substance” in which collagens and glycoproteins are embedded.
The polysaccharide gel **resists compressive forces on the matrix** while **permitting the rapid diffusion of nutrients**, metabolites, and hormones between the blood and the tissue cells.

The **collagen fibers** strengthen and **help organize the matrix**, while **other fibrous proteins**, such as the rubberlike elastin, give it **resilience**.

Finally, the many **matrix glycoproteins** help cells migrate, settle, and differentiate in the appropriate locations.

Cells interact with the extracellular matrix **mechanically** as well as **chemically.**
Studies in culture suggest that the mechanical interaction can have dramatic effects on the architecture of connective tissue.

Thus, when fibroblasts are mixed with a meshwork of randomly oriented collagen fibrils that form a gel in a culture dish, the fibroblasts tug on the meshwork, drawing in collagen from their surroundings and thereby causing the gel to contract to a small fraction of its initial volume.

Fibroblasts may have a similar role in organizing the extracellular matrix inside the body.
First they **synthesize the collagen fibrils** and deposit them **in the correct orientation**.
Then they **work on the matrix they have secreted**, crawling over it and tugging on it so as to create tendons and ligaments and the tough, dense layers of connective tissue that surround and bind together most organs.

Cell-ECM organization and cancer

Unlike free-living cells such as bacteria, which compete to survive, **the cells of a multicellular organism are committed to collaboration:** the cells send, receive, and interpret an elaborate set of extracellular signals that serve as social controls, directing cells how to act.
As a result, **each cell behaves in a socially responsible manner**—resting, growing, dividing, differentiating, or dying—as needed for the good of the organism.

In a human body with more than 10^{14} cells, billions of cells experience **mutations** every day, potentially disrupting the social controls. Most dangerously, a mutation may give one cell a selective advantage, allowing it to grow and divide slightly more vigorously and survive more readily than its neighbors and in this way to become a founder of a growing mutant clone.

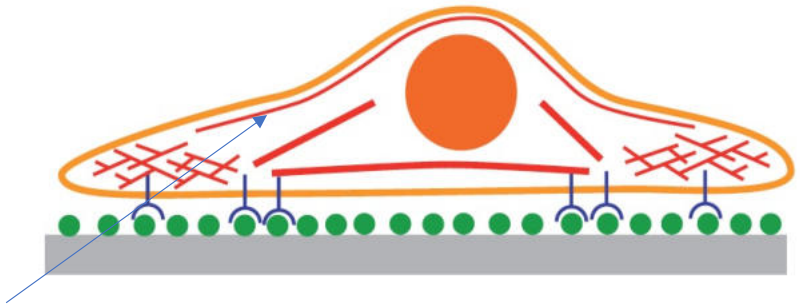
Over time, **repeated rounds of mutation**, **competition, and natural selection** operating within the population of somatic cells can cause matters to go from bad to worse.

These are the basic ingredients of cancer: it is a disease in which an individual mutant clone of cells begins by prospering at the expense of its neighbors. In the end—as the clone grows, evolves, and spreads—it can destroy the entire cellular society.

Role of Focal Adhesions in Cell Movement:

Focal adhesions act as signaling hubs that allow cells to sense and respond to mechanical stimuli from their environment.
They provide **traction forces** that **enable cells to migrate by dynamically forming and disassembling during movement.**

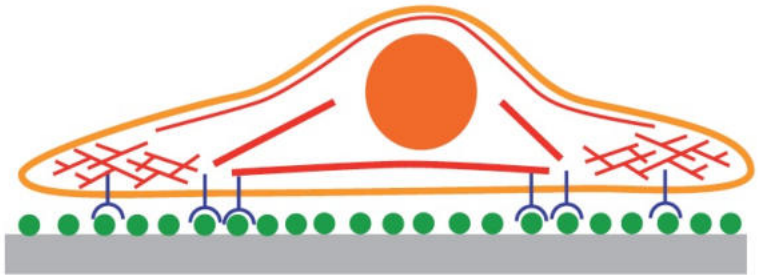
On one side, they collect the cell membrane to the cytoscheleton while, on the other side, anchor to the extracellular matrix



Underneath the plasma membrane is the **actin cortex**, a relatively thin (100 nm) dynamic layer of cross-linked actin filaments whose mechanical properties dominate the elastic response in reaction to deformations of the cell. On the other side, cells adhere to the extracellular environment.

Inside the cell, there exists a highly cross-linked and entangled network of three different types of polymers (**actin filaments, microtubules, and intermediate filaments**) collectively called **the cytoskeleton (CSK**).

On the outside, the cell is coupled to another **multicomponent, gel-like network** (including fibrous protein components such as collagen or fibronectin) called the **extracellular matrix (ECM**).



If **subjected to mechanical forces**, the biological material **initially (a few seconds) responds like a passive elastic body**; thus elasticity theory is an essential element of the physics of cells and tissues. Stress-strain relationship through the cell elastic Young modulus E (**stiffness, Pa**):

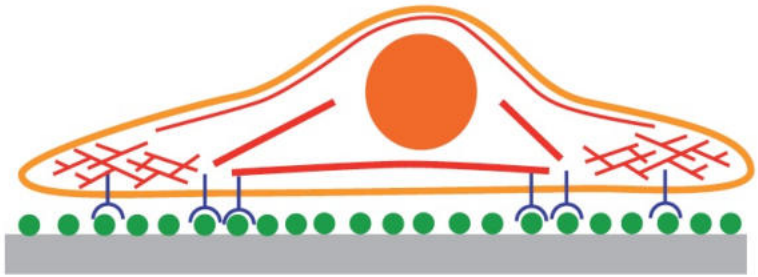
$$\sigma = E\epsilon$$

At longer time scales, the cell responds to mechanical perturbations by **actively reorganizing the structure of its CSK** (and, to a certain extent, its ECM as well).

Typical values: 10 kPa

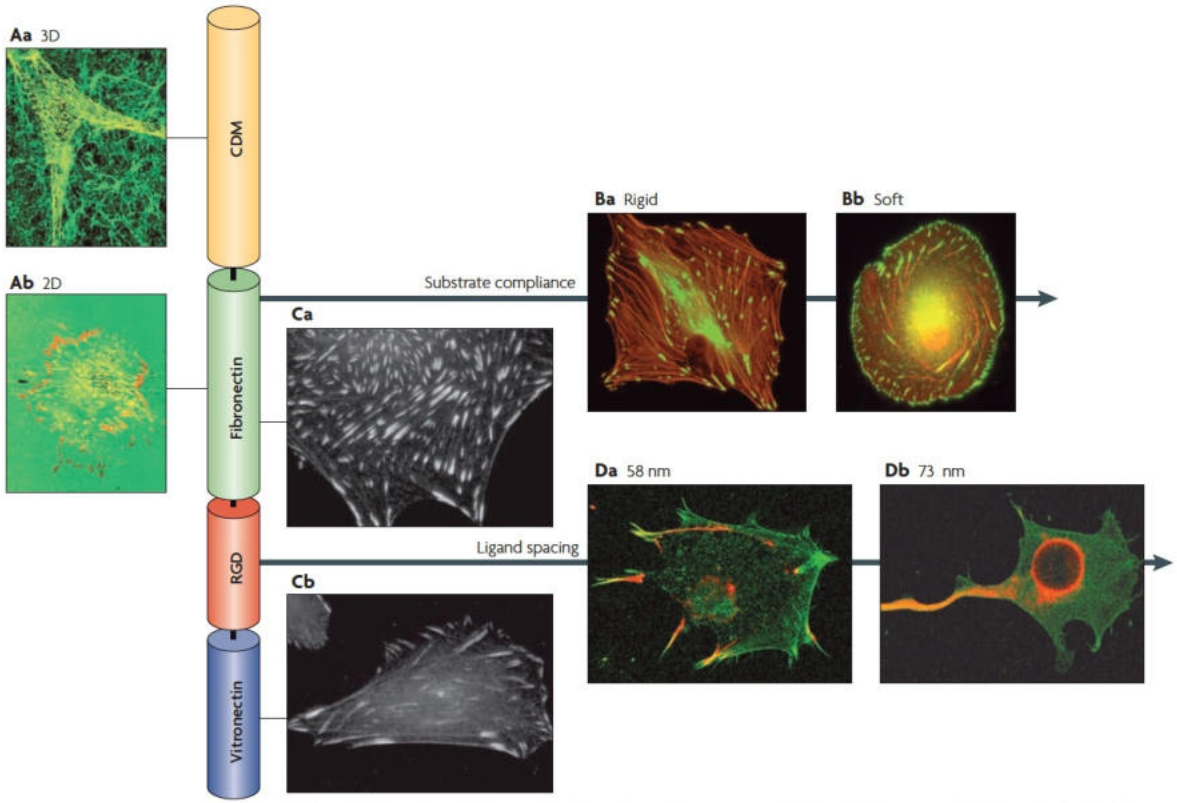
Typical crystal moduli: 100 GPa.

With a typical size of the supramolecular assembly of 10 nm, simple scaling predicts that the typical energy scale for cells is in the range of of 10 kPa x $(10\ nm)^3 = 10^{-20}$ J, which is close to the thermal energy scale $k_BT = 4.1 \times 10^{-21}$ J = 4.1 pN nm (for T = 300 K).



the cohesive interactions that stabilize cells are weak.

These are mainly electrostatic attractions between charges or charge distribution (mainly dipoles) that are screened by water and relatively high salt concentration (100 mM corresponding to a Debye screening length of about 1 nm), hydrogen bridges, hydrophobic interactions due to the special properties of water, and entropic forces such as depletion interactions, all of which operate on an energy scale of a few k_BT

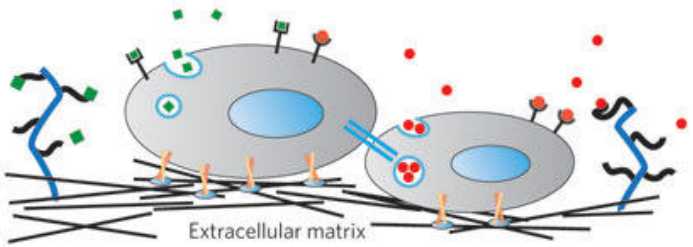


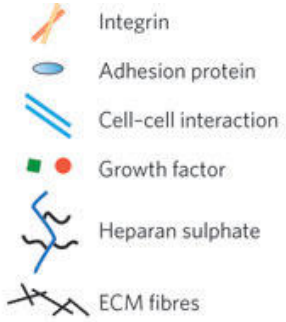
Adhesion is induced only if it is promoted by specific molecular signals that are found on the substrate.

The specificity of cell-matrix adhesion is implemented by transmembrane adhesion receptors (in humans, these are mainly the 24 variants of the integrin family), which bind to complementary ligands of the extracellular matrix (including collagen, fibronectin, vitronectin, and laminin).

The early stages of cell adhesion and spreading can be strongly determined by viscoelastic processes, e.g., the deformation of the rim of the developing contact region. Later stages are more strongly determined by **remodeling of the cytoskeleton and the establishment of localized sites of specific adhesion.**

Extracellular Matrix





The ECM promotes a unique microenvironment that fosters tissue organization.

ECM key factors are:

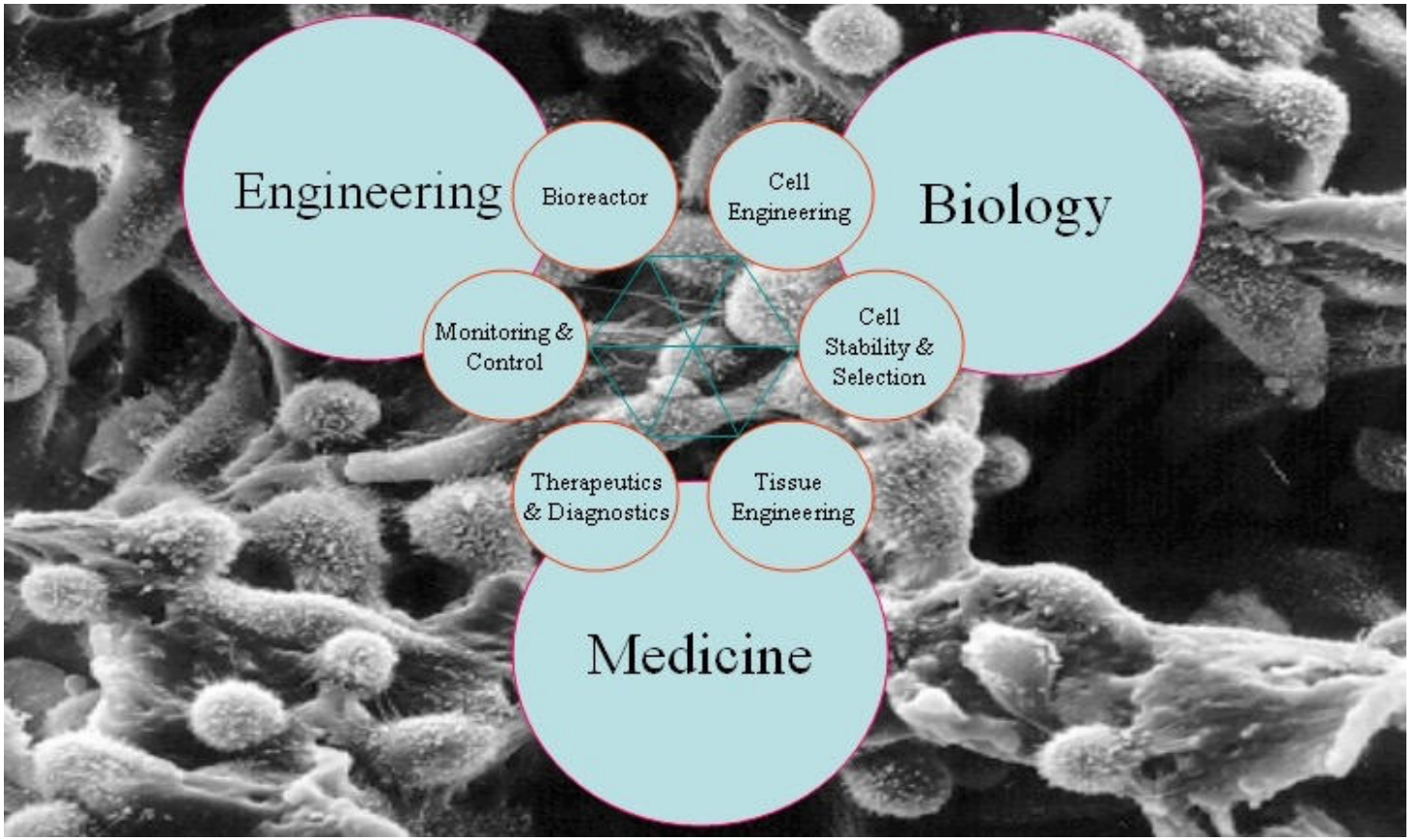
- **ECM Molecules**
- **Growth Factor Concentration**
- **Topography**
- **Mechanical Properties**

- Protein fibres (collagen, elastin)
- Adhesive protein (laminin, fibronectin)
- Polysaccharides (hyaluronic acid, heparan sulphate)
- Cell adhesion (integrin, cadherin)

control the ECM → control the tissue

Dvir, Tal, *et al*. Nanotechnological strategies for engineering complex tissues. Nature Nanotechnology. 2011.

Controlling ECM impacts on different fields…

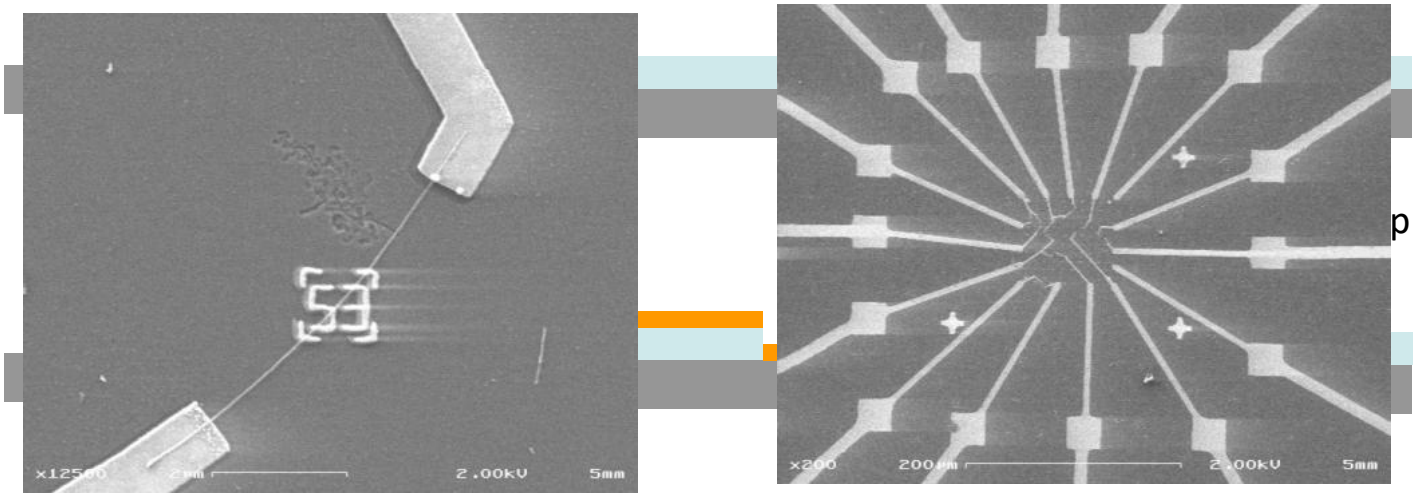


Nanotechnology can offer different ways to modify substrates (for 2D cell cultures) both physically and chemically (patterning)

Physical patterning of the substrate

Micro- and nanofabrication: PHOTOLITHOGRAPHY

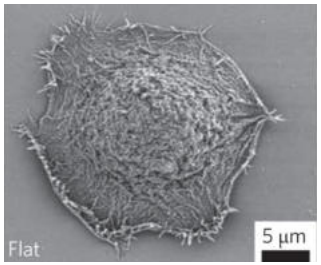
Microfabrication, through its role in microelectronics and optoelectronics, is an indispensable contributor to information technology.



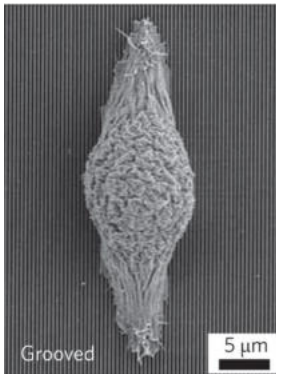
Photolithoghaphy: ~ 1 micrometer (resolution limited by diffraction of light)
E-beam lithography: < 50 nm

Drawback: expensive, time consuming (e-beam), poorly suited for patterning nonplanar surface

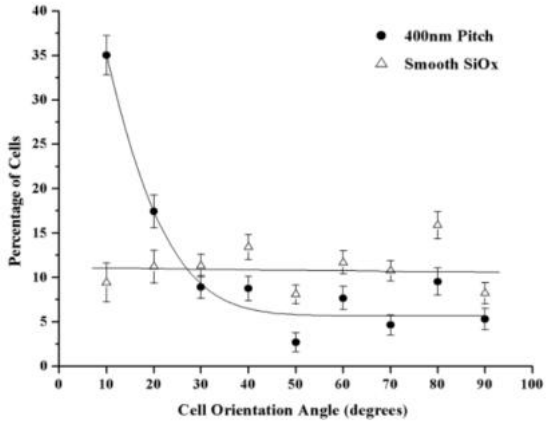
Epithelial contact guidance on well-defined micro- and nanostructured substrates



Flat topography

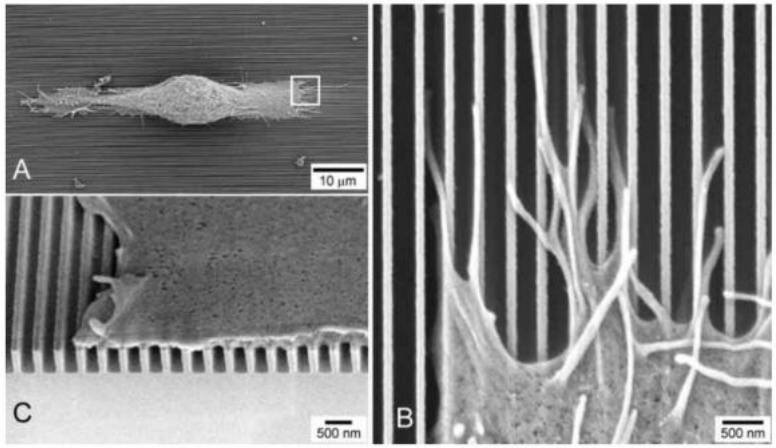


Grooved topography

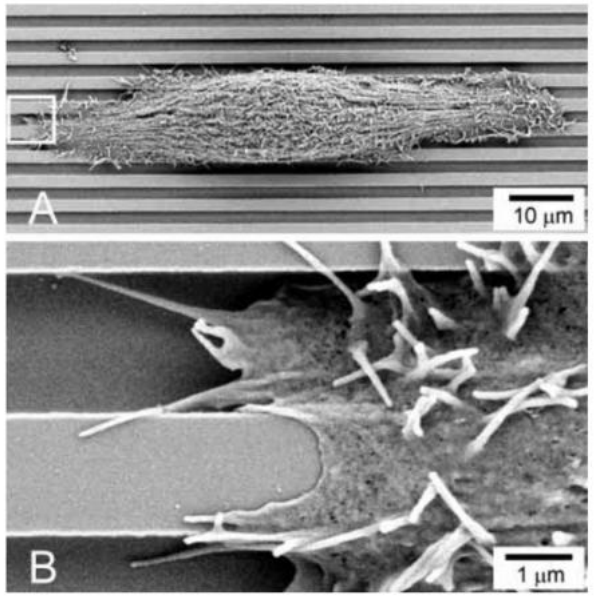


4 μm pitch (1.9 μm ridge 2.1 μm groove) 600 nm depth

400 nm pitch (70 nm ridge 330 nm groove) 600 nm depth

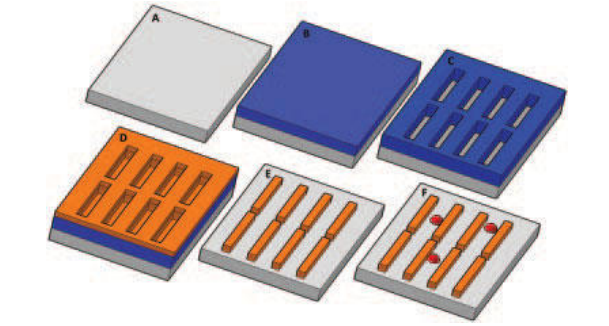


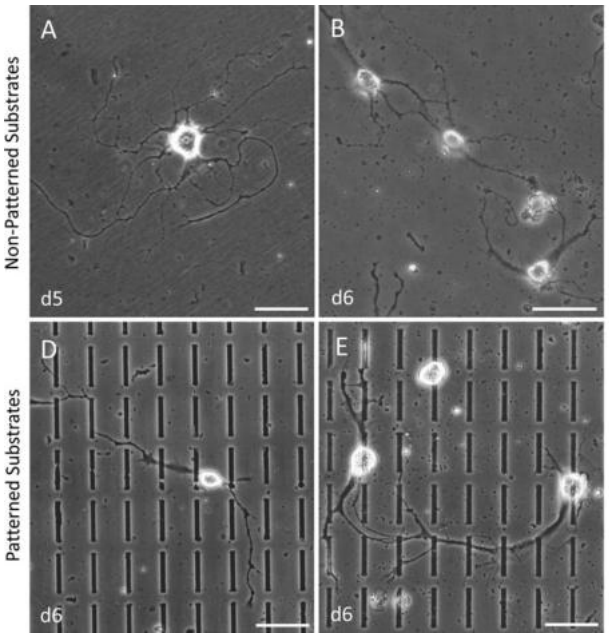
Teixeira, AI. *et al.* J. Cell. Sci. 2003.

