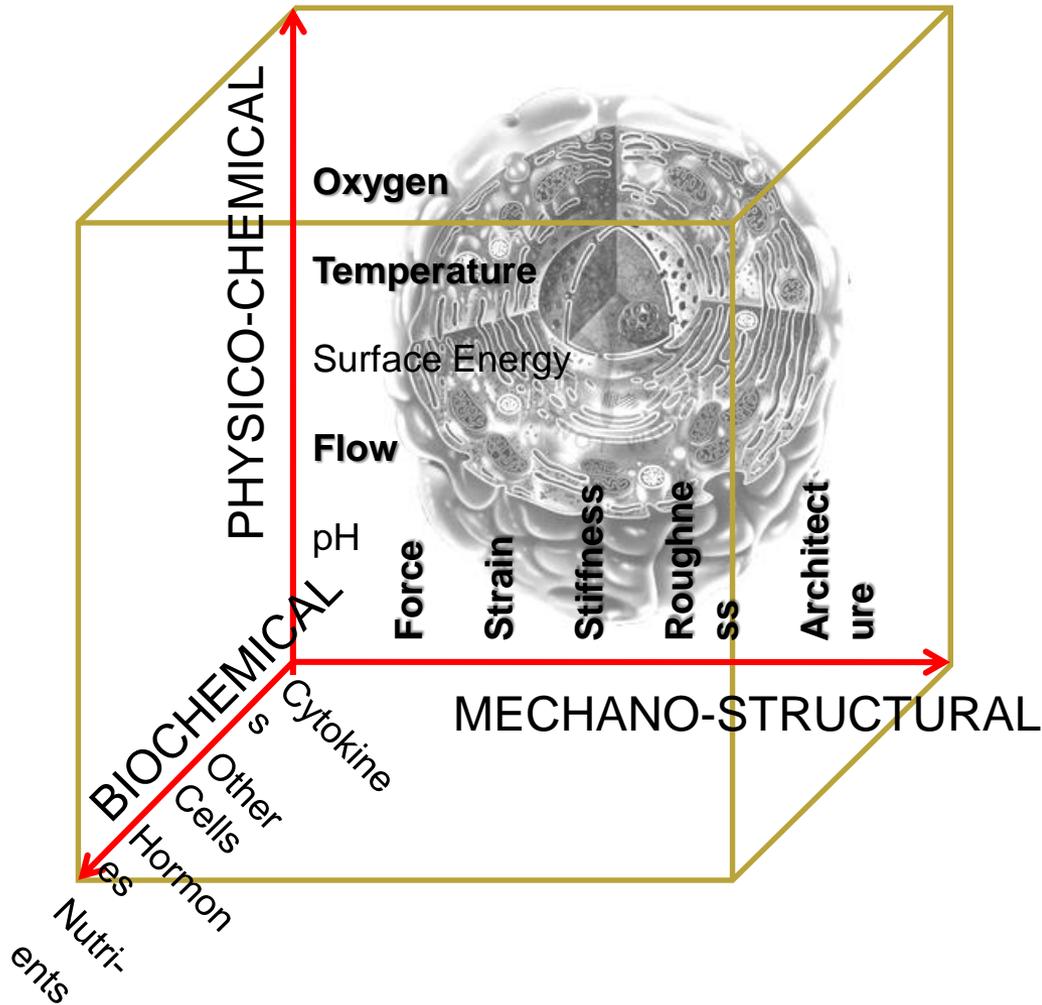


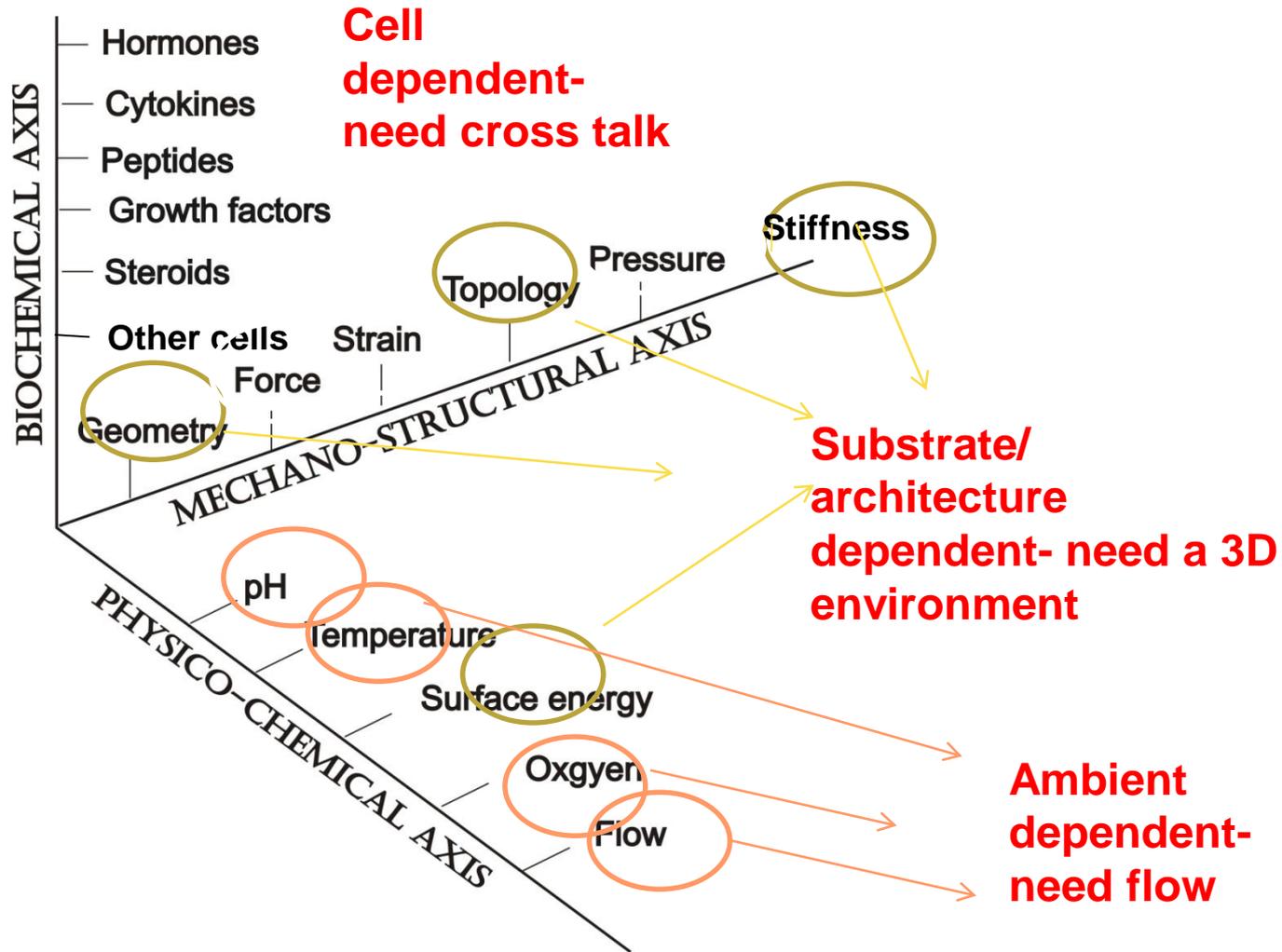
Cell response to micro-environment

Cells sense and respond to their environment.

The cell microenvironment



Decomposition of the cell microenvironment



Mechanotransduction

- The process by which cells sense and respond to **mechanical stimuli** .
- Stress, shear, stiffness, viscosity....

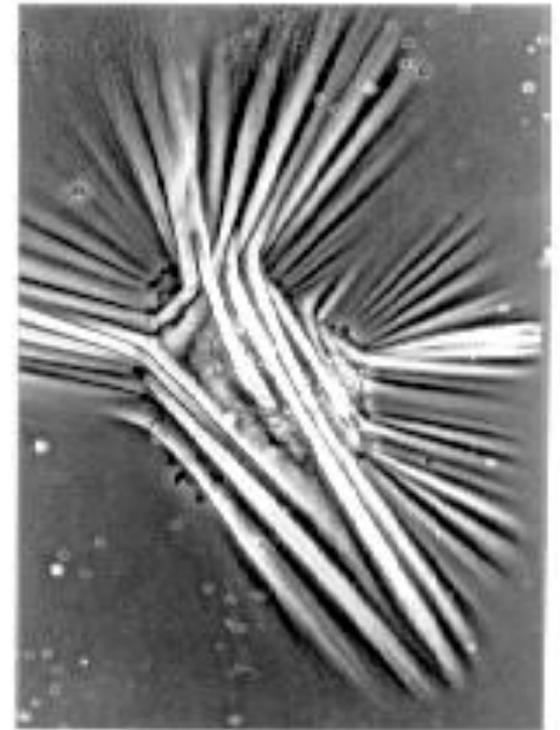
Adhesion, Motility

Adhesion and motility are closely linked and important for

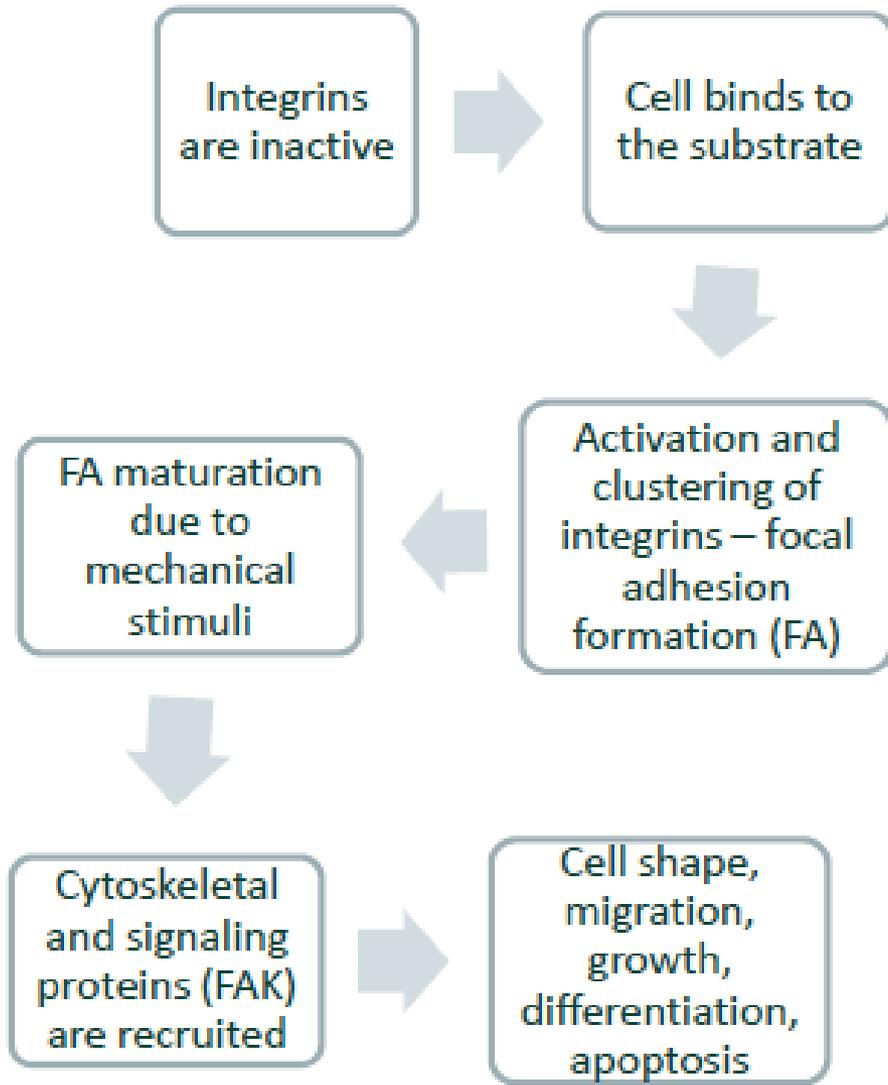
- Scaffold colonization
- Integration of host-construct
- Morphogenesis
- Wound healing
- Inflammation and repair
- Metastasis

