Mechanotransduction

Cells sense and exert mechanical forces

Mechanotransduction

- The process by which cells sense and respond to **mechanical stimuli** .

- Stress, shear, stiffness, viscosity, haptics….

Adhesion, Motility

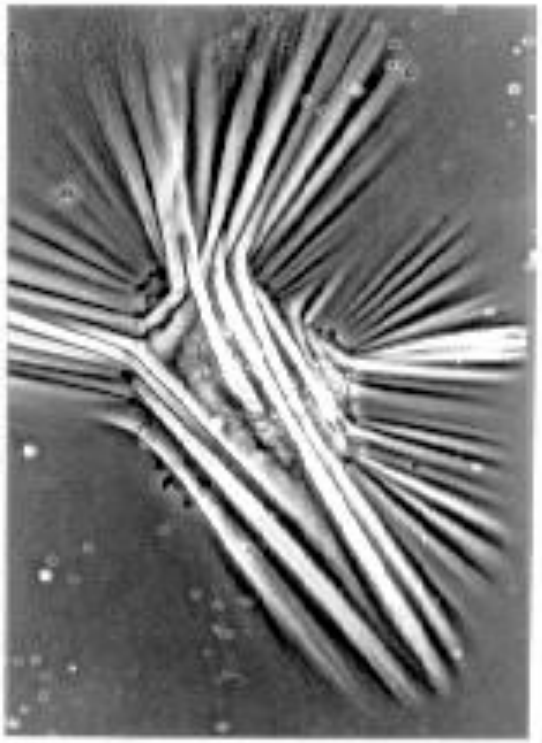
Adhesion and motility are closely linked and important for

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

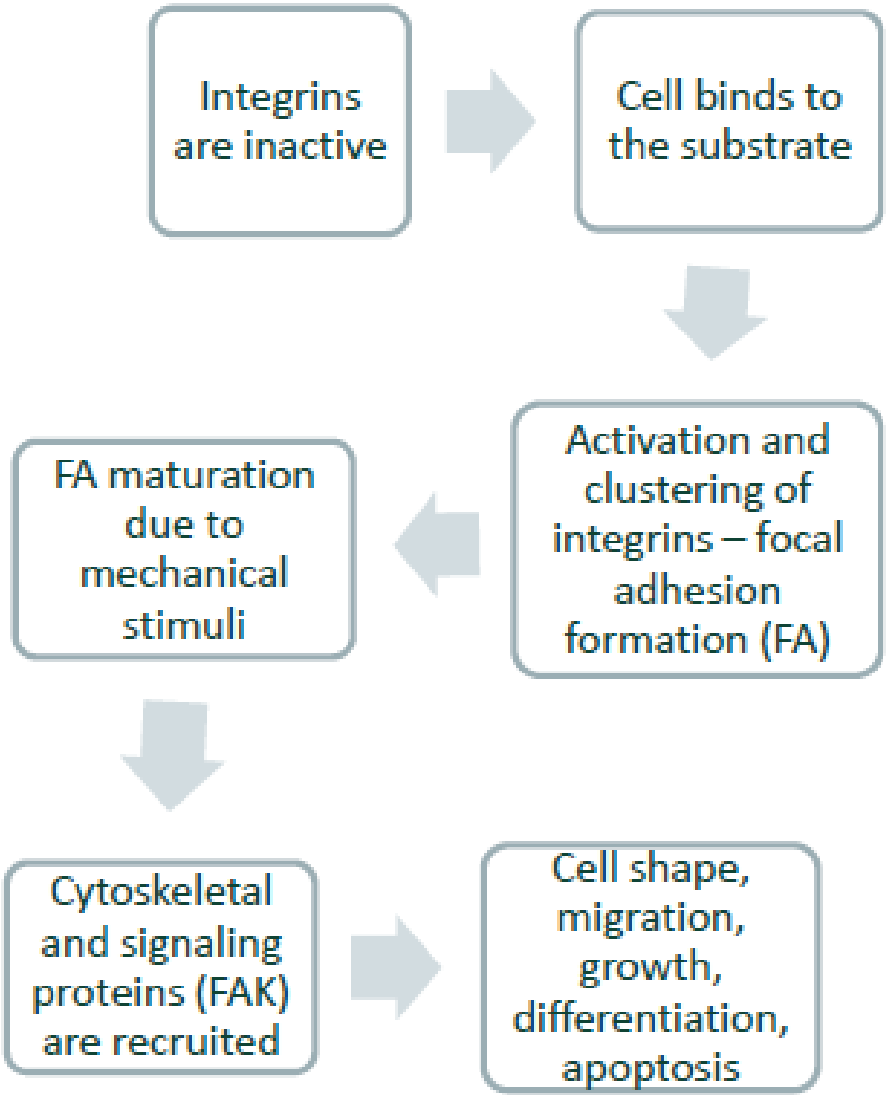
Adhesion

- 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)