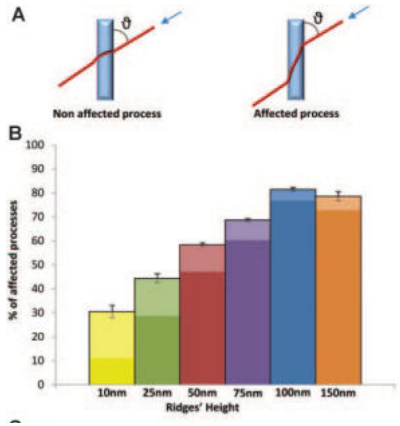


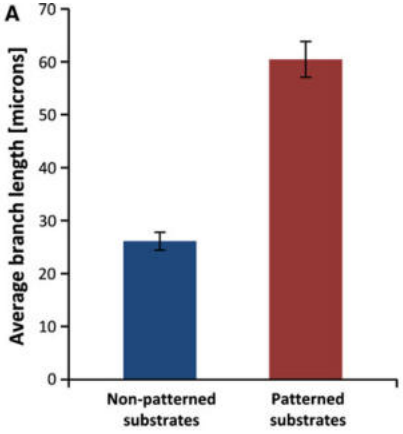
Topographic Cues of Nano-Scale Height Direct Neuronal Growth Pattern

Leech neurons are plated atop substrates with line-pattern ridges

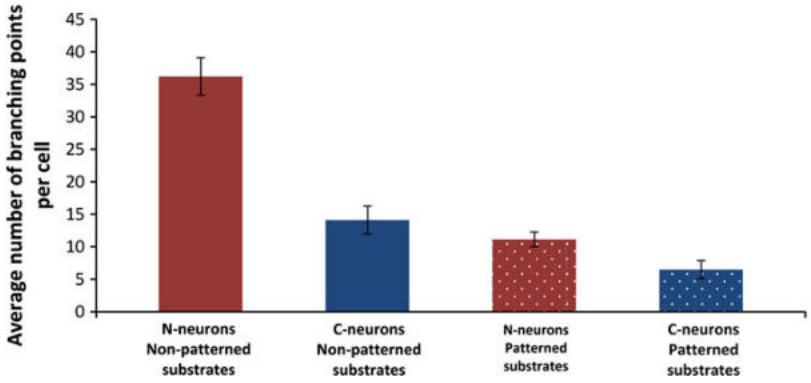








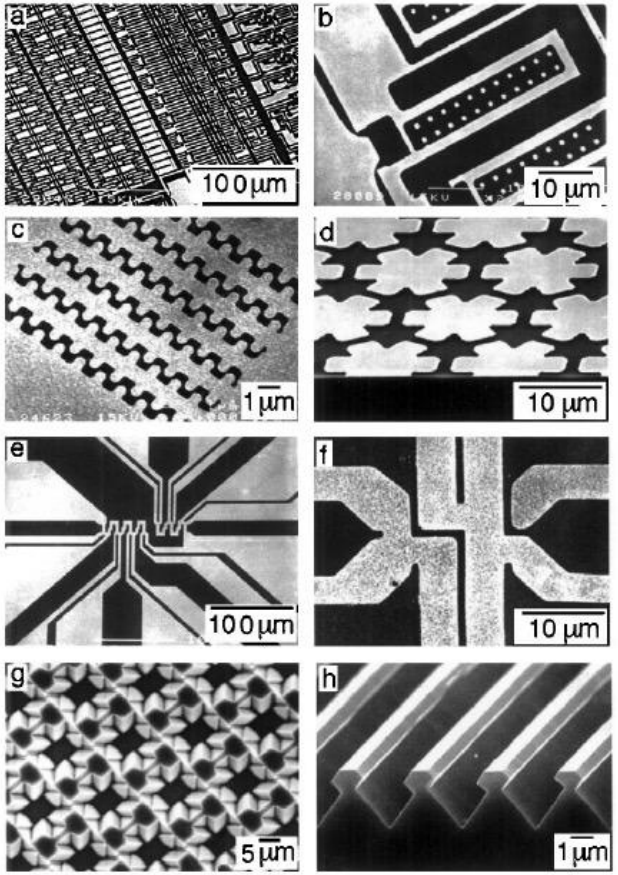
Line patterned substrates affect the typical neuronal tree length and bifurcation frequency



Barades *et al.* Biotech Bioeng 2012; J Mol Hist 2012

Chemical patterning of the substrate

Soft Lithography



Xia, Y. N. and Whitesides, G. M. "Soft Lithography". *Annu. Rev. Mater. Sci.* **28**, 153-184 (1998)

Soft lithography represents a *non-photolithographic* strategy based on self assembly, microcontact printing and replica molding for carrying out micro- and nanofabrication

1. Generate patterns and structures with feature sizes ranging from 30 nm to 100 mm

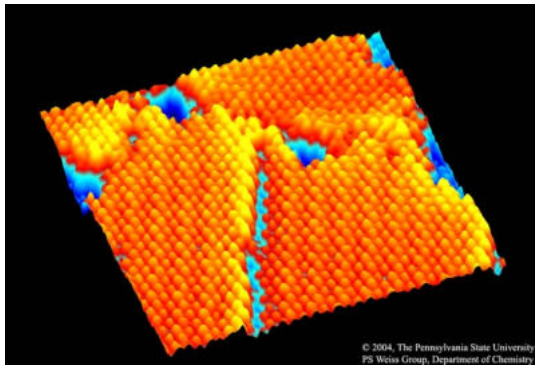
2. Convenient, effective, and low-cost

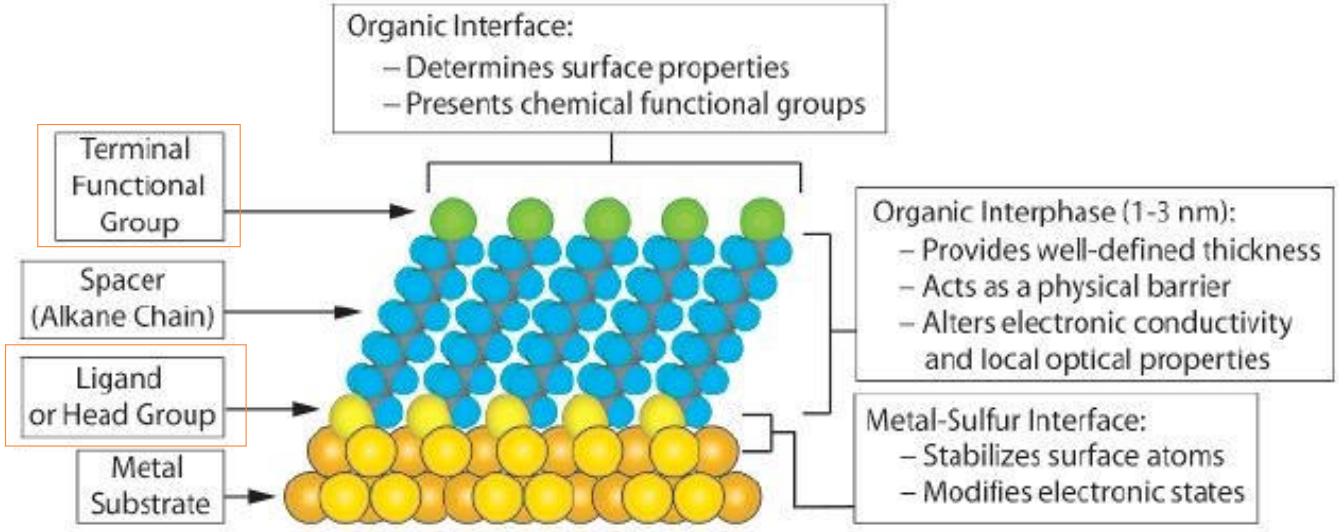
One strategy employed for patterning SAMs on surfaces is physical transfer of the molecular components of a SAM to the substrate in an imposed pattern:

- Microcontact printing (μCP)
- scanning probe lithography

Self Assembled Monolayers

Self-assembled monolayers (SAM) of organic molecules are molecular assemblies formed spontaneously on surfaces by adsorption and are organized into more or less large ordered domains.
Example: Alkanethiols on Gold





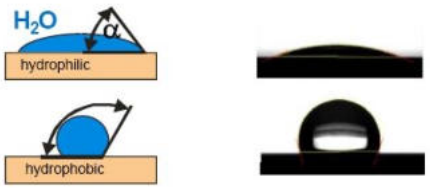
J. C. Love et al., Chem. Rev. **2005**, 105, 1103-1169

SAMs can be used to…

Modify wetting properties (e.g. water)

→ Selective adhesion

→ “Bio-functionalizing”

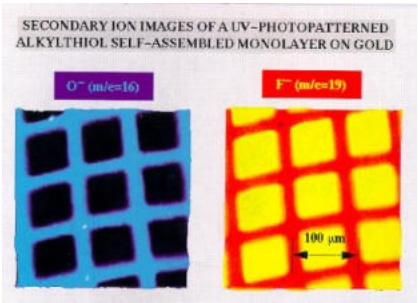


Prepare functional films

→ Corrosion protection

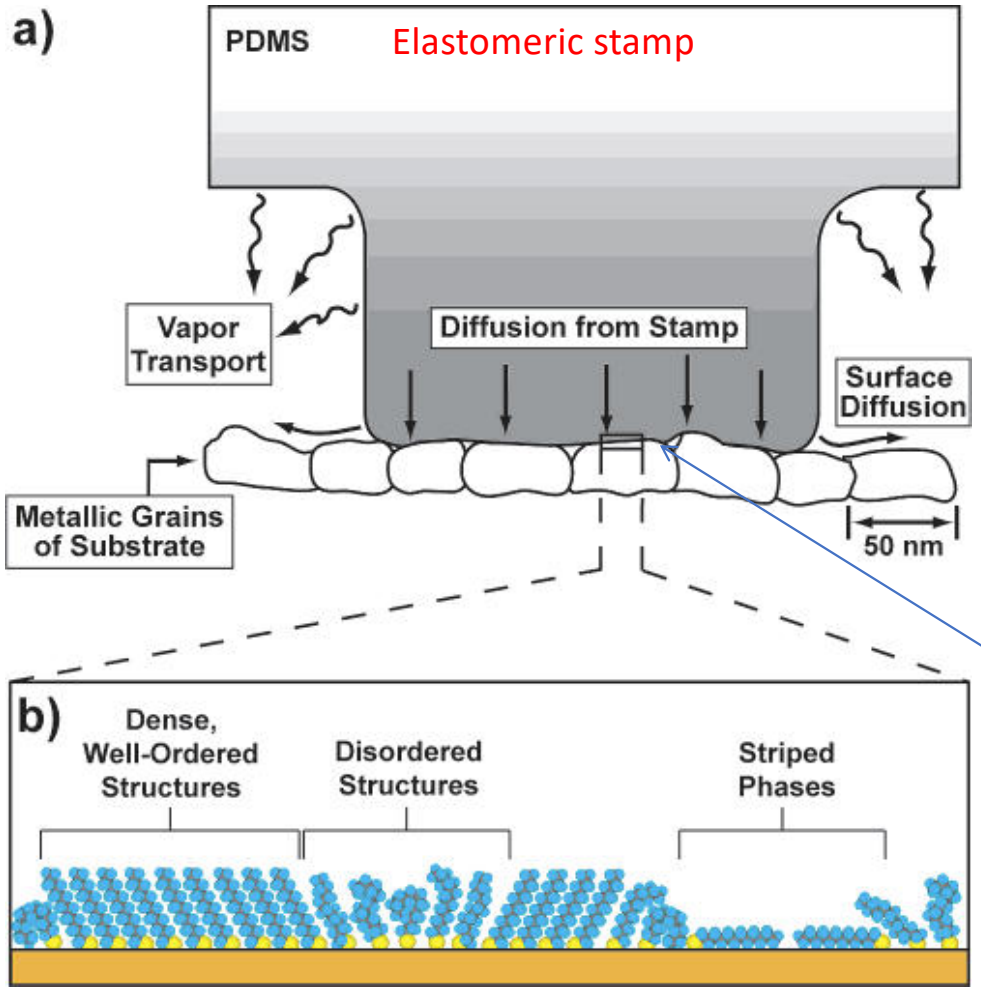
→ Patterning

→ Electronic devices



[1] E. V. Romanova et al., Biomaterials, **2006**, Vol. 27, 1665

Microcontact Printing

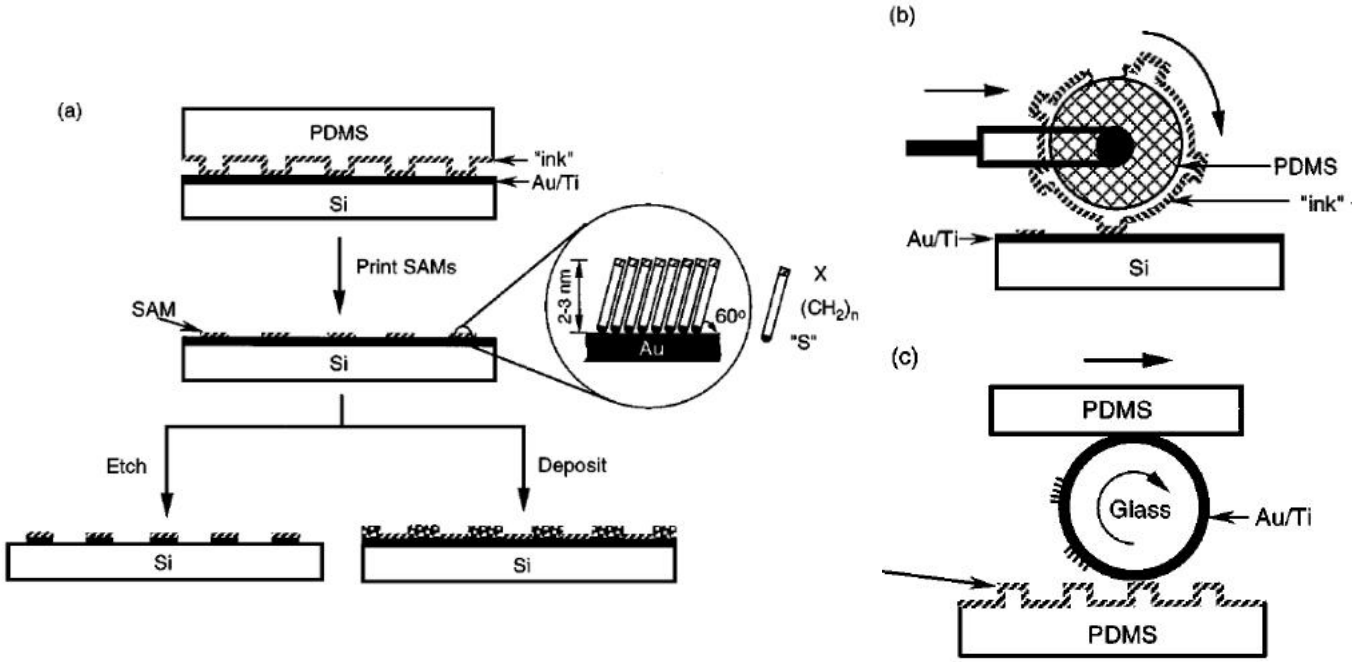


Microcontact printing is a method for patterning SAMs on surfaces that is operationally analogous to printing ink with a rubber stamp on paper

Contact for 5-10 s
Total area: 0.1-100 cm^2
Reachable feature size: 50 nm

Microcontact Printing

The concept of microcontact printing is use the relief pattern on the surface of a PDMS stamp to *form patterns of SAMs* on the surfaces of substrates by contact. For example, alkylthiol on Au and Ag surfaces.



Simple and suitable for non-planar substrates!

Xia, Y. N. and Whitesides, G. M. "Soft Lithography". *Annu. Rev. Mater. Sci.* **28**, 153-184 (1998)

Poly(dimethylsiloxane) (PDMS)

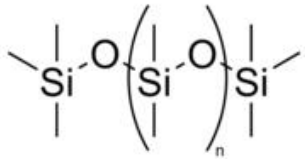
Poly(dimethylsiloxanes):

1.A unique combination of properties resulting from the presence of an inorganic siloxane backbone and organic methyl groups attached to silicon. Biocompatible and transparent!

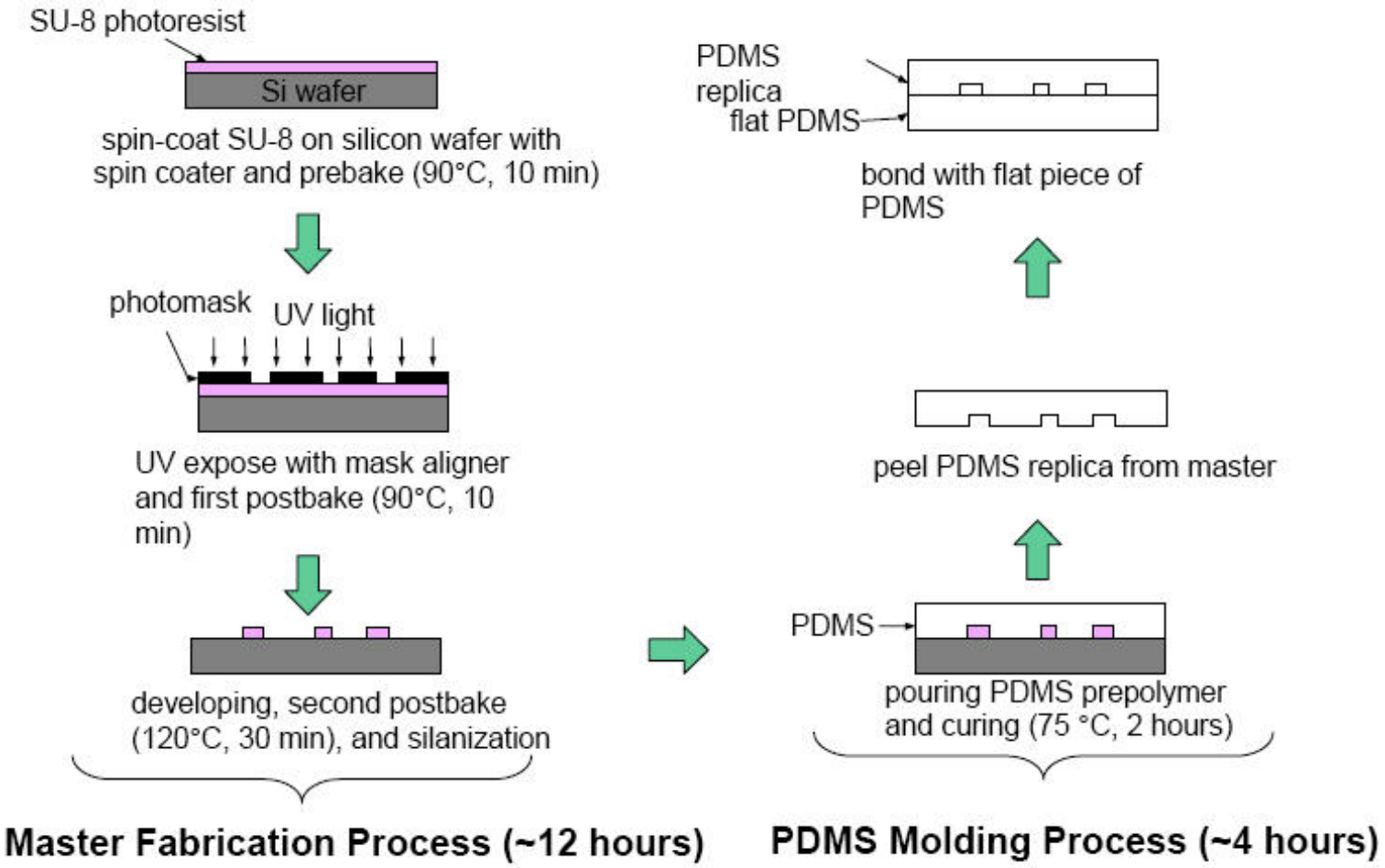
2.Very low glass transition temperatures and hence are fluids at room temperature.

3.Can be readily converted into solid elastomers by cross-linking.

PDMS:

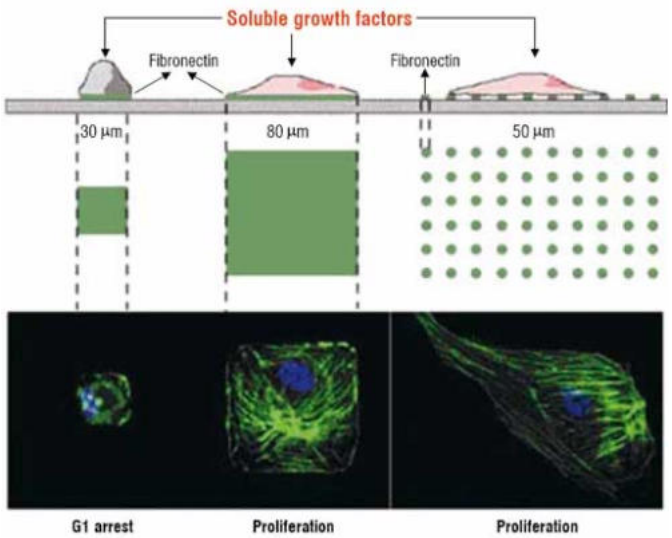


PDMS conventional fabrication

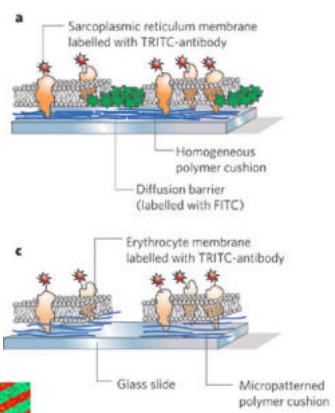


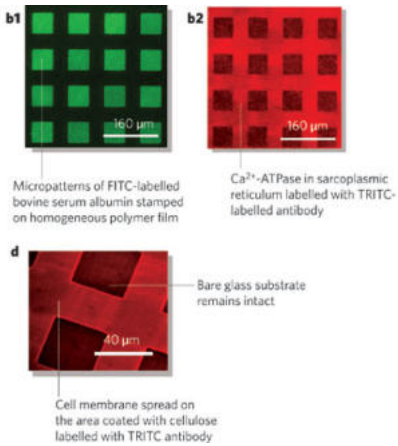
Microcontact Printing:Examples

- Cell growth

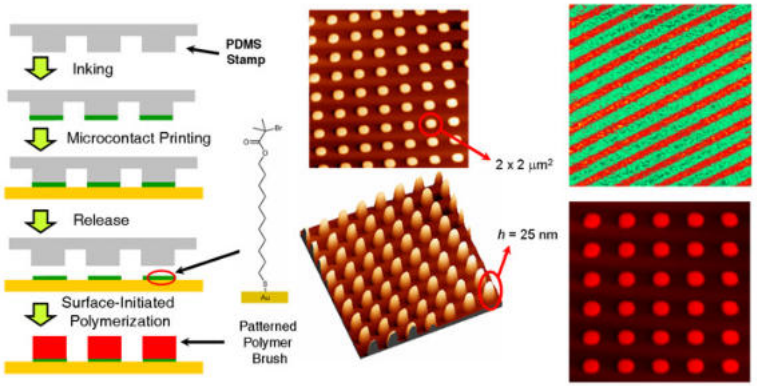


- Supported membrane model



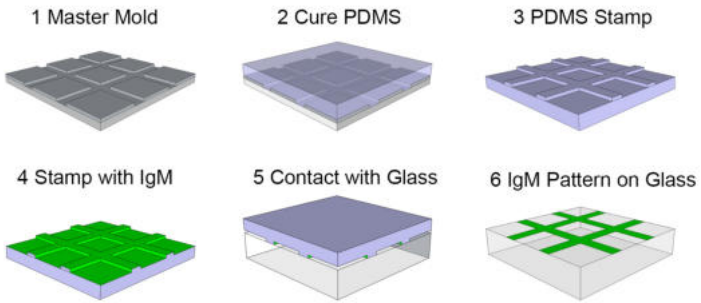


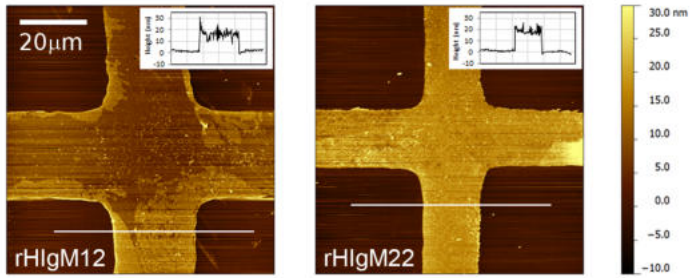
- Nanostructured polymer interfaces



A patterned recombinant human IgM guides neurite outgrowth of CNS neurons

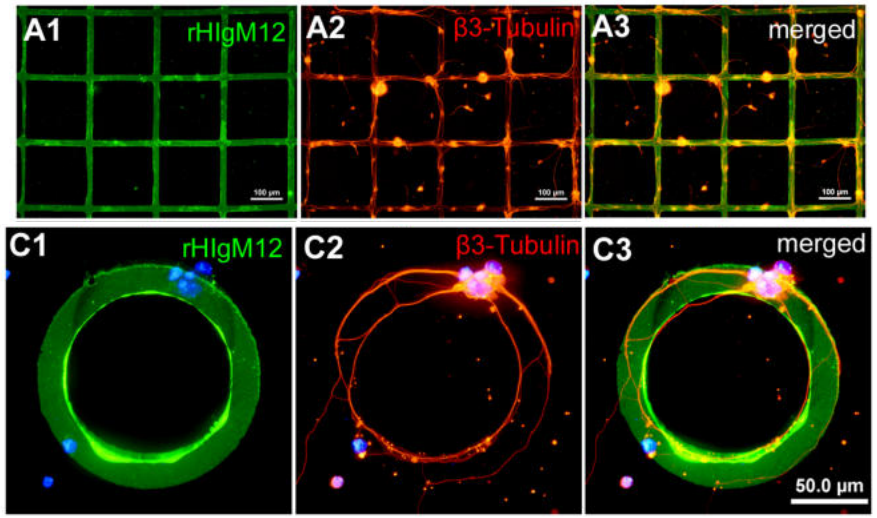
Recombinant human IgMs (rHIgM) bioengineered to have variable regions capable of binding to neurons can guide neurite outgrowth of CNS neurons





The patterned recombinant IgM, maintained the bioactivity to promote neuronal attachment and neurite outgrowth. Processes from spinal neurons followed grid patterns of IgM and formed a physical network.

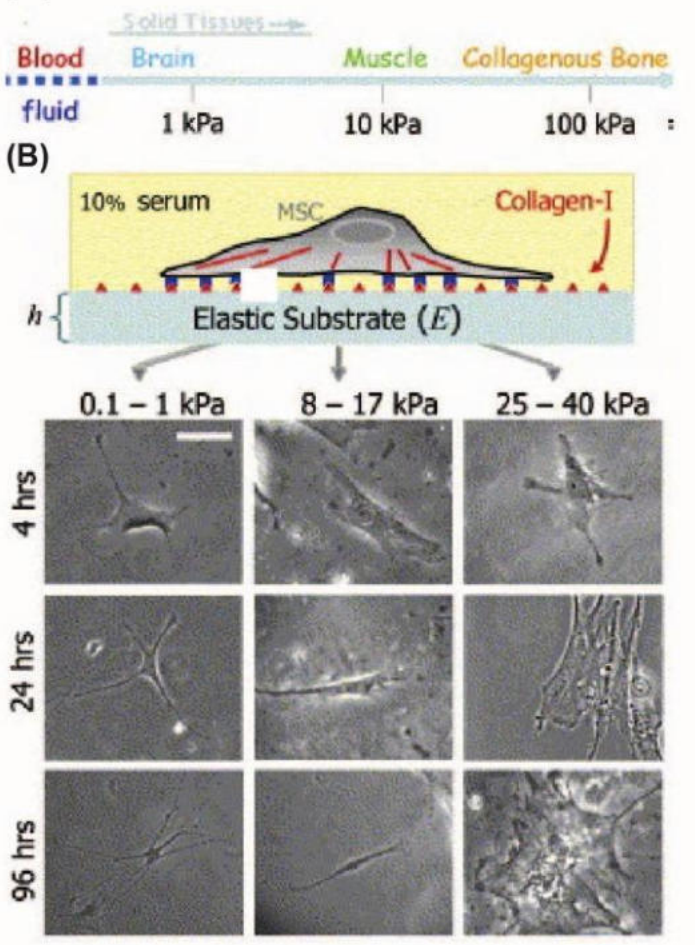
Neurons project neurites in the direction of higher antibody concentration.