Adhesion

- Adhesion ligand (eg RGD Arg-Gly-Asp)
- Cell adheres and spreads
- Cytoskeleton contracts
- Substrate is deformed (if soft)
- Tension develops in cell
- Tension will develop in substrate if it is soft enough
- Mechanical stress transmitted to nucleus.....LINC complex (Linker of Nucleoskeleton to the Cytoskeleton)



This is mechanobiology-transduction of mechanical forces into biological responses



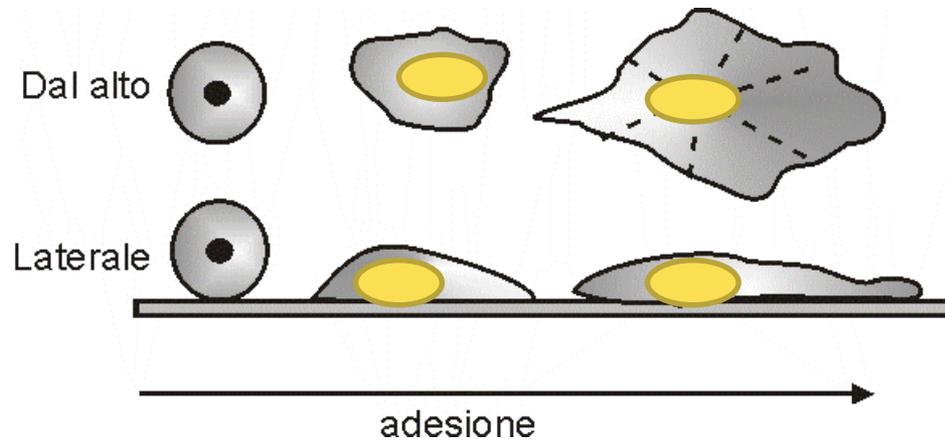
Form
Function
Phenotype

Remember: Adhesion, motility, cell shape and phenotype are closely linked:

FORM-FUNCTION-PHENOTYPE

Adhesion and motility ^{known to be} are[^] modulated by:

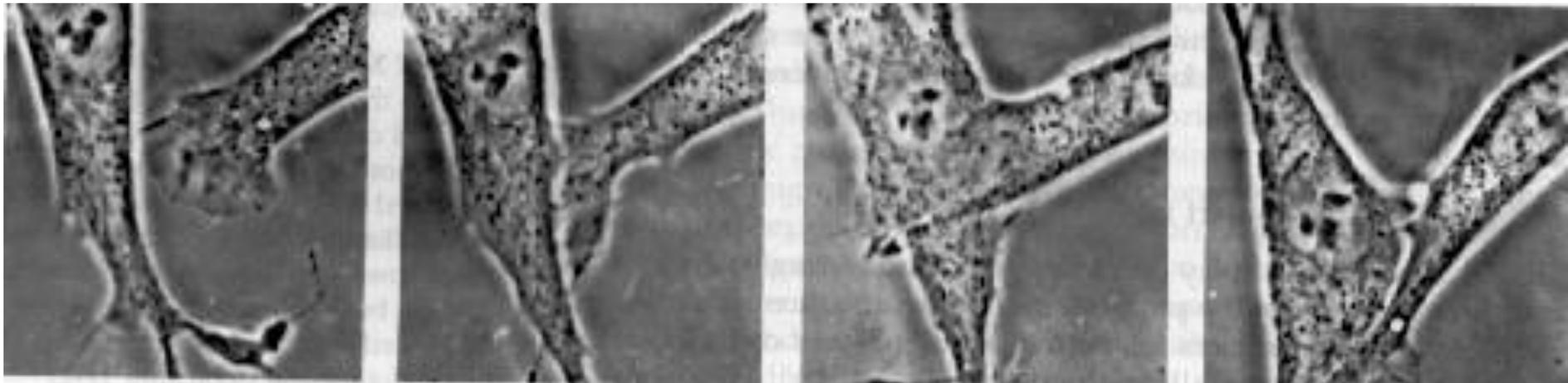
- **Chemotactic gradients (nutrients, growth factors etc)**
- **Haptotactic gradients (Adhesion ligands eg RGD, YIGSR)**
- **Contact inhibition**
- **Galvanotaxis**
- **Contact guidance from a substrate**
- **Durotaxis**



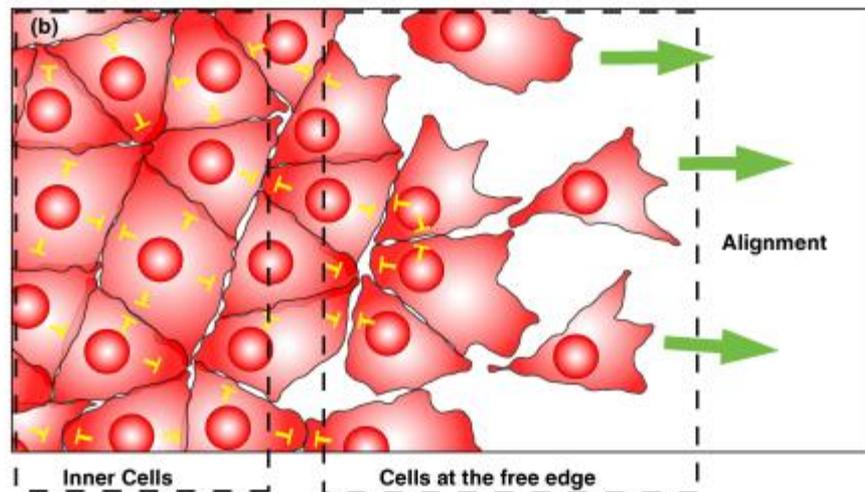
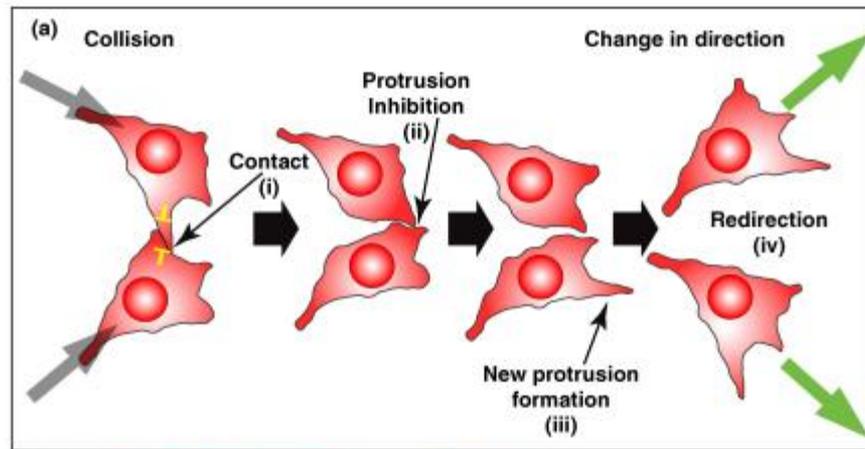
Cell shape changes with adhesion (and the nature of the ECM).

Contact inhibition is the natural process of arresting cell growth. Cells growing in –vitro will generally continue to replicate until they form a continuous monolayer on the vessel surface. In this case, they would be subject to contact inhibition. Thus, cells in culture need to be repeatedly passaged (placed into new vessels at a lower density) to maintain normal growth.

Tumor cells typically lose this property and thus grow in an uncontrolled manner even when in contact with neighbouring cells.

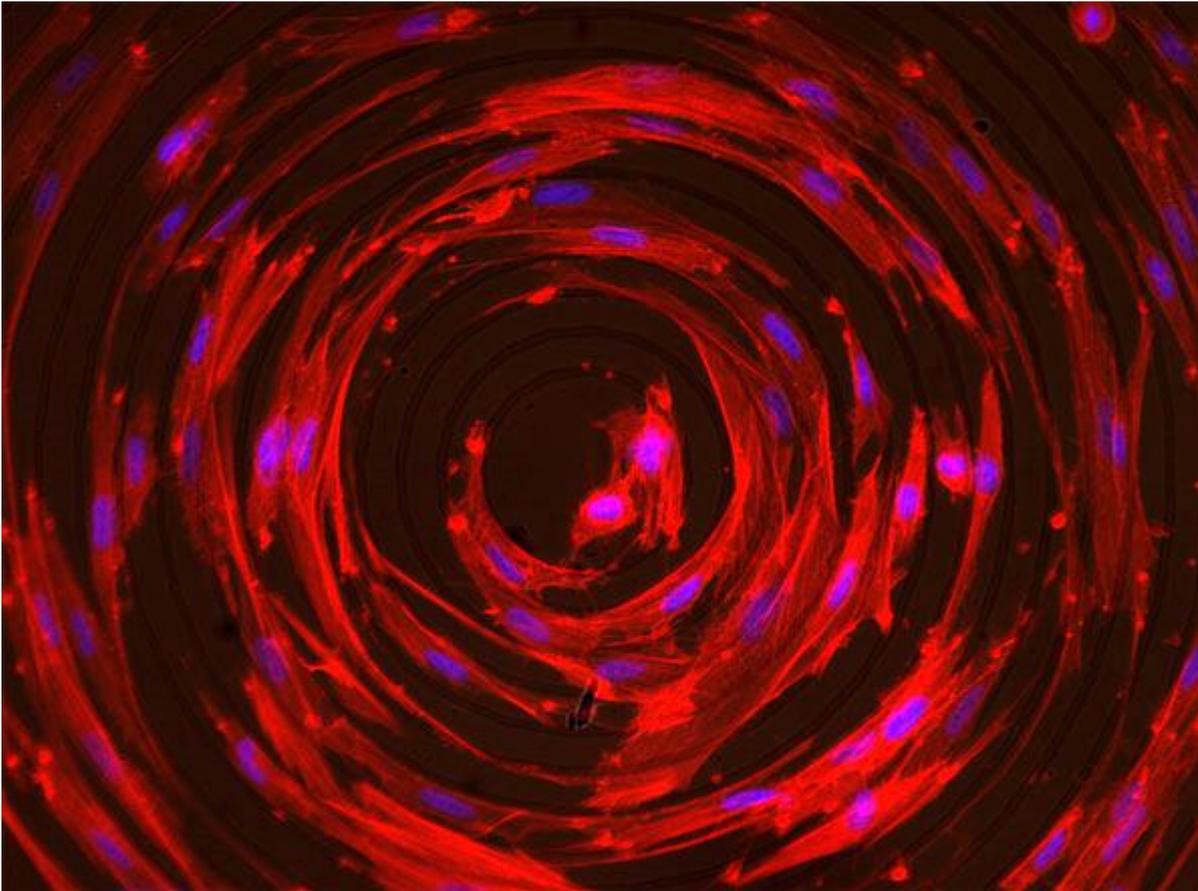


Contact inhibition of locomotion is the process by which cells in vitro change their direction of migration upon contact with another cell



Contact Guidance

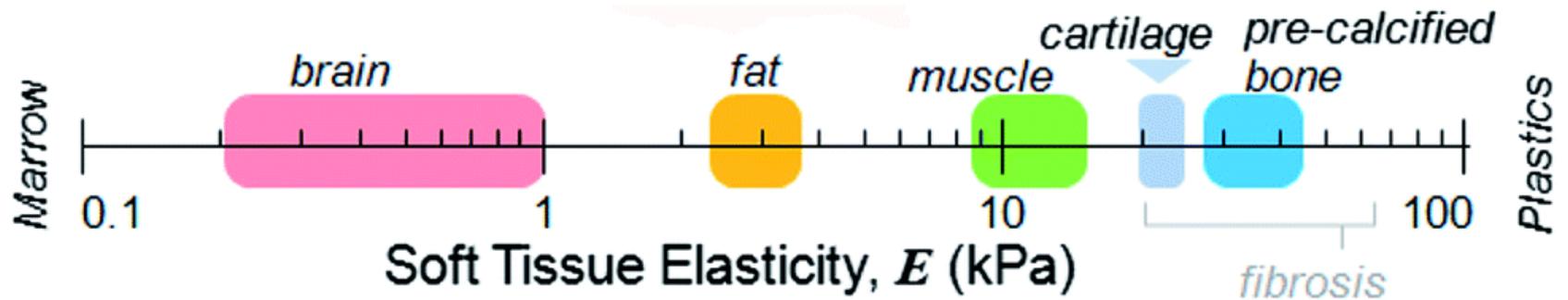
- Various cell types display contact guidance when cultured on groove and ridge patterns with lateral dimensions in the micrometer range. Muscle cells, neurons, epithelial cells.