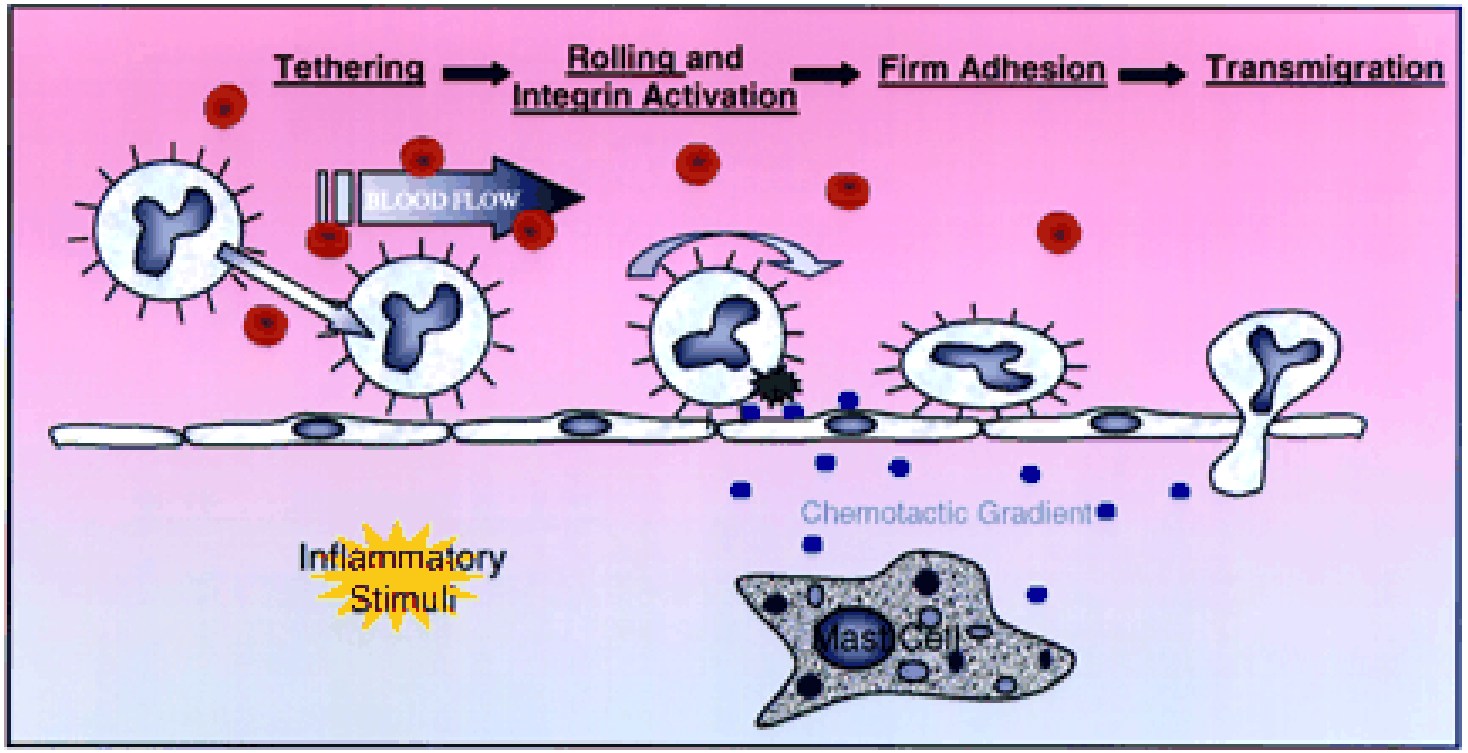


This is mechanobiology-

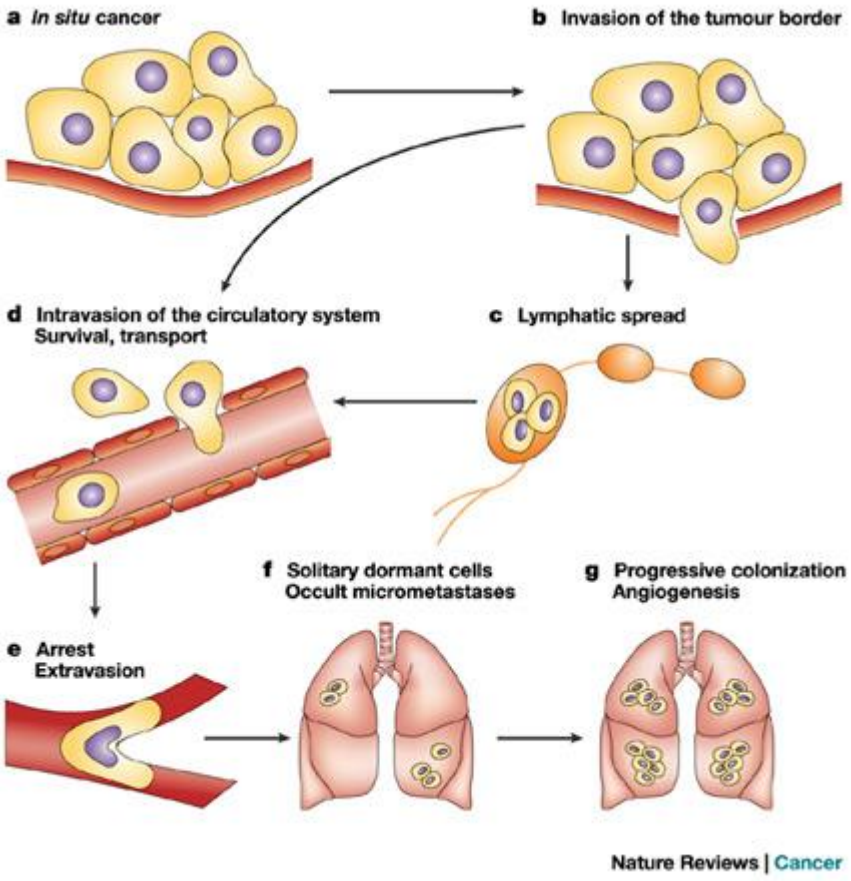


Form
Function
Phenotype

Inflammatory response

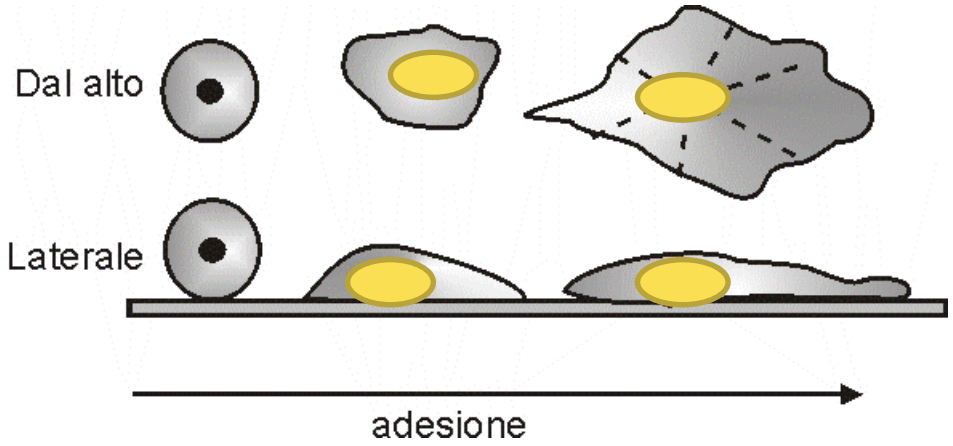


Metastasis


a In situ cancer
b Invasion of the tumour border
c Lymphatic spread
d Intravasion of the circulatory system
Survival, transport
e Arrest
Extravasion
f Solitary dormant cells
Occult micrometastases
g Progressive colonization
Angiogenesis
Nature Reviews | Cancer

Remember: Adhesion, motiltity, cell shape and phenotype are closely linked:
FORM-FUNCTION-PHENOTYPE
Adhesion and motility are^ known to be modulated by:

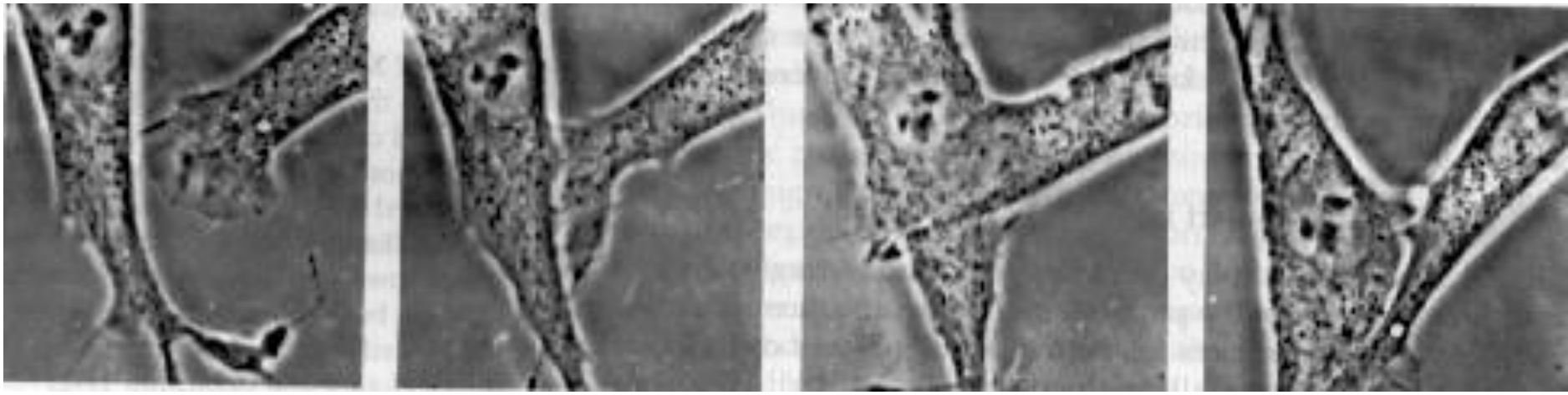
- **Chemotatic gradients (nutrients, growth factors etc)**
- **Haptotatic gradients (Adhesion ligands)**
- **Contact inhibition**
- **Galvanotaxis**
- **Contact guidance from a substrate**
- **Durotaxis**



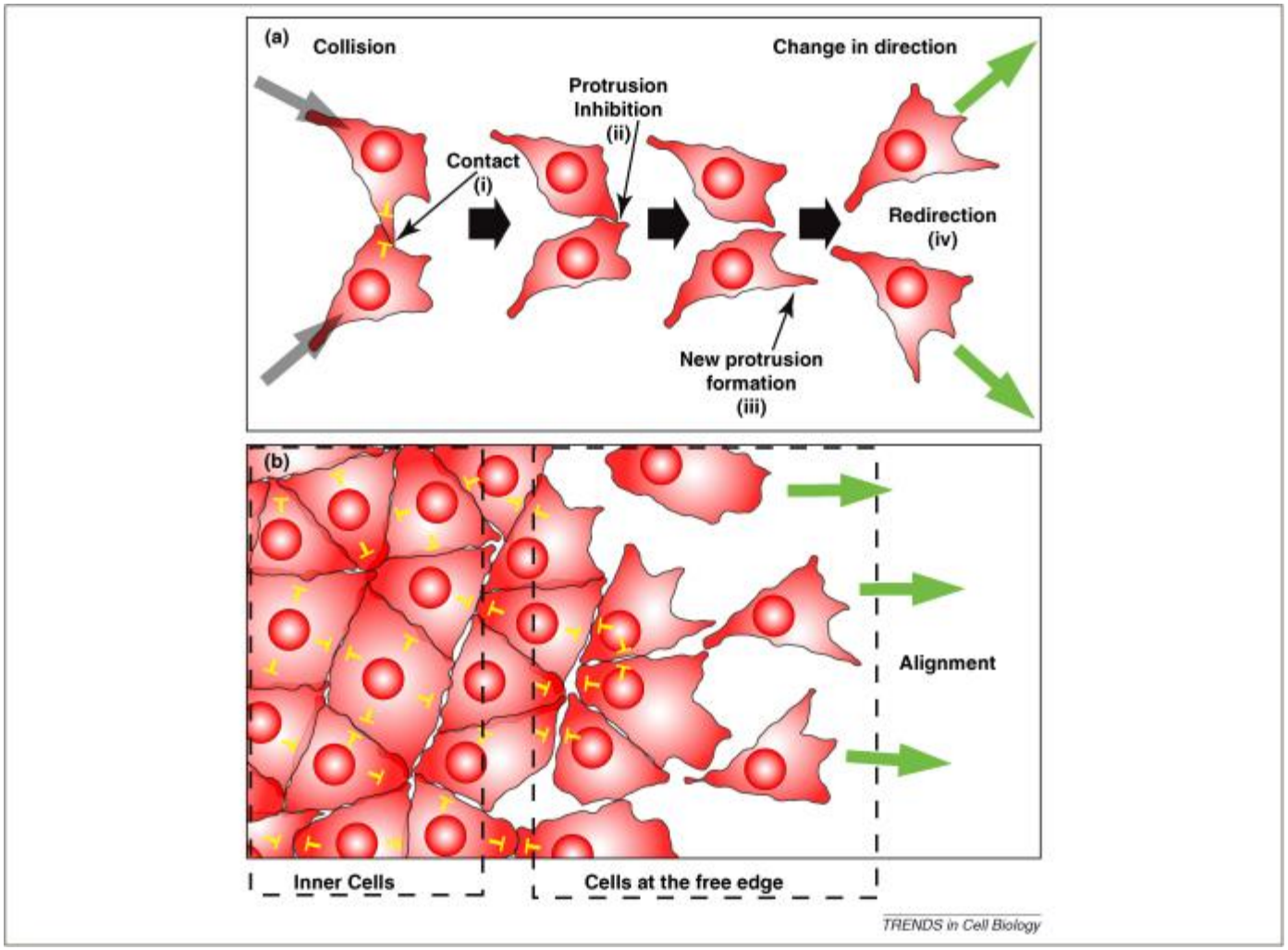
Cell shape changes with adhesion (and the nature of the ECM).

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.