Xu *et al.* Sci. Rep. 2013.

Elastic properties of the substrate

Substrate stiffness affects cellular fate



First evidences in the ’80s (Emerman, J.T.; Burwen, S.J.; Pitelka, D.R. Tissue Cell 1979)

hMSCs cultured on a polyacrylamide gel homogeneously coated with collagen I ligands.

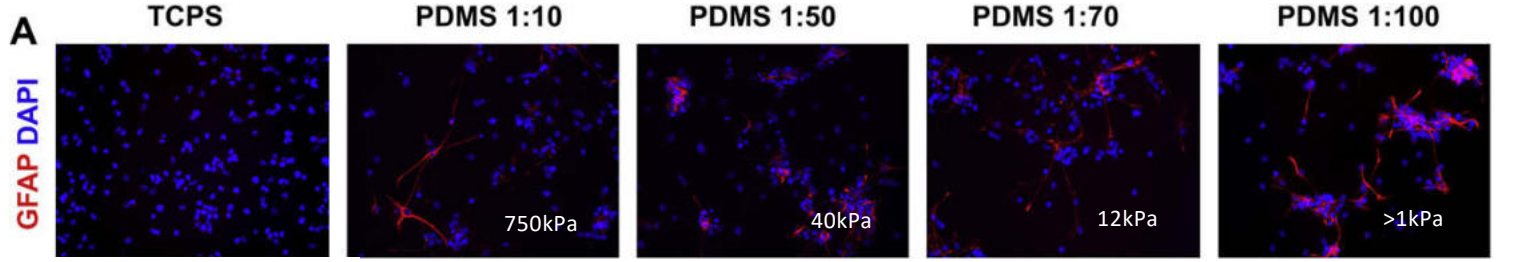
The substrates had variable stiffness representing that of nerve (Young Modulus about 0.1–1 kPa), muscle (8–17 kPa) and bone tissue (25–40 kPa) and it was it was observed that the hMSCs differentiated along the neurogenic, myogenic and osteogenic lineage, respectively

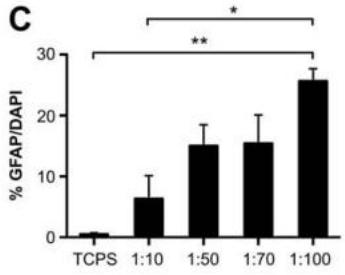
Engler *et al.* Cell 2006

Playing with PDMS elastic properties:

The promotion of neuronal maturation on soft substrates

Embrional NeuralSCs cultured as monolayers on PDMS were able to maintain the stem cell state (when supported with FGF2) and differentiate in response to soluble factors, according to established protocols.

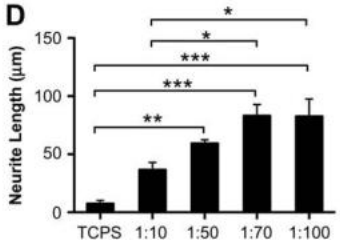


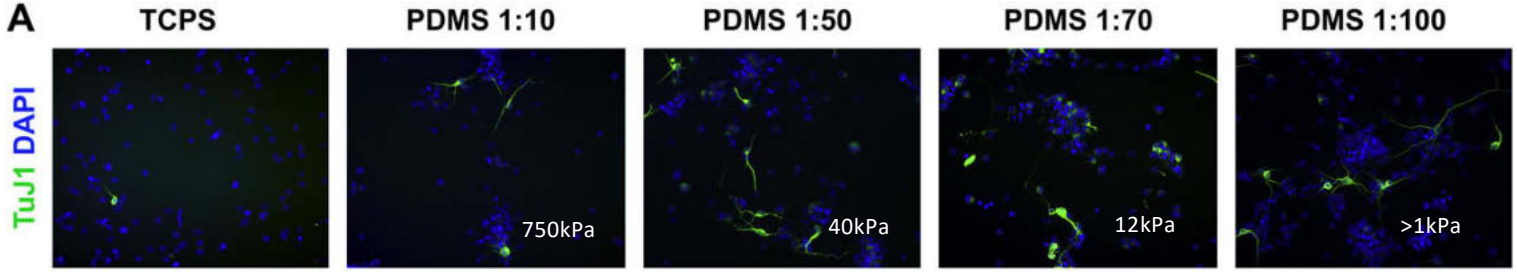


Less crosslinking agent (1:10>>1:100) impacts on rigidity of the PDMS (softer substrate)

Soft substrates promote astrocytic differentiation…

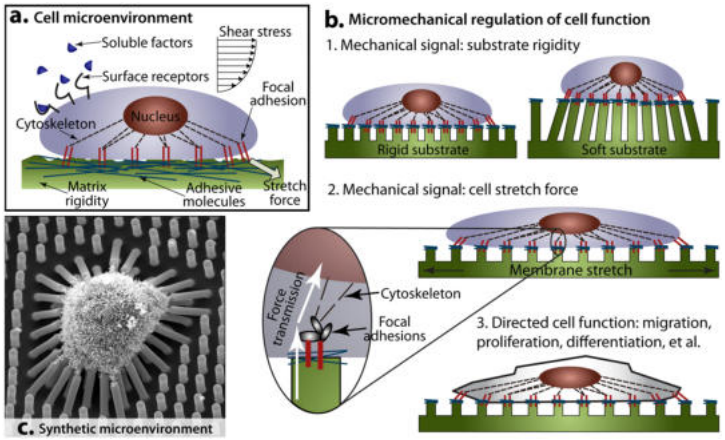
… and neurite length on neurons





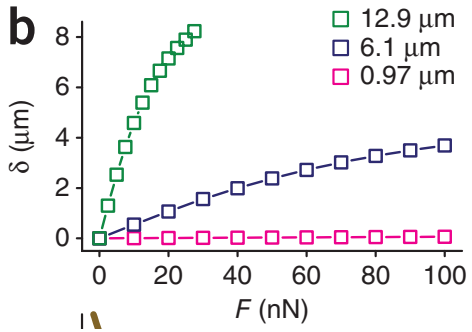
Teixeira AI et al. Biomaterials 2009.

Mechanical regulation of cell function with geometrically modulated elastomeric substrates

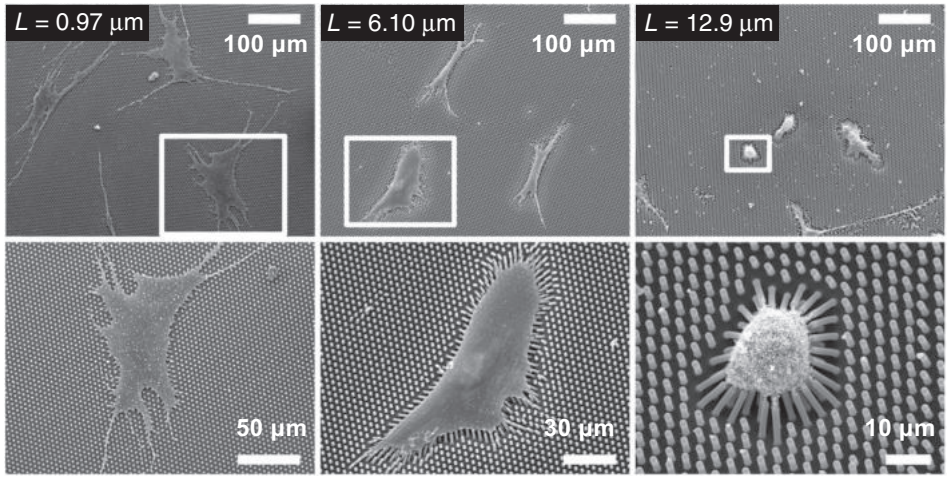


- Human Mesenchimal Stem cells
- growth medium or bipotential differentiation medium supportive of both osteogenic and adipogenic fates
- Micropost rigidity shifted the balance of hMSC fates: osteogenic lineage was favored on rigid micropost arrays whereas adipogenic differentiation was enhanced on soft ones.

Decouple substrate rigidity from adhesive and surface properties

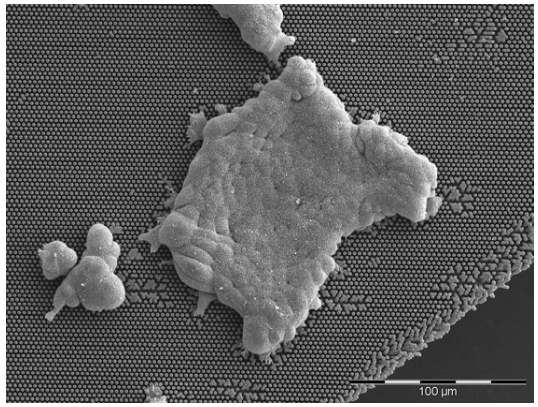


Higher Pillar → Softer substrate



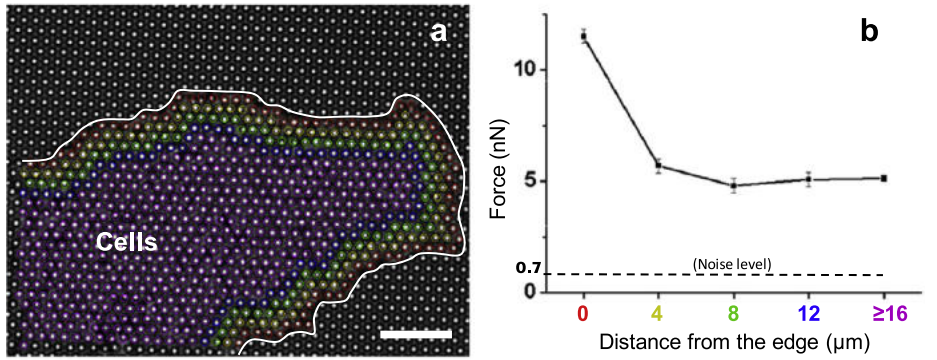
Fu J. *et al.* Nature Methods 2010.

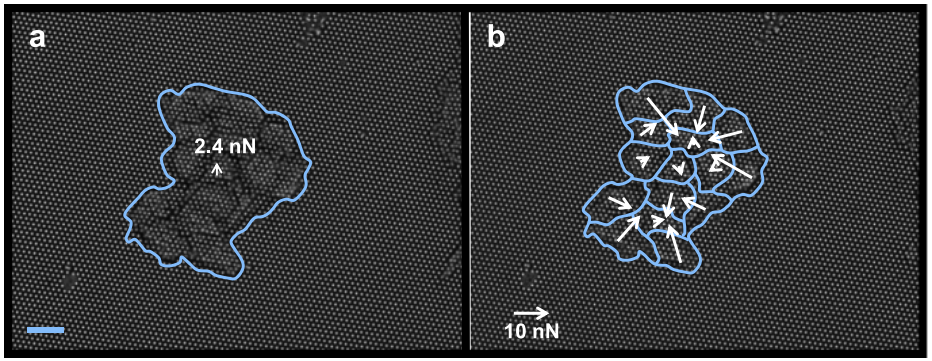
Traction forces exerted by epithelial cell sheets



Epithelial cells act collectively in the transmission of forces within the group and exert large traction forces mostly localized at the periphery

Forces exerted by Madin–Darby Canine Kidney (MDCK) epithelial cells using microfabricated substrates covered by an array of flexible micropillars

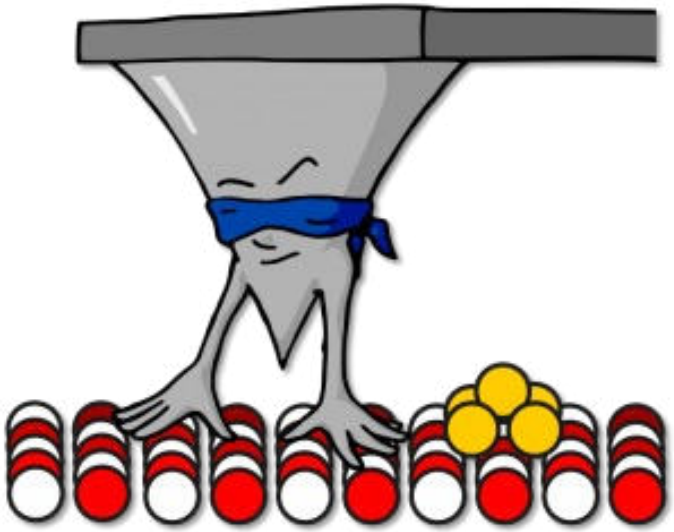




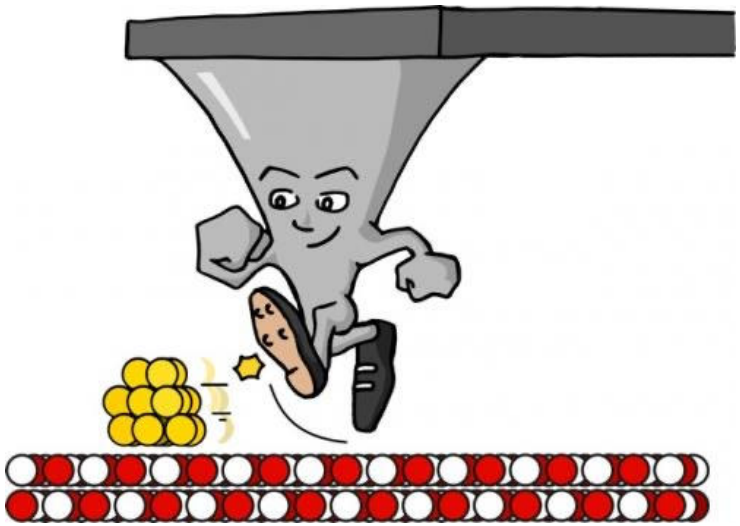
Chemical patterning of the substrate…

…WITH AFM!!

AFM: a Versatile Tool

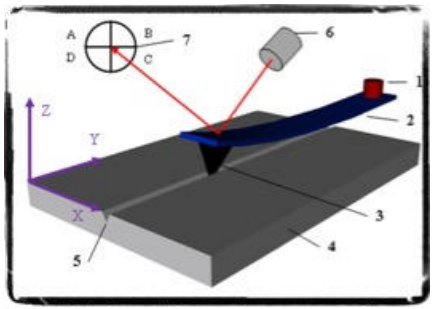


IMAGING

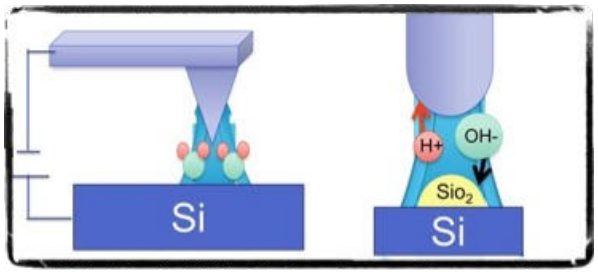


MANIPULATION

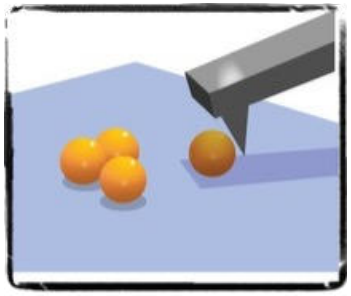
Nanolithography with AFM



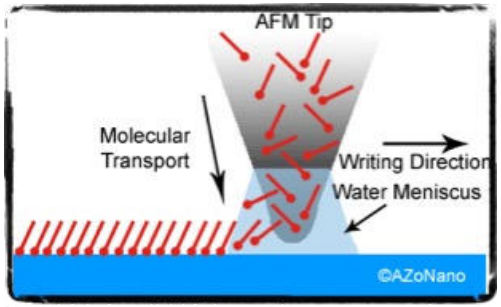
Nanoscratching/nanoshaving



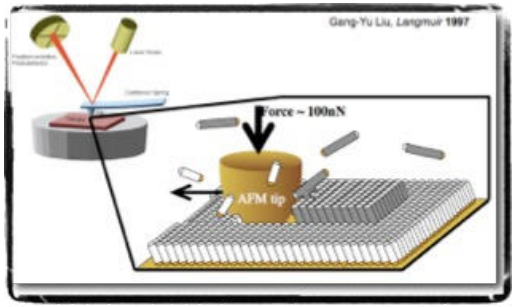
Local Anodic Oxidation



Nanomanipulation



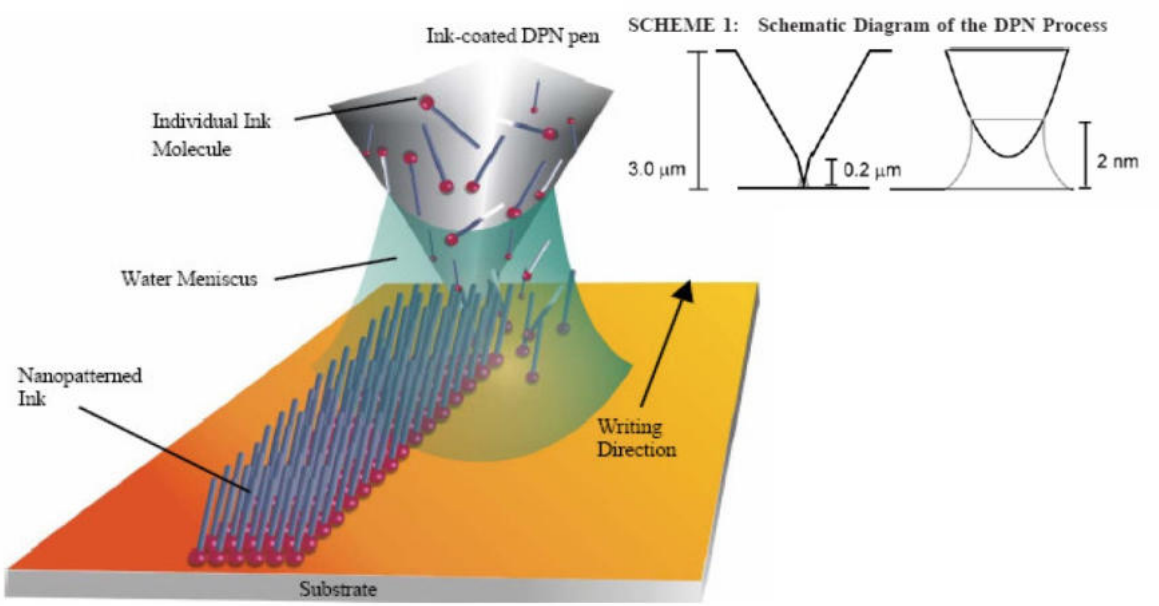
Dip-pen nano lithography



Nanografting

Dip-Pen Nanolithography

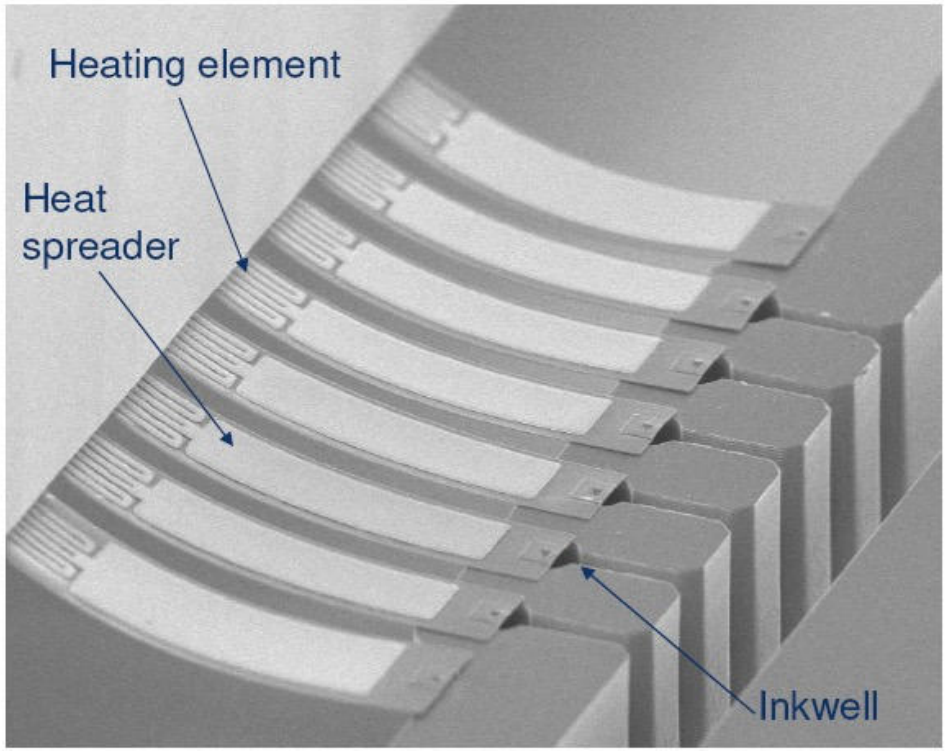
- AFM Tip used to write molecules
- SAMs, DNA, Proteins, etc…
- Serial procedure (need an array of cantilevers for parallel writing)
- Continuous source of molecules – microfluidcs
- Being commercialized by Nanoink. Inc.



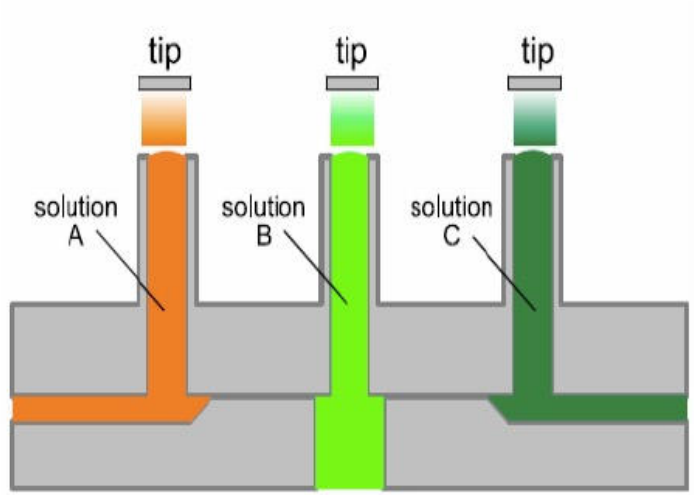
Dip-Pen Nanolithography

Active Pen Arrays

- 8 pen array
- 6 writing pens
- 2 reader probes

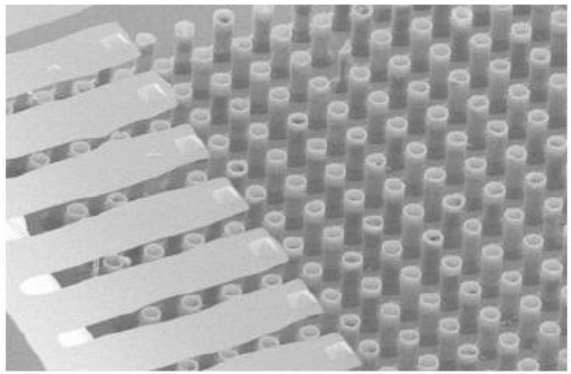


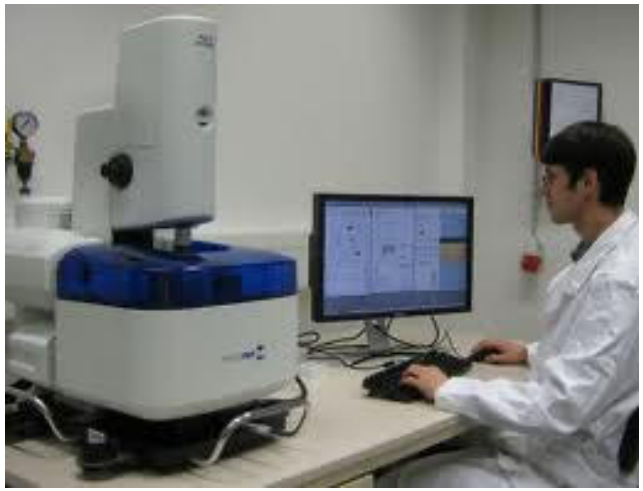
Active Pen Arrays



Ink wells are in registry with a tip array.

Microfluid system delivers chemicals to inking apertures





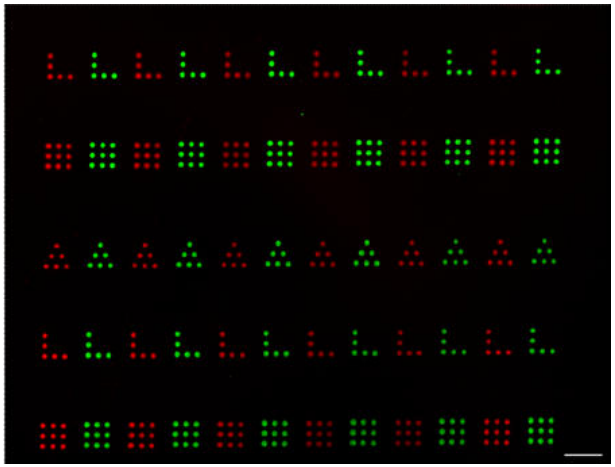


Figure 1. Fluorescent image of a multiplexed pattern of various shapes of laminin (green) and fibronectin (red) created with the NLP 2000 System. Scale bar = 50 µm.