Human fibroblasts growing on a circularly patterned elastomer surface. The cells aligned themselves along the edge of the rings, which served as the contact guidance. The cells were stained with Rhodamine-Phalloidin and DAPI <http://microscopy.berkeley.edu>.

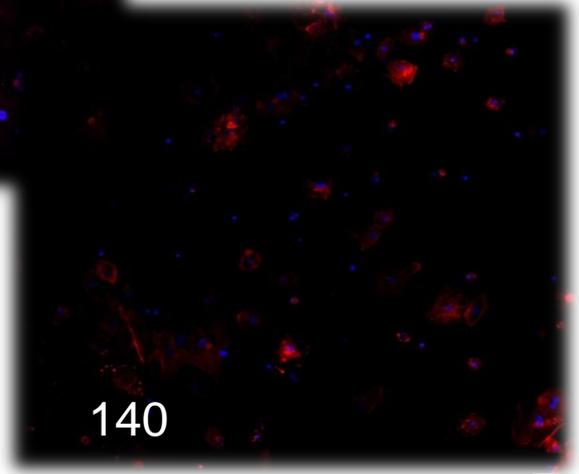
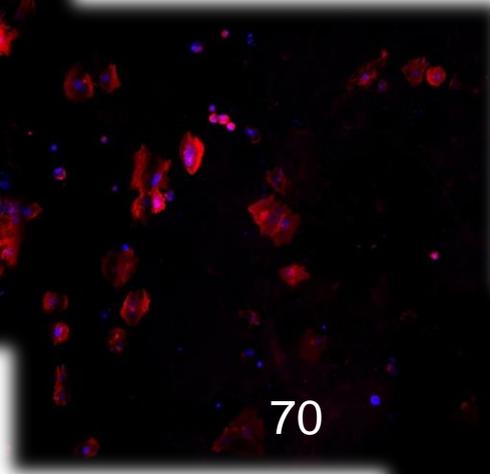
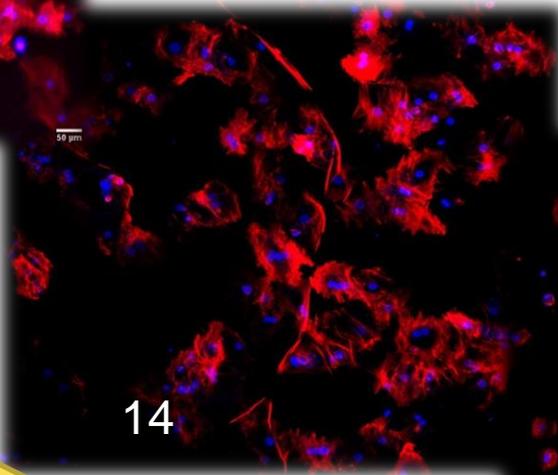
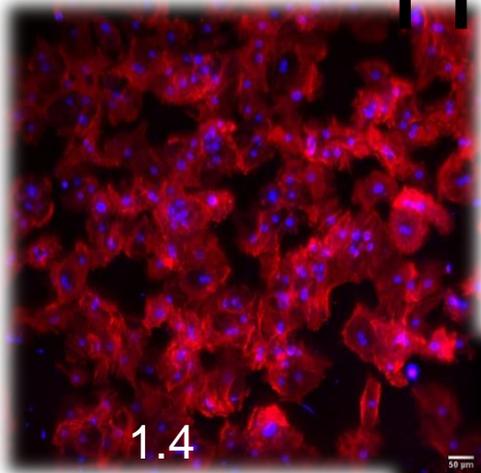
Durotaxis/Durosensing

The range of elastic moduli found in tissues

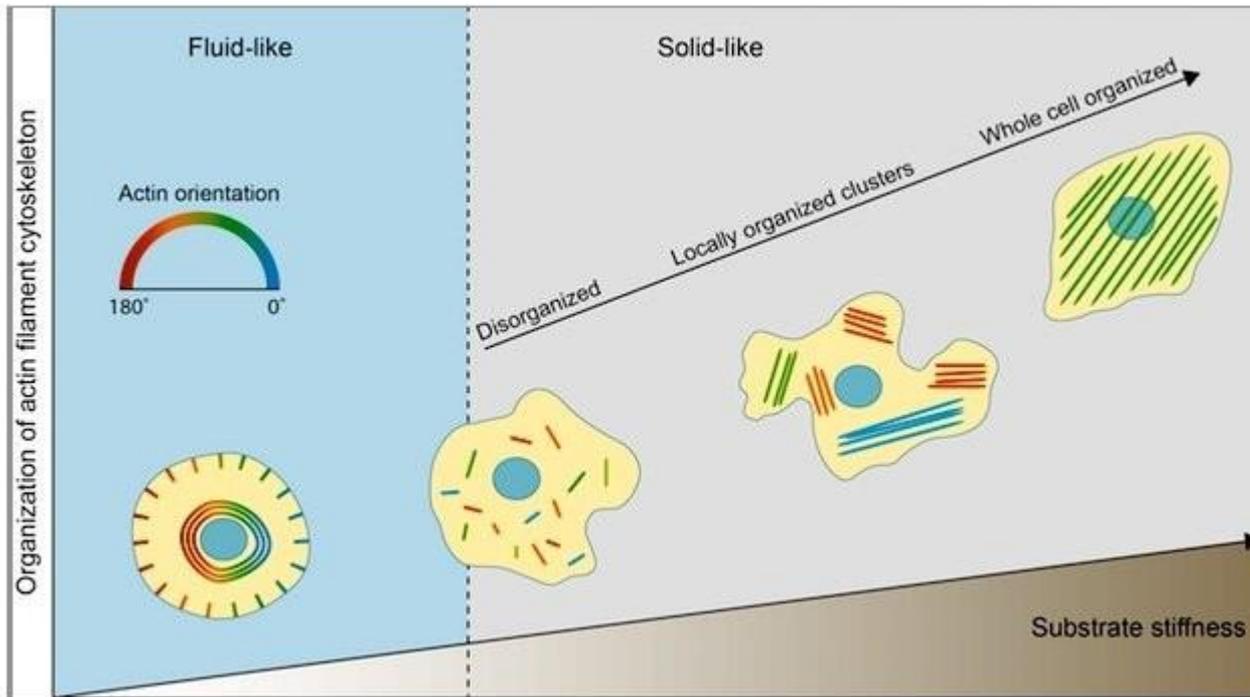


NB: these are estimates- all biological tissues are viscoelastic

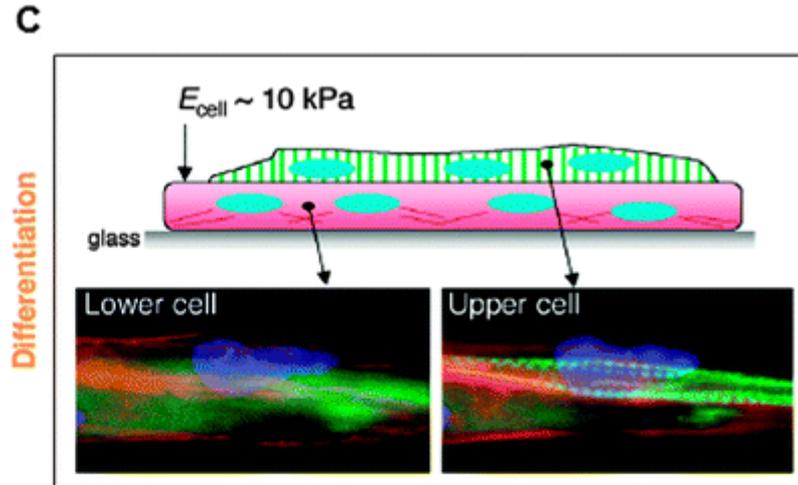
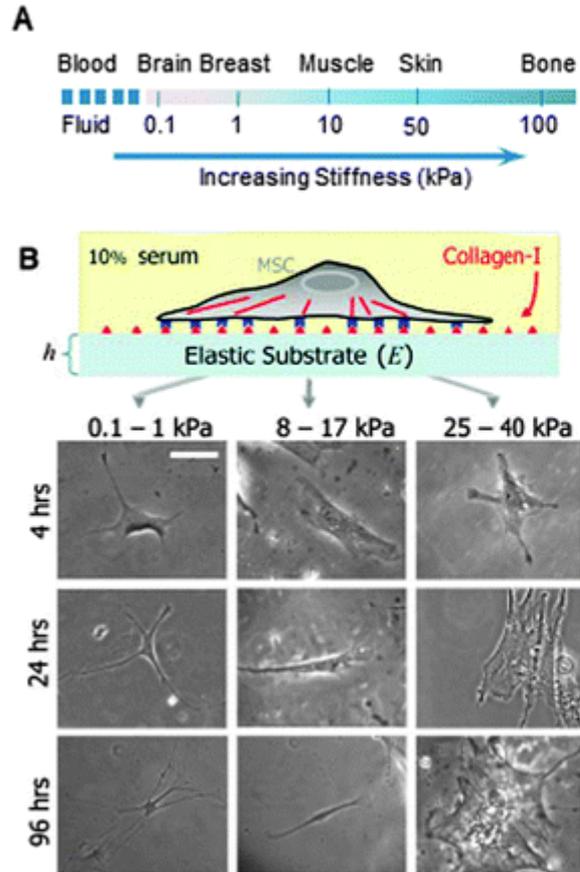
Human hepatocytes

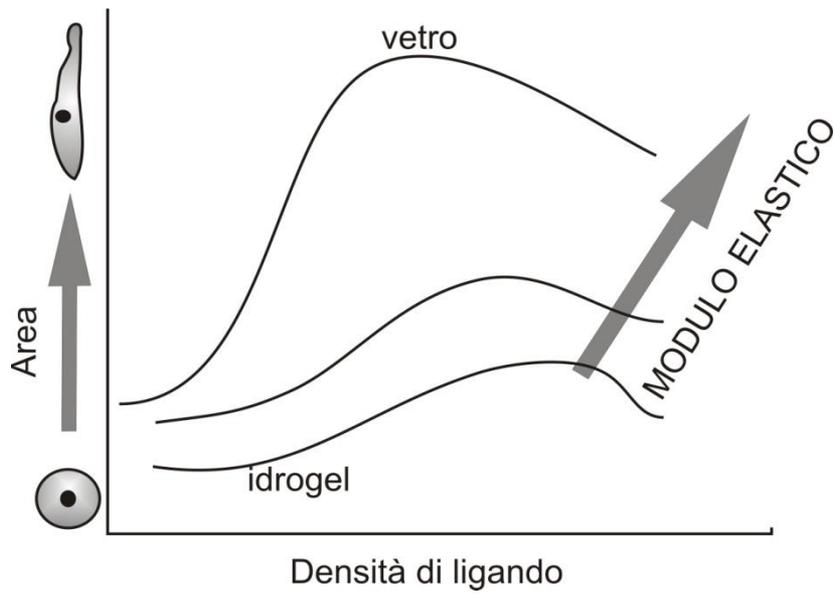
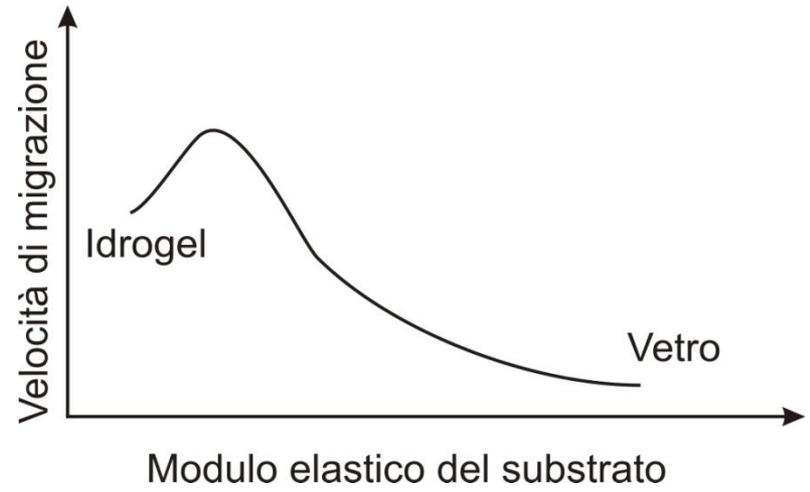