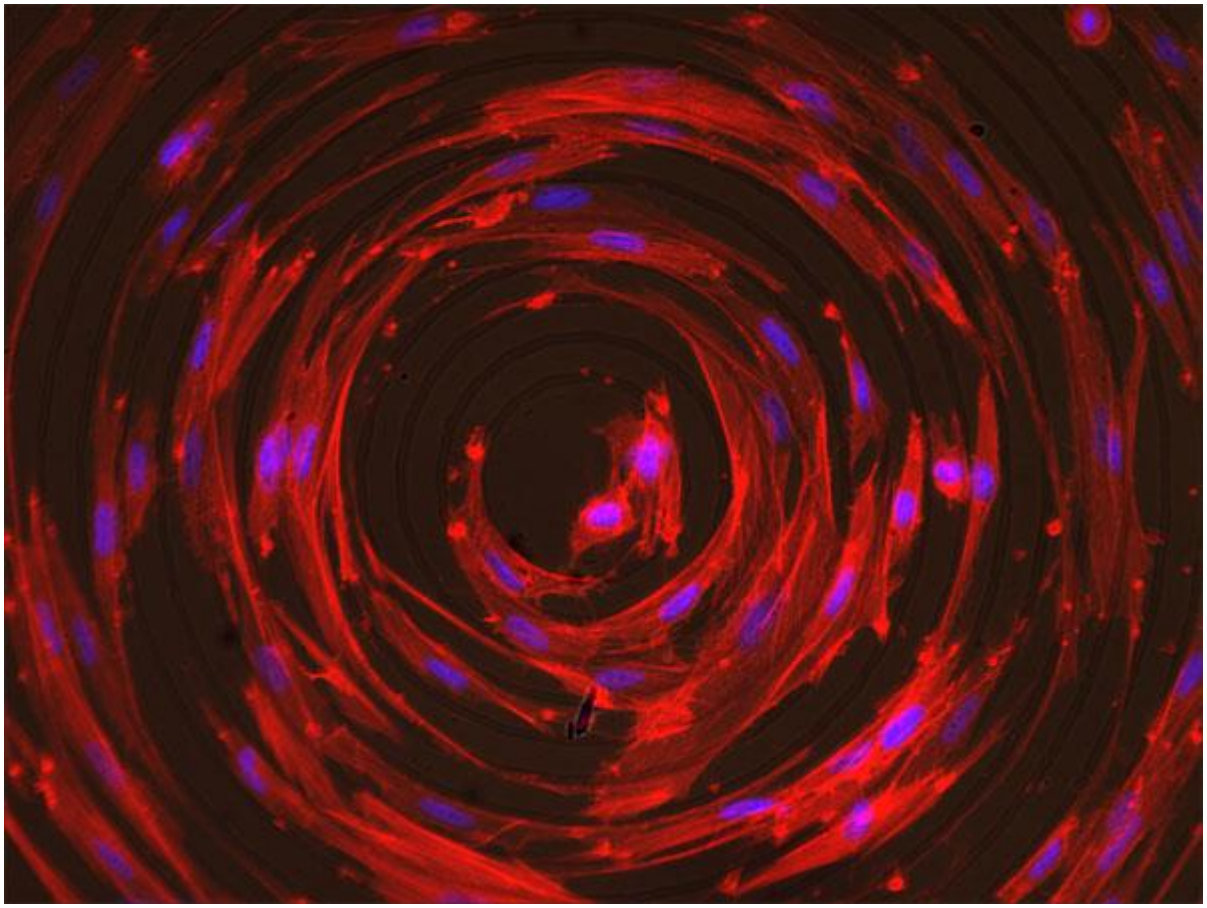


Contact inhibition of locomotion is the process by which cells in vitro change their direction of migration upon contact with another cell



Contact Guidance

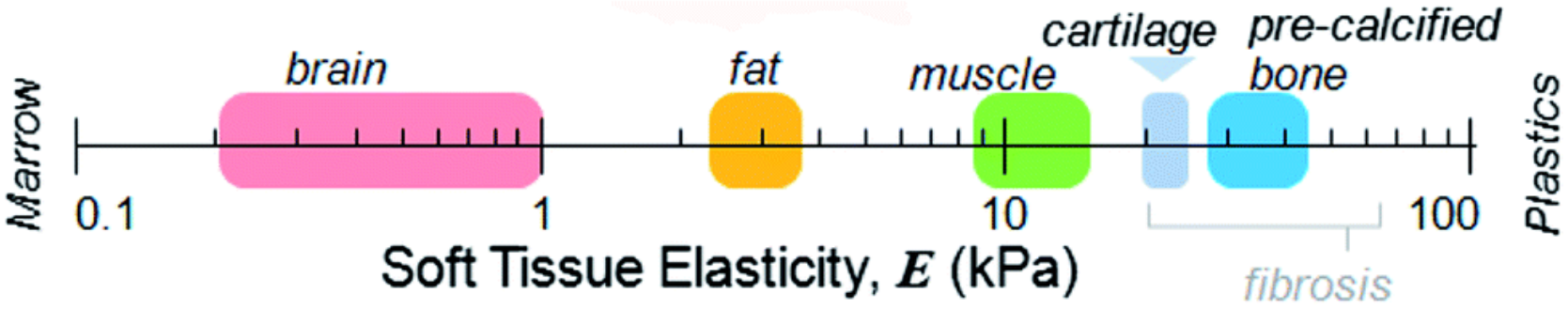
- Various cell types display contact guidance when cultured on groove and ridge patterns with lateral dimensions in the micrometer range. Muscle cells, neurons, epithelial cells.



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 Rhodamin-Phalloidin and DAPI http://microscopy.berkeley.edu.

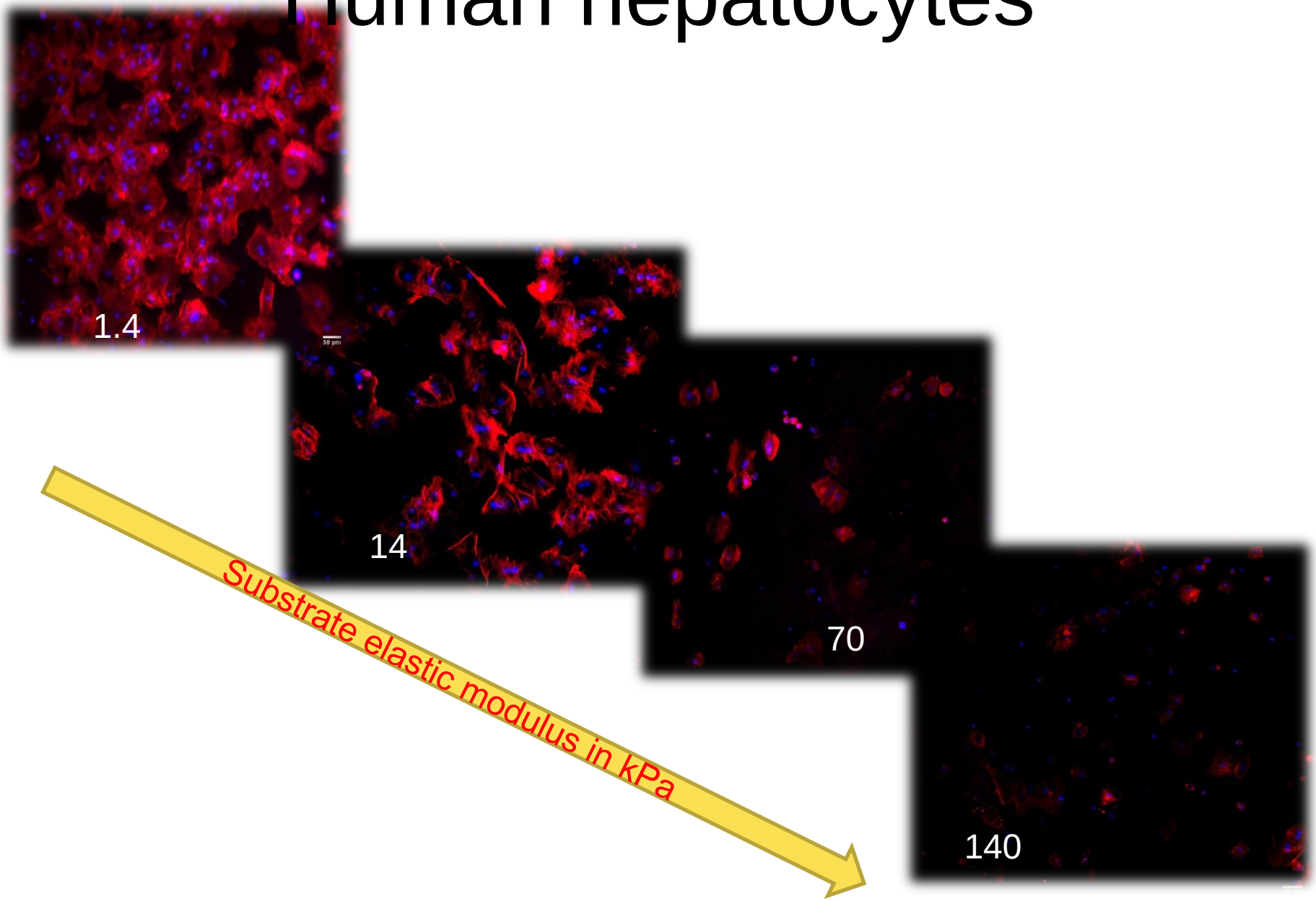
Durotaxis/Durosensing

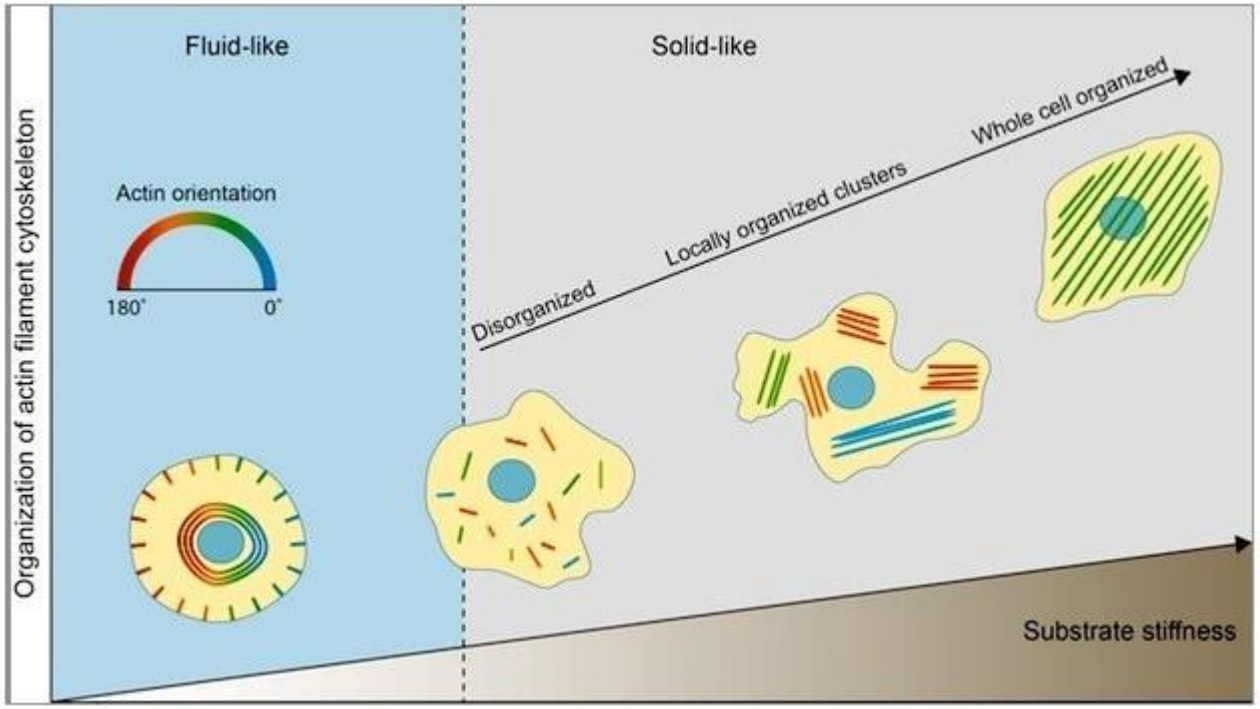
The range of elastic moduli found in tissues