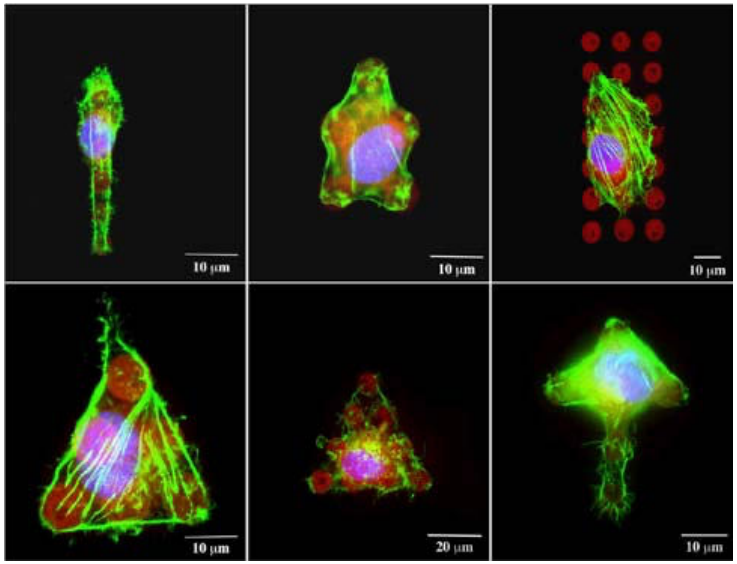
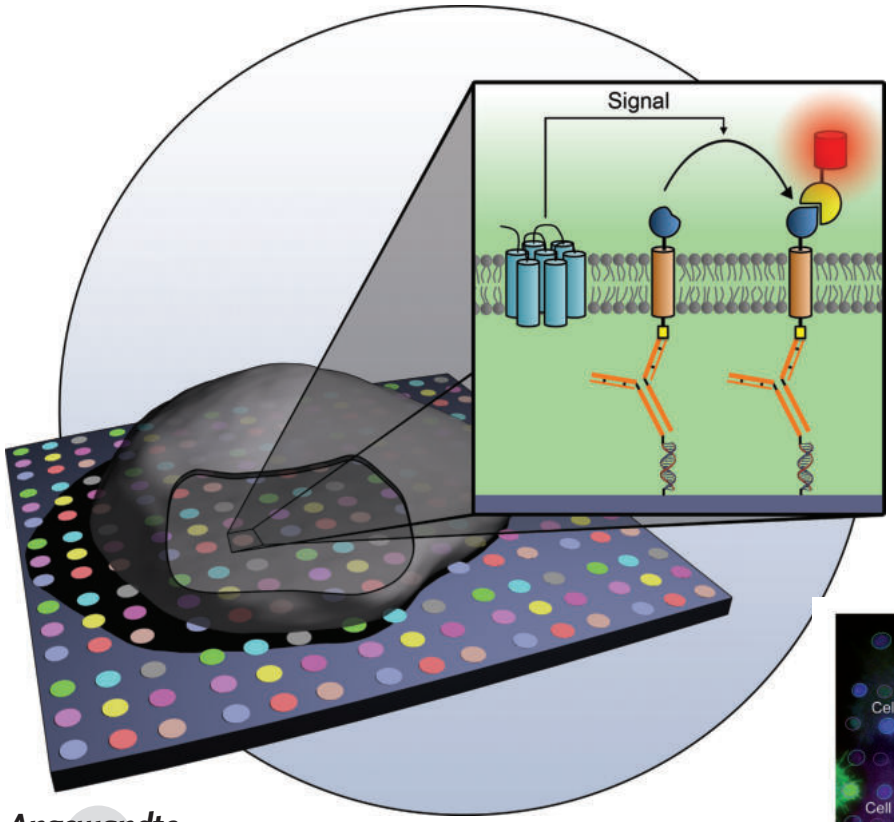


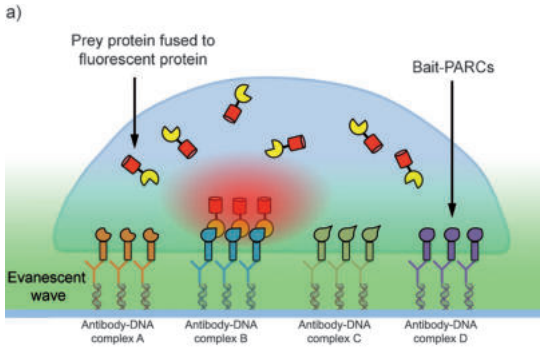
Figure 3. 63x fluorescent images of fibroblasts attached to fibronectin (red) patterned on epoxy-functionalized glass slides, showing actin (green) and nuclei (blue).

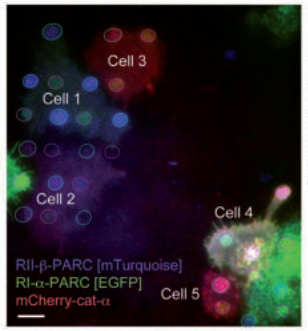
A Protein-Interaction Array Inside a Living Cell**

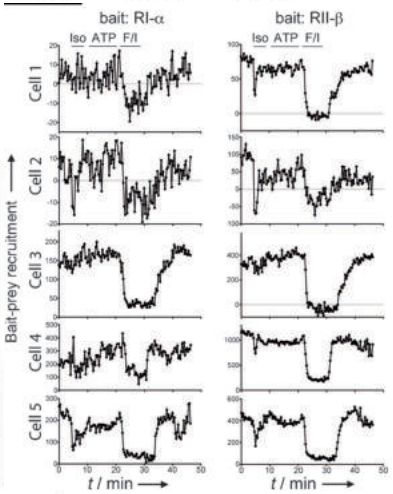
Silke Gandor, Stephanie Reisewitz, Muthukumaran Venkatachalapathy, Giuseppe Arrabito, Martina Reibner, Hendrik Schröder, Katharina Ruf, Christof M. Niemeyer, Philippe I. H. Bastiaens, and Leif Dehmelt**

A microstructuring approach allowed for analysis of the interaction of one naturally occurring receptor type with one of its interaction partners inside cells.



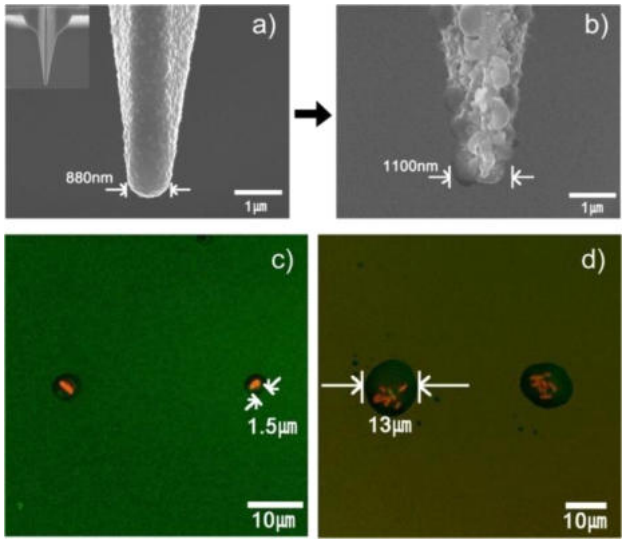


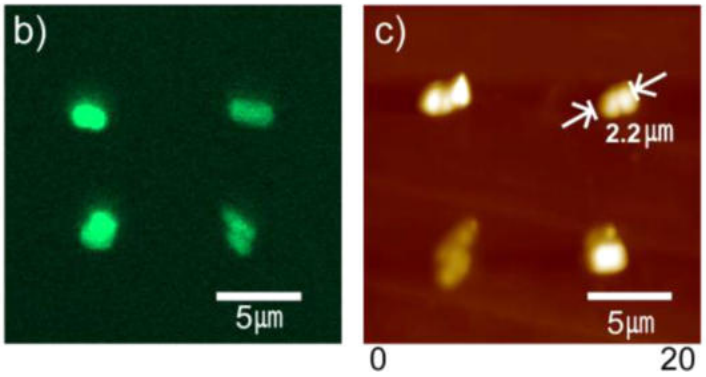


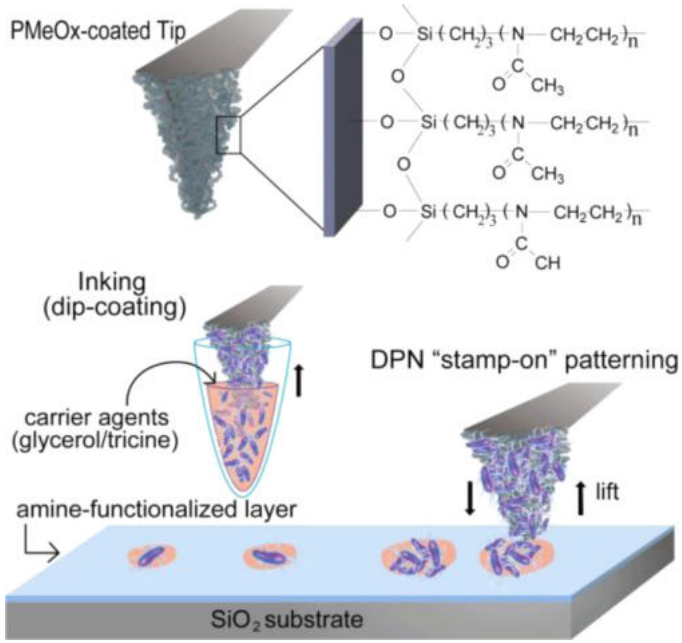


Angewandte International Edition Chemie

Direct-Write Patterning of Bacterial Cells by Dip-Pen Nanolithography

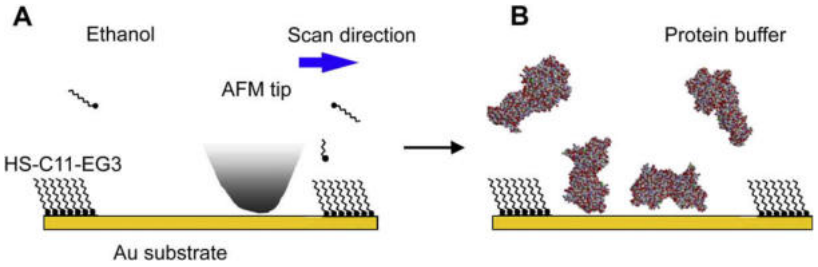


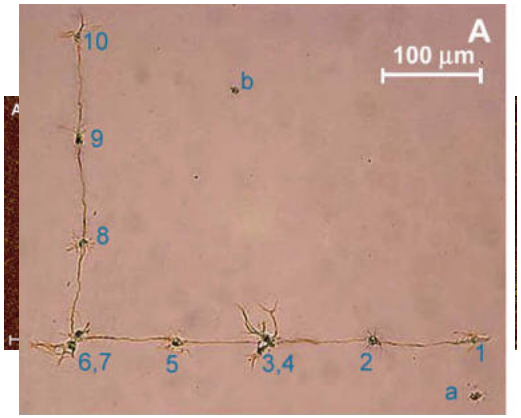




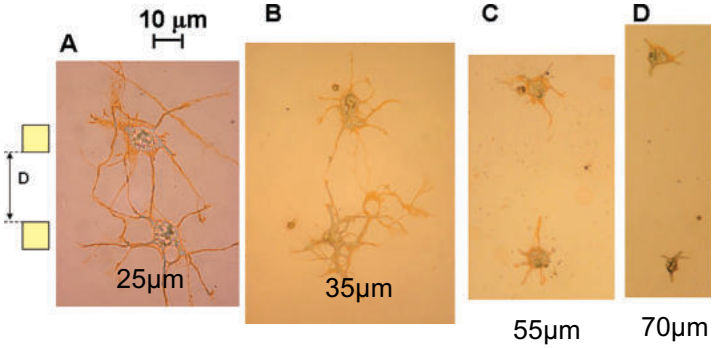
Playing with the solvent (wetting properties), the force and the time of contact are able to tune the number of bacteria on the array spot

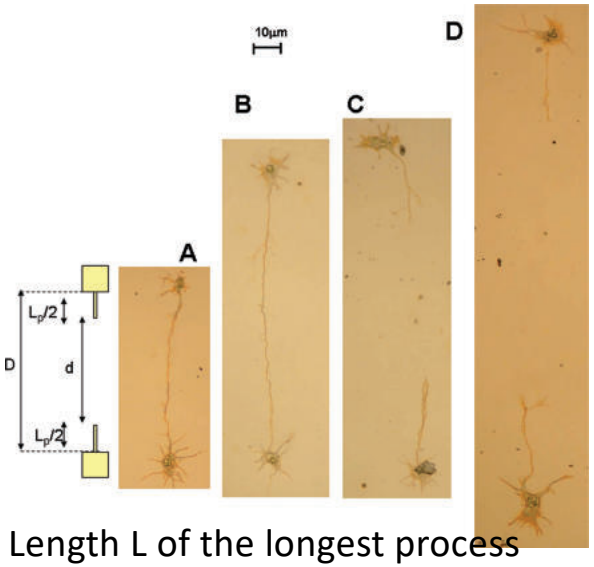
Nanoshaving

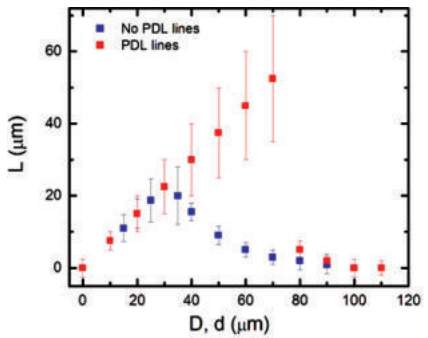


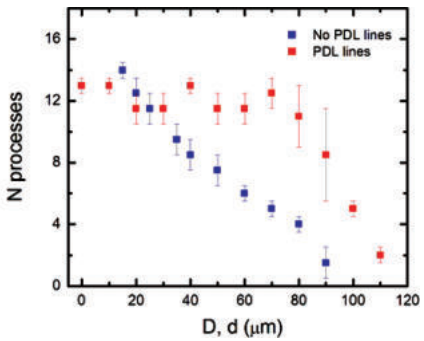


Laminin or Poly-D-Lysine





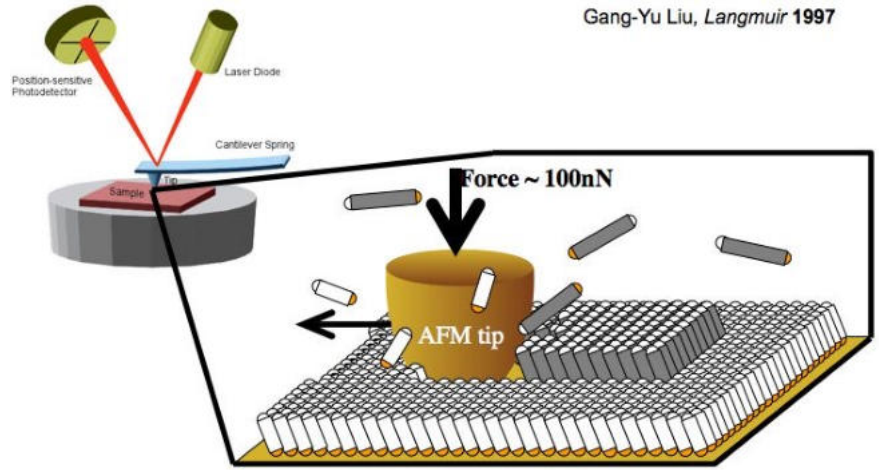




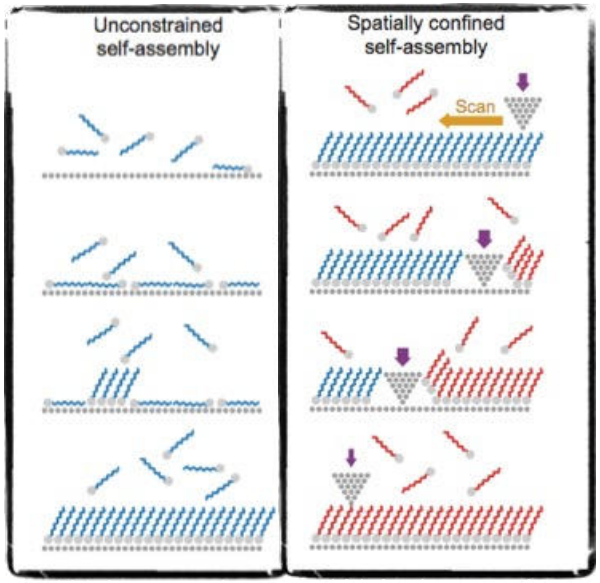
Length L of the longest process grown over the inhibitory PEG substrate vs separation distance for two types of patterns

Staii C. *et al.* Biomaterials; Lagmuir 2009

Nanografting

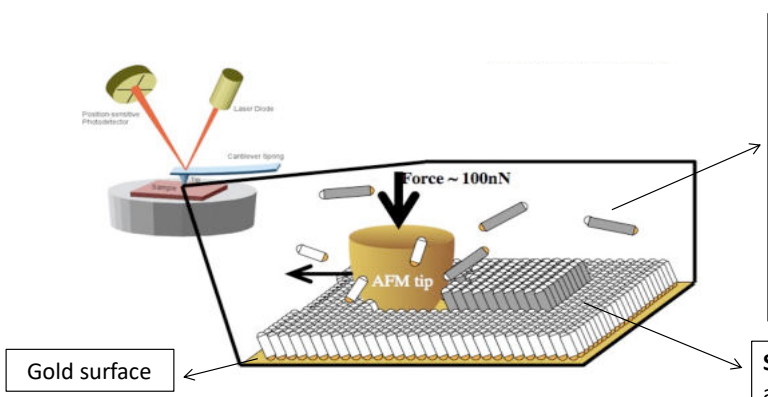


Gang-Yu Liu, *Langmuir* **1997**

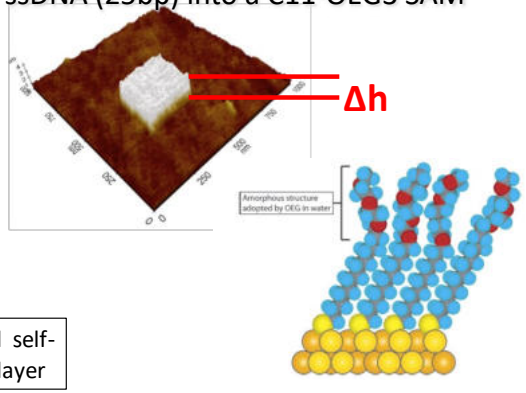


- AFM Tip used to graft molecules within molecule monolayers
- SAMs, DNA, Proteins, etc…
- Serial procedure (need an array of cantilevers for parallel writing)
- Faster kinetics with respect to SAM counterpart
- Higher order
- Nanometer size
- Play with densities (concentration of the grafting solution, etc…)
- Multiplexing

DNA Nanografting



ssDNA (25bp) into a C11-OEG3 SAM

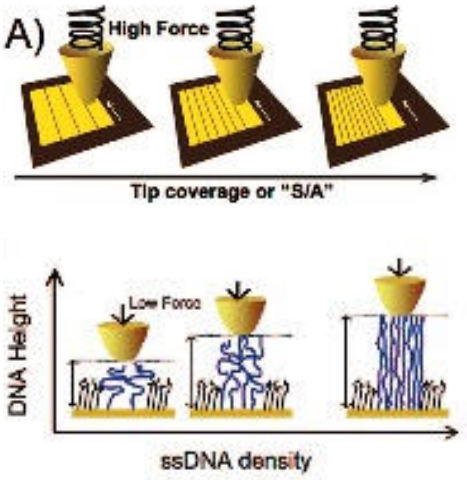


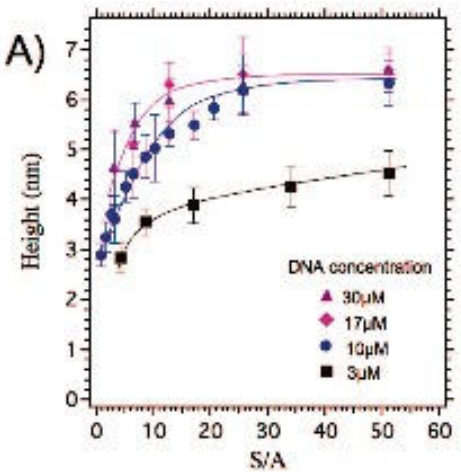
GRAFTING

(Load ~ 100 nN)

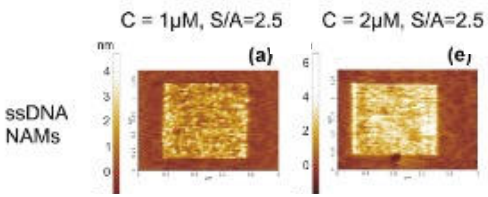
IMAGING

(Load < 1 nN)



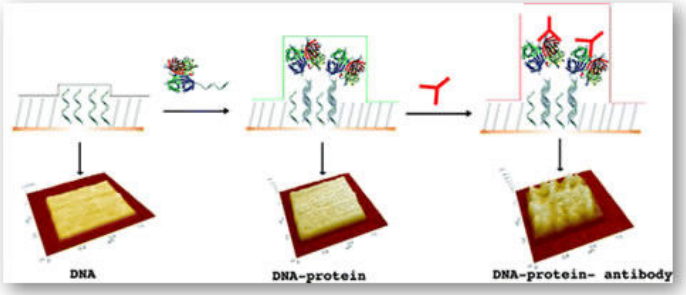


ssDNA, Low *S/A*
[DNA]=1-2μM
TE buffer 1M NaCl



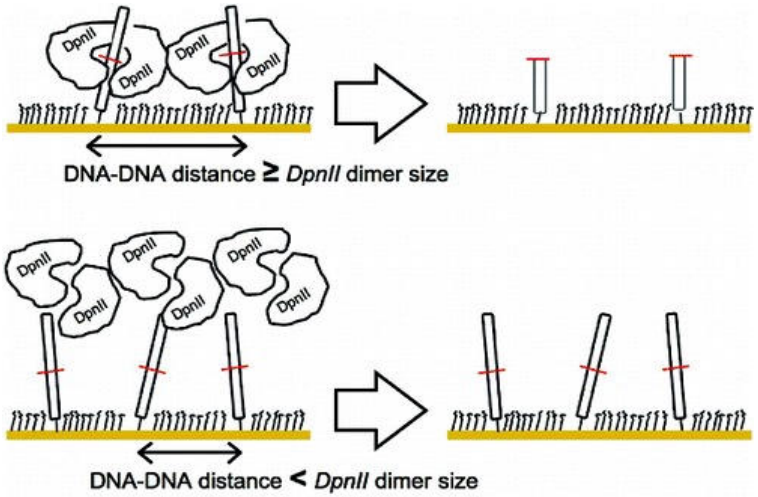
Mirmomtaz E. et al. Nano Letters, **8** 4134 (2008)

Protein/Antibody nanodevices



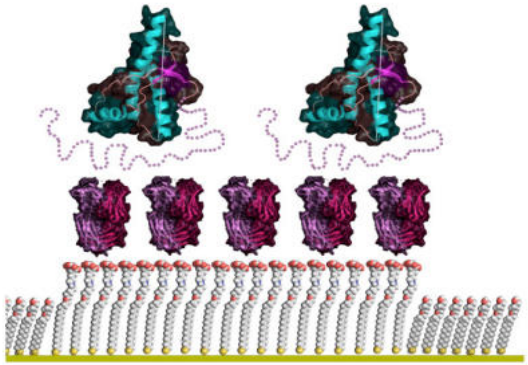
Bano et al. *Nano Lett.* , **9**, 2614 (2009)

Spatially confined biochemical reactions



Castronovo et al., *Nano Lett.* **8**, 4140 (2008)

Artificial confinement of proteins in a selective and oriented manner

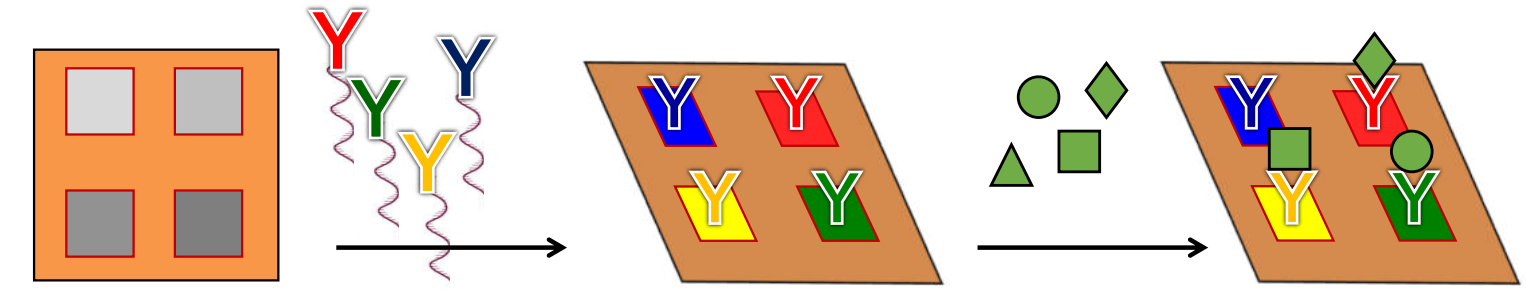


Sanavio *PhD Thesis* (2010)

Nanostructures in Biodiagnostics

Rosi and Mirkin, *Chem. Rev.*, **105**, 1547 (2005)
Niemeyer, *Angew. Chem. Int. Ed.*, **49**, 1200 (2010)

