# Mechano-transduction & Bioreactors

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What is the difference between stress and strain?

Strength? Stiffness? What are the crucial characteristics for an osteosynthesis?

# Basic concepts & definitions in mechanics

[Cordey, J 2000 Injury]

KHU

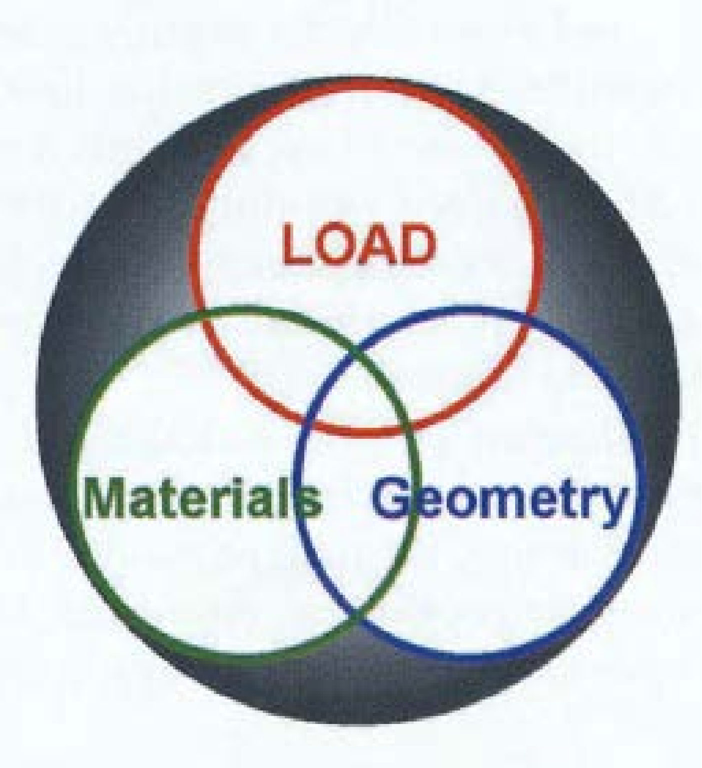
# Key Aspects in Mechanical Analysis

## Beam

- Long piece of material, whereby the cross-section does not vary much along its long axis.
- We will assume that this is nearly the case for long bones.

## Three key aspects in mechanical analysis: Load, material, & geometry

- Load applied by physiological motion
- Material properties of bones indicate that bones are optimized for this function
- Bone cross-sectional and structural geometry indicate functional aspect of bone
    - Size (bone is too small)
    - Osteoporosis (structurally degraded)
    - Fluorosis (bone become fragile)



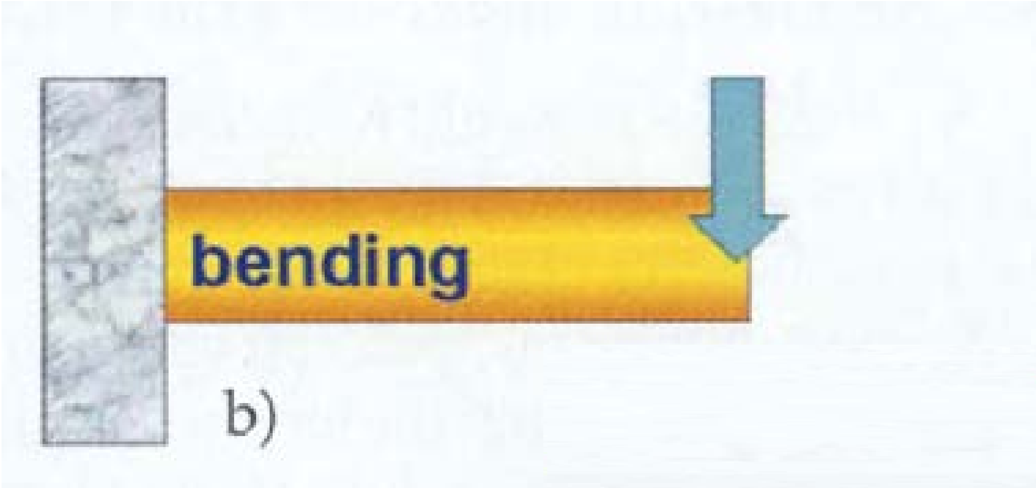
# The main loads: axial load, bending, & Torque