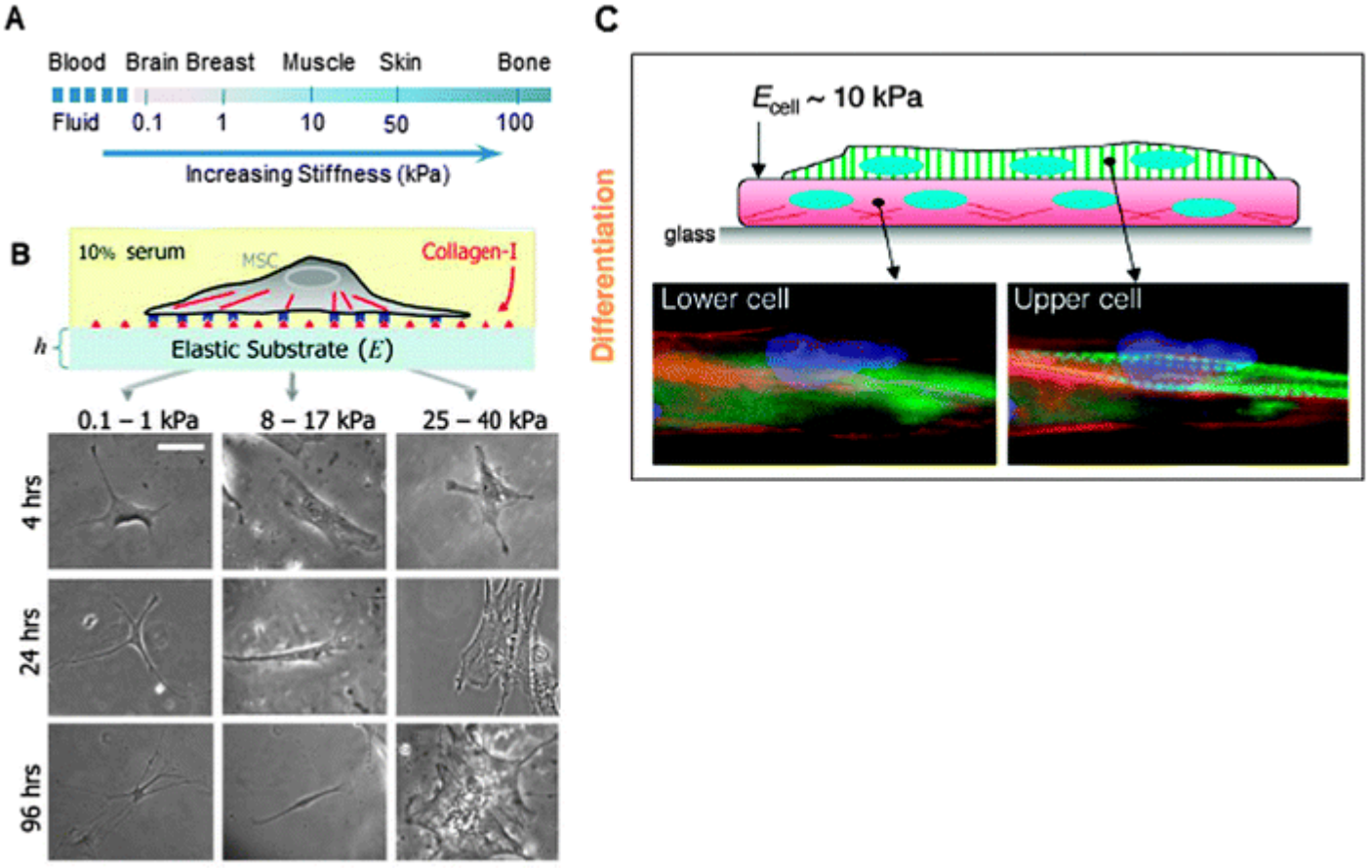
NB: these are estimates- all biological tissues are viscoelastic

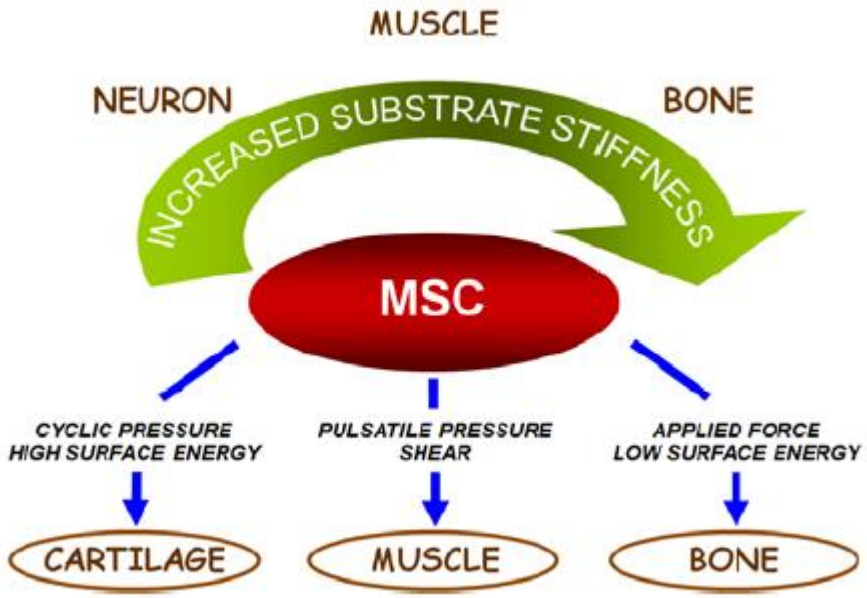
Human hepatocytes



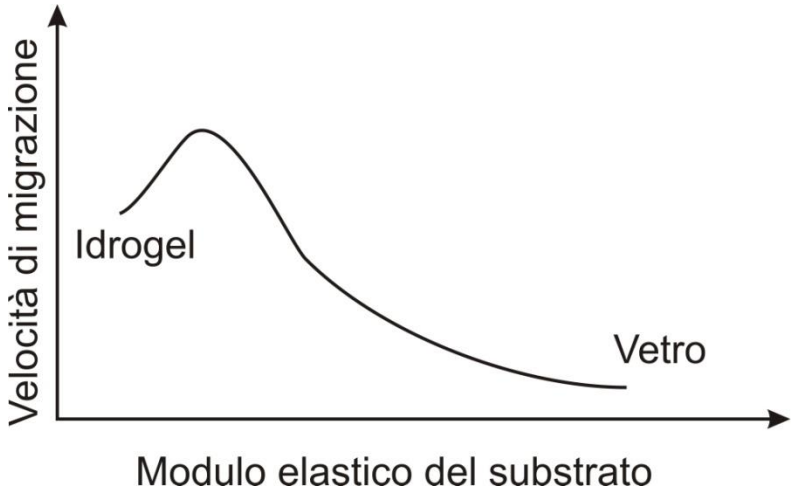

Fluid-like
Solid-like
Organization of actin filament cytoskeleton
Actin orientation
180°
0°
Disorganized
Locally organized clusters
Whole cell organized
Substrate stiffness

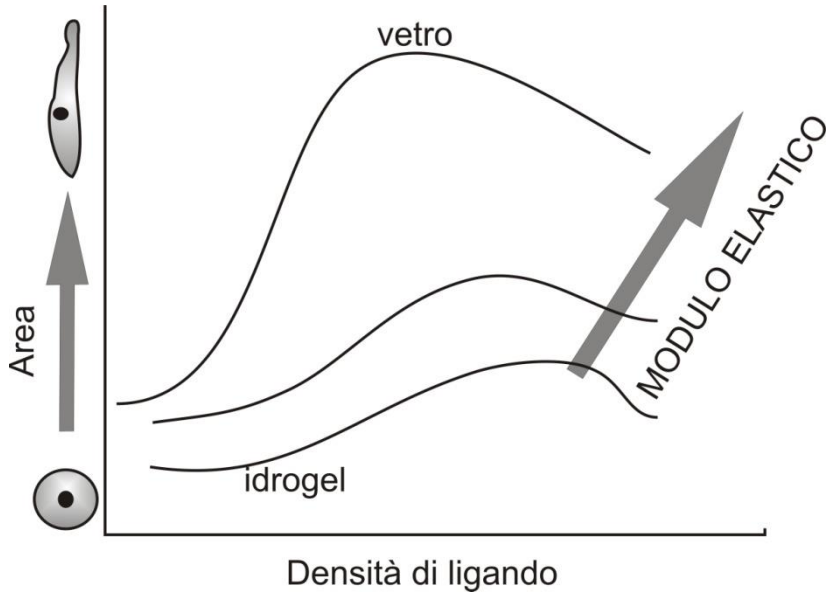
Adam Engler


A
Blood Brain Breast Muscle Skin Bone
Fluid 0.1 1 10 50 100
Increasing Stiffness (kPa)
B
10% serum
MSC
Collagen-I
h
Elastic Substrate (E)
0.1 – 1 kPa
8 – 17 kPa
25 – 40 kPa
4 hrs
24 hrs
96 hrs
C
E_{cell} ~ 10 kPa
glass
Differentiation
Lower cell
Upper cell