PROTEIN/ANTIBODY NANOARRAY



Grafting of different oligo sequences

Immobilization of different antibodies

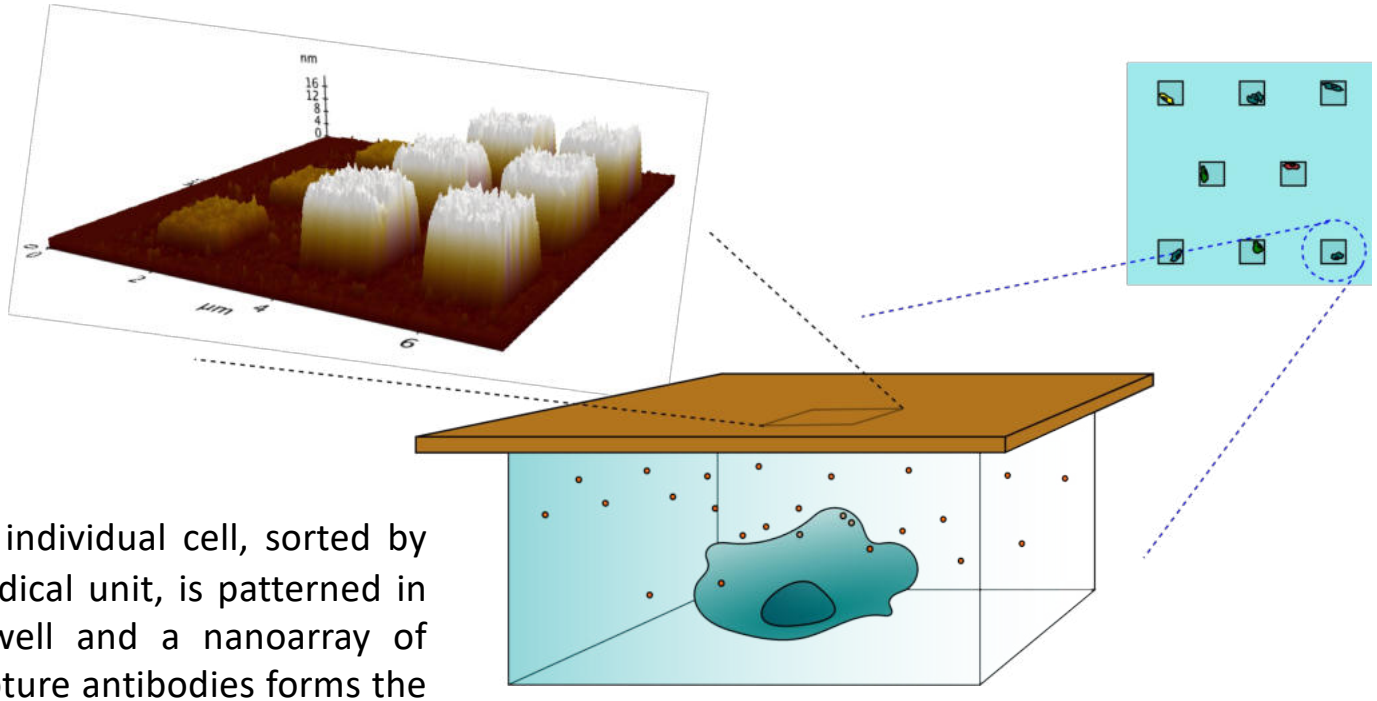
Detection of different cancer biomarkers

PROs - different Abs on the same surface

→ Multiplexing protein nanoarray

- small volume detection system

NANODEVICE FOR THE ANALYSIS OF THE PROTEIN CONTENT OF ONE OR FEW CELL

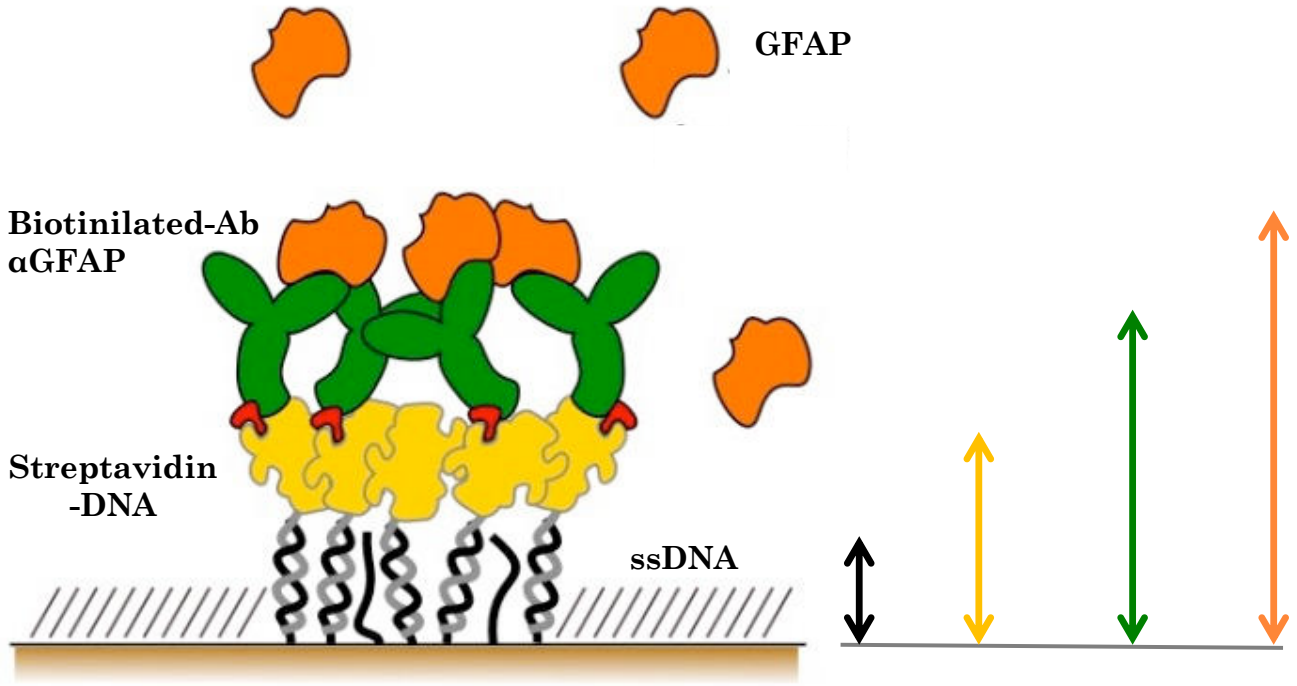


An individual cell, sorted by medical unit, is patterned in a well and a nanoarray of capture antibodies forms the roof of the well.

Bosco Ganau *et al.* Nanomedicine 2014

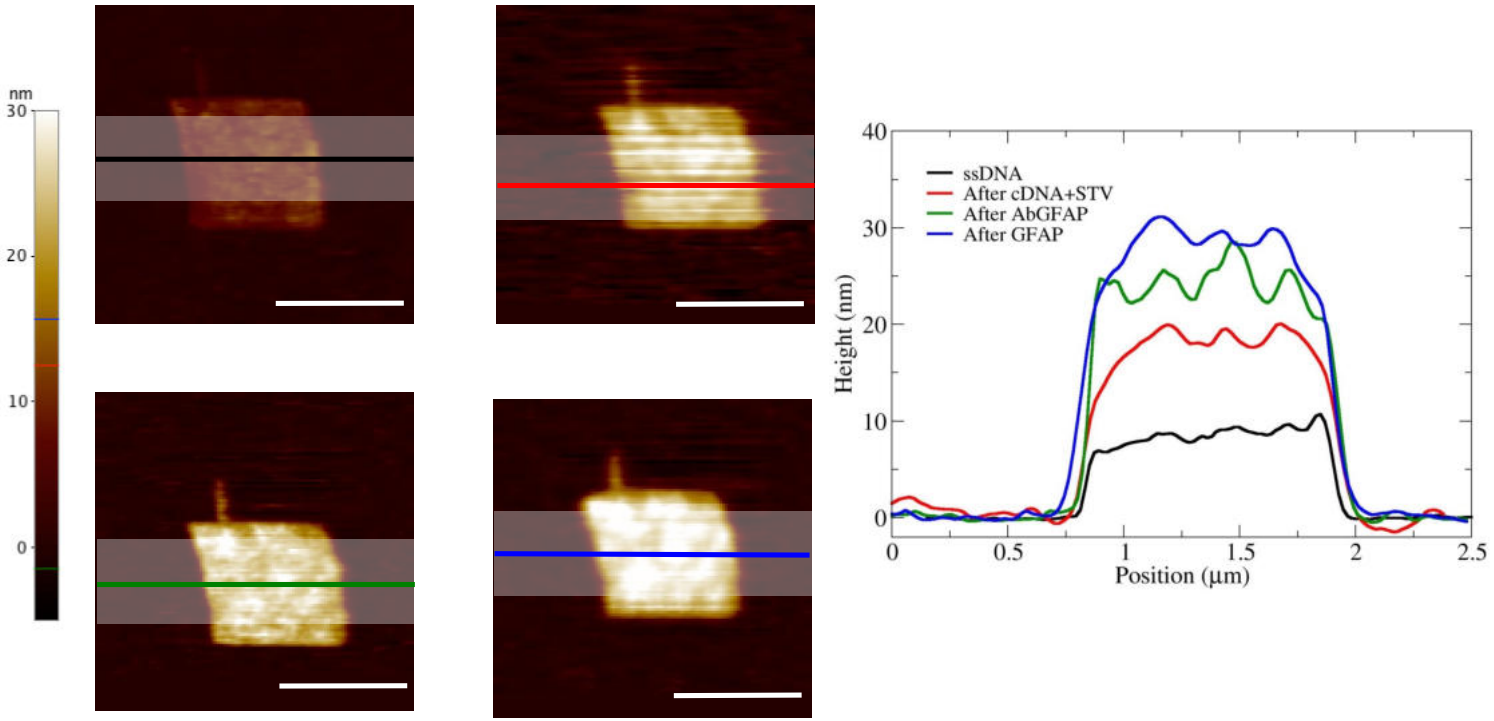
LABEL FREE NANO-IMMUNO ASSAY FOR GLIOMA BIOMARKER DETECTION

Glial Fibrillary Acidic Protein (**GFAP**) represents a marker for astrocytic differentiation; it belongs to a group of 100 proteins that show alterations in tumor



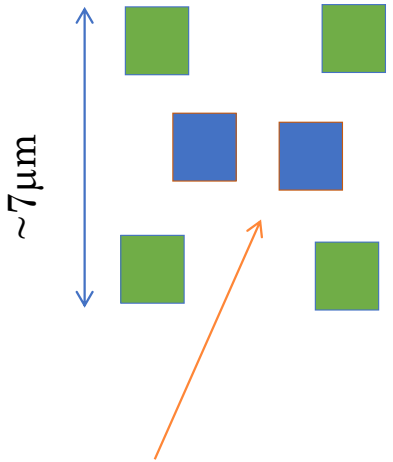
NANO-IMMUNO ASSAY FOR GLIOMA BIOMARKER DETECTION

Analysis of AFM topographic images of GFAP detection by nanopatches

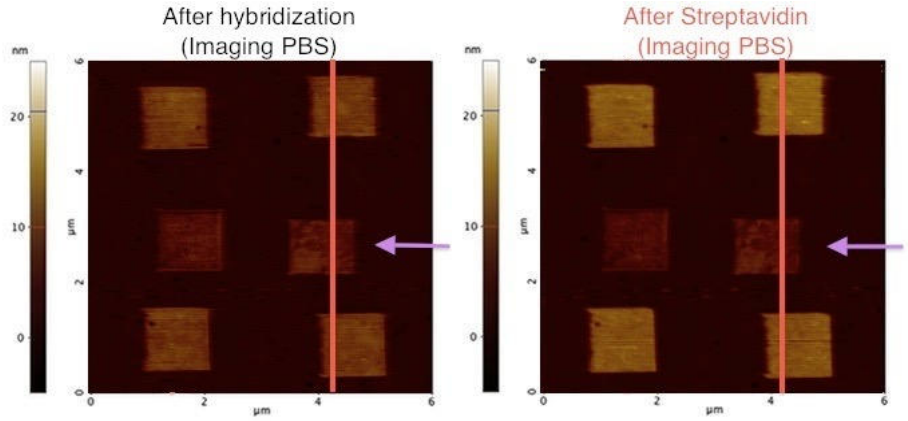


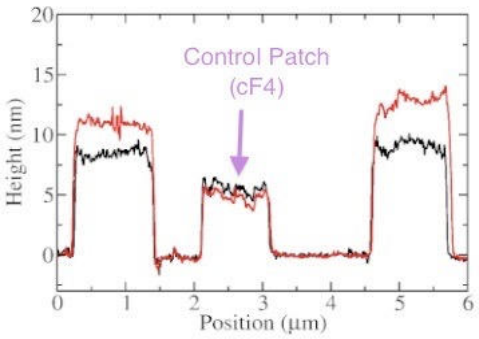
ELISA-like protocol for immobilization
(with Blocking for preventing aspecific binding)

TOWARDS A MORE ACCURATE NANOBIOASSAY: CONTROL PATCHES



Control patches



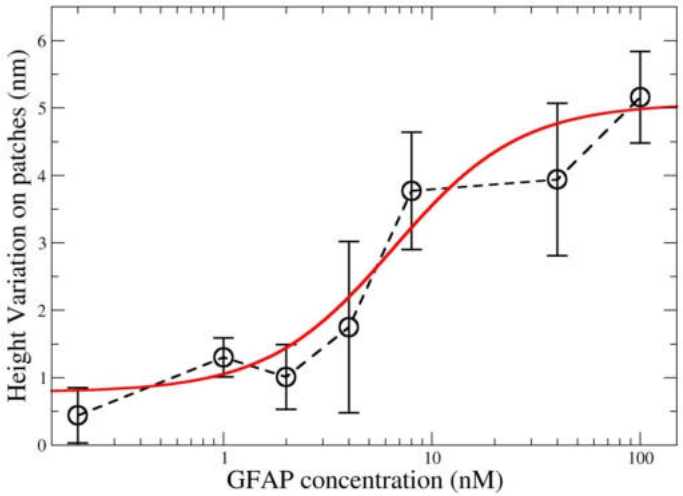


Line Profiles

NANO-IMMUNO ASSAY FOR GLIOMA BIOMARKER DETECTION

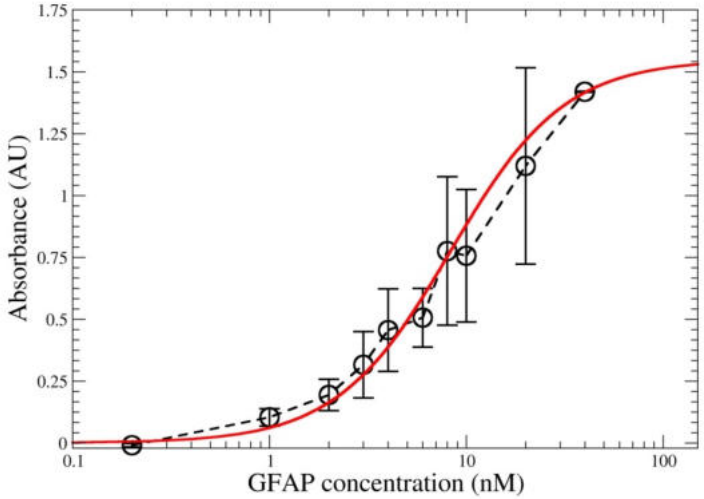
Nanoarray vs ELISA

NANOARRAY



K_D= 6.6±3.8 nM

ELISA



K_D= 8.30±1.04 nM

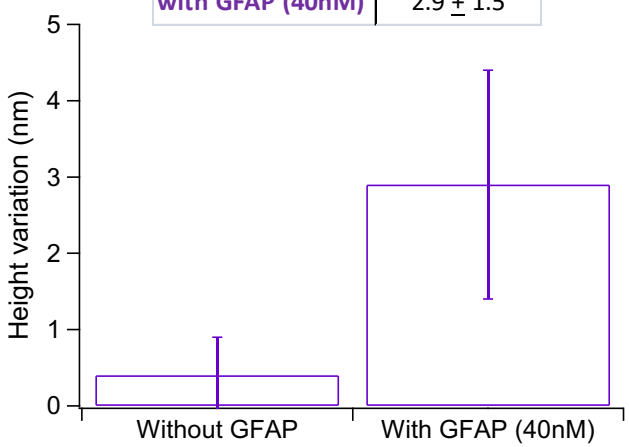
Comparable K_D values have been obtained with the two different assays

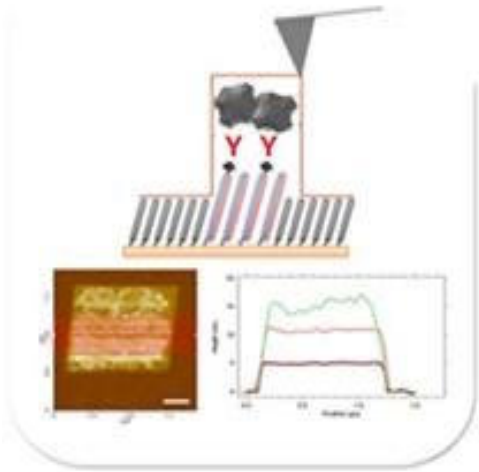
NANO-IMMUNO ASSAY FOR GLIOMA BIOMARKER DETECTION

Testing the device in multicells's lysate + recombinant GFAP (40nM)

Incubation with cell lysate

	Height (nm)
without GFAP	0.4 ± 0.5
with GFAP (40nM)	2.9 ± 1.5



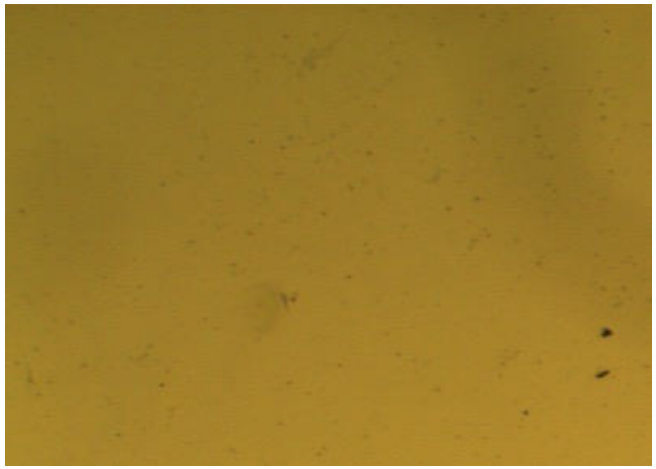


Progressive increase in the height of the nano-immuno patches with final values comparable to those obtained in standard conditions

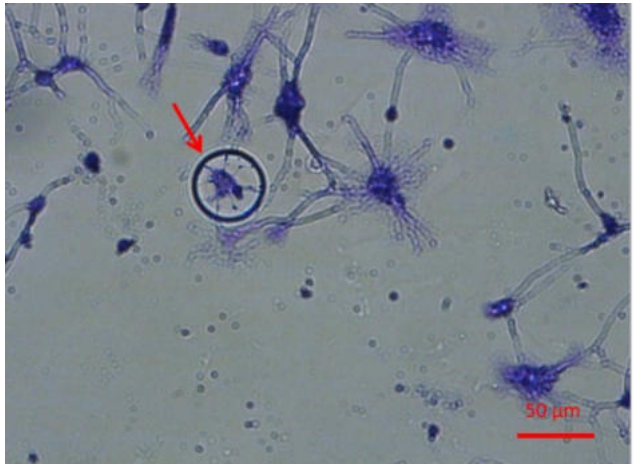
Functionalization of microwells

More efficient cell colture requires PDMS treatment with **polyornithine**

Tests with Hyppocampal Cells

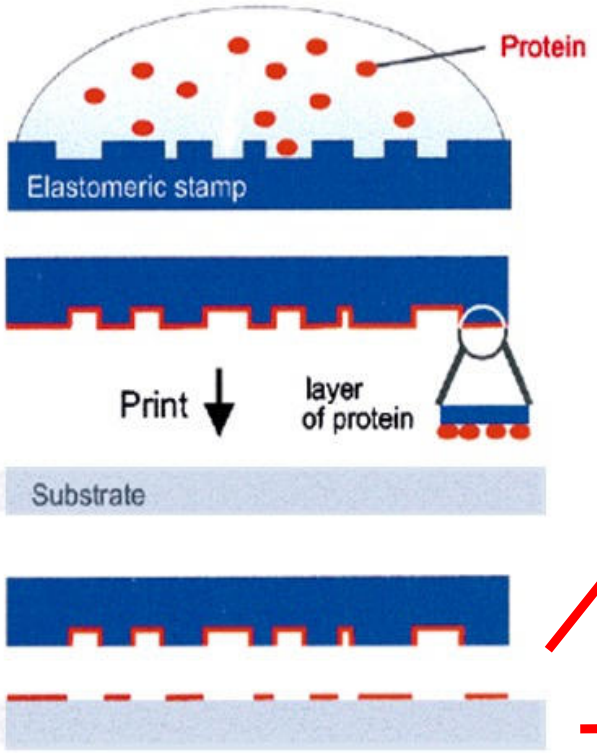


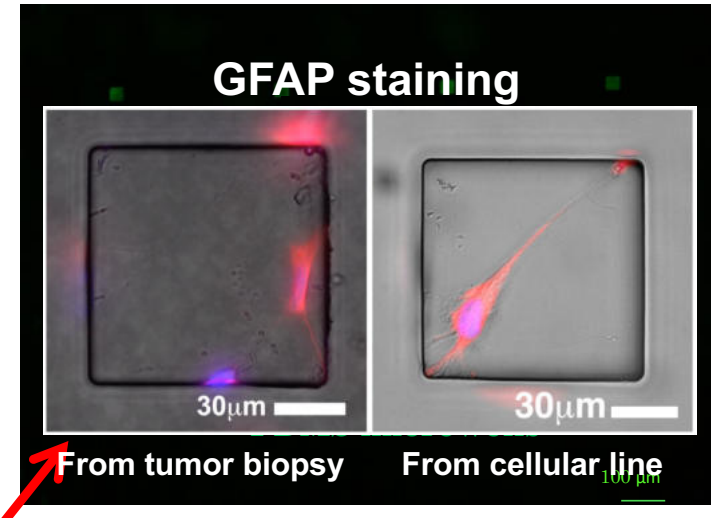
PDMS not treated (control)



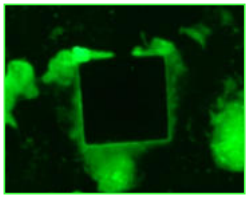
PDMS polyornithine treated

Microcontact printing





Fluorescence only outside on the glass slide



(in collaboration with University of Udine, Italy)

The Molecular Clutch Model of Focal Adhesions

or: How cells convert cytoskeletal motion into mechanical traction

The Molecular Clutch is:

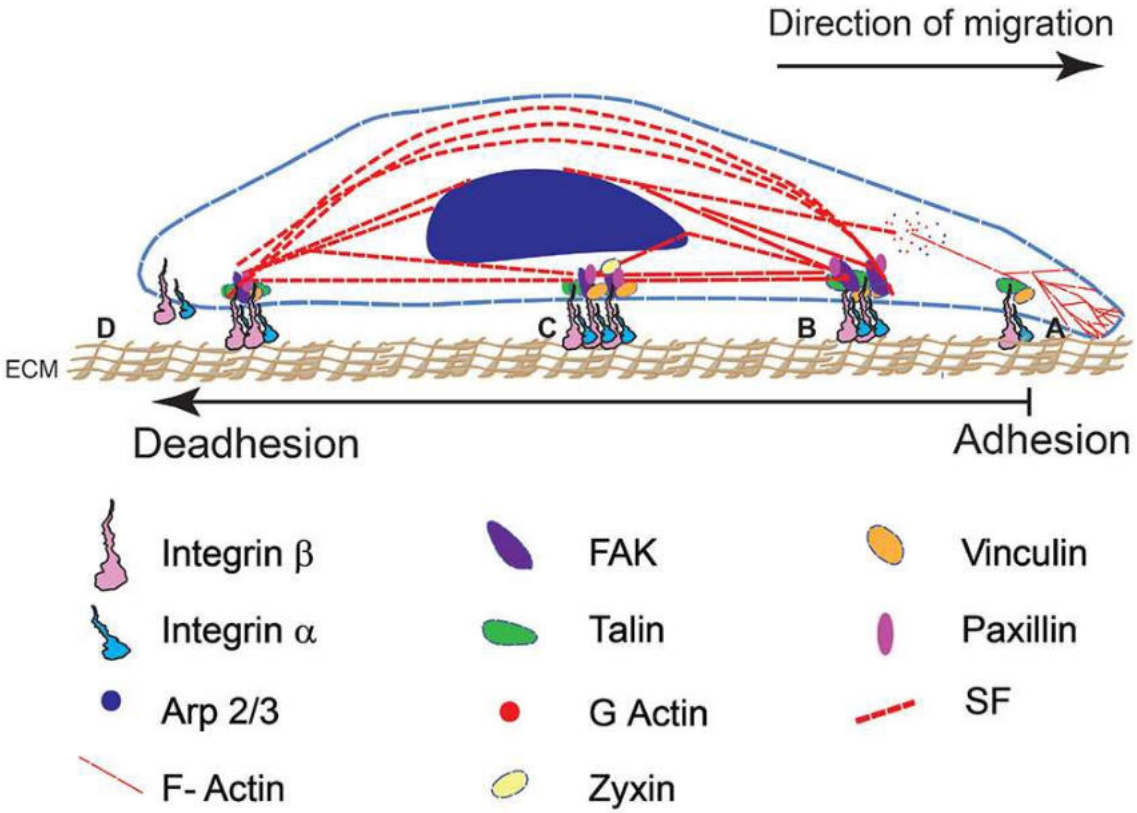
•A model describing how cells **transmit force** to the ECM (and viceversa)

•Focal adhesions act as a **“clutch”** between **actin retrograde flow ↔ integrins bound to ECM**.

•Engagement = traction; slipping = no traction.

Key Players:

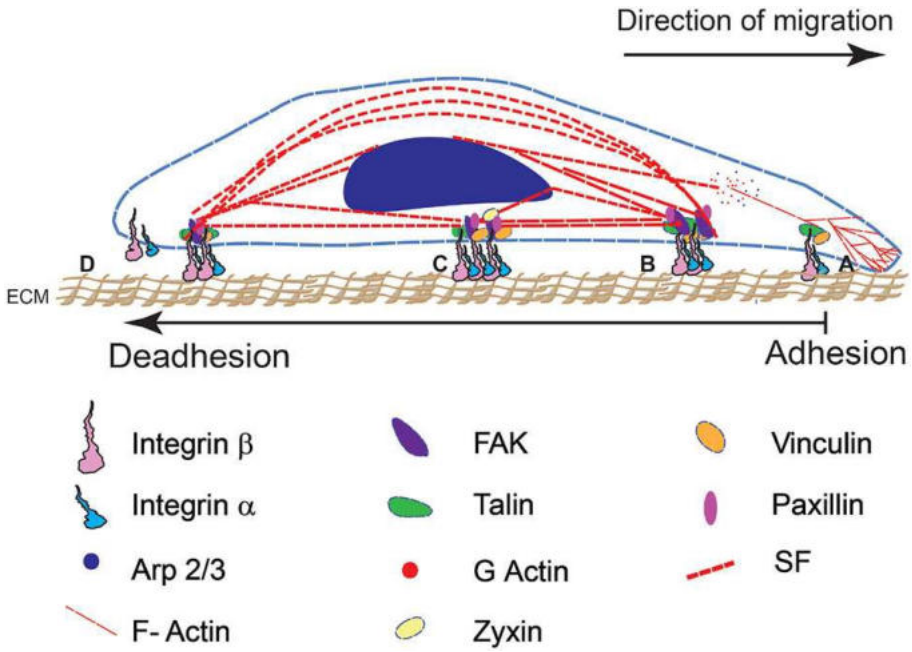
- **Integrins** – bind ECM
- **Talin** – mechanosensitive linker
- **Vinculin** – reinforces under tension
- **Actin cytoskeleton** – generates force
- **Myosin II** – contractility
- Dynamic binding/unbinding = the “clutch.”