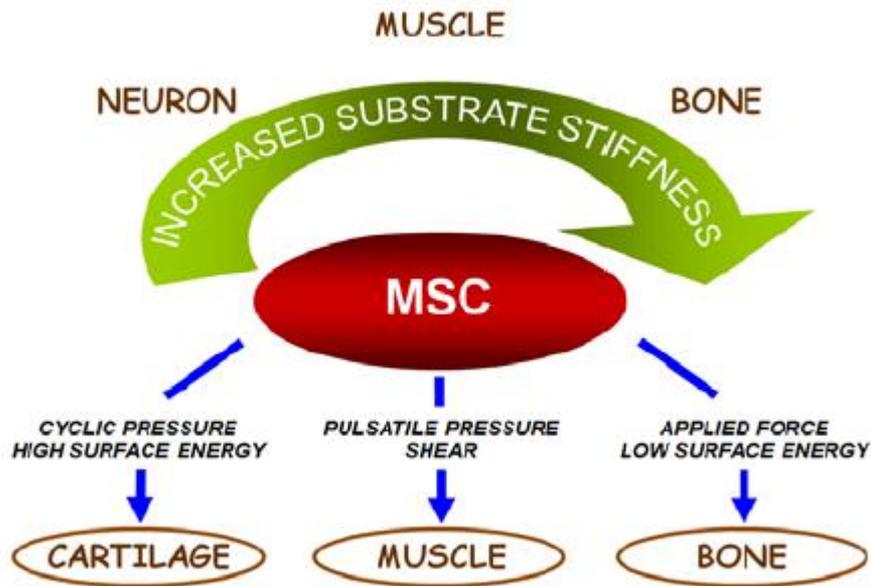
Substrate elastic modulus in kPa



Adam Engler

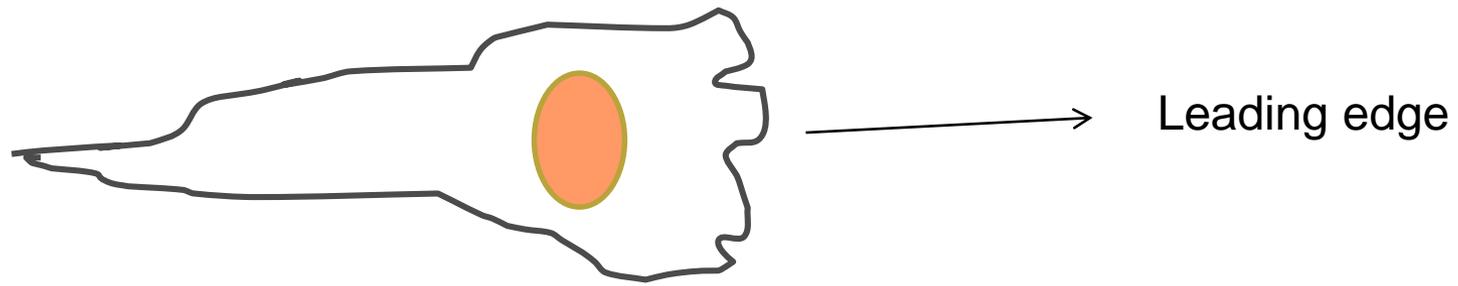
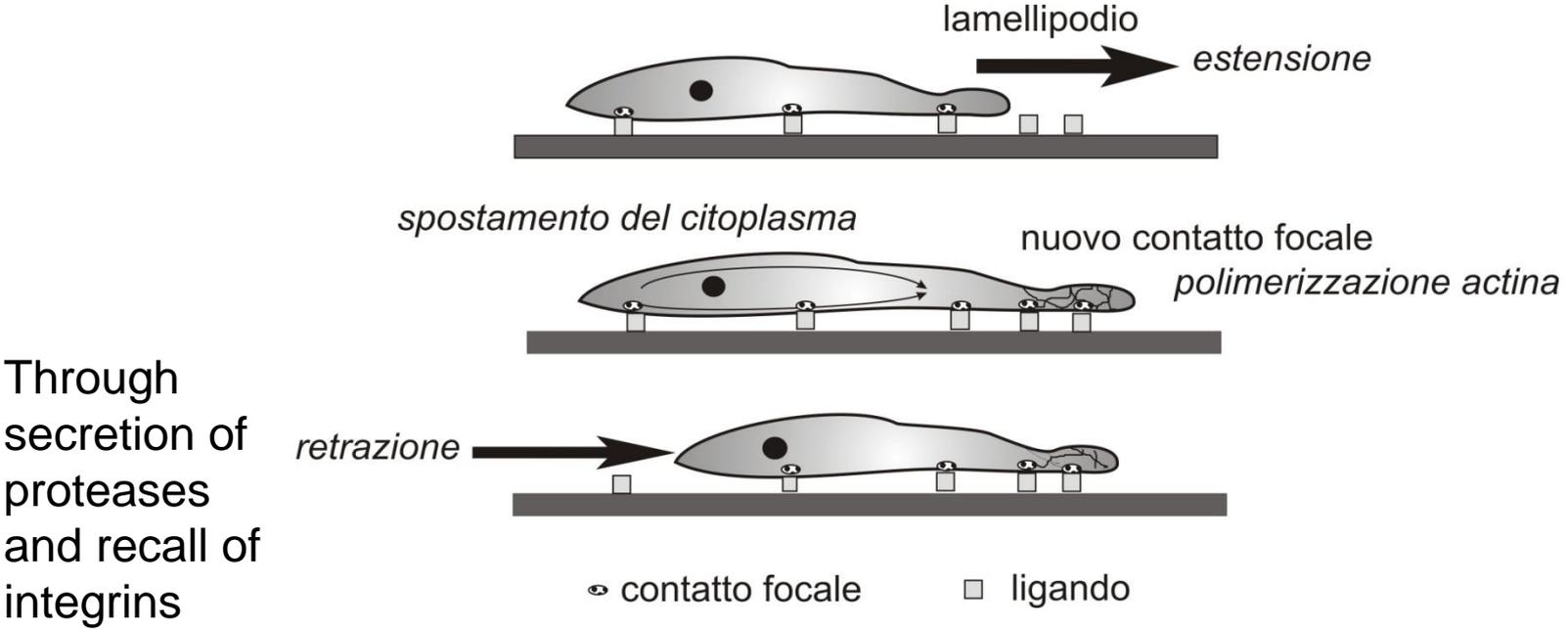






Stem cells are sensitive to physical stimuli: MSC (Mesenchymal stromal cells) can differentiate towards different phenotypes depending on the type of stimulus

Migration



Migration

- Cells need adhesion for traction
- Should not be too strong (stick), or weak (slip)
- Remember most studies performed in 2D but
- Cells live and move in 3D

Migration

- When observed over **long** times in the absence of gradients, cells move similar to Brownian particles (change in direction due to elastic collisions).
- Over short periods cells move in one direction. This is known as “persistence”. No instantaneous momentum transfer but mechanisms which require energy: attachment, contraction, cytoskeletal organization, binding, unbinding.

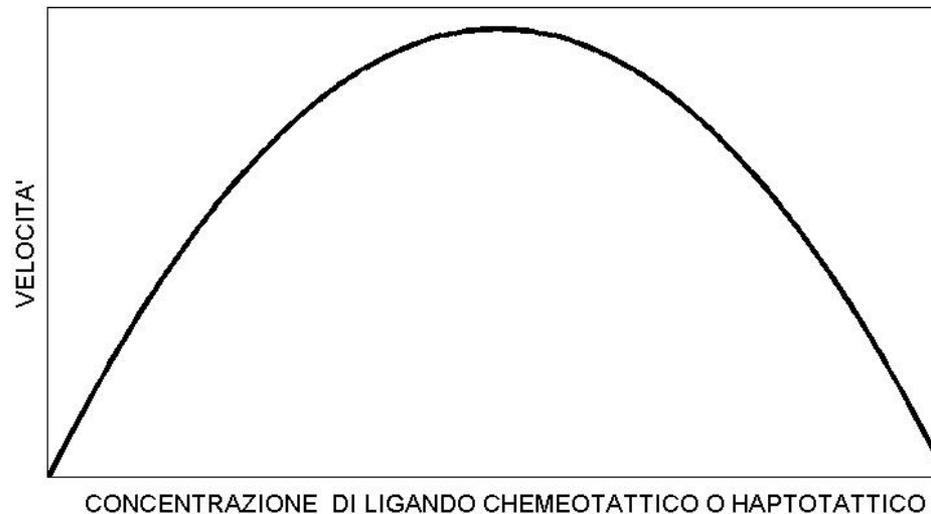
Motility

S=velocity

P=persistence time= time required for significant changes in direction

Cellula	Funzione	s (velocità)	P (tempo di persistenza)
Neutrofili	Fagocitosi batteri	20 $\mu\text{m}/\text{min}$	4 min
Linfociti	Distruzione cellule	4 $\mu\text{m}/\text{min}$	20 min
Macrofagi	Sviluppo antigeni	2 $\mu\text{m}/\text{min}$	30 min
Cellule endoteliali	angiogenesi	0.5 $\mu\text{m}/\text{min}$	4-5 ore
Cellule embrioniche	morfogenesi	0.16 $\mu\text{m}/\text{min}$	
Fibroblasti	Guarigione	0.5 $\mu\text{m}/\text{min}$	1 ora

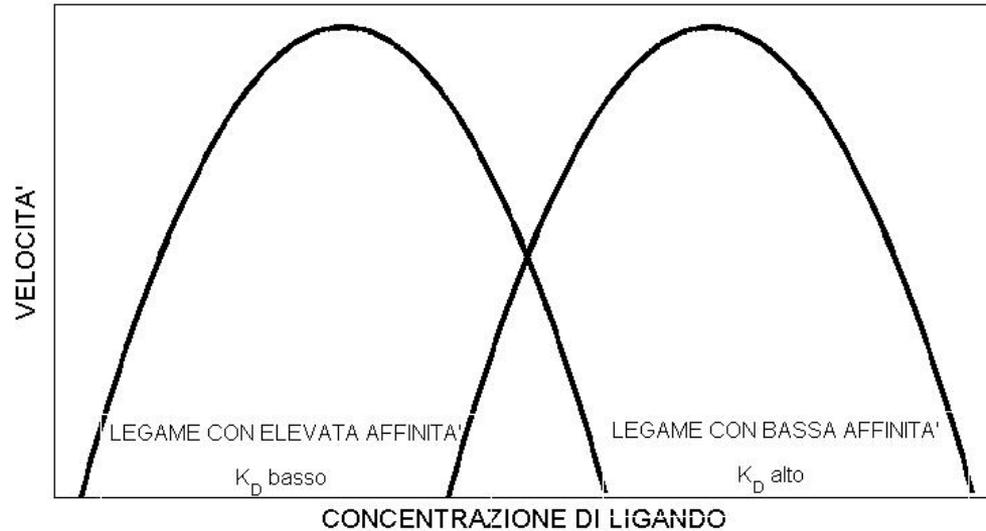
Velocity/motility vs. ligand conc



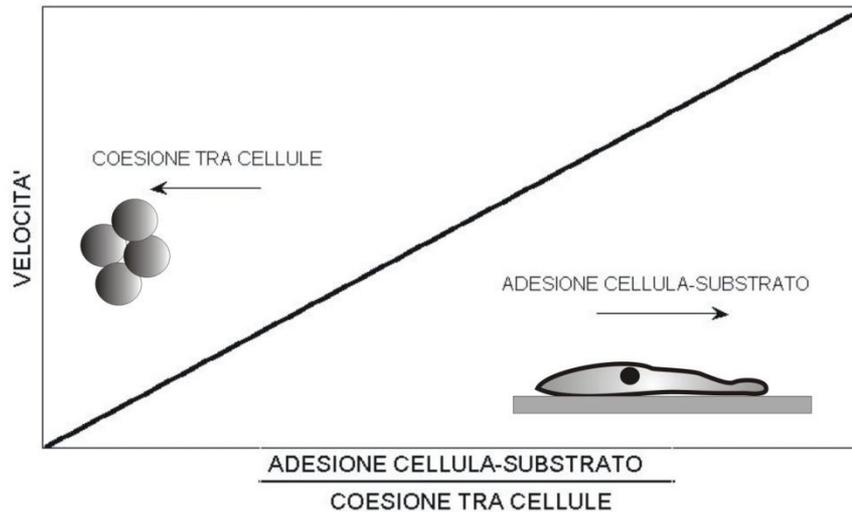
Andamento bifasico.