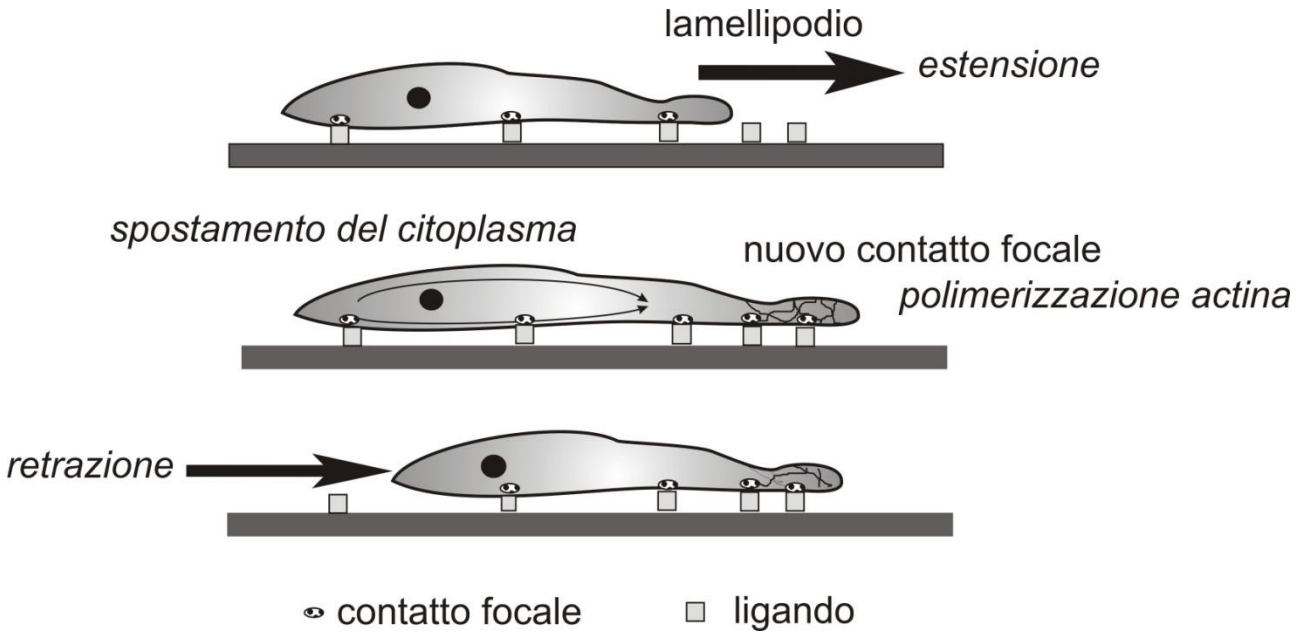


Stem cells are sensitive to physical stimuli: MSC (Mesenchymal stromal cells) can differentiate towards different phenotypes depending on the type of stimulus

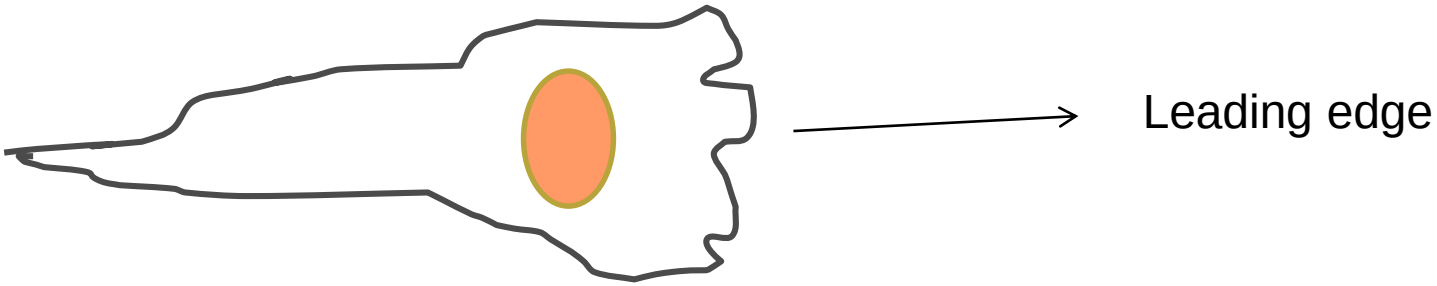

Velocità di migrazione
Idrogel
Vetro
Modulo elastico del substrato


vetro
MODULO ELASTICO
Area
idrogel
Densità di ligando

Migration



Through secretion of proteases and recall of integrins



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

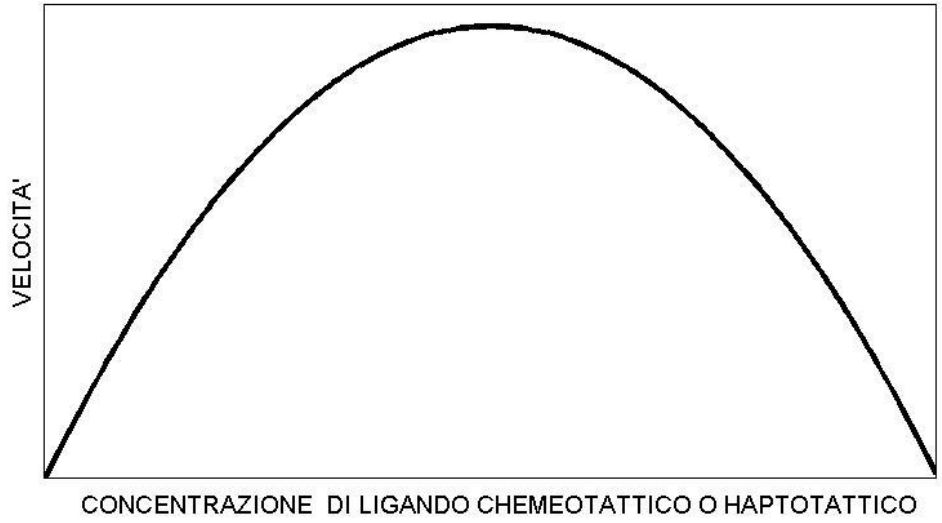
- When observed over **long** times in the absence of gradients, cells move similar to Brownian particles (change in direction due to elastic collisions).
- Over short periods cells move in one direction. This is known as “persistance”. No instantaneous momentum transfer but mechanisms which require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

Motility

S=velocity
P=persistance time= time required for significant changes in direction

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

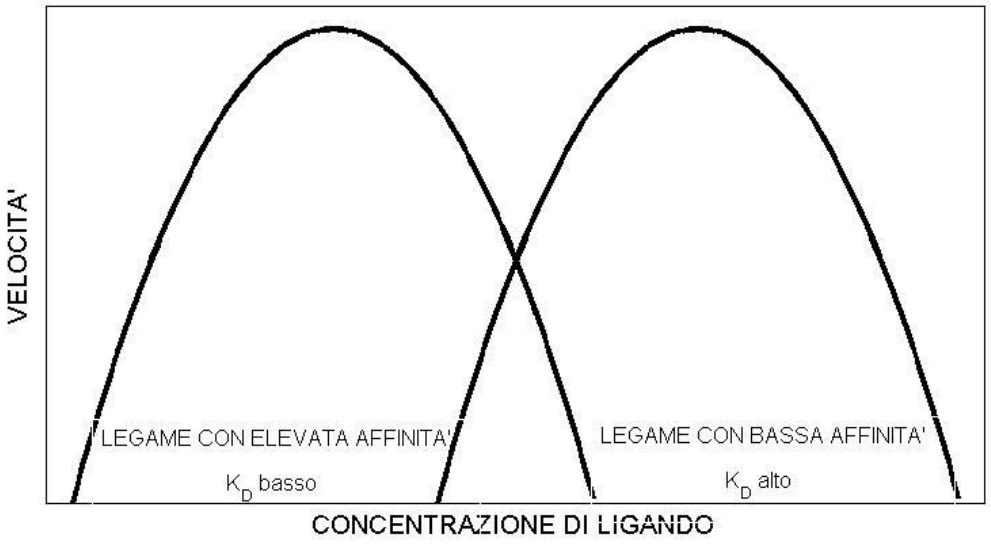
Velocity/motility vs. ligand conc



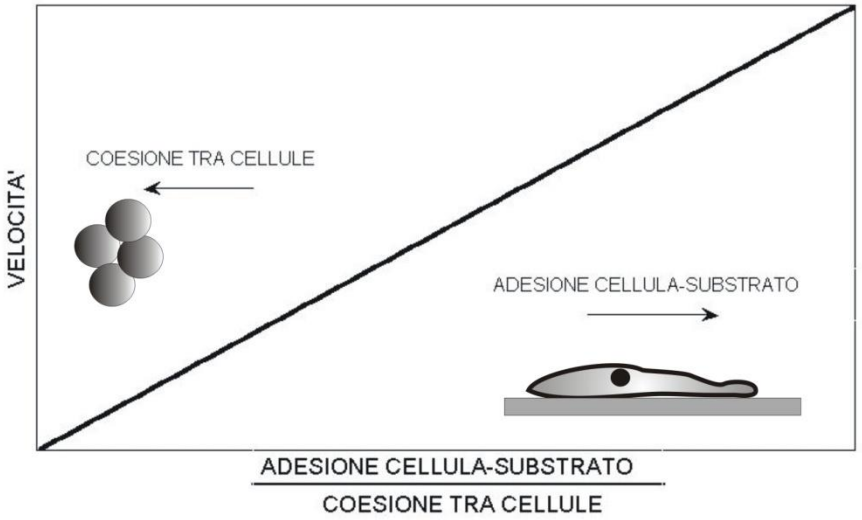
Andamento bifasico.

1. Down regulation
2. Substrato troppo appicicoso

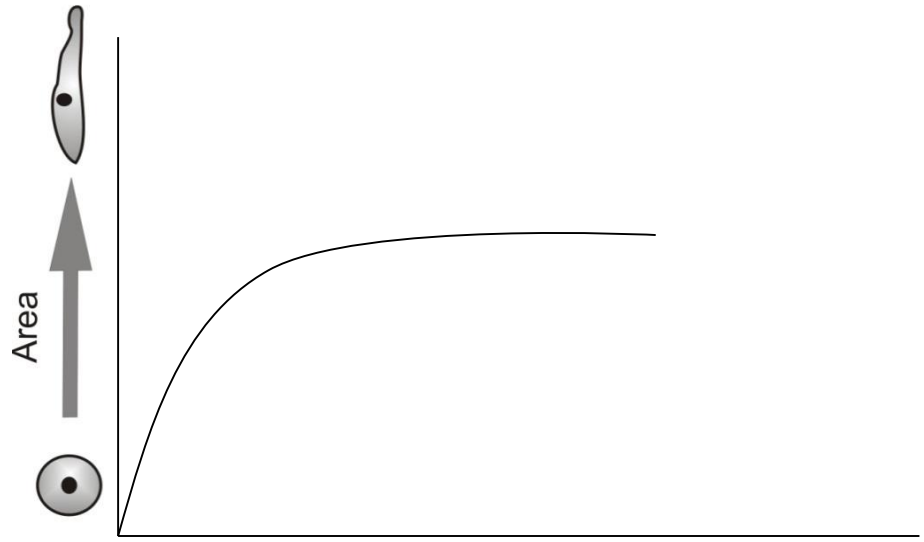
Velocità e concentrazione di ligando adesivo