Cells encounter mechanical forces through their contacts with other cells in tissue as well as from flows in the vasculature.

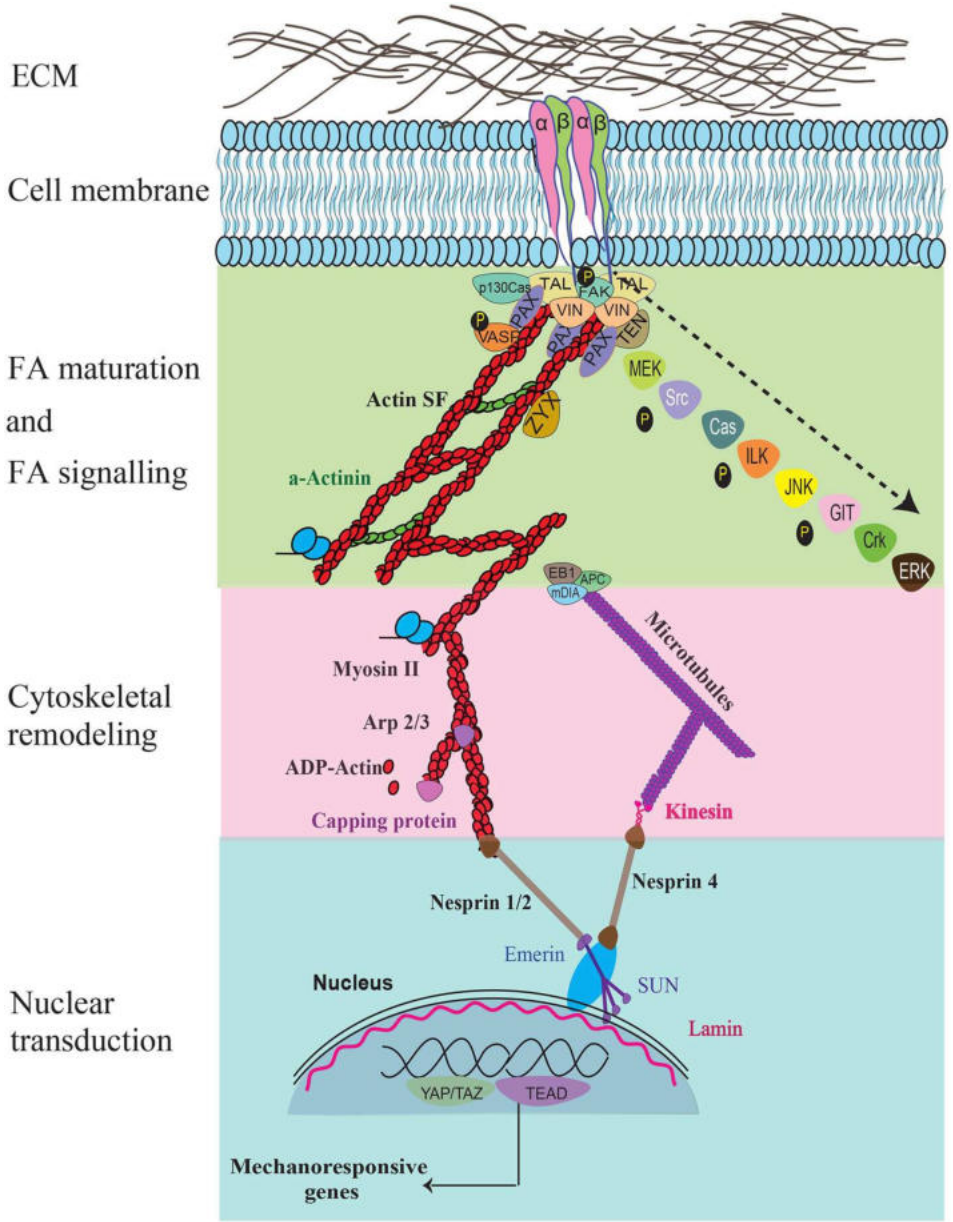
They respond to these forces through multiple levels of feedback, often altering their shape and orientation in response.

Change of shape is accompanied by a similar alignment in the underlying actin cytoskeleton

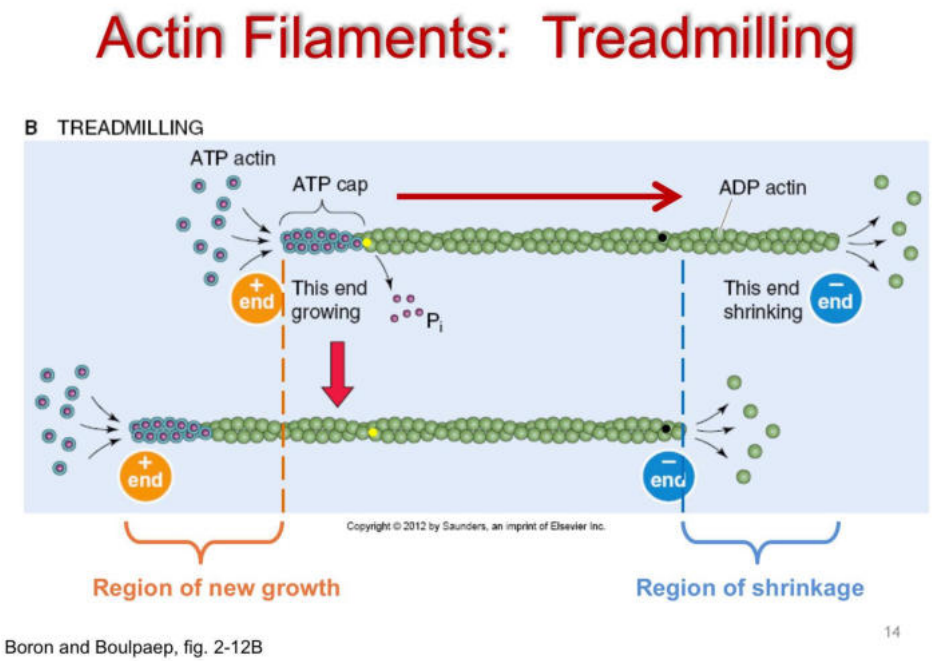


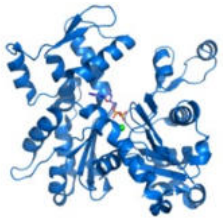
How the Clutch Works

1.Actin polymerization generates **retrograde flow**.
2.Integrins bind ECM → **initial adhesion**.
3.Talin connects integrins to actin → **clutch engages**.
4.Tension stretches talin → **vinculin recruitment**.
5.Actin flow slows → **traction force increases**.
6.Excess force breaks bonds → **clutch slips**.



1.Actin polymerization generates **retrograde flow**.





The energy released by ATP hydrolysis is used to modify the conformations of actin, that is bound to ATP in its lowest energy state. The **resulting conformational changes** destabilizes polymerization at **increased or decreased bonding of the molecules to other molecules;** in the case of actin, hydrolysis destabilizes polymerization at its plus end.

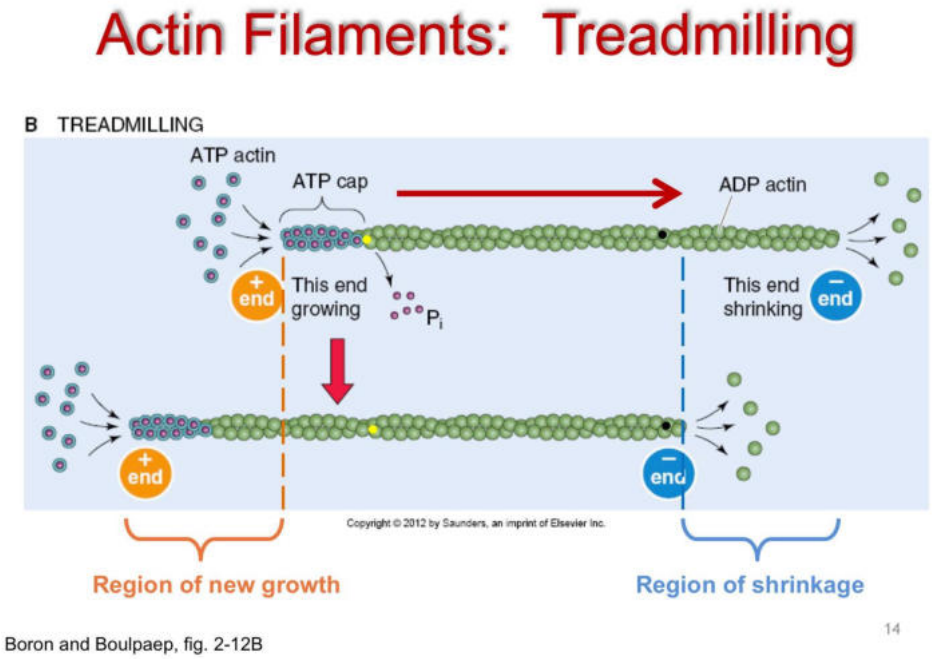
Cytoskeletal filaments are not at equilibrium in cells --- fast addition and subtraction of subunits. Non-equilibrium requires an energy source ---- hydrolysis of ATP (actin) and GTP (tubulin).

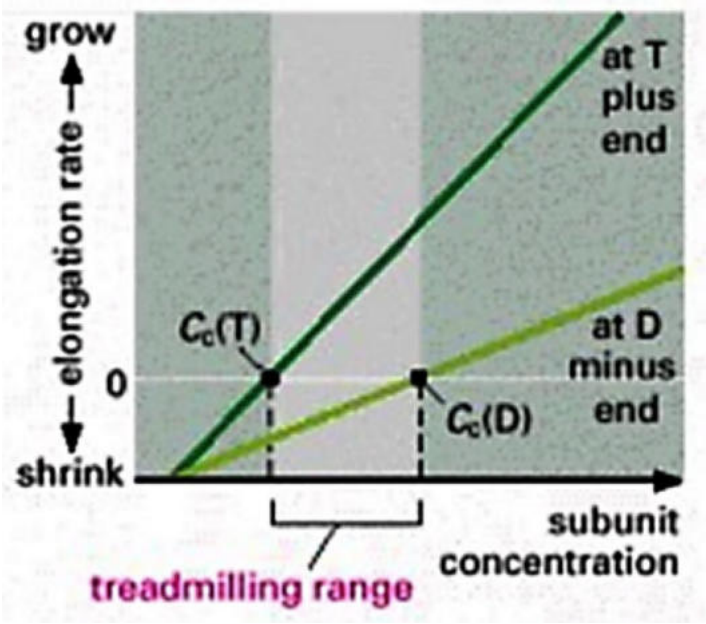
Actin filaments grow mostly at the **barbed end** and shrink at the **pointed end**. **Treadmilling** is a dynamic behavior of actin filaments in which:

- **Subunits are added at the barbed end (+ end)**
- **Subunits are removed at the pointed end (– end)**
- The **filament maintains a constant length**, while **individual actin monomers flow through the filament.**

In other words: **the filament stays in place, but the subunits move.**

1.Actin polymerization generates **retrograde flow**.





Each end of an actin filament has a different **critical concentration (Cc)**:

- **Barbed end Cc ≈ 0.1 µM** → polymerization favored
- **Pointed end Cc ≈ 0.6 µM** → depolymerization favored

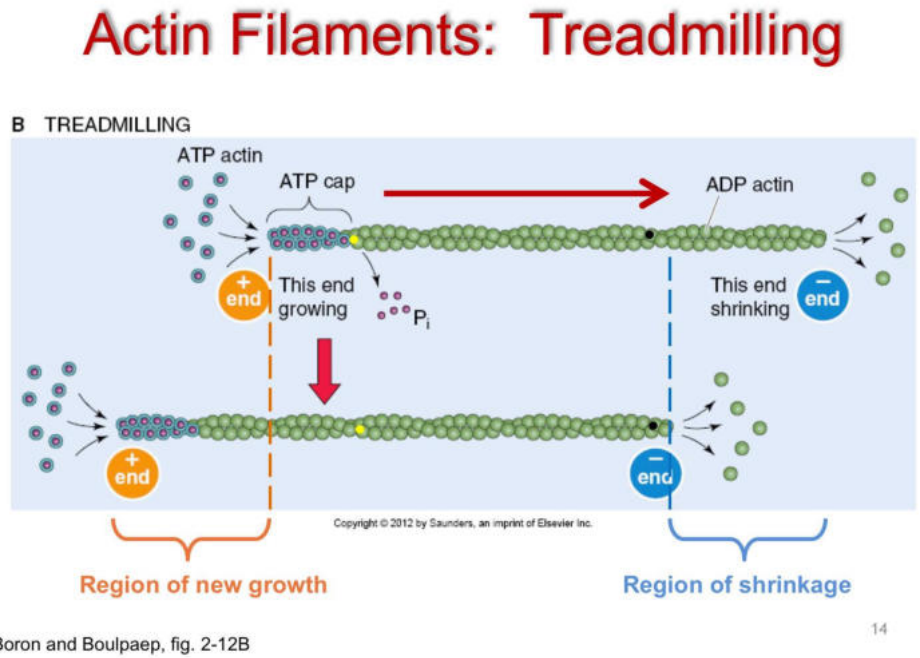
So at intermediate actin concentrations (0.1–0.6 µM):

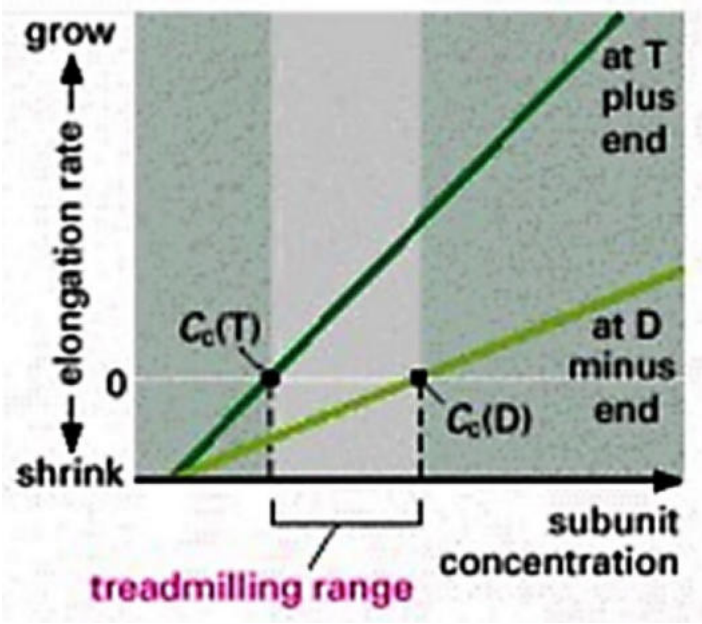
- the barbed end **grows**
- the pointed end **shrinks**

→ **treadmilling**

ATP hydrolysis on F-actin occurs in two steps: 1) rapid cleavage (forming ADP-Pi–actin) followed by slow Pi release (producing unstable ADP-actin); 2) creating nucleotide "caps" that control filament stability and turnover.

1.Actin polymerization generates **retrograde flow**.





This behavior is only possible because:

•Subunits bind as **ATP-actin** at the barbed end

•Hydrolysis converts them to **ADP-Pi** and then **ADP-actin**

•ADP-actin has **weaker filament binding**, especially at the pointed end

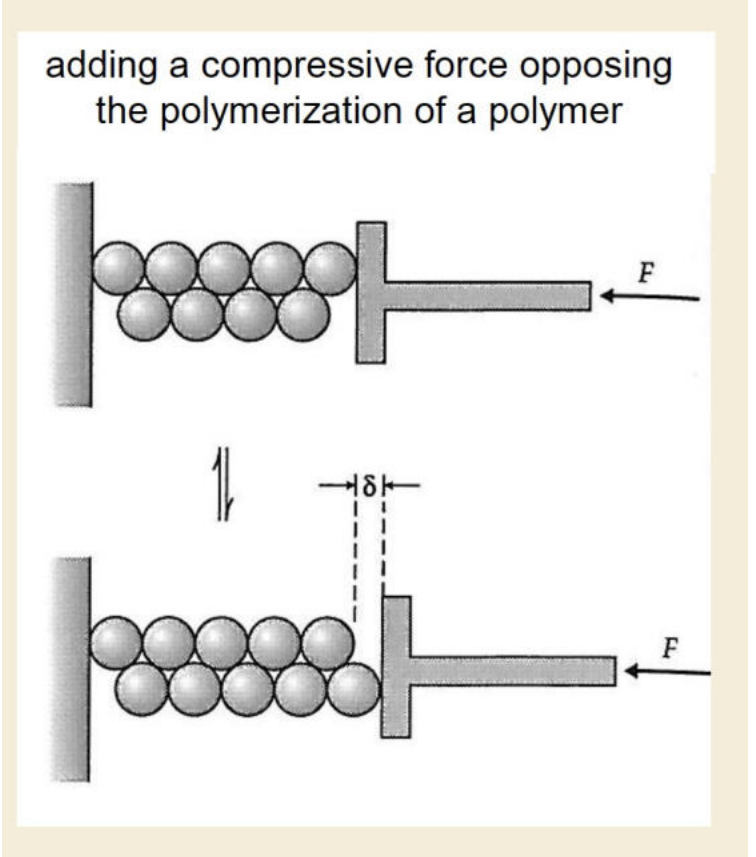
Thus, ATP hydrolysis creates a **nucleotide gradient** that drives the directional flow of subunits.

ATP hydrolysis on F-actin occurs in two steps: 1) rapid cleavage (forming ADP-Pi–actin) followed by slow Pi release (producing unstable ADP-actin); 2) creating nucleotide "caps" that control filament stability and turnover.

1.Actin polymerization generates **retrograde flow**.

A **growing actin filament generates a *pushing force*** against the plasma membrane.
More precisely, this is called the **polymerization force** or **protrusive force**, and it arises from the addition of new actin subunits at the **barbed end**.

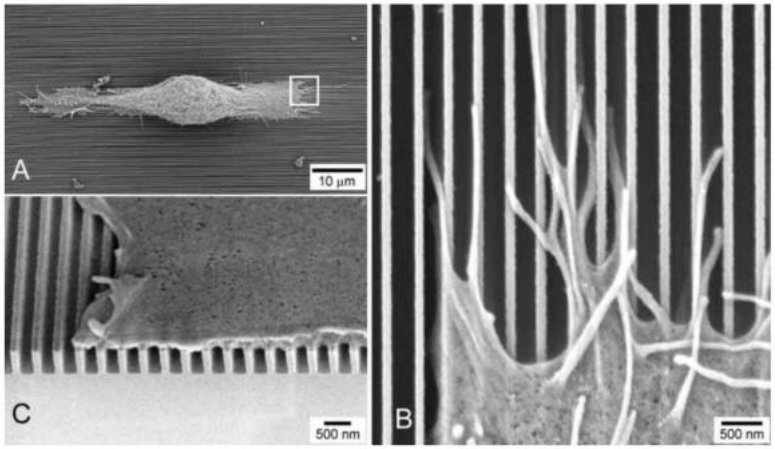
The **pushing force is perpendicular to the membrane**.
This force is strong enough to:
•push the membrane forward
•drive lamellipodial protrusion
•overcome membrane tension
•initiate cell movement
This is the **primary protrusive force in migrating cells**.

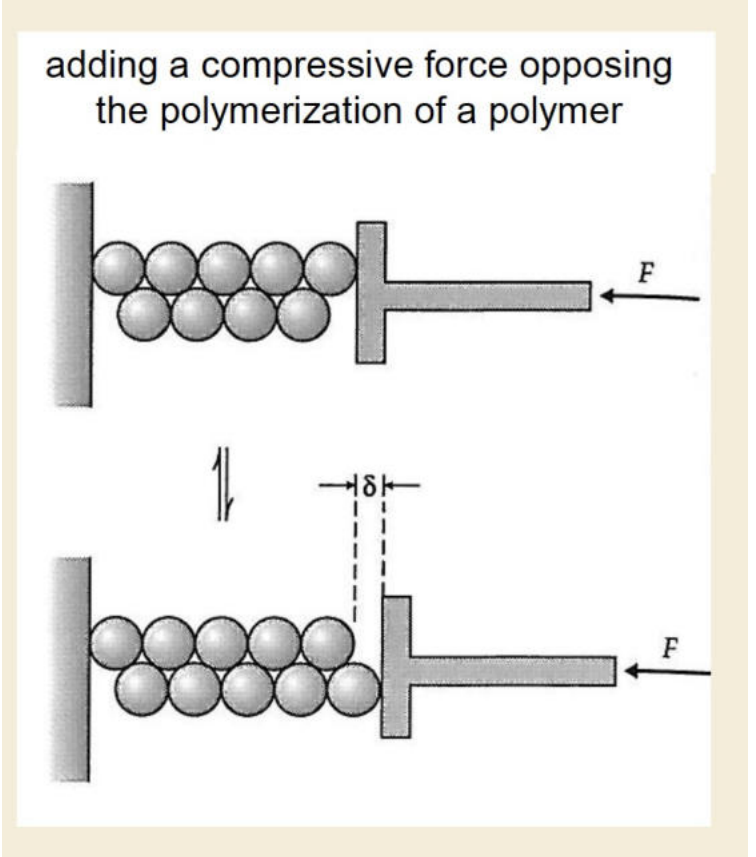


1.Actin polymerization generates **retrograde flow**.

The Brownian racket model:

1.The membrane fluctuates outward due to **thermal motion**.
2.When a tiny gap opens between the membrane and the filament tip:
→ an actin monomer inserts into the gap.
3.This "ratchets" the membrane forward.
4.Repeated additions push the membrane outward.
Thus, **polymerization converts chemical energy (ATP hydrolysis) into mechanical work**.





1.Actin polymerization generates **retrograde flow**.

A single actin filament can generate **1–7 piconewtons (pN)** of pushing force. But cells use **branched networks** of many filaments. Together, they can generate **tens to hundreds of pN per µm²** of leading edge.

This is enough to overcome:
- membrane tension
- membrane bending rigidity
- resistance of the extracellular environment

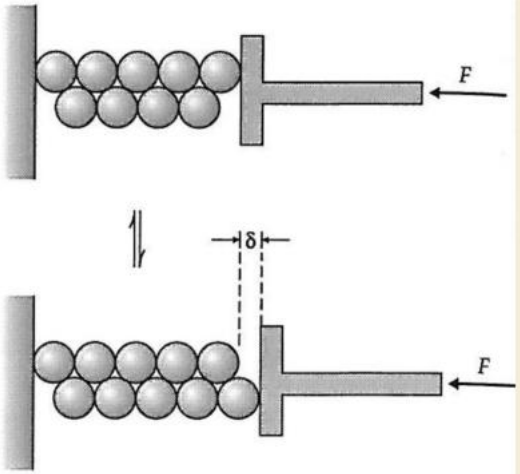
The work done to add one actin subunit against a load F equals at the equilibrium, the **free energy gained by polymerization**:
$F \cdot \delta \approx \Delta\mu$

- $k_BT \approx 4.1$ pN nm (room temperature)
- $\delta \approx 2.7$ nm (axial rise per actin subunit)
- reasonable ratios of C/Cc in cells ≈ 2–20

2

How much force can be generated by polymerization and depolymerization ?

adding a compressive force opposing the polymerization of a polymer



Polymerization against the force *F* → the mechanical energy of the n+1 mer exceeds that of the n-mer by ***F δ***.

Ex. Actin: δ = 5.5 /2 = 2.75 nm (monomer size / 2 - the number of strands);
Microtubules: δ = 8 /13 ≈ 0.6 nm (monomer size / 13 – number of monom / ring)

At equilibrium in the presence of force, application of Boltzmann's law gives a dissociation constant *K(F)*:

$$K(F) = K_c \exp\left(\frac{F \cdot \delta}{kT}\right)$$

K(F) - the concentration at which there is no net elongation of the filament.

Kc - the critical concentration in the absence of force.

At quilibrium concentration [A1] with force, the system is at equilibrium (i.e. there is no net polymerization) when ***F= F_{eq}***:

$$F_{eq} = \frac{kT}{\delta} \ln \frac{[A_1]}{K_c}$$

Ex: for a concentration [A1] of monomer [A1] = 100 Kc → $F_{eq} \approx 7$ pN for actin and $F_{eq} \approx 30$ pN for microtubule

Some comments:

1. **The equilibrium force is independent of the polymerization mechanism**.
   i.e. does not matter whether the polymer pushes directly on the particle or whether the polymer is coupled to the particle via accessory proteins (e.g. Arp2/3 complex for actin, kinetochore for microtubules).

2. **The maximum force exerted by a real polymer will be less than the equilibrium force.**
   i.e. polymerization might be so slow at higher forces that growth is effectively stalled at forces significantly smaller than the equilibrium force (polymerization stalls at forces < 7 pN for actin and 30 pN for microtubules).

3. **Are the cytoskeletal filaments strong enough to exert forces while polymerizing ?**
   Polymerization-based motility requires that the filaments be in compression, and there is only so much compressive force that a filament can withstand before it buckles. This does not represent a problem until the filaments are not too long (< 1 um).

The polymerization mechanism can generate sufficiently large forces to account for cellular processes. Both actin and microtubules filaments are rigid enough to support the polymerization forces that are observed in cells.

Another question is: **is the polymerization fast enough** ?

To answer this question, kinetic mechanisms by which polymerization is coupled to force generation should be considered.

It is difficult to envision how the end of a growing or shrinking filament could push or pull on a particle.
e.g. how a depolymerizing polymer could maintain the contact with the particle being pulled ?

A possible answer are the **accessory proteins** which may **connect** the end of **the filament** to **the particle**.
However, experiments in vitro demonstrated that the additional proteins are not a necessary condition for force generation.