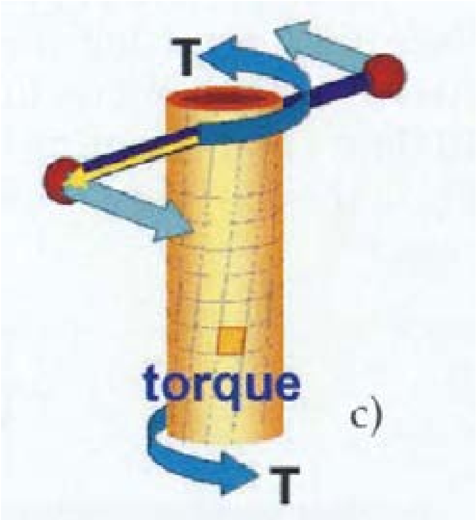
**Axial load produce compression or tension in the beam.**

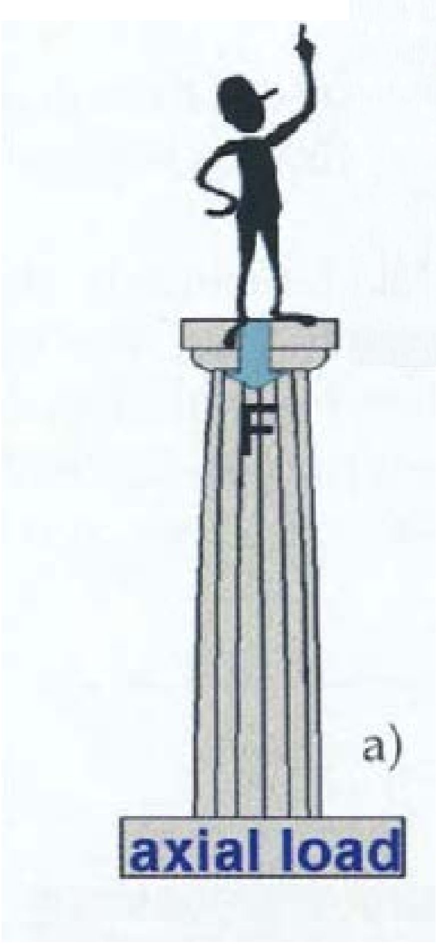
- Centric axial load produce a homogeneous compression deformation of the column
- When the compressing lead is eccentric, the deformation within the column is not to simple and vending is produced.

**Bending is the most important load in bio-mechanics.**

**Torque: Long bones overloaded in torsion results in spiral fractures.**

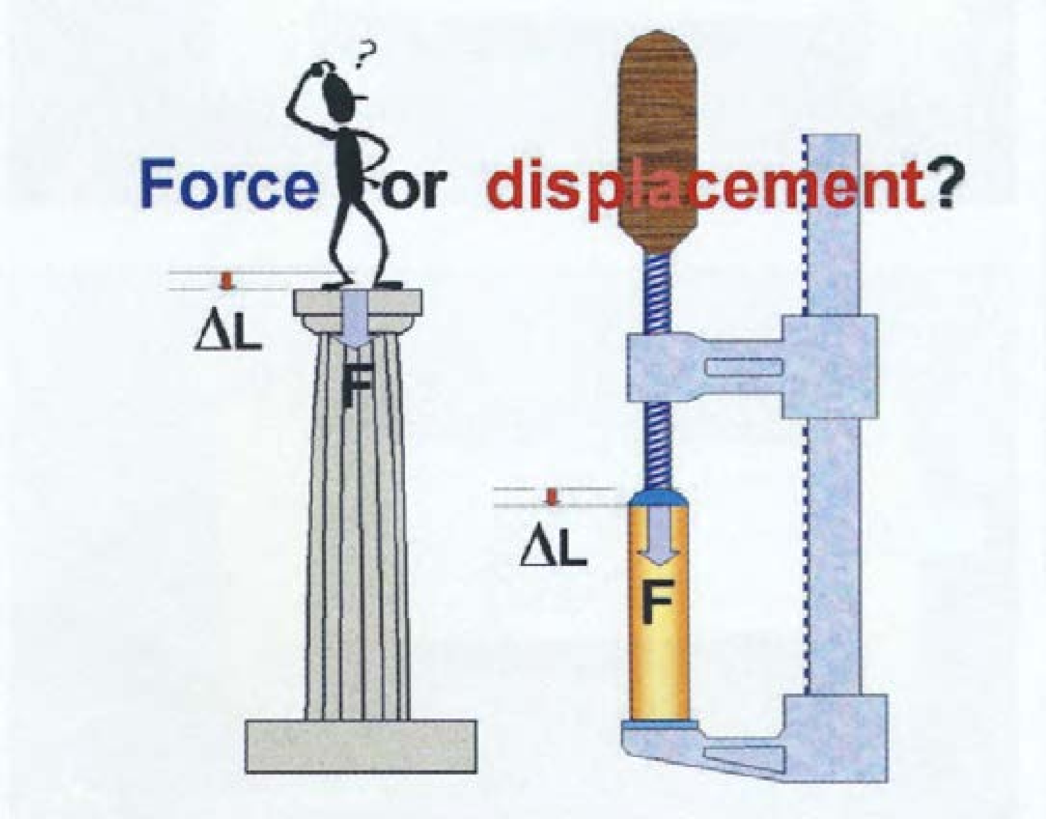


# Load & deformation

**Load produces a deformation of the beam.**
**The cause is the load, the consequence is the deformation.**
**However, it is not always the case.**



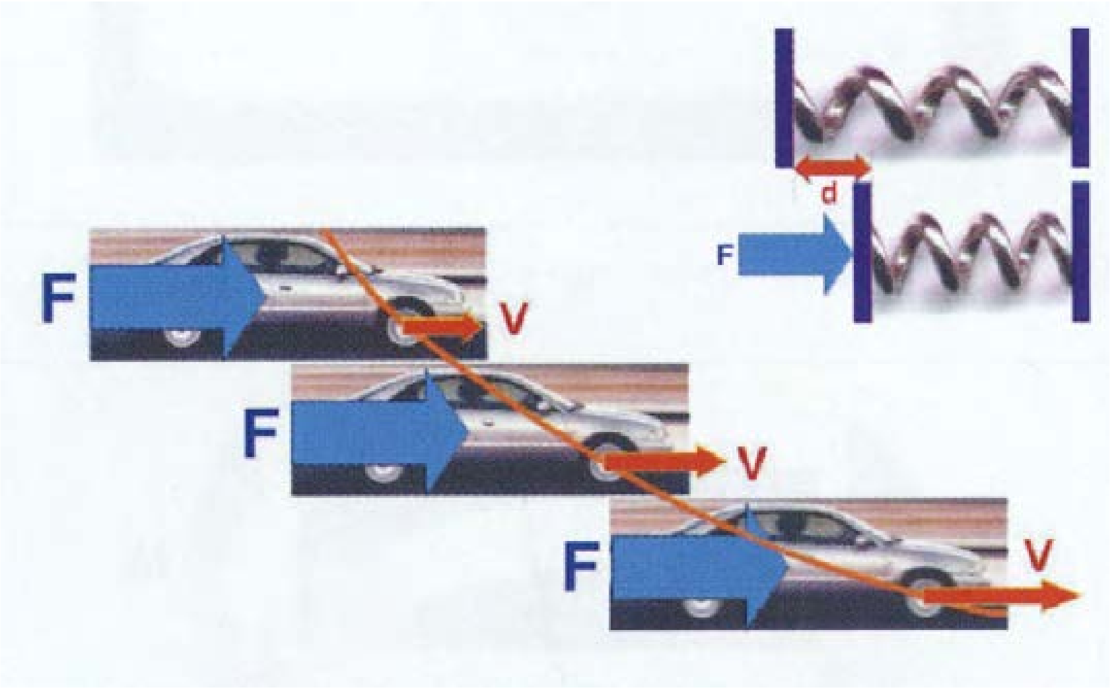
# Forces & moments

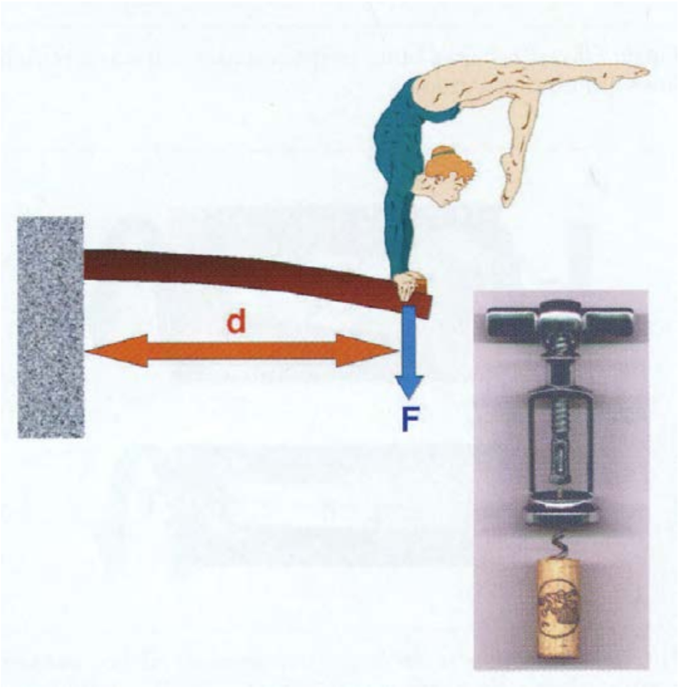
**Force: something which causes the acceleration of a moving body.**

- When it is blocked, it results in deformation.

**Moment: the effect of a force acting on a lever arm.**

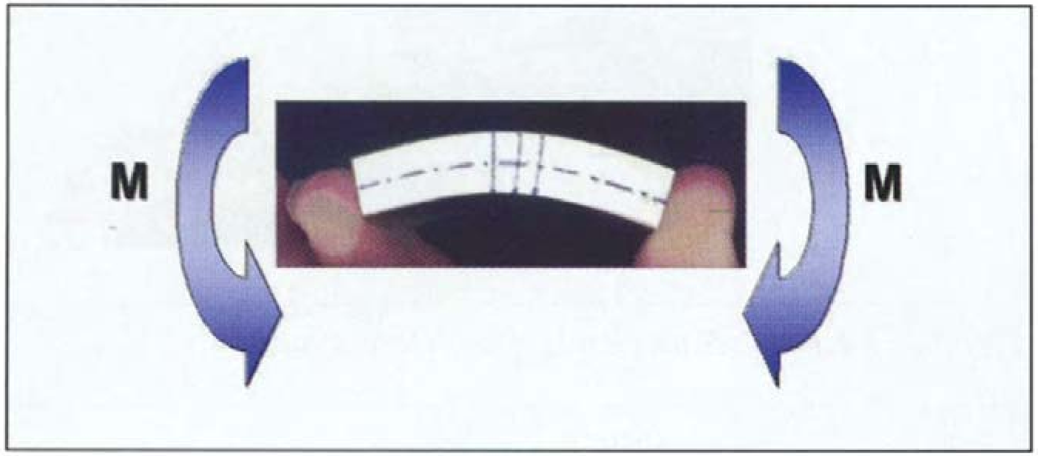
- It can act as a bending moment or as torque.

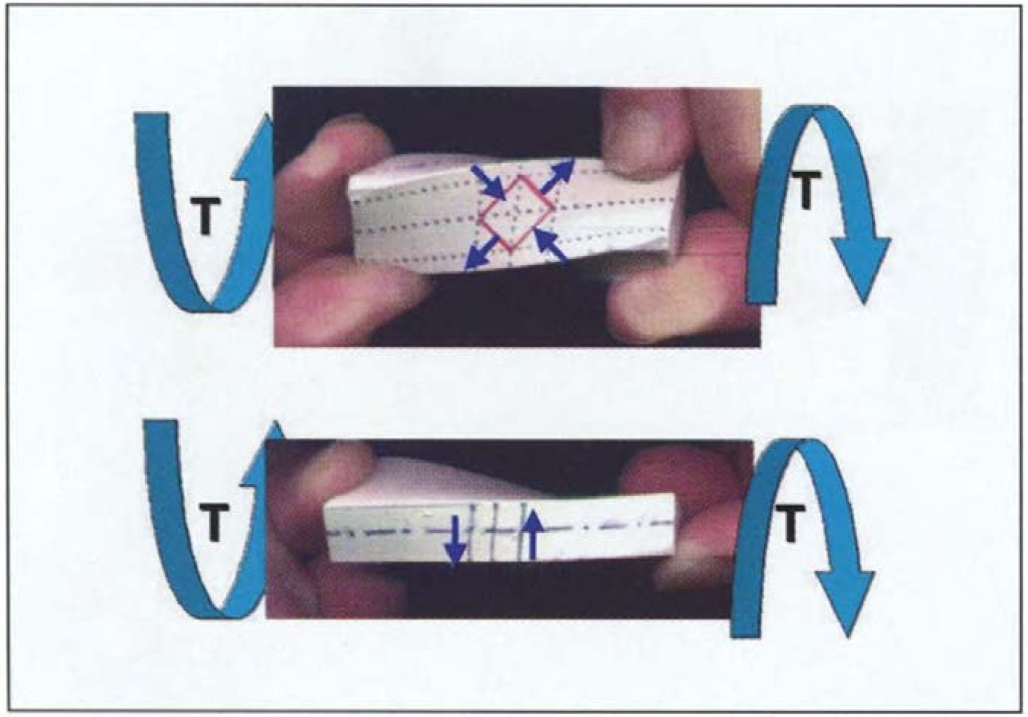


# Effect of bending

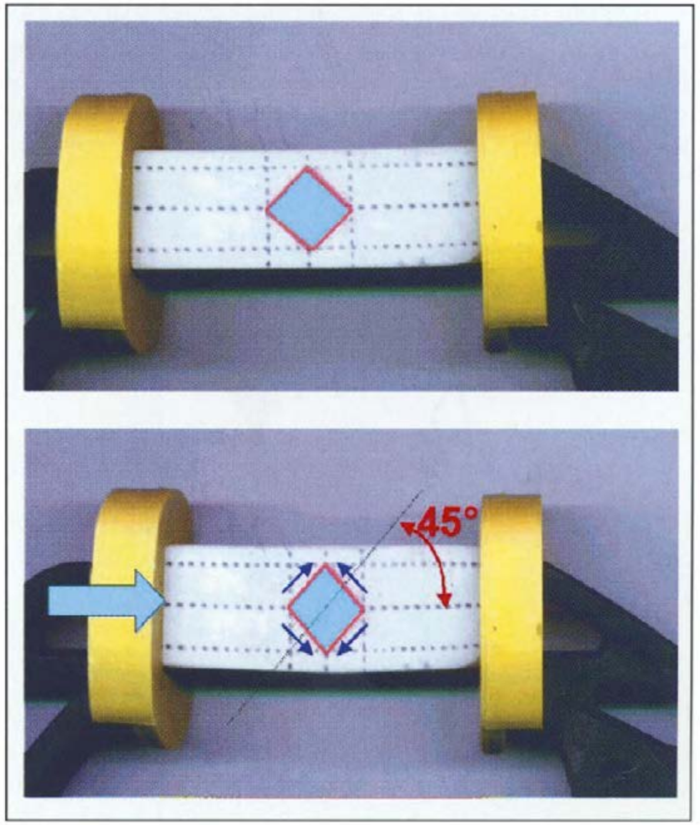
Torque produces almost pure shear, leading to tension and compression at 45° to the axis of the eraser



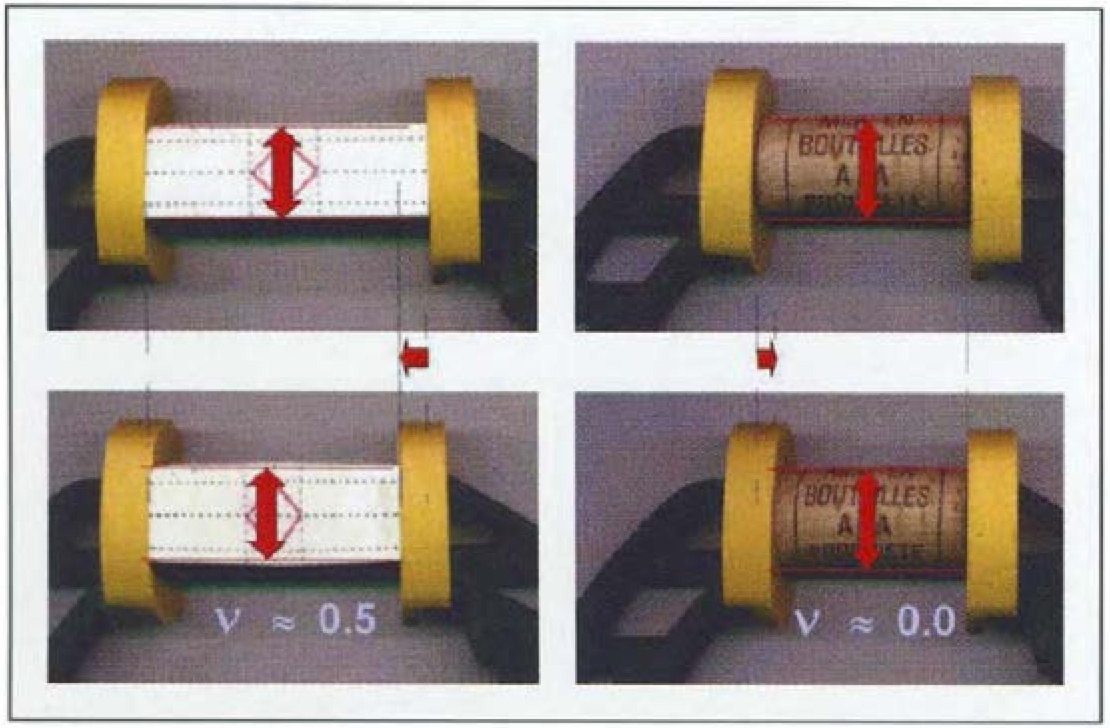



# Axial load

**The simplest load**
**Load is homogenous**
**When this load is applied to very flexible piece of rubber, the vertical dotted lines converge.**

When a piece of material is subjected to compression, it is shortened; but also, a small amount of widening occurs.
Some materials widen more than others.
(Rubber > Cork)
The relationship between the axial force applied and the deformation relates to the material properties and to the cross-sectional geometry of the loaded object



# Material Properties

**Assumption:**

- The load is not to high.
- The relationship between load and deformation is linear (elastic)

- **When axial load is applied…**
  - The piece shortens by a length of $\Delta L$ and widens slightly.
  - The local mechanical values are the stress & strain

**Stress $\sigma$ = F/A**

**F: force applied**

**A = cross-sectional area of the prismatic material**

**Strain $\varepsilon$ 1 = $\Delta$L/L (relative deformation)**

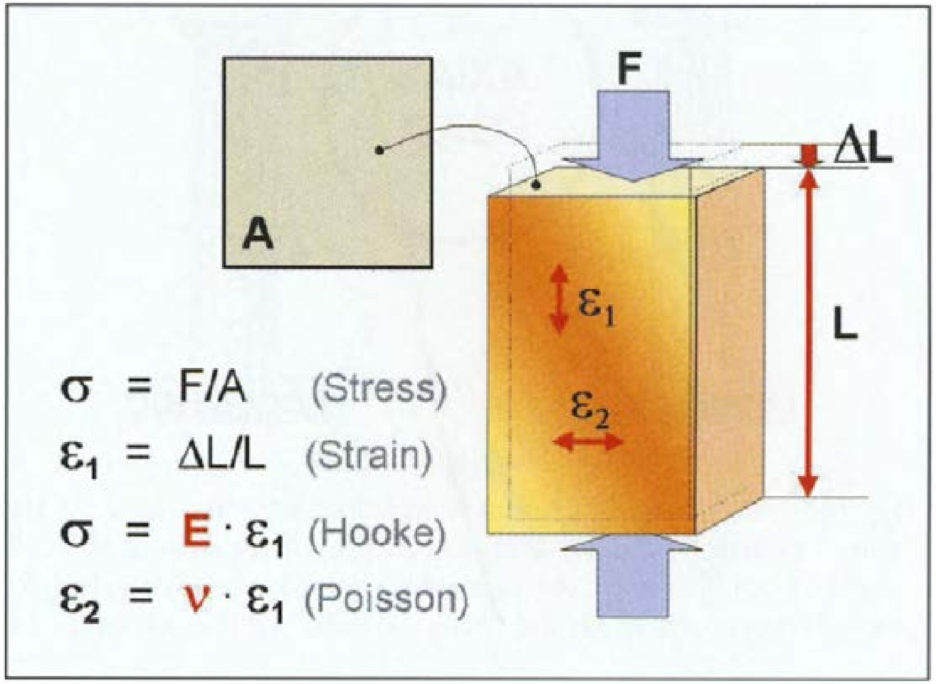
- **strain along the loading axis**

- Hooke's law:

  the relationship between stress and strain

$\sigma = E \cdot \varepsilon 1$

E: Young's modulus or elastic modulus