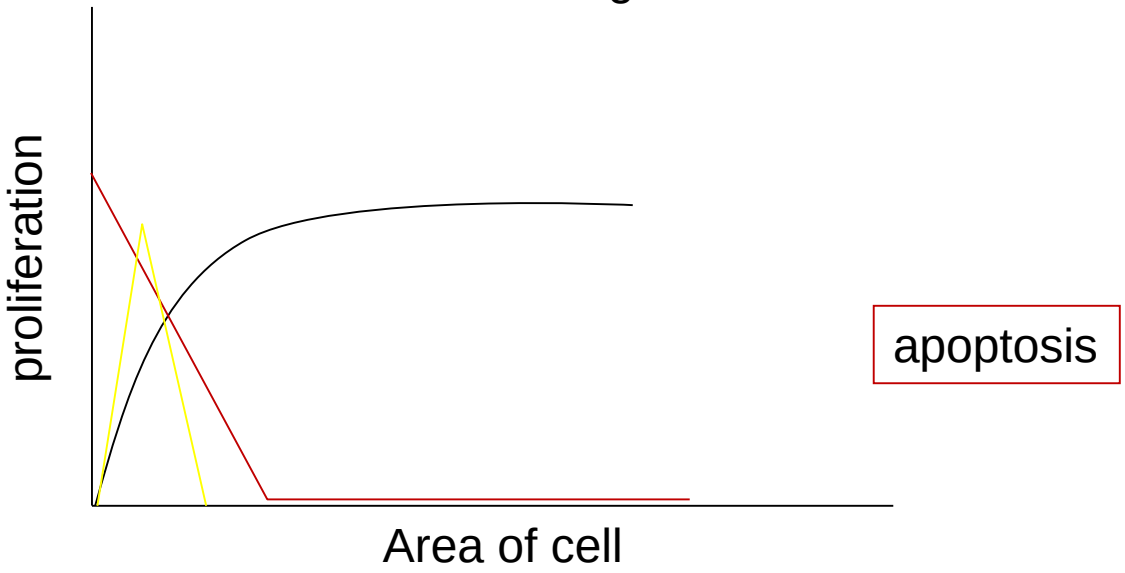
Motilità cellulare in funzione dell'adesivita e affinità di una superficie. Se il legame integrina-ligando (o recettore-ligando chemeotattico) ha un elevata affinità, basta una piccola concentrazione per aumentare la velocità e saturare. Invece se il valore di KD è elevato, sono necessarie più ligandi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



Motilita 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.



No of adhesion ligands/area



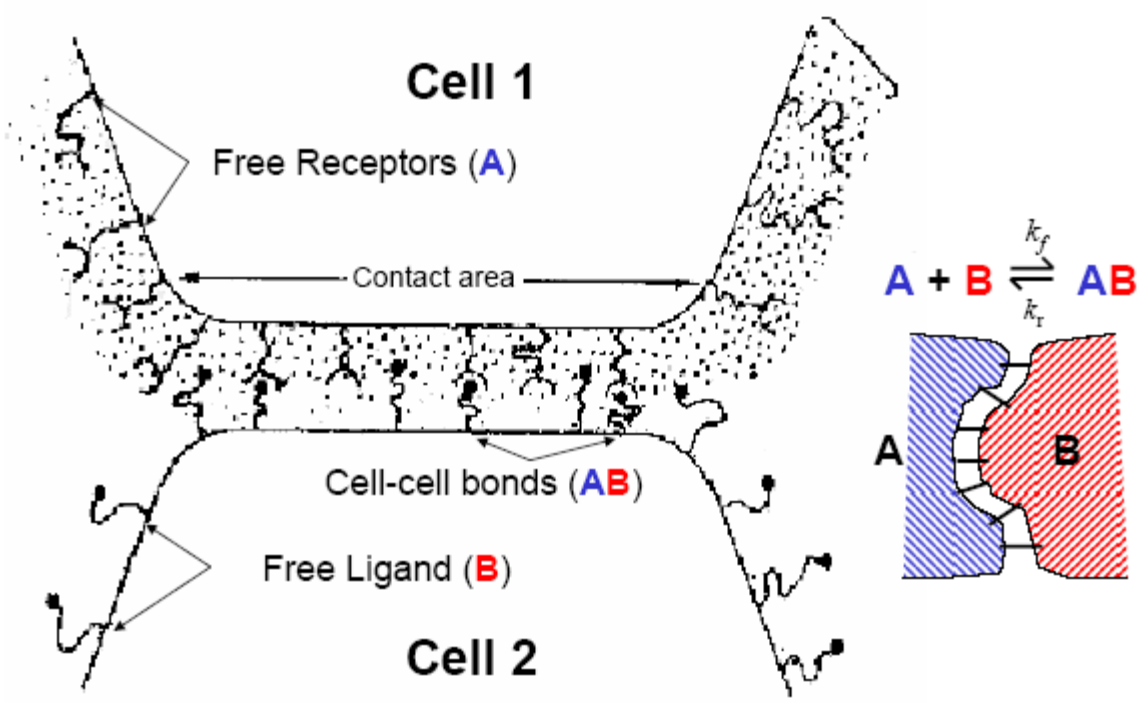
Le forze microscopiche

Forze meccaniche: adesione di cellule in presenza di forze di shear
Migrazione cellulare
Deformazione e orientazzione
Contrazione muscolare

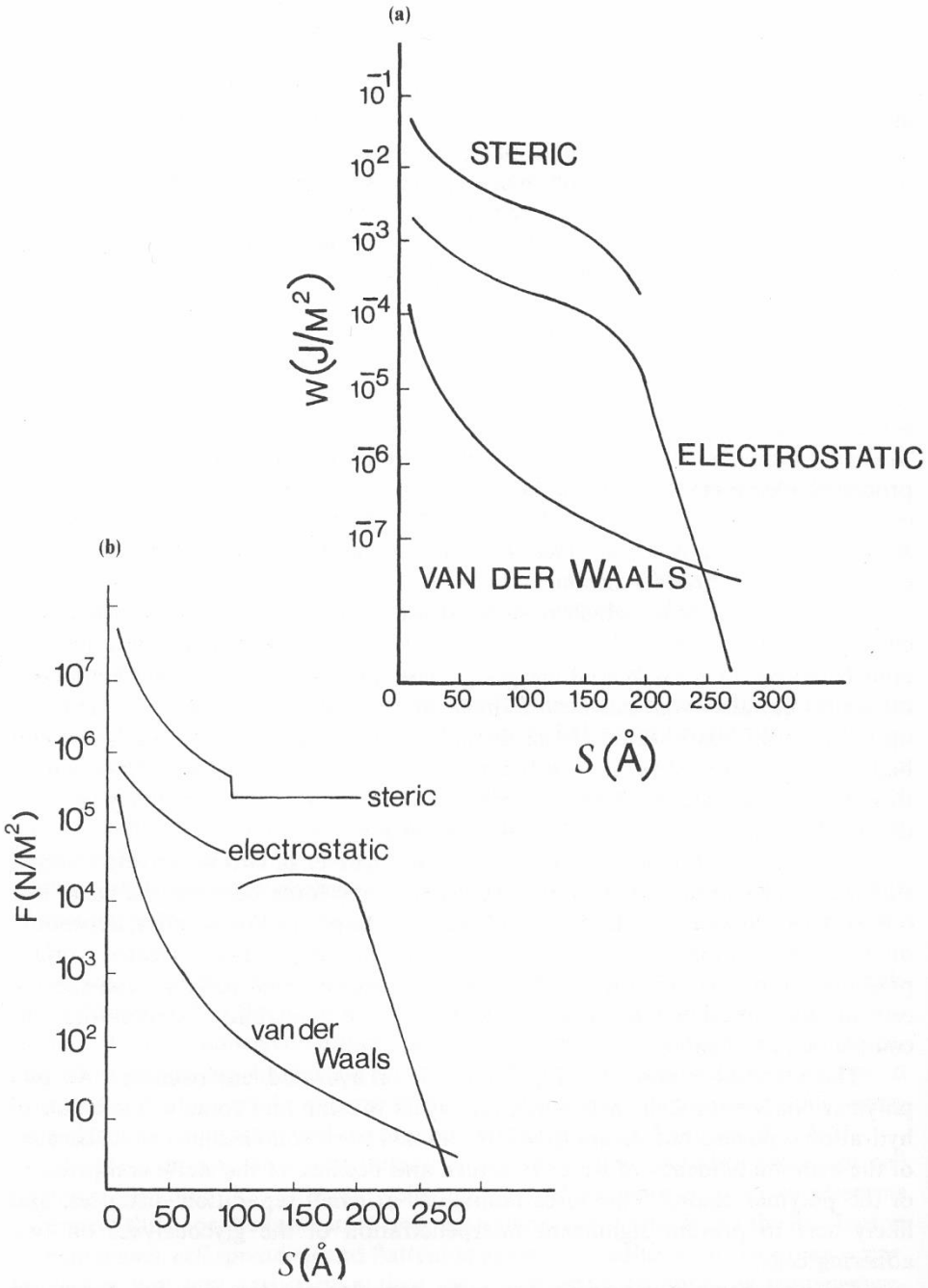
Forze esterne: viscose, elastiche

Forze intermolecolari
Interazione elettrostatiche
Interazione VDW e legami idrogeno
Idrofobiche –tensioni superficiali
Entropia