This has lead to the idea that in the case of a growing polymer pushing against an opposing force, the particle being pushed must be able to undergo thermal noise sufficiently large to unblock the adjacent filament end and permit subunit addition. → mechanism termed a Brownian ratchet model.

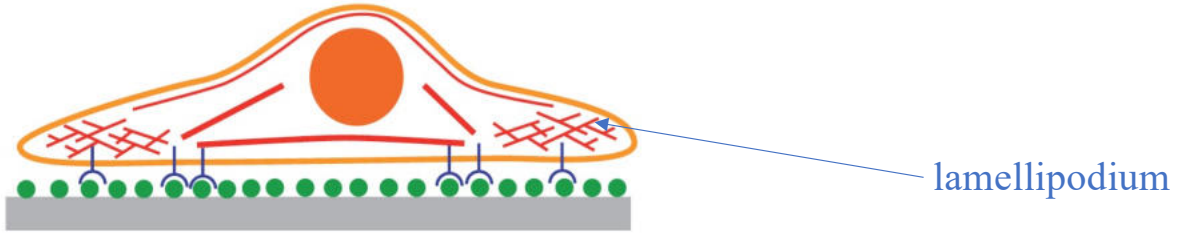
Treadmilling actin filaments in the vicinity of the cell membrane have their motion impeded by the restoring forces (due to surface tension and curvature energy) of the membrane.

However, one important aspect regarding actin networks and bundles in cells is the fact that **these networks are under tension due to the contractile activity of myosin motors.**

In cells, the myosin motors generate **internal forces in the actin network** which are transmitted to its surroundings due to the ‘‘glue’’ the cell produces in the form of proteins that assemble into **focal contacts or focal complexes.**

Also in this case, the production of force is a nonequilibrium process that requires energy input via ATP hydrolysis that causes conformational changes in the myosin molecular motors.
Motor activity also means that the **cell can exert forces on itself** and this, along with polymerization of actin, plays an important role in **cell motility**.

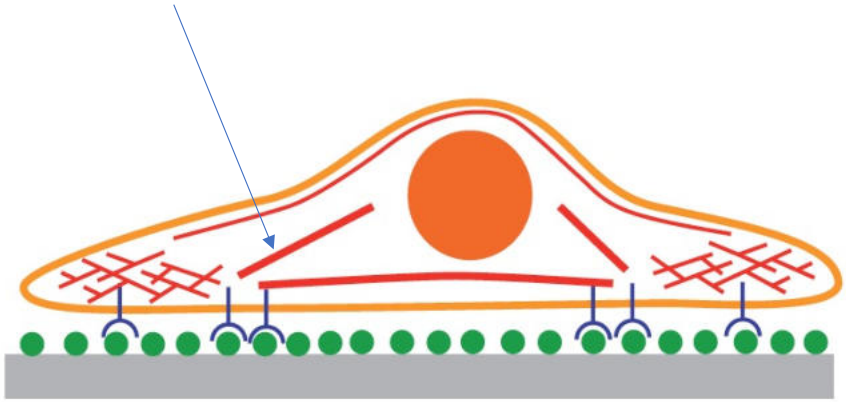
During the **remodeling** process, **the actin system is organized into additional networks** extending throughout the cytoplasm.
Because these networks are cross-linked, the **actin cytoskeleton provides the cell with elastic restoring forces that resist shear deformations** and is thus essential in determining the shape, stability, and mechanical response of cells.
While the volume of a cell tends to stay constant during adhesion and spreading, the surface can increase by up to 50%, which occurs via the flattening of the undulated membrane as well as by the addition of new lipid material (Gauthier et al., 2011).



The main mechanism that leads to outward expansion of the plasma membrane and thus to the development of a contact area with the substrate is the **rapid polymerization of an actin network at the cell periphery (lamellipodium)**. Lamellipodia grow through the elementary processes of actin filament polymerization, branching, capping, and cross-linking. The exact organization of the lamellipodium varies as a function of cell type, motility state, and external signals.

One of the most important aspects of **lamellipodia growth** is its **force-velocity relation**, for which conflicting experimental evidence exists.

Other types of actin structures that develop in cell adhesion are **bundles and networks that are contractile** due to the action of molecular motors that tend to slide actin filaments relative to each other. If the filaments are sufficiently anchored to their surroundings, they can no longer move; thus, instead of motion, tension is developed in the actin bundles or network by the forces exerted by the molecular motors. In adhesive cells, this is mainly achieved by the molecular motor protein myosin II, which organizes into myosin minifilaments that typically contain only dozens of nonskeletal myosins II molecules. The most prominent myosin-based contractile structures in adhesion-dependent cells are **stress fibers.**

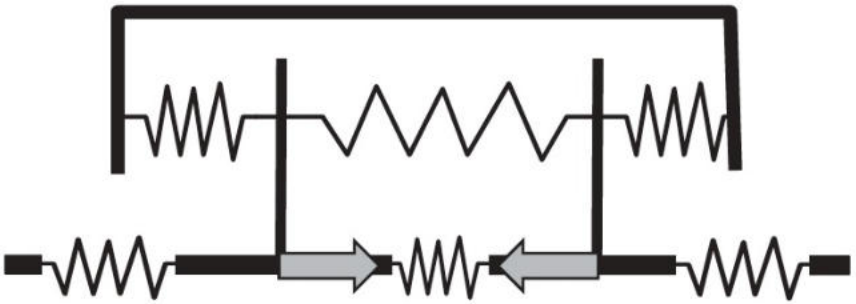


Stress fibers are thought to serve as the main sources of cellular forces that are exerted on the substrate, since their end points are often found at large adhesion sites that correlate with large forces.

The **lamellipodium** and **stress fibers** are actin assemblies that create pushing and pulling forces, respectively; hence, they are the two main force-generating mechanisms for cells that adhere to flat substrates.

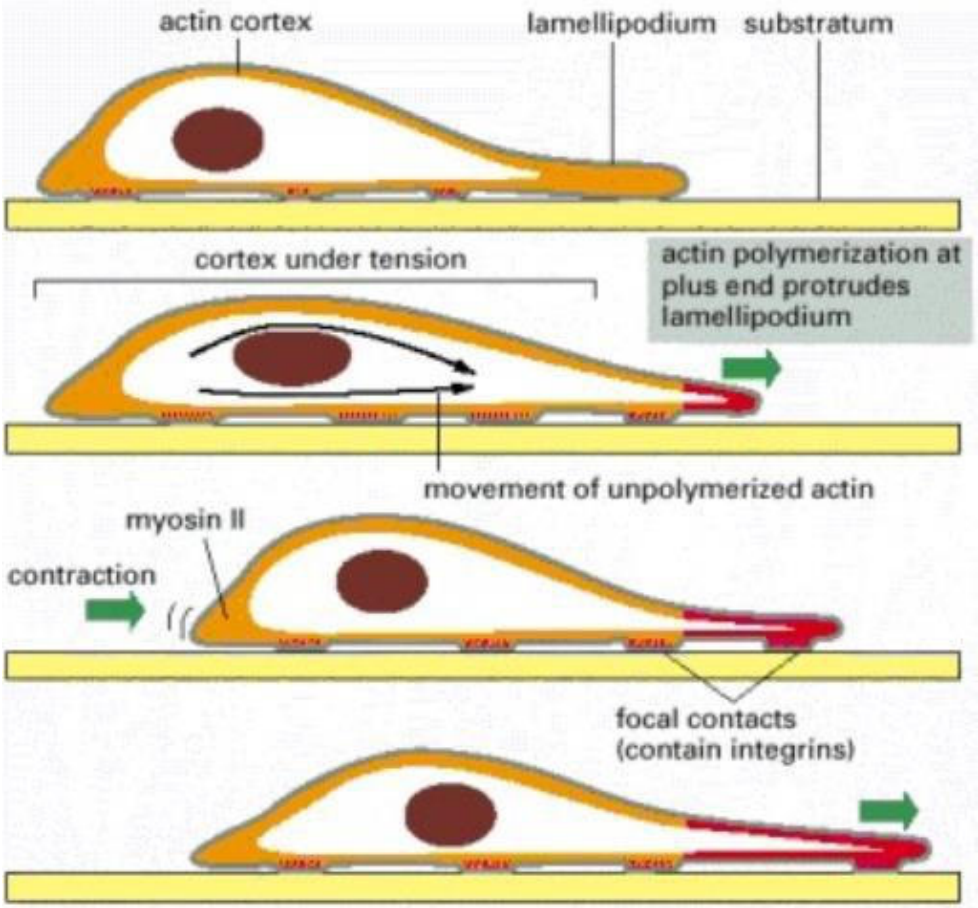
Although its effect is rather indirect, the **plasma membrane** plays an important role in this context.

Apart from acting as host for the transmembrane receptors from the integrin family, it also controls the polymerization of the lamellipodium and the contraction of the stress fibers by triggering biochemical signals that regulate these processes (Ridley, 2011).
Equally important, the plasma membrane plays an important role in the overall force balance in the cell, since its tension and curvature elasticity provide the counterforces to actin-generated forces that tend to extend and deform the membrane. An imbalance in these forces is especially important in cell migration.



To first order, the **lamellipodium** can be depicted as a **compressed spring** that pushes outward on the cell membrane and inward on the focal adhesion.
The **stress fibers** appear as **stretched springs** that pull inward on the adhesion.

Cells Can Crawl Across A Solid Substratum



A model of how forces generated in the actin-rich cortex move a cell forward

The actin-polymerization-dependent **protrusion** and firm **attachment** of a lamellipodium at the leading edge of the cell moves the edge and stretches the actin cortex.
Contraction at the rear of the cell propels the body of the cell forward to relax some of the tension (**traction**).
New **focal contacts** are made at the front, and old ones are disassembled at the back as the cell crawls forward.
The same cycle can be repeated, moving the cell forward. The newly polymerized cortical actin is shown in red.

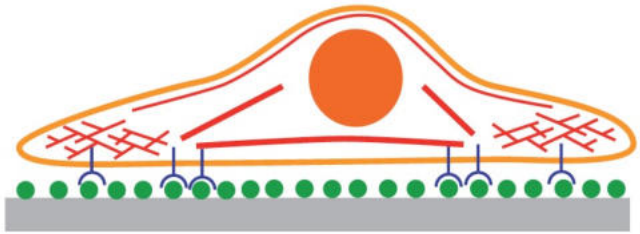
Cells migrate through a **push–pull cycle**:

1.Lamellipodium pushes the membrane forward (actin polymerization)
2.Nascent adhesions form under the lamellipodium
3.These adhesions **mature** and become anchor points
4.Stress fibers connect to these adhesions and **pull the cell body forward** using myosin II contractility
5.Old adhesions at the rear release → movement continues
Thus:
•**Lamellipodia = push**
•**Stress fibers = pull**
Together they create coordinated cell motility and mechanosensing.

Stress fibers are **thick, contractile actomyosin bundles** found deeper in the cell body.

Key Features
•Composed of **unipolar or antiparallel actin bundles** crosslinked by **α-actinin**.
•Powered by **myosin II**, producing **contractile tension**.
•Anchored at **focal adhesions** at their ends.
•Generate **traction forces** needed for movement, ECM remodeling, and maintaining cell shape.

actin filaments + myosin II motors working together.
This system is the **core contractile machinery** of eukaryotic cells - analogous to muscle fibers, but present in nearly all cell types.



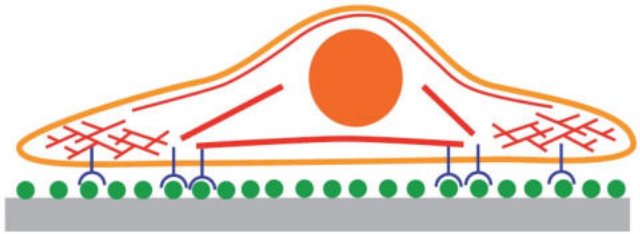
actin filaments + myosin II motors working together.
This system is the **core contractile machinery** of eukaryotic cells - analogous to muscle fibers, but present in nearly all cell types.

Myosin II uses ATP to “walk” on actin filaments.
Because filaments are antiparallel in stress fibers or networks, this produces **contraction**.
Cells need contractile force for:
•pulling on focal adhesions
•maintaining tension
•cell body translocation
•actin retrograde flow in lamella
•cytokinesis (contractile ring)

Actomyosin tension determines:
•cortical stiffness
•membrane tension
•cell rounding
•cell polarization

Without actomyosin, cells collapse into a soft, flaccid state.

Actomyosin:
•retracts the trailing edge
•stabilizes the lamella
•couples adhesions to traction
•sets the balance between protrusion and contraction



2. Integrins bind ECM → **initial adhesion**.

While the response of cells to external forces or other mechanical perturbations can necessitate the disassembly and rebuilding of the actin cytoskeleton, the stable coupling of the cell to the surrounding elastic matrix is due to sites of adhesion called **focal adhesions** that connect the actin cytoskeleton to transmembrane adhesion receptors from the integrin family.

These are then connected, on the extracellular side, to the substrate or extracellular matrix.

The spatial distribution of the adhesion structure of cells is very heterogeneous. It is mainly localized at the cell periphery, because it is strongly coupled to the growth processes of the lamellipodium.

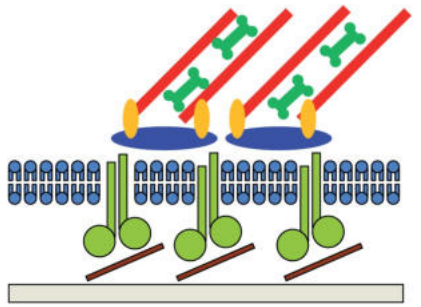
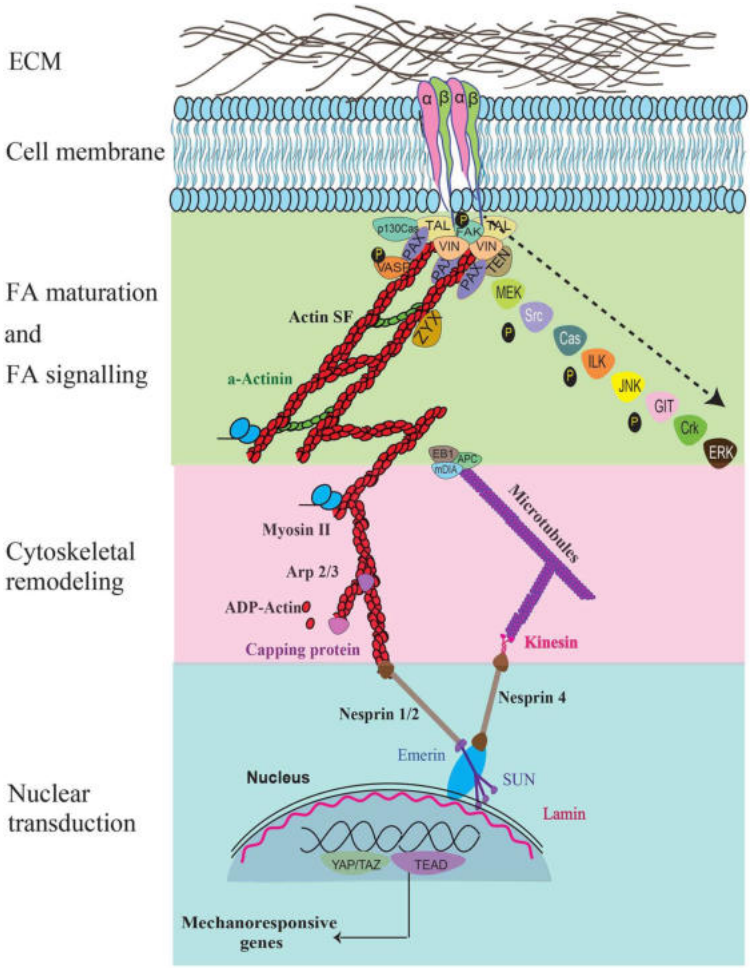


FIG. 7 (color online). Schematic view of a focal adhesion. The transmembrane adhesion receptors from the integrin family (a heterodimer with two subunits) bind to the extracellular matrix (for example, collagen) on the outside and are cross-linked by cytoplasmic proteins such as talin in the inside. Talin binds to actin and this binding is further strengthened by proteins such as vinculin. The contractility of the actin cytoskeleton is determined by the activity of myosin II minifilaments.

2. Integrins bind ECM → **initial adhesion**.



Integring are heterodymers, with α and β subunits.
There are 24 different integrins in mammalian cells.

Examples:
- **α5β1** → fibronectin receptor
- **α1β1, α2β1** → collagen receptors
- **α3β1, α6β1** → laminin receptors

Or
Important for immune trafficking:
- **α4β7** → directs lymphocytes to the gut

Target of anti-inflammatory drug vedolizumab.

Focal Adhesion (FA) are key sites of transmembrane integrine clustering, which mediate intracellular force transmission through dynamic mechano-sensitive complexes.

FA are connected, both mechanically and biochemically, to the cytoskeleton.
FA generate upon input from the ECM and transmit signal by generating a cytoskeleton tension, up to the nucleus, and vice-versa.

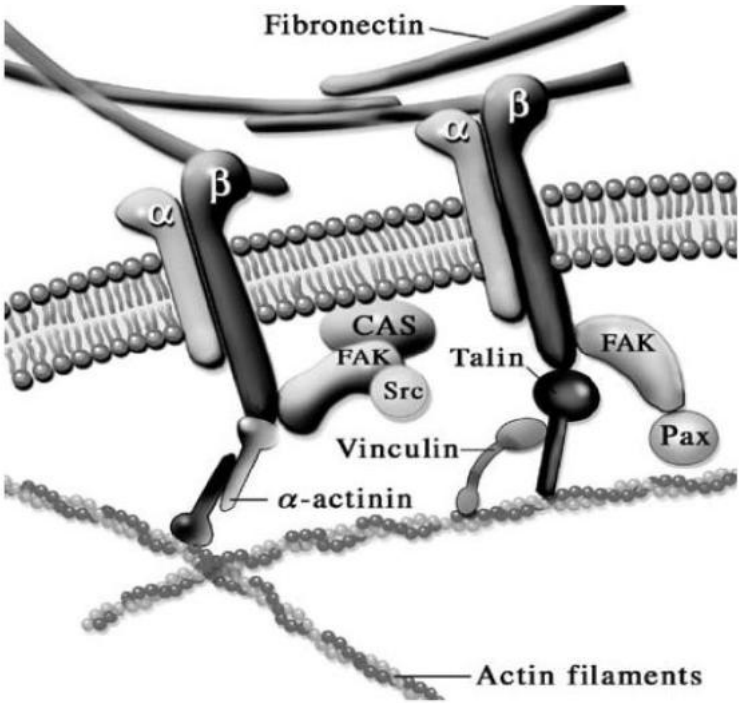
Focal adhesions are composed of three main types of components:

1. **Integrins**: Transmembrane receptors that mediate cell-ECM attachment.
    - Integrins form the main link between the ECM and the internal cytoskeleton of the cell.
    - They exist in active (open) and inactive (closed) conformations, with activation often triggered by ECM binding or intracellular signaling.
2. **Adaptor Proteins**: These proteins connect integrins to the actin cytoskeleton and include talin, vinculin, paxillin, and kindlin.
    - **Talin**: Binds directly to integrins and recruits actin, playing a crucial role in force transmission.
    - **Vinculin**: Reinforces the connection between talin and actin, stabilizing focal adhesions under force.
3. **Actin Cytoskeleton**: The internal scaffold that generates forces and helps in cell shape changes.
    - Actin fibers are tethered to focal adhesions, allowing cells to "pull" on their surroundings by contracting actin-myosin bundles (stress fibers).

2. Integrins bind ECM → **initial adhesion**.

Integrins bind ECM ligands

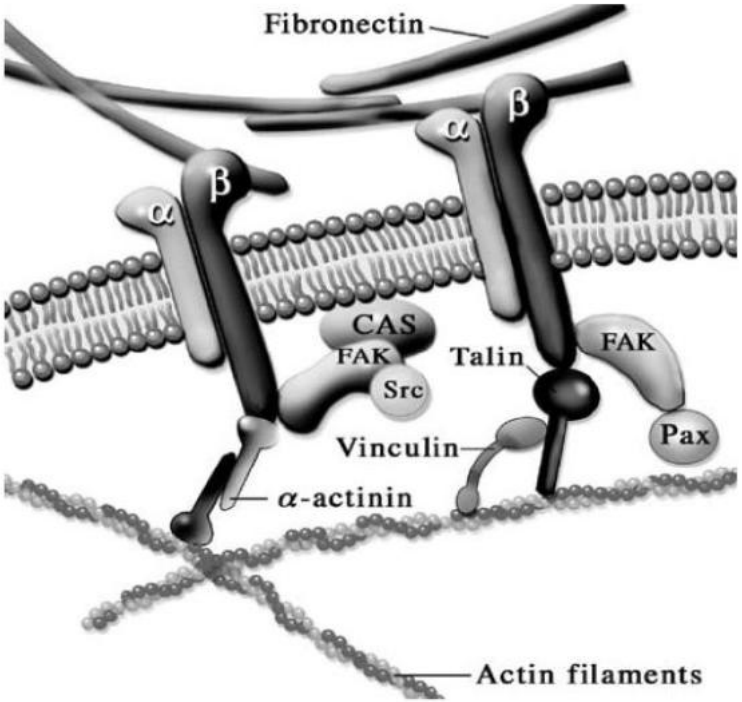
Examples: fibronectin, collagen, laminin, vitronectin.
This forms a weak, nascent adhesion
•These adhesions are small (tens of nanometers)
•They form within seconds
•They do *not* yet transmit significant force



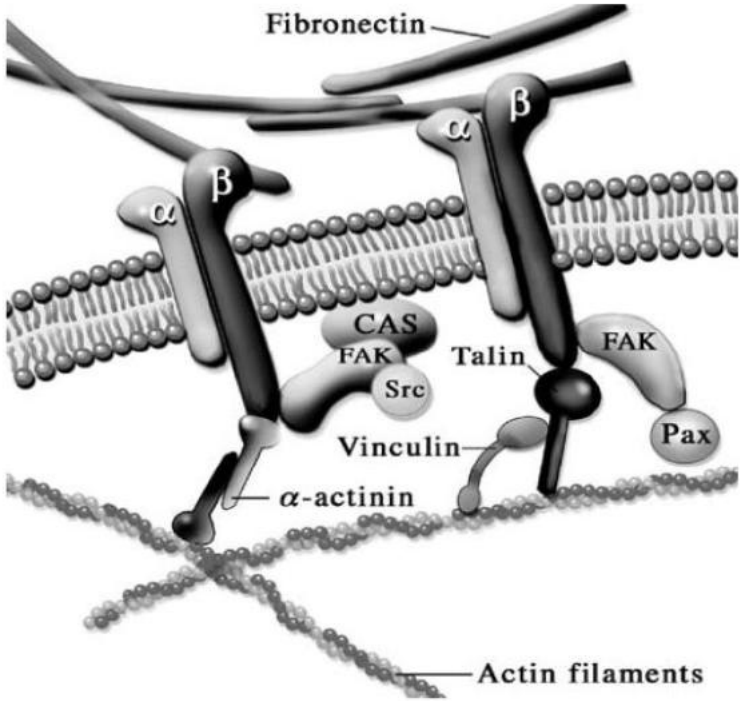
3. Talin connects integrins to actin → **clutch engages**

Talin and kindlin start to bind the integrin tails

•Talin binding begins to link integrins to the actin cytoskeleton
•Kindlin helps stabilize the active integrin conformation
This sets the stage for clutch engagement
Once actin flow couples to these initial adhesions, forces build and the adhesion can mature.



4. Tension stretches talin → vinculin recruitment.



This work demonstrates how stretching of Talin induces a biochemical effect (Vinculin binding)

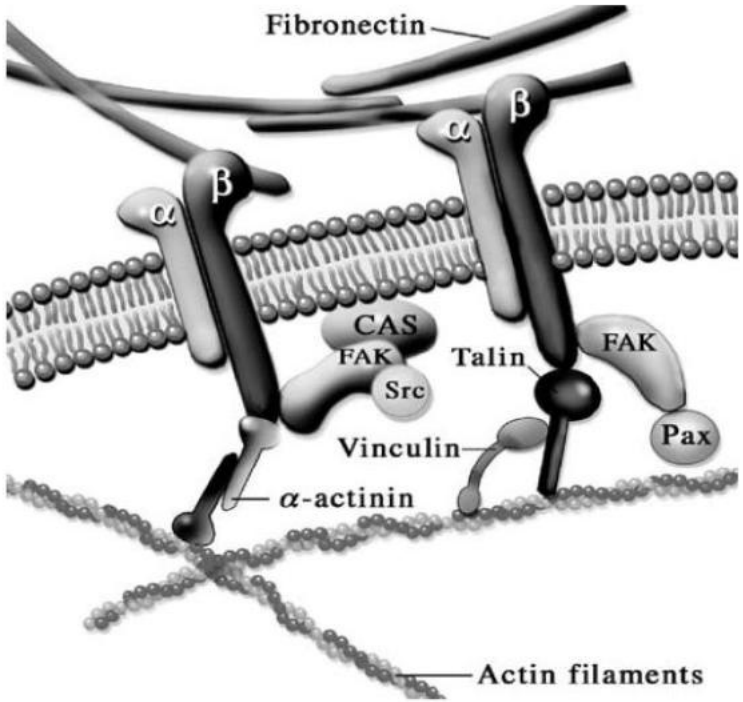
Known:
a. Mechanical forces increase the accumulation of vinculin at the focal adhesions

b. Talin can bind to the actin cytoskeleton

Hypotesis:
a, b suggest that force induced by actomyosin contraction could stretch the talin rod exposing talin binding sites to the vinculin head.

Example . Vinculin Binding Stretching Activates Single TalinRod Molecules
Science 323, 638 (2009); Armando del Rio, et al.