1. Down regulation
2. Substrato troppo appiccicoso

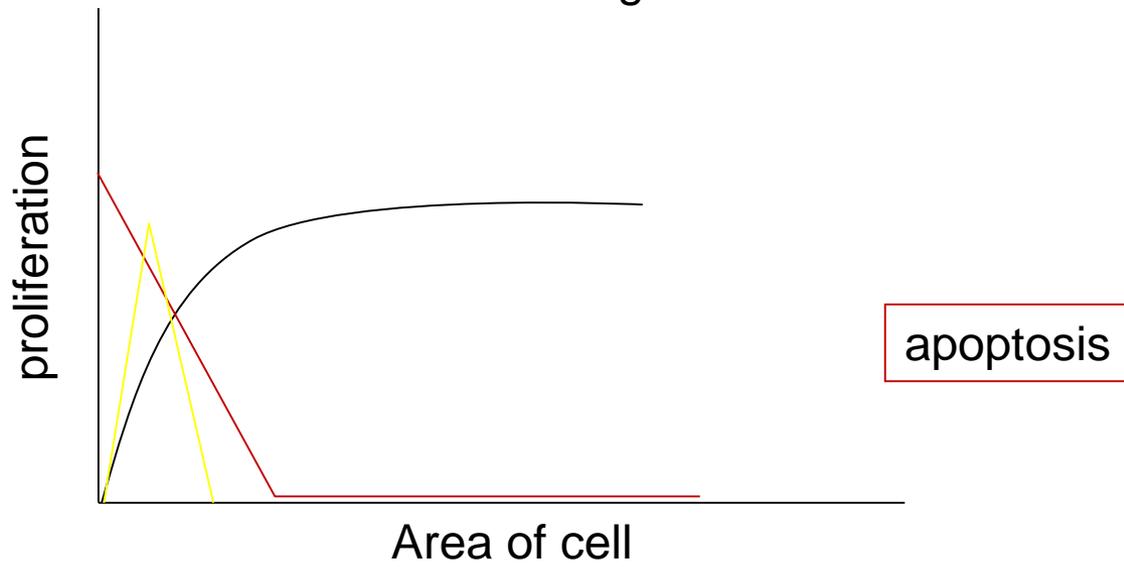
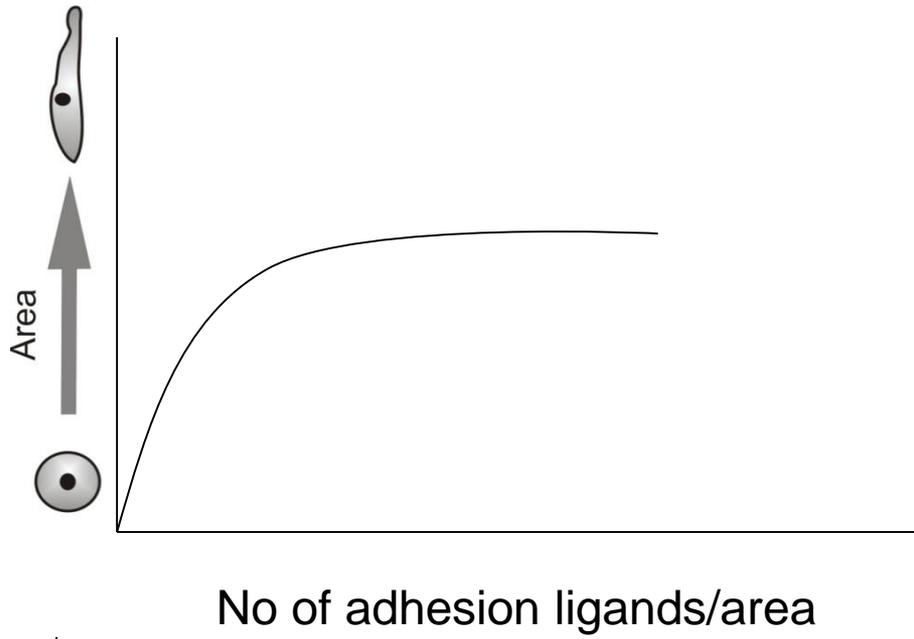
Velocità e concentrazione di ligando adesivo



Motilità cellulare in funzione dell'adesività e affinità di una superficie. Se il legame integrina-ligando (o recettore-ligando chemotattico) ha un'elevata affinità, basta una piccola concentrazione per aumentare la velocità e saturare. Invece se il valore di K_D è elevato, sono necessarie più ligandi per stimolare la migrazione.



Motilità cellulare in funzione del rapporto adesività del substrato/coesività cellulare. Se le cellule sono coesive tendono a migrare meno. Invece cellule che interagiscono con la matrice sono più motili, e più sensibili a gradienti haptotattici.



Le forze microscopiche

Forze intermolecolari

Interazione elettrostatiche

Interazione VDW e legami idrogeno

Idrofobiche –tensioni superficiali

Entropia

Energia= KT , a 37 °C calcolare l'energia in pN. Nm

Forze biomolecolari specifiche

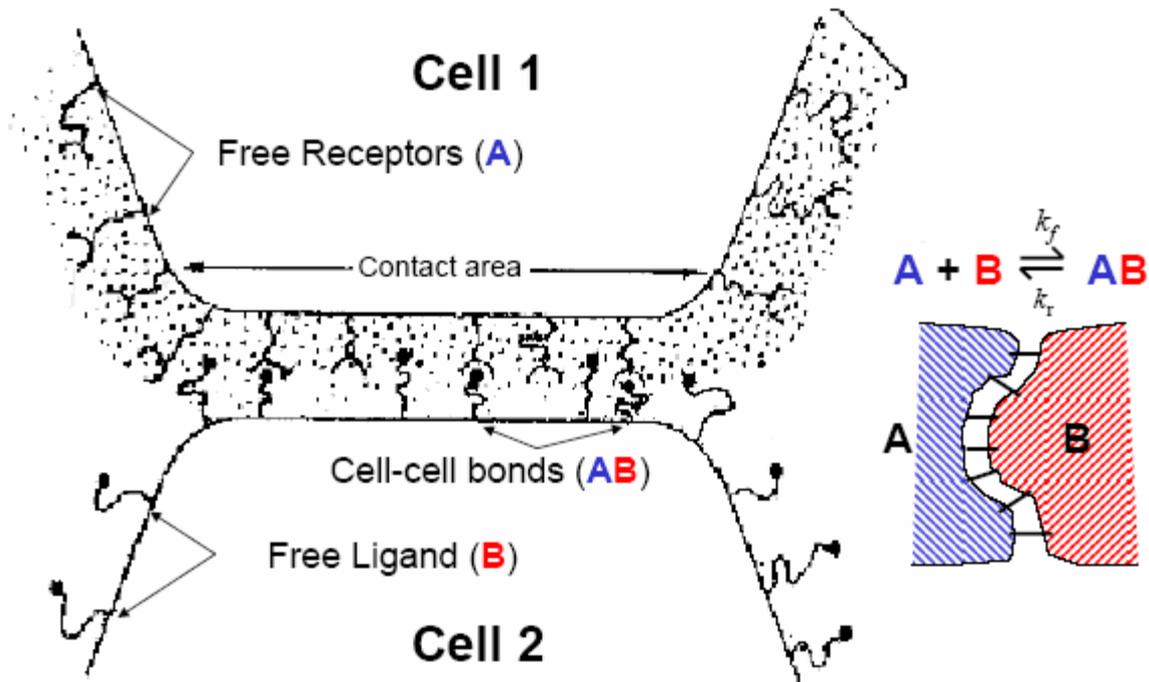
Forze meccaniche: adesione di cellule in presenza di forze di shear

Migrazione cellulare

Deformazione e orientazione

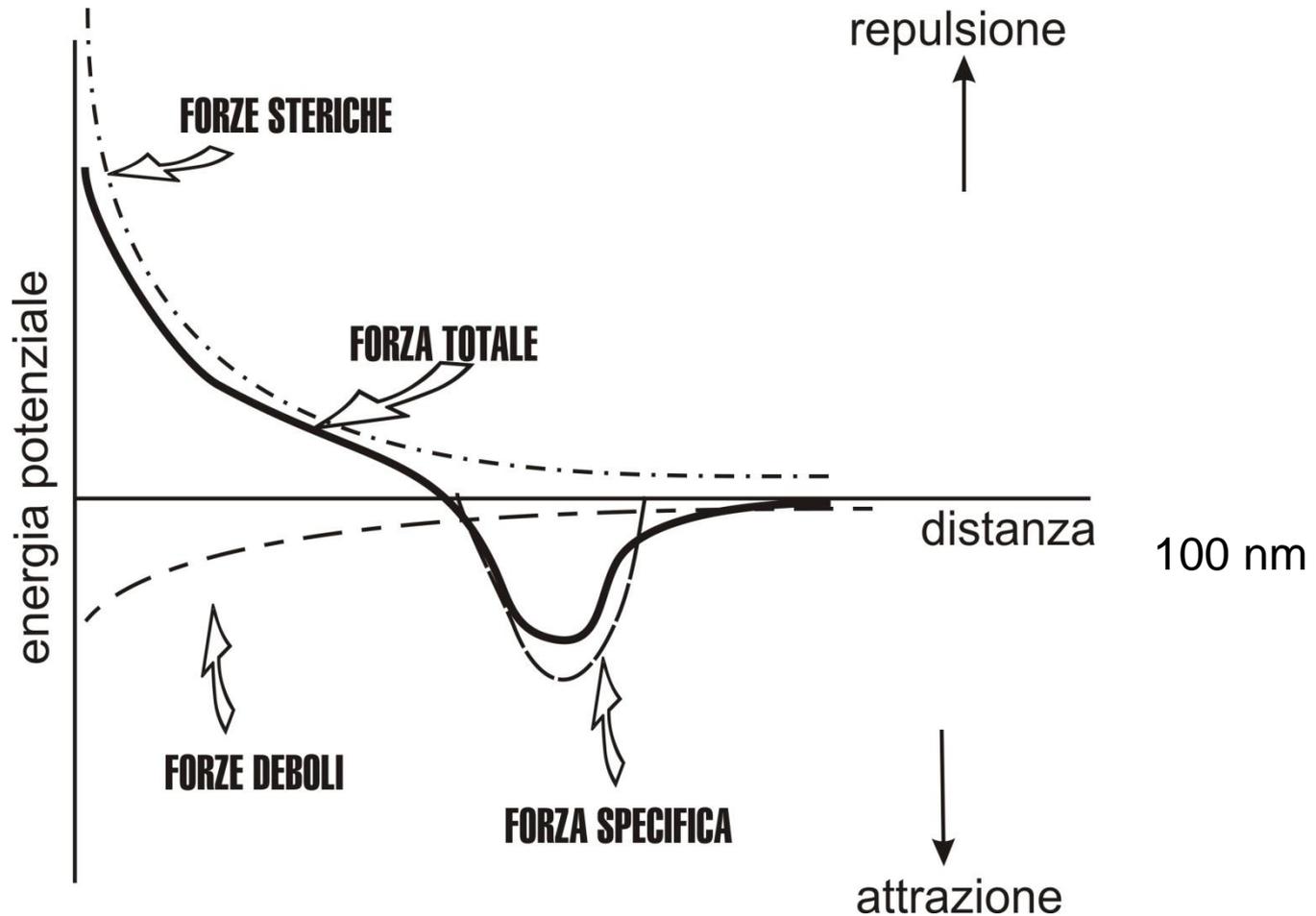
Contrazione muscolare

Forze esterne: viscosi, elastiche



Bell

Energia potenziale o energia libera in funzione della distanza dalla superficie di una cellula. Il buco di potenziale dovuto al legame Recettore-Ligando che si trova a circa 25 nm dalla superficie



Il modello di Bell- parte da Zhurkov

KINETIC CONCEPT OF THE STRENGTH OF SOLIDS*

S. N. Zhurkov**

ABSTRACT

An examination of the time to failure for uniaxial tensile specimens of some 50 materials, measured in some cases over ten decades of time, has suggested a universal rate relation between lifetime, stress, and temperature of the form $\tau = \tau_0 \exp [(U_0 - \gamma\sigma)/kT]$. The constant τ_0 is essentially the reciprocal of the natural oscillation frequency of atoms in the solid, U_0 is the binding energy on the atomic scale, and γ is proportional to the disorientation of the molecular structure. Assuming the kinetic nature of bond destruction through the thermofluctuation mechanism, direct experimental verification of the phenomenon for polymers has been obtained using electron paramagnetic resonance.

Int J, Fracture Mech, 1965

Il modello di Zurkhov

This result indicates that the localization of fracture in a crack does not substantially change kinetics of the fracture process, which also turns out to obey the principal equation (1) in this case. Hence it permits the evaluation of the limiting rate of crack growth, which will be determined by the condition that the bond fluctuation breakage possibility is unity. In this case, during one oscillation the tip of the crack will displace by an interatomic distance. Therefore, the maximum crack growth rate is $V \approx 10^{-8}/10^{-13} = 10^5$ cm/sec or 1 km/sec. This value is close to the velocity of sound in solids, and it is in good agreement with direct measurements of the crack growth rate carried out by a number of investigators.

The condition of reaching the limiting rate is set by the equality $\exp[-(U_0 - \gamma\sigma)/kT] = 1$ or $(U_0 - \gamma\sigma)/kT = 0$. It is seen from this condition that at a conventional testing temperature the maximum rate of fracture propagation is reached at $U_0 - \gamma\sigma = 0$. The rate of crack propagation under this condition will be characterized as a barrierless process which does not depend on temperature.

One deviation from Eq. (1), which is observed experimentally, should be noted. It concerns a variation of lifetime at very small stresses and a high temperature. Here a systematic deviation is revealed from the exponential law. For different solids, as shown in Figure 8, the log lifetime begins to grow faster with diminishing tensile stress than is specified by formula (1a). At a stress approaching zero, the lifetime becomes infinitely large. The reason for such deviation, which is common for different solids, has not yet been elucidated. One of the reasons consists undoubtedly in the reversibility of the process, that is, in the recombination of the ruptured bonds. One may expect that the reverse process will play an even greater role with the decrease of tensile stress. At a zero stress, the recombination of ruptured bonds will become equal to the rate of their rupture. The total bond rupture effect becomes zero, and lifetime under

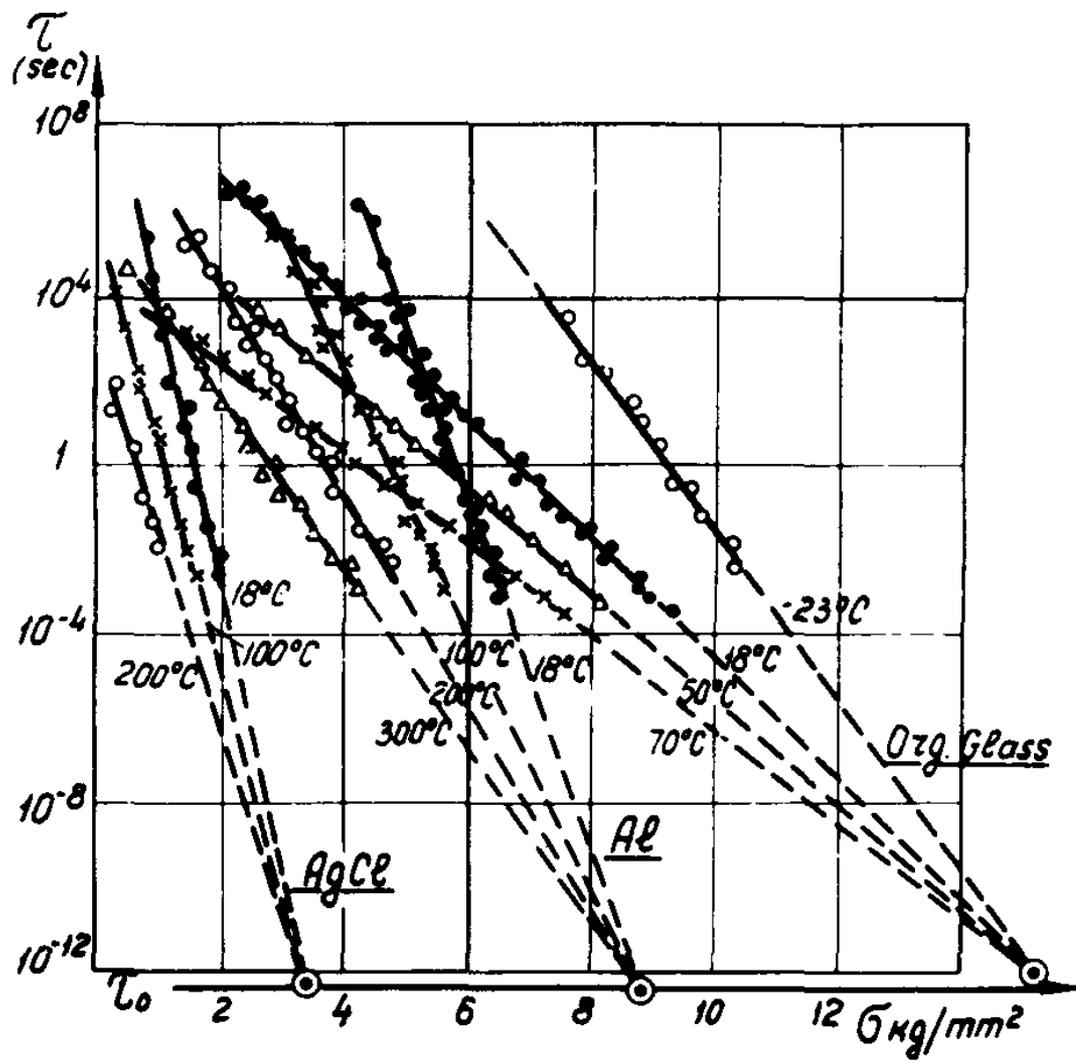


Fig. 5. Time and temperature dependence of the lifetime of solids on stress.

1. Silver chloride (Reference 4)
2. Aluminum (Reference 5)
3. Plexiglas (Reference 6)

S. N. Zhurkov

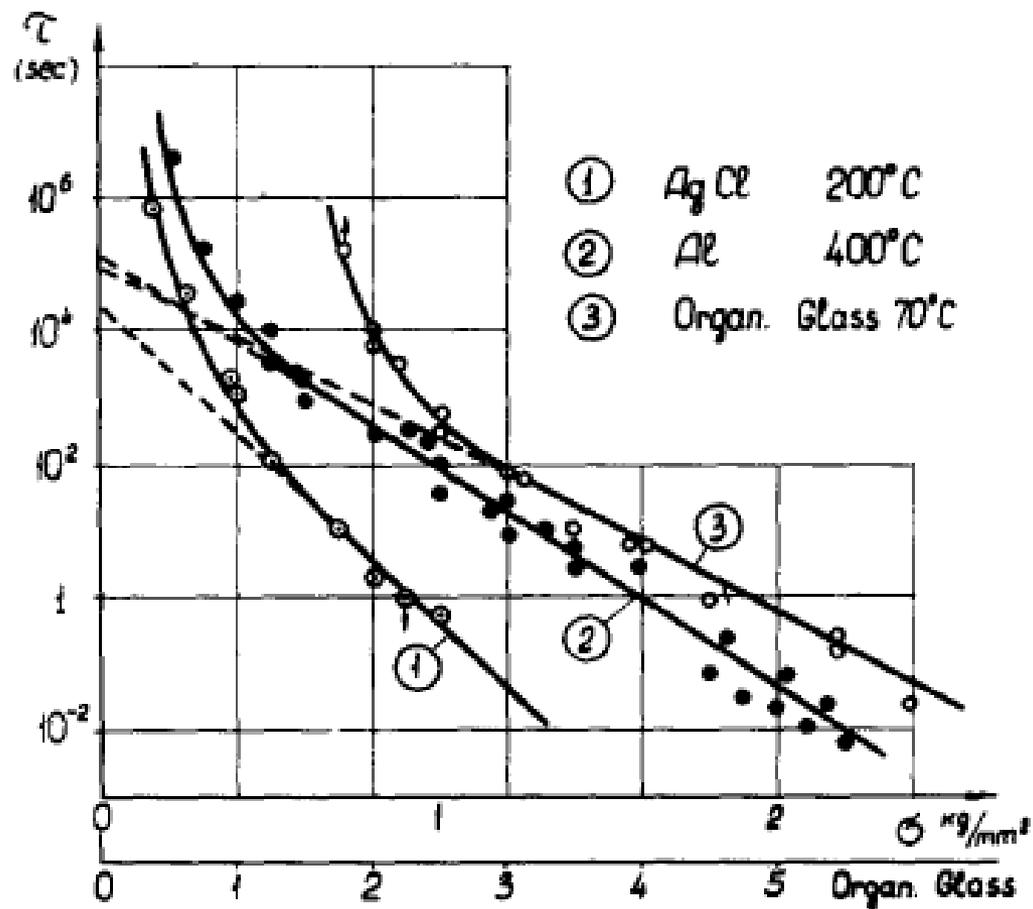
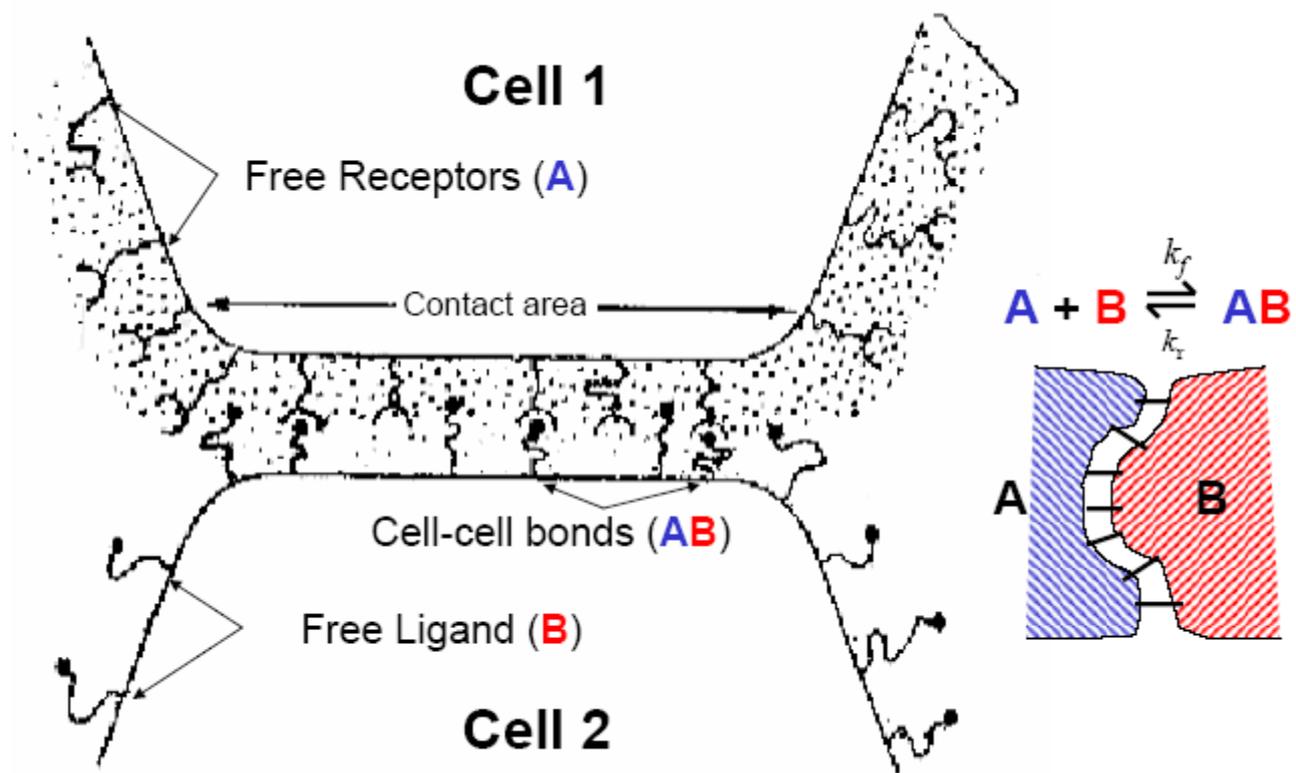
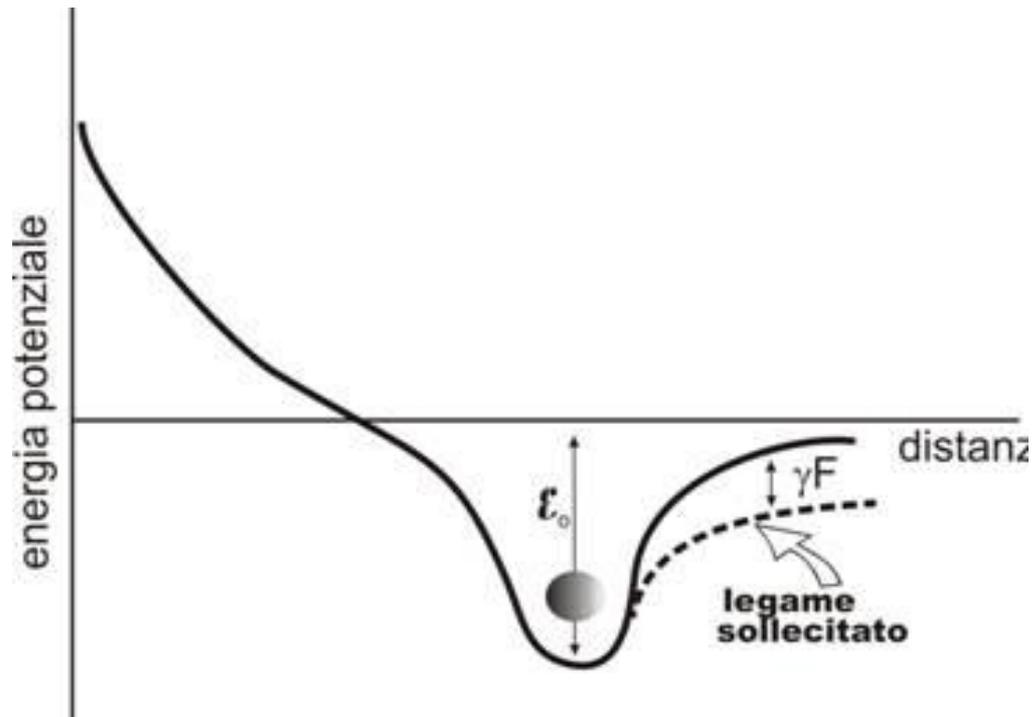


Fig. 8. Variation of the lifetime of solids at small stresses and high temperatures.



I. Bell et al., *Biophys. J.* 45:1051, 1984

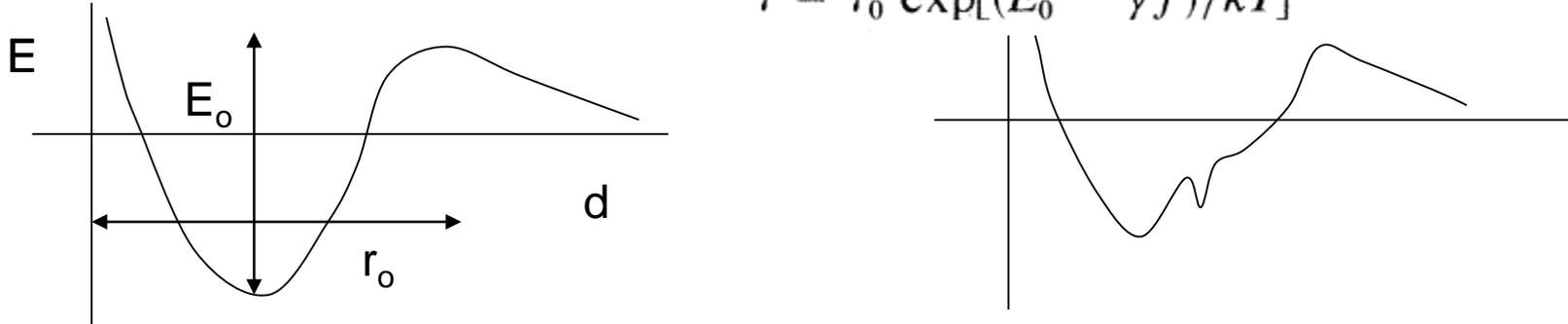
Abbassamento della barriera di energia potenziale in seguito alla sollecitazione del legame con a una forza pari a F .



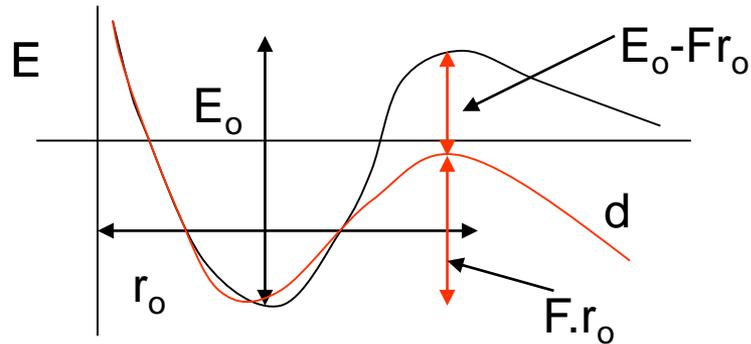
The Bell model

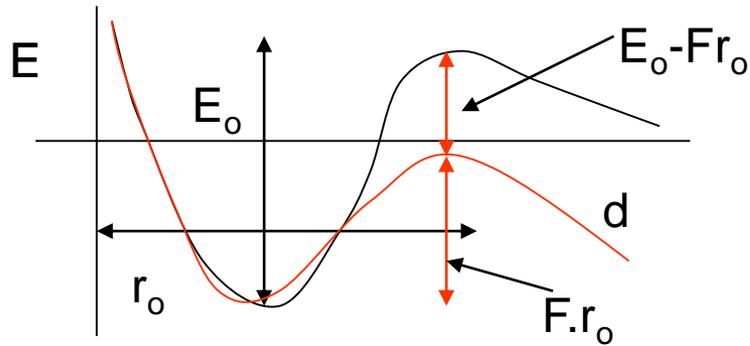
Bell (Science 1978, vol 200) had the insight to relate cell ligand binding kinetics to the lifetime of bond formation previously used in metals.

$$\tau = \tau_0 \exp[(E_0 - \gamma f)/kT]$$



In resting conditions the bond energy is E_0 . If a force is applied, the energy barrier moves down.





So now the energy required to break the bond is less, $E_0 - F.r_0$

Then the lifetime of an unstressed bond is $\tau = \tau_0 \exp(E_0 / KT)$

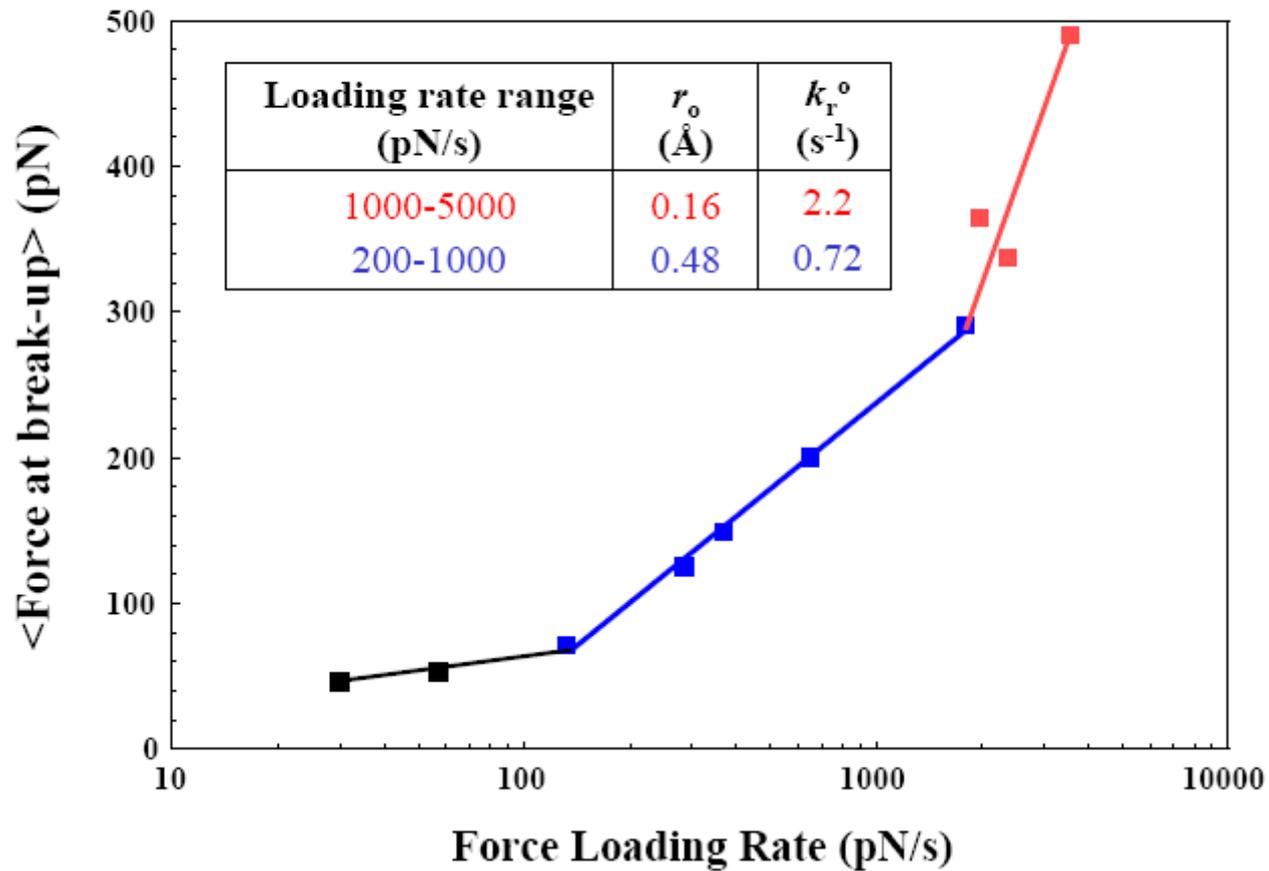
And when stressed $\tau = \tau_0 \exp([E_0 - F.r_0] / KT)$

According to Bell, now accepted and verified experimentally: $\tau \propto \frac{1}{k_r}$
 This means that the reverse reaction rate is proportional to the lifetime of a bond. High rate, low bond lifetime.

$$k_r = k_{r_0} \exp(F.r_0 / KT)$$

k_r reverse reaction rate, k_{r_0} reverse rate when unstressed. F force applied, r_0 bond length

Observed Force vs Loading Rate



November 24, 2015 | [Research](#) | [No comments](#)

Measuring what cells feel using the nano-epsilon dot method

When we walk on the sand, a trampoline or a concrete pavement, we can feel the hardness, softness and springiness of the ground and respond by changing the way we move. The way we respond depends on how heavy we are, as well as on the size of our feet. In the same way, cells in our body are also responsive to the mechanical features of the tissues around them, changing their behavior. A good example is that of aged tissues, which are harder and less compliant than younger ones. Cells on stiffer materials which resemble older tissue usually move and grow slowly. Some studies suggest that stiffer and less compliant tissues also inhibit tissue healing and regeneration.

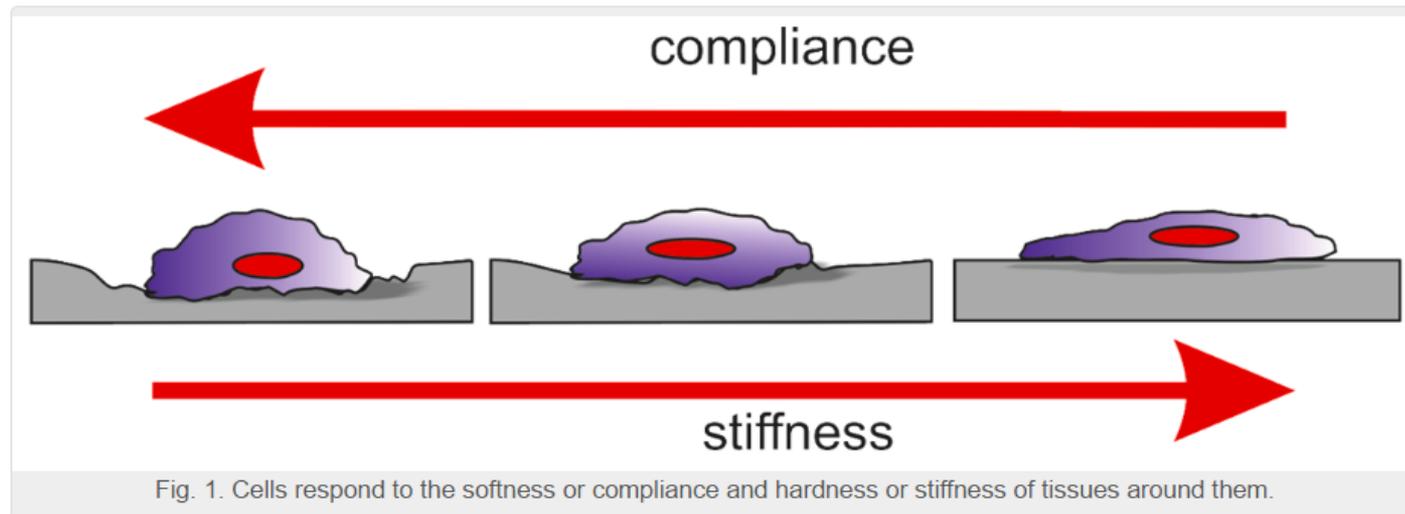
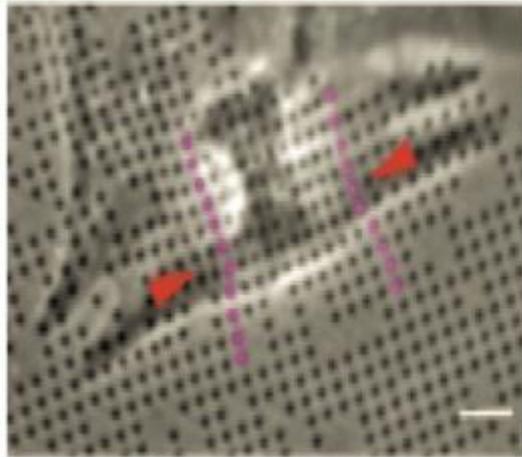


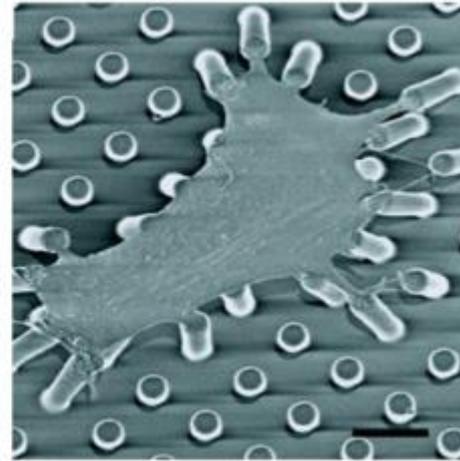
Fig. 1. Cells respond to the softness or compliance and hardness or stiffness of tissues around them.

To better understand what cells feel beneath them, it is important to determine the mechanical properties of tissues and materials at the same scales as cells themselves because larger scale measurements may miss local feature and characteristics. Biological tissues are typical biphasic materials in which a solid network made up of different components (e.g proteins, glycosaminoglycans) is swollen and surrounded by water. Their resultant mechanical behavior is therefore viscoelastic, that is in between that of purely

How do we measure cell forces?

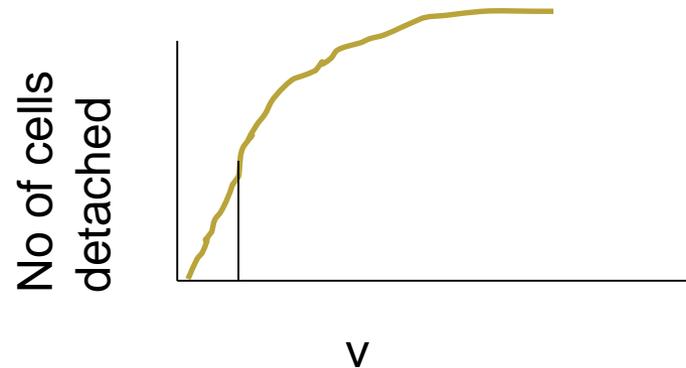
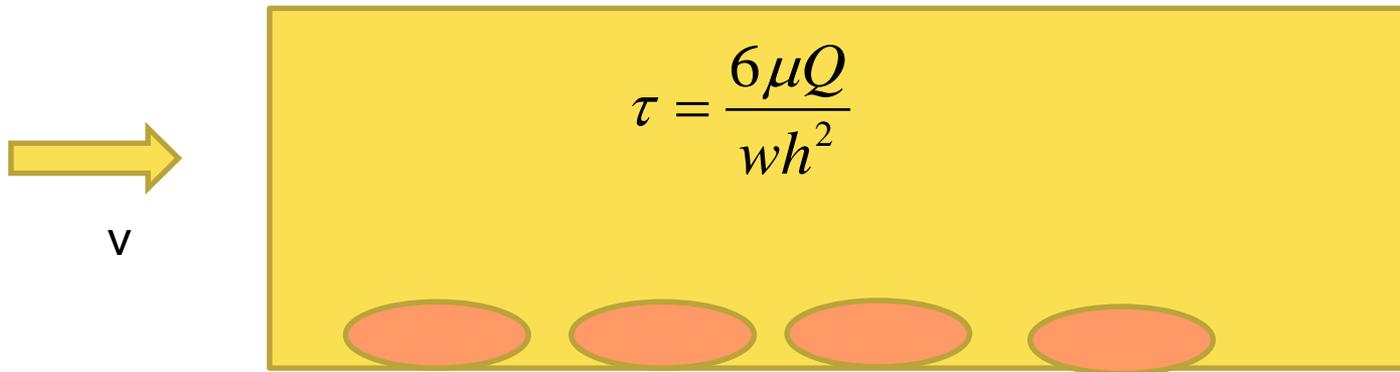


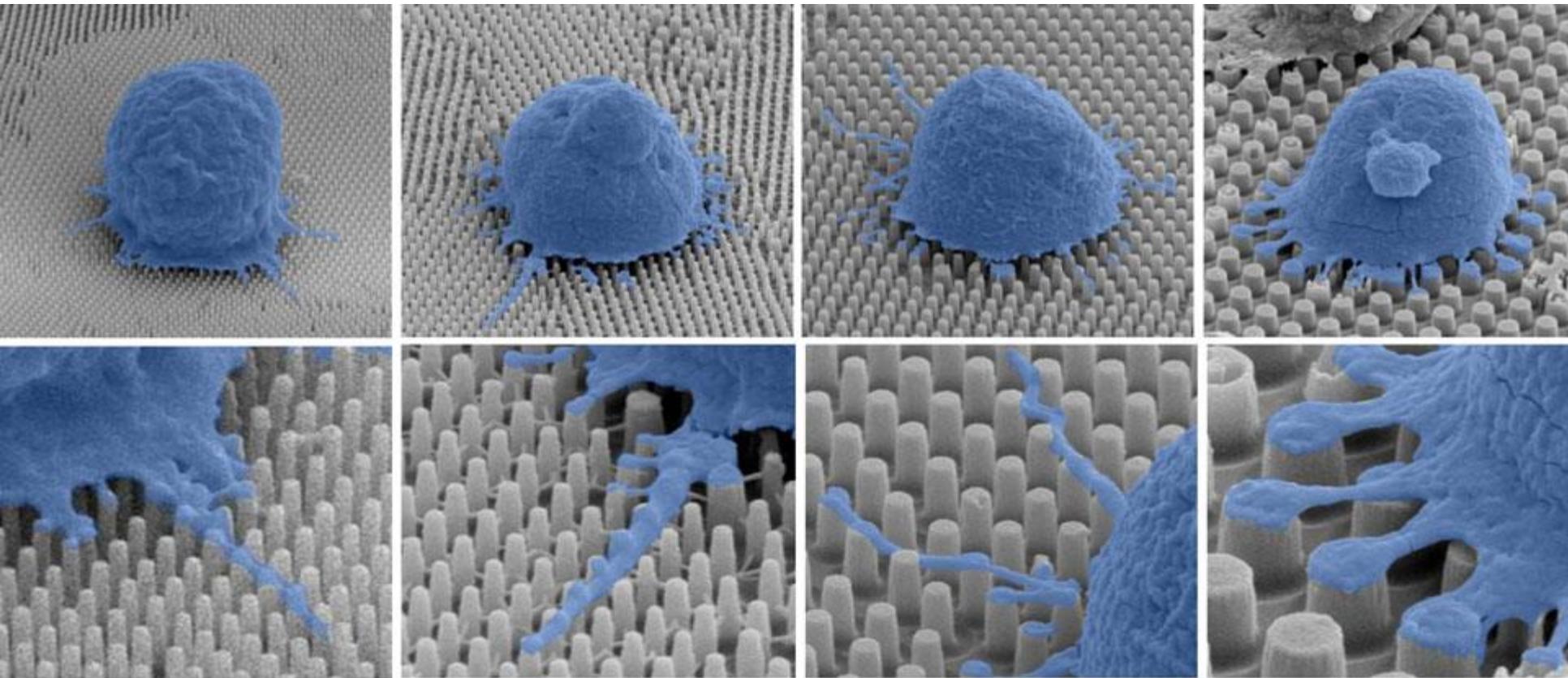
(a)



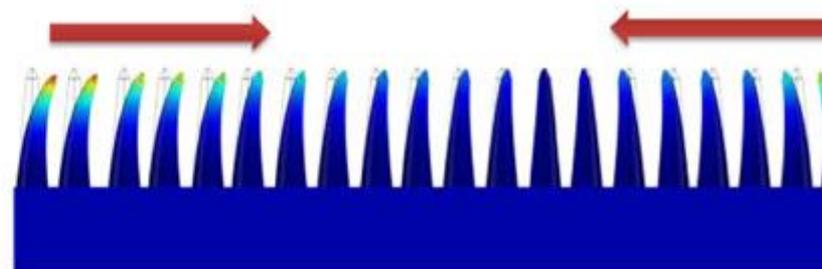
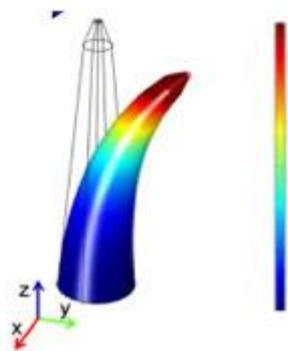
(b)

Laminar flow chamber





10-40 pN/integrin
10 microN/cell



NANOPILLARS/MICROPOSTS

Kim et al, Nanoscale Research Lett. 2013

Read these papers

- Sensors 2010, 10, 9948-9962;
doi:10.3390/s101109948
- Microsystems for cellular force measurement: a review
- The Forces Behind Cell Movement
- Review on Cell Mechanics: Experimental and Modeling Approaches

Answer these questions

1. What is the difference between cell traction and cell adhesion?
2. What is the magnitude of forces exerted by a cell on a substrate?