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

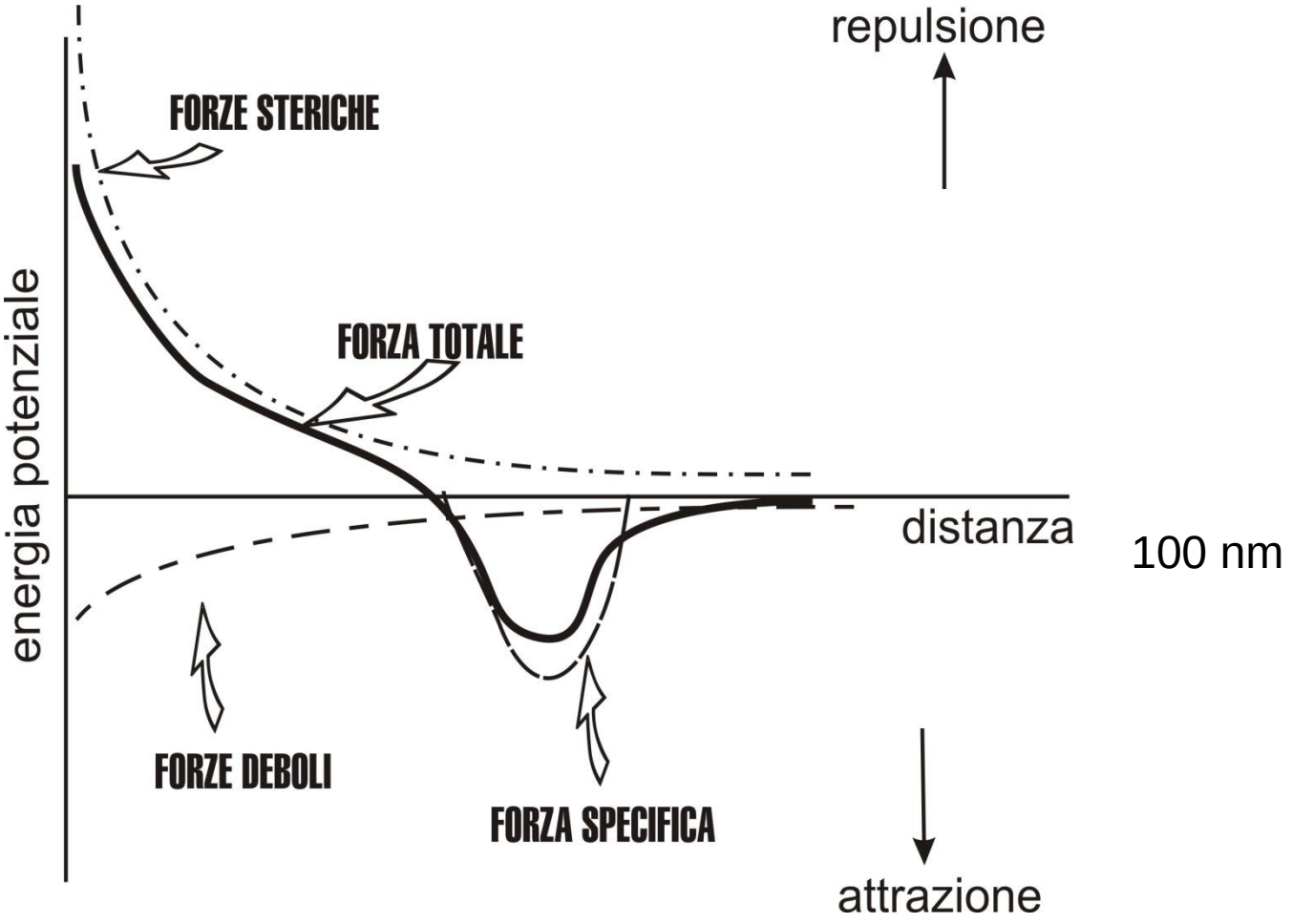

Cell 1
Free Receptors (A)
Contact area
Cell-cell bonds (AB)
Free Ligand (B)
Cell 2
A + B
AB
A
B

Bell



Specific interactions- receptor mediated
Non specific interactions
Electrostatic forces -rep
Steric stabilisation-rep
Van der Waals (electrodynamic) –att
Calculate kT,

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 $\exp[-(U_o - \gamma\sigma)/kT] = 1$ or $(U_o - \gamma\sigma)/kT = 0$. It is seen from this condition that at a conventional testing temperature the maximum rate of fracture propagation is reached at $U_o - \gamma\sigma = 0$. 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 load becomes infinite.

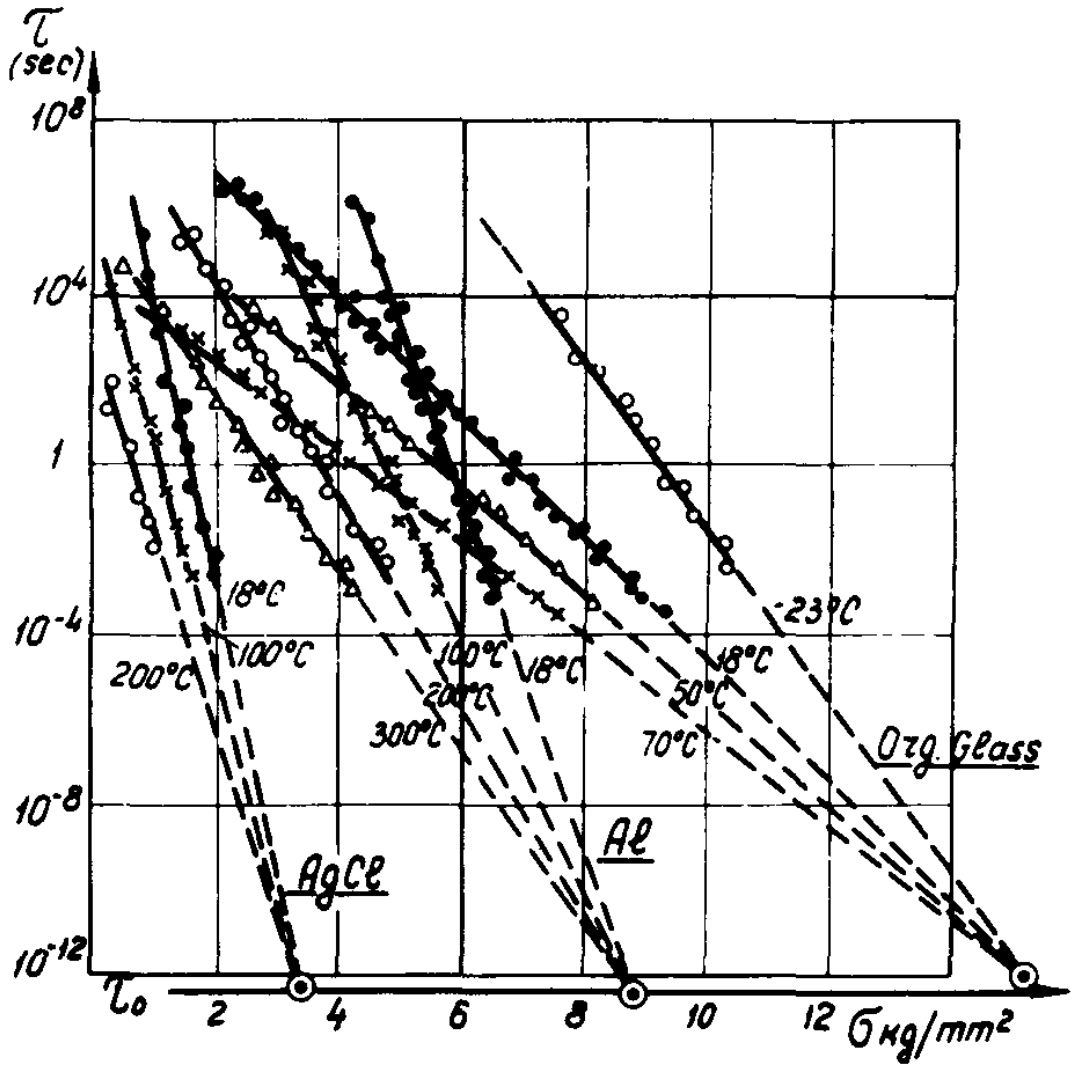
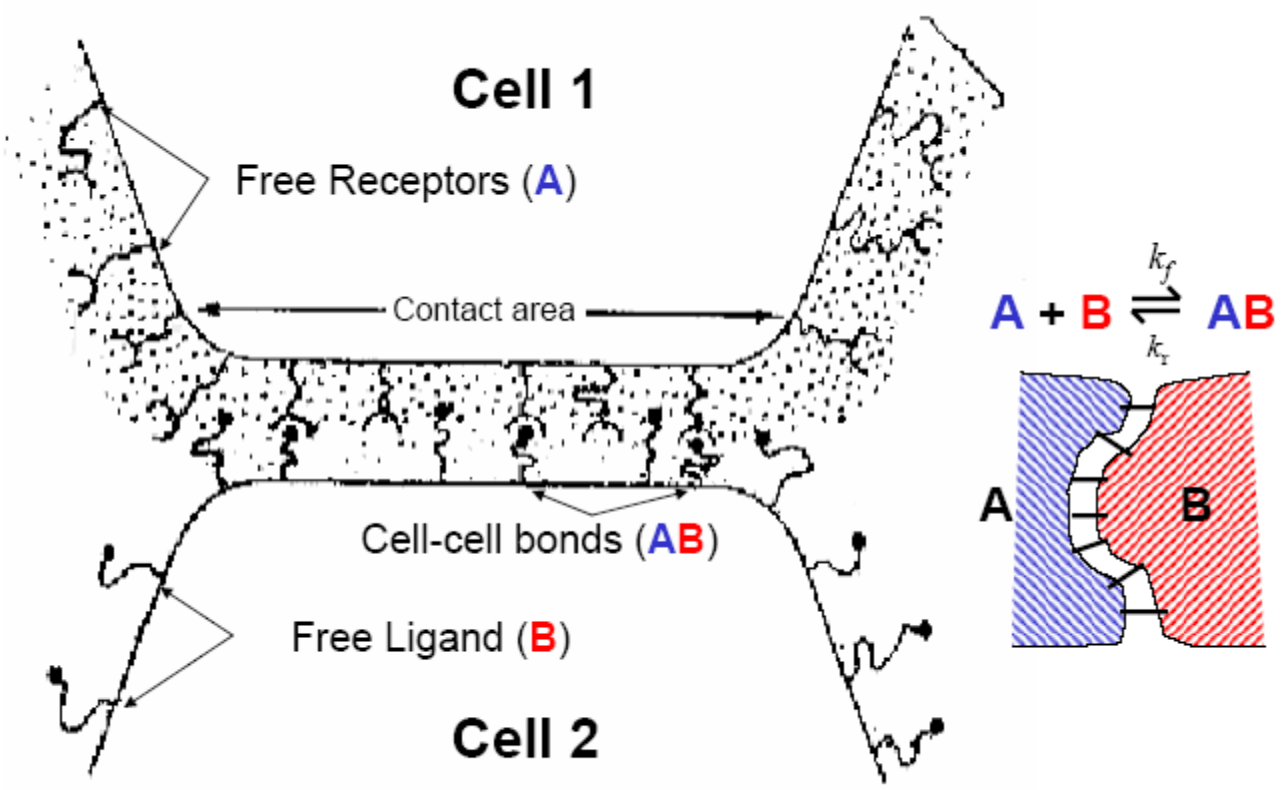
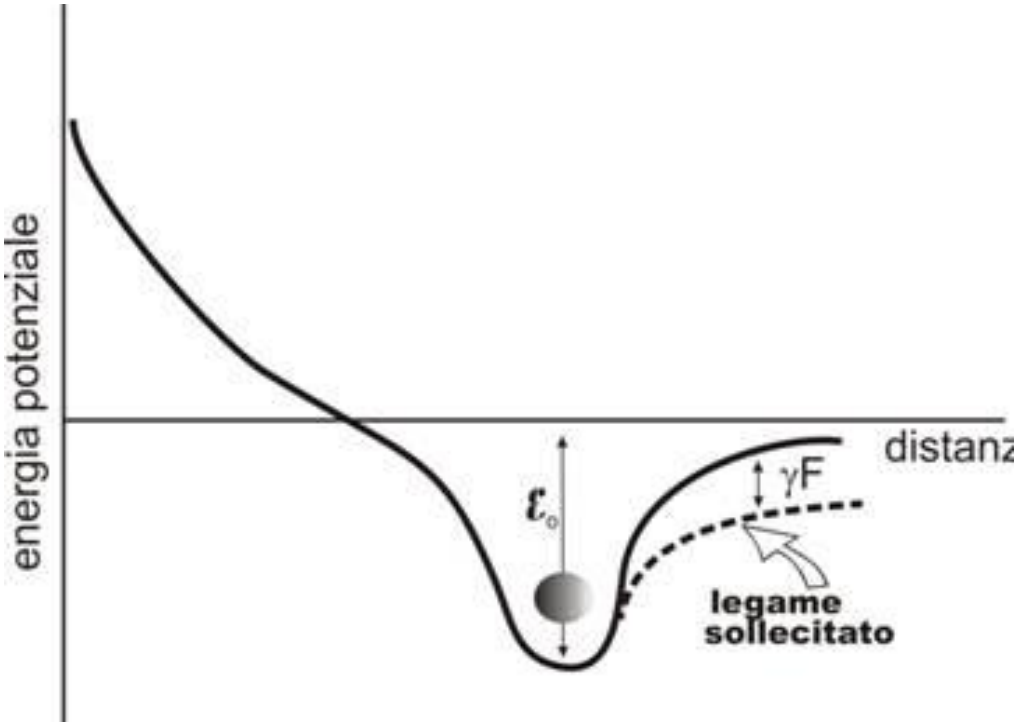


Fig. 5. Time and temperature dependence of the lifetime of solids on stress.
1. Silver chloride (Reference 4)
2. Aluminum (Reference 5)
3. Plexiglas (Reference 6)



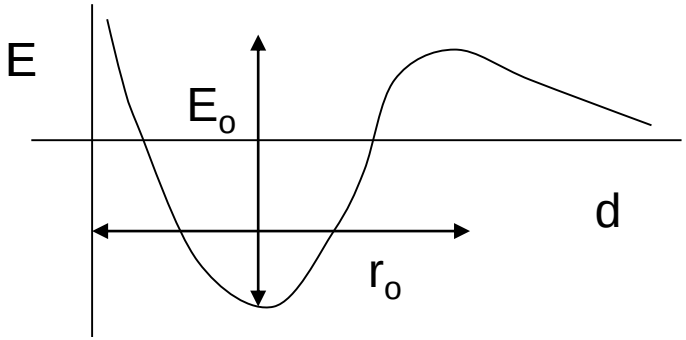
I. Bell et al., *Biophys. J.* 45:1051, 1984

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

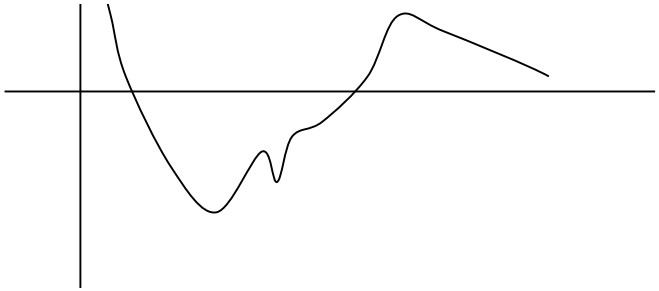


The Bell model

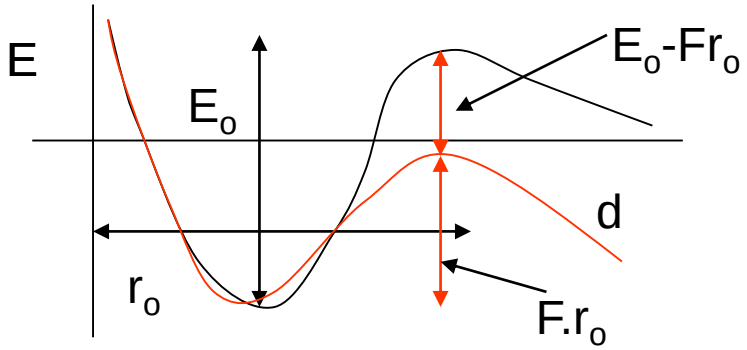
Bell (Science 1978, vol 200) had the insight to relate cell ligand binding kinetics to the lifetime of bond formation previously used in metals.

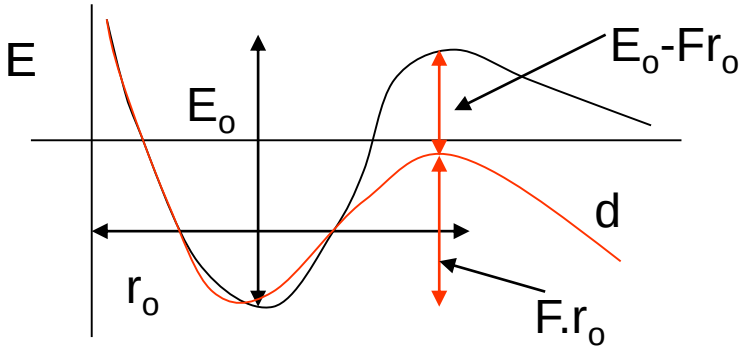


$$\tau = \tau_0 \exp[(E_0 - \gamma f)/kT]$$



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





So now the energy required to break the bond is less, E_o-Fr_o

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

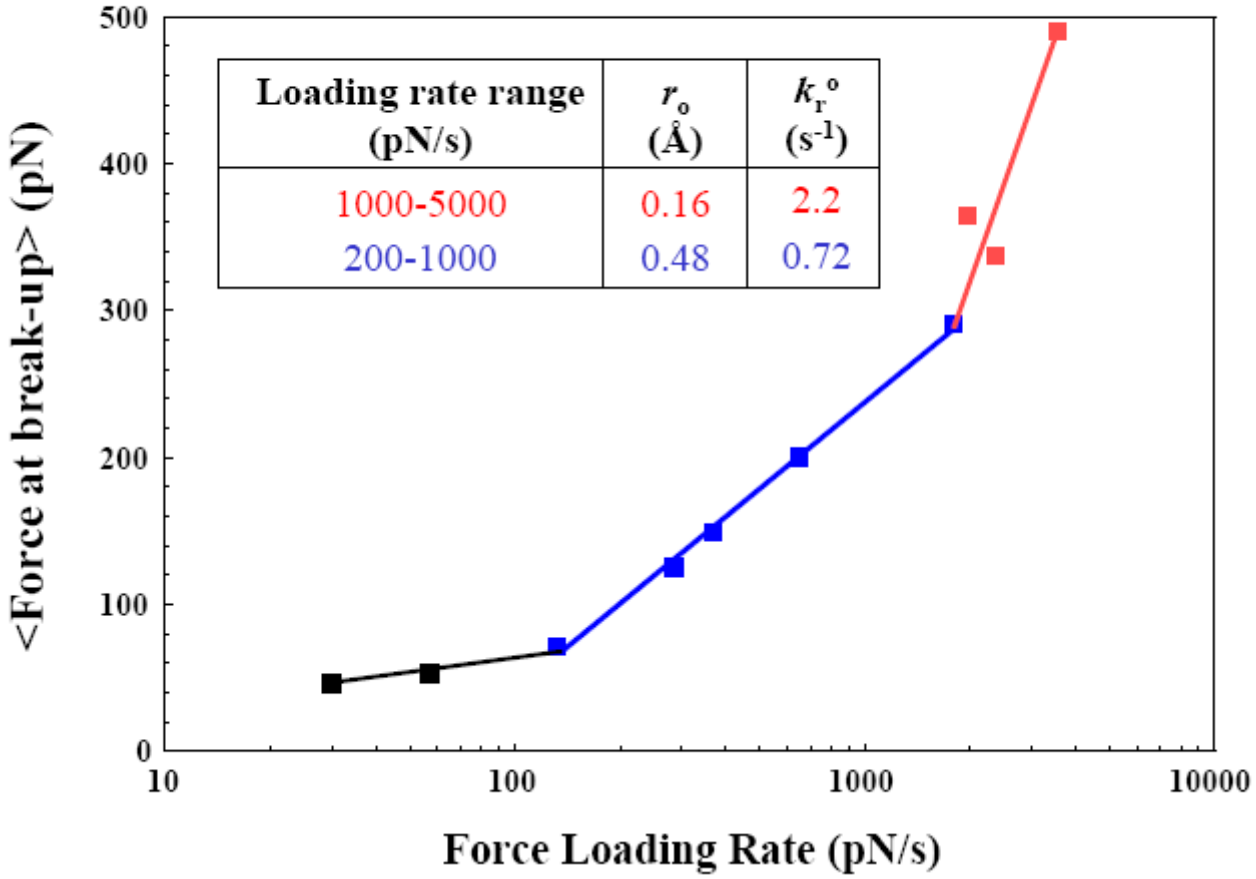
And when stressed $\tau = \tau_o \exp([E_o - F.r_o] / KT)$

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.

$$k_r = k_{r0} \exp(F.r_0 / KT)$$

k_r reverse reaction rate, k_{r0} reverse rate when unstressed. F force applied, r_0 bond length

Observed Force vs Loading Rate



How is adhesion measured?

method	Forces, pN	Distances, nm	Loading rate pN/s
Laser tweezers	1-200	100	10^{-1}-10^{2}
Micropipette aspiration	10-1000	100	10^{1}-10^{3}
AFM	10-10000	0.1	10^{3}-10^{6}
Flow chamber	?	?	?
Sedimentation -detachment or centrifugation	1-1000	100	10^{0}-10^{3}

Laminar flow chamber