4. Tension stretches talin → vinculin recruitment.



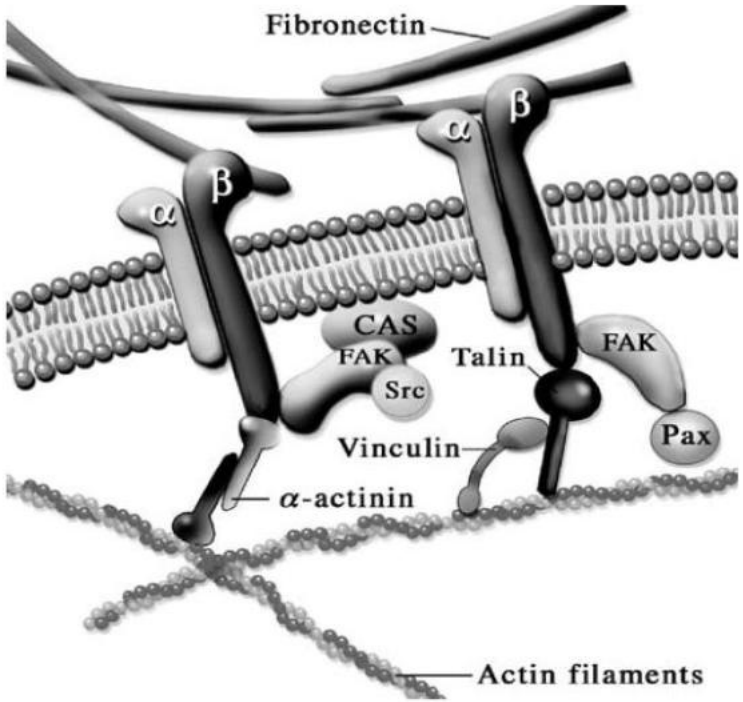
Focal adhesions not only anchor cells but also act as mechanosensors, converting mechanical cues into biochemical signals in a process known as **mechanotransduction**. When external forces are applied to a cell, focal adhesions sense these forces through integrins, which trigger signaling cascades within the cell.

- **External Force Transmission**: When ECM stiffness increases, focal adhesions can grow and recruit more proteins, thereby strengthening their attachment.
- **Internal Force Generation**: Actomyosin contractility generates forces within the cell that are transmitted outward through focal adhesions, affecting how the cell responds to mechanical changes in the ECM.

Example . Vinculin Binding Stretching Activates Single TalinRod Molecules

Science 323, 638 (2009); Armando del Rio, et al.

4. Tension stretches talin → vinculin recruitment.



Talin is a long, multidomain protein that connects:
•**Integrins** (extracellular adhesion receptors)
to
•**Actin filaments** (the cytoskeleton)
Talin is normally **folded**, with many of its vinculin-binding sites (VBSs) *hidden* inside compact helical bundles.

As the actin cytoskeleton pulls on integrins through talin, the force causes talin to **stretch and partially unfold**.
This happens when:
•the molecular clutch engages
•traction forces build
•actomyosin contractility increases

When talin stretches, buried VBSs become **exposed**.
This is the key mechanosensing step:
Force → Talin unfolds → New binding sites open
These newly exposed sites are recognized by **vinculin**.

Control of Mechanotransduction by **Molecular Clutch Dynamics**

Alberto Elosegui-Artola, Xavier Trepat, and Pere Roca-Cusachs, Trends in Cell Biology, 2018

The linkage of cells to their microenvironment is mediated by a series of bonds that dynamically engage and disengage, in what has been conceptualized as the molecular clutch model. Whereas this model has long been employed to describe actin cytoskeleton and cell migration dynamics, it has recently been proposed to also explain mechanotransduction (i.e., the process by which cells convert mechanical signals from their environment into biochemical signals). Here we review the current understanding on how cell dynamics and mecha-notransduction are driven by molecular clutch dynamics and its master regulator, the force loading rate. Throughout this Review, we place a specific emphasis on the quantitative prediction of cell response enabled by combined experimental and theoretical approaches.

5. Actin flow slows → **traction force increases**.

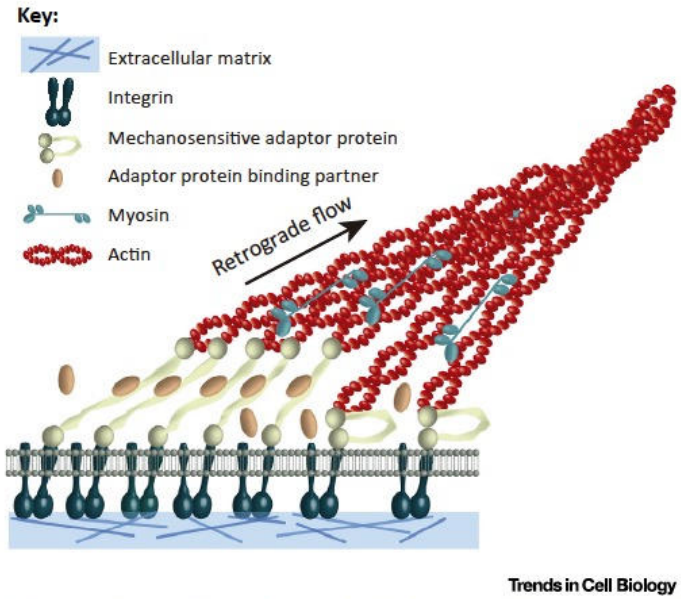


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

At the leading edge, branched actin polymerization pushes the membrane forward, but the network itself moves **rearward** due to:
•polymerization-driven push,
•myosin II contractility,
•membrane resistance.
This is **fast** when adhesions are weak.

Some integrins bind ECM strongly and connect to actin via:
•talin (mechanosensitive linker),
•vinculin (reinforcement),
•α-actinin,
•FAK/paxillin.
This forms a **stronger link** between the cytoskeleton and the substrate.

Because the actin network is now mechanically coupled to the ECM through adhesions:
•when the clutch **engages**,
•part of the retrograde flow is **absorbed** by the adhesions.
So actin flow speed **decreases**.

5. Actin flow slows → **traction force increases**.

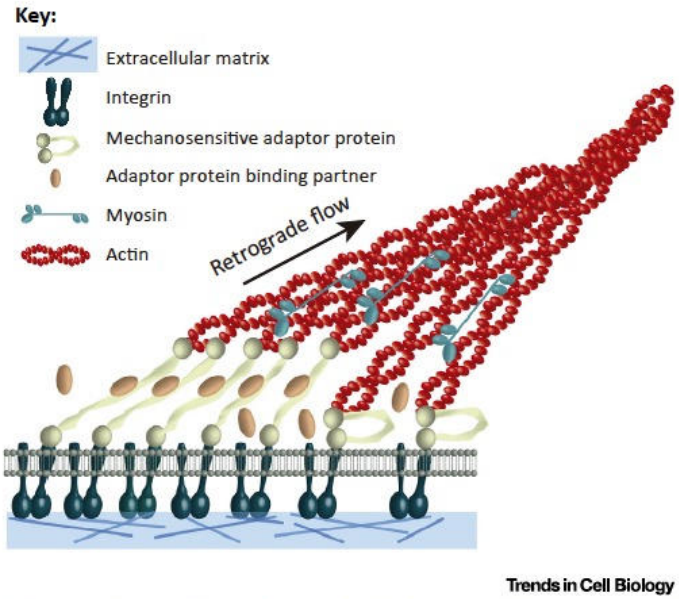


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

At the leading edge, branched actin polymerization pushes the membrane forward, but the network itself moves **rearward** due to:
•polymerization-driven push,
•myosin II contractility,
•membrane resistance.
This is **fast** when adhesions are weak.

Some integrins bind ECM strongly and connect to actin via:
•talin (mechanosensitive linker),
•vinculin (reinforcement),
•α-actinin,
•FAK/paxillin.
This forms a **stronger link** between the cytoskeleton and the substrate.

When actin flow slows:
•more of the force generated by polymerization and myosin is transmitted to the substrate,
•tension in talin increases (→ unfolding),
•focal adhesions strengthen,
•traction forces rise.

5. Actin flow slows → **traction force increases**.

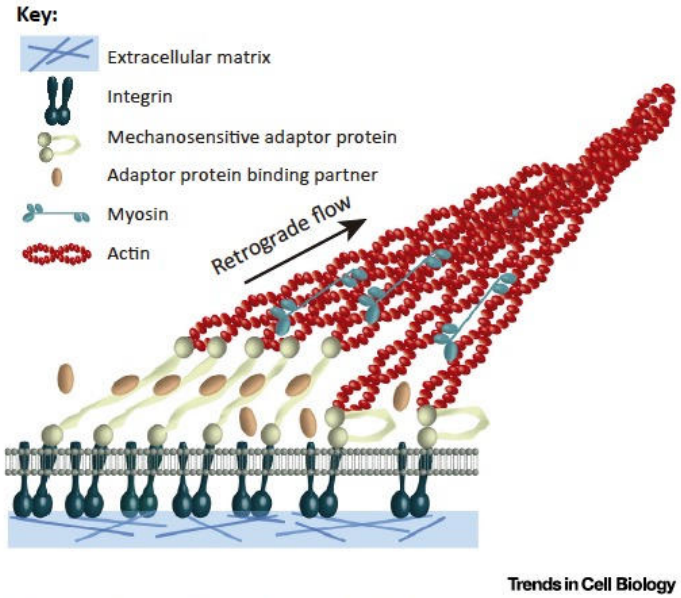


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

Force is redirected from internal flow to outward traction.
Mathematically:
•Fast flow → low force
•Slow flow → high force
•No flow (stall) → maximal force

When the molecular clutch engages, actin retrograde flow slows, allowing more cytoskeletal force to transmit through adhesions to the ECM — increasing traction.

6. Excess force breaks bonds → **clutch slips**.

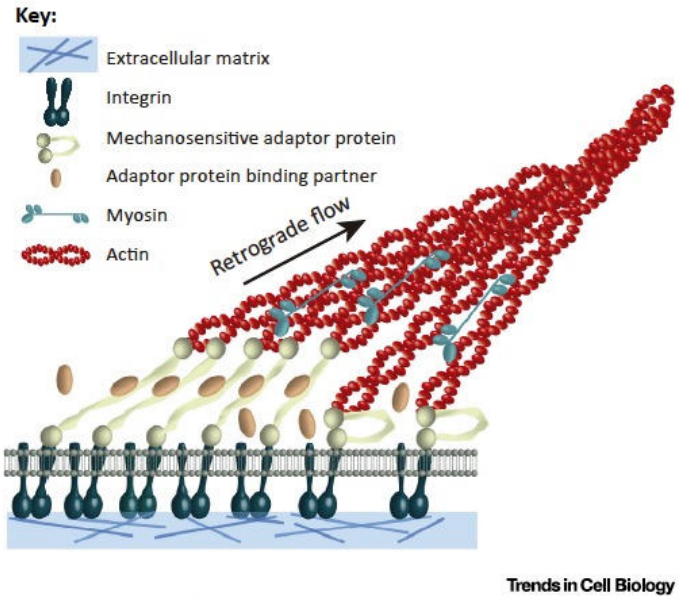


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

The integrin–talin–actin linkage is **force-dependent**, but it is not infinitely strong.
Each component has:
•a maximum load it can sustain,
•a force-dependent unbinding rate.
As tension rises (from actomyosin contraction or resisting membrane load), the probability of bond rupture **increases sharply**.

If the applied force becomes greater than what the integrin–talin–vinculin–actin connections can hold:
•**integrin–ECM bonds unbind**, or
•**talin refolds / detaches**, or
•**actin–linker bonds disconnect**.
This is called **slip**.
It happens stochastically — one bond ruptures, then load shifts to others, causing a cascade.

6. Excess force breaks bonds → **clutch slips**.

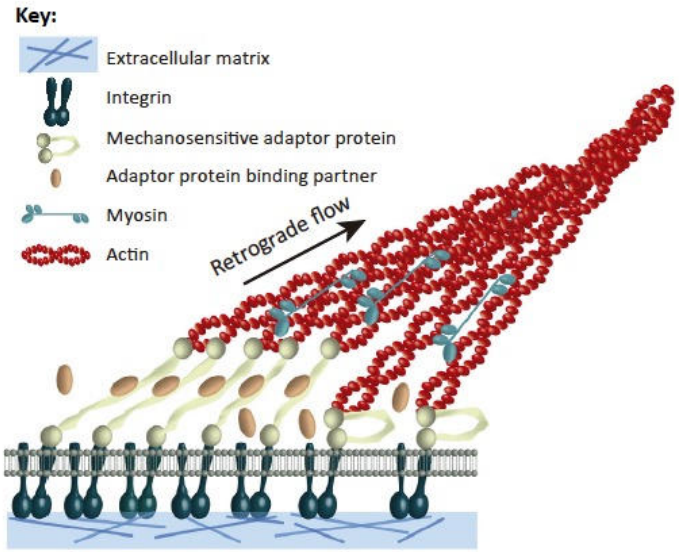


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

If force rises above the strength of integrin–ECM and talin–actin bonds, the clutch disengages, causing bonds to break, traction to drop, and actin to slip backward rapidly.

Slip occurs under conditions such as:

•**Soft ECM**
(more deformation → high local strain → early rupture)
•**Excess actomyosin contraction**
(too much internal tension)
•**High membrane resistance**
(e.g., filopodium unable to push forward)
•**Immature adhesions**
(too few integrins/talin molecules engaged)

Slip is an essential part of the clutch's **force–velocity relationship**.

Slip helps explain:

•why cells cannot generate traction on very soft substrates
•why migration speed peaks at intermediate stiffness
•why lamellipodia sometimes "stall" or "retract"
•how adhesions turn over during motility
•why too much myosin II activity *impairs* protrusion

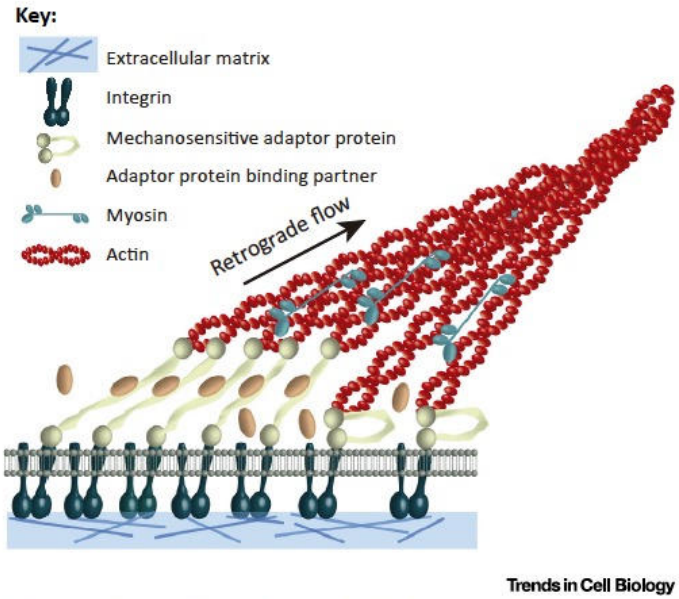


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

The molecular clutch model helps explain why cells respond differently depending on the stiffness of the ECM:

On Stiff Substrates: The clutch remains engaged for a longer period, resulting in stronger force transmission and larger, more stable focal adhesions.

On Soft Substrates: The clutch disengages more quickly, leading to weaker force transmission, smaller adhesions, and different migration behavior.

6. Excess force breaks bonds → **clutch slips**.

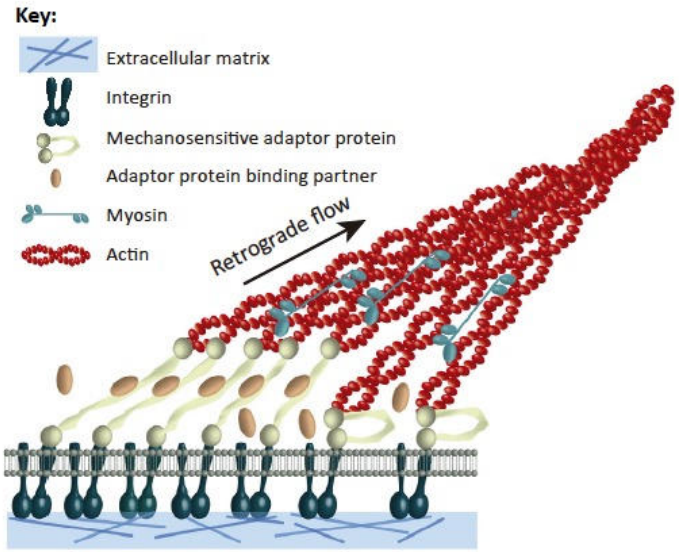


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

The **molecular clutch model** describes how focal adhesions control the connection between the actin cytoskeleton and the ECM, allowing cells to move in a controlled and efficient manner.

- **Clutch Engagement and Slippage**: In this model, the "clutch" is a molecular linkage that can dynamically engage or disengage between the actin cytoskeleton and integrins bound to the ECM.
  - **Engagement**: When engaged, the clutch transmits forces generated by actin polymerization to the ECM, allowing the cell to "push" against its environment.
  - **Slippage**: When the load becomes too high, the clutch disengages (or slips), releasing some of the tension and allowing the cell to reset.

Glossary

Adaptor proteins: term usually employed to refer to the proteins linking actin to either integrins or cadherins in cell–matrix or cell–cell adhesion complexes, respectively.

Binding/unbinding rates: for a given binding event [such as an integrin–extracellular matrix (ECM) bond], this is the inverse of the average time required to bind/unbind the bond, respectively. Binding occurs at zero force, whereas unbinding rates depend on the force applied to the bond.

Catch bond: more precisely defined as a catch–slip bond, a catch bond is a bond in which unbinding rates decrease with applied force up to a given threshold, and then increase. Catch bonds thus have an optimal stability (minimum unbinding rate) when a specific value of force is applied to the bond. Importantly, this concept can also be applied to molecular events other than unbinding, such as protein unfolding.

Contractility: ability of a cell to contract its actin cytoskeleton via myosin motors. In a situation with very low cell adhesion, contractility would power fast retrograde flows. In a context of high adhesion, contractility is transmitted to the substrate, leading to cell–matrix (or cell–cell) force transmission.

Durotaxis: directional cell migration towards areas of increased substrate rigidity.

Frictional slippage: regime with low cell–matrix adhesion, in which transient clutch engagement is unable to significantly slow retrograde flow.

Load and fail/stick–slip: regime with high cell–matrix adhesion, in which simultaneous engagement of several clutches leads to repeated cycles of progressive buildup of force, followed by complete disengagement and force release.

Loading rate: in units of force/time, rate at which applied force increases for a given clutch or clutch ensemble.

Load and fail/stick–slip: regime with high cell–matrix adhesion, in which simultaneous engagement of several clutches leads to repeated cycles of progressive buildup of force, followed by complete disengagement and force release.

Loading rate: in units of force/time, rate at which applied force increases for a given clutch or clutch ensemble.

Molecular clutch: link between actin and an ECM ligand (or a neighboring cell) which can be bound (engaged) or unbound. Usually assumed to represent the serial link between actin, an individual

Dynamic Regulation of Clutch Components

1. **Talin Stretching and Vinculin Binding**: Talin undergoes conformational changes when stretched, exposing binding sites for vinculin, which stabilizes the link between integrins and actin.
2. **Actin Flow Rate and Focal Adhesion Lifetime**: Actin polymerization rate and retrograde flow (backward movement) can influence clutch engagement and disengagement dynamics, directly affecting focal adhesion lifetime and maturation.

Implications of the Molecular Clutch Model

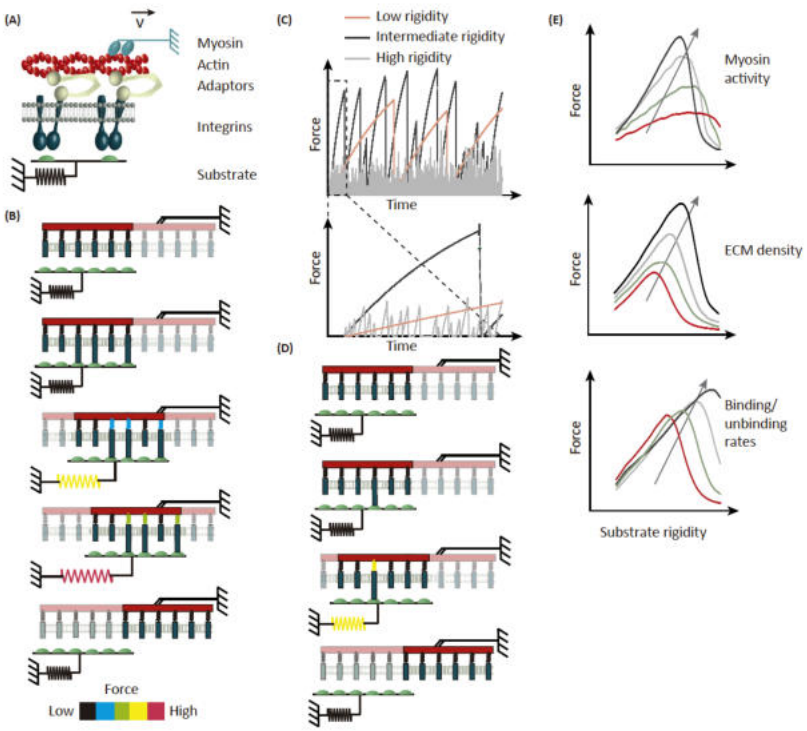
The molecular clutch model highlights the balance between ECM rigidity and cytoskeletal dynamics, explaining why cells exhibit different behaviors on different substrates. This balance is crucial in processes such as:

- **Cell Migration**: Efficient clutch engagement and disengagement allow cells to move directionally.
- **Cancer Metastasis**: Cells with dysregulated focal adhesion dynamics may migrate more aggressively, aiding in metastasis.
- **Stem Cell Differentiation**: Stem cells can sense ECM stiffness and differentiate accordingly, with stiffness affecting clutch engagement.

Applications and Importance of Understanding Focal Adhesions

Studying focal adhesions and the molecular clutch model provides insight into many biological and medical applications:

- **Tissue Engineering**: Understanding focal adhesion dynamics helps in designing biomaterials that mimic natural ECM properties, guiding cell behavior in engineered tissues.
- **Cancer Research**: Targeting focal adhesion components, such as integrins or talin, has therapeutic potential in limiting cancer cell migration and invasion.
- **Stem Cell Therapy**: Manipulating ECM properties can direct stem cell fate decisions, aiding in regenerative medicine.



Trends in Cell Biology

Figure 2. Force Transmission through the Molecular Clutch. (A) Cartoon summarizing the fundamental elements of the system. (B) From top to bottom, sequence of events in a typical 'load and fail' cycle of a molecular clutch (rectangles represent integrins). As clutches bind, myosin contractility deforms the substrate, building force on the substrate and each bound clutch. At some point, force leads to bond destabilization, all clutches disengage, and the cycle starts again. (C) Typical plots of force exerted versus time for molecular clutches on low, intermediate, and high rigidity. (D) From top to bottom, sequence of events in a typical 'frictional slippage' cycle of a molecular clutch, observed on a high-rigidity regime. As a clutch binds, myosin contractility builds force very quickly due to the high rigidity, leading to clutch disengagement before others have time to bind. This limits overall force transmission to the substrate. (E) Clutch model predictions of average force transmission to the substrate as a function of substrate rigidity. Top, middle, and bottom graphs show the changes in the curve induced by increasing myosin activity, increasing ECM ligand density, and simultaneously increasing binding and unbinding rates, respectively. Abbreviation: ECM, Extracellular matrix.

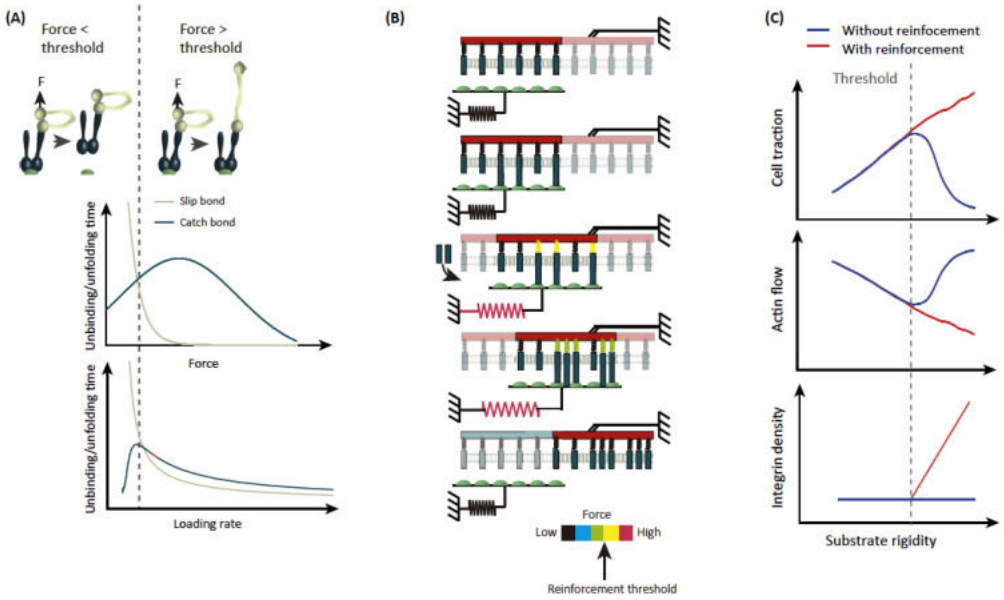


Figure 3. Force Transduction through the Molecular Clutch. (A) Bottom, effect of either a constant force or a constant force loading rate on the average times required for protein unfolding or bond unbinding. Typical curves for a slip or catch bond are shown. Top, expected effect on a system in which force is applied to a serial link between a molecule that unfolds as a slip bond (such as talin) and a bond that unbinds as a catch bond (such as an integrin–fibronectin bond). Unbinding occurs first when force is below the threshold, and unfolding (and subsequent mechanotransduction) occurs first when force is above the threshold. (B) From top to bottom, sequence of events in a typical 'load and fail' cycle of a molecular clutch, including mechanotransduction (reinforcement) events. As clutches bind and force builds, some clutches surpass the threshold force required for mechanotransduction, leading to the recruitment of additional integrins. This increases the number of bound clutches, reducing the force applied per clutch, delaying the failure of the system, and increasing average force transmission. (C) Examples of predicted force/rigidity curves in the presence and absence of reinforcement. Reinforcement only affects force transmission above a threshold in rigidity, which corresponds to the loading rate threshold from (A). Then, the increase in integrin recruitment prevents the reduction in force (and increase in actin flows) normally expected in a molecular clutch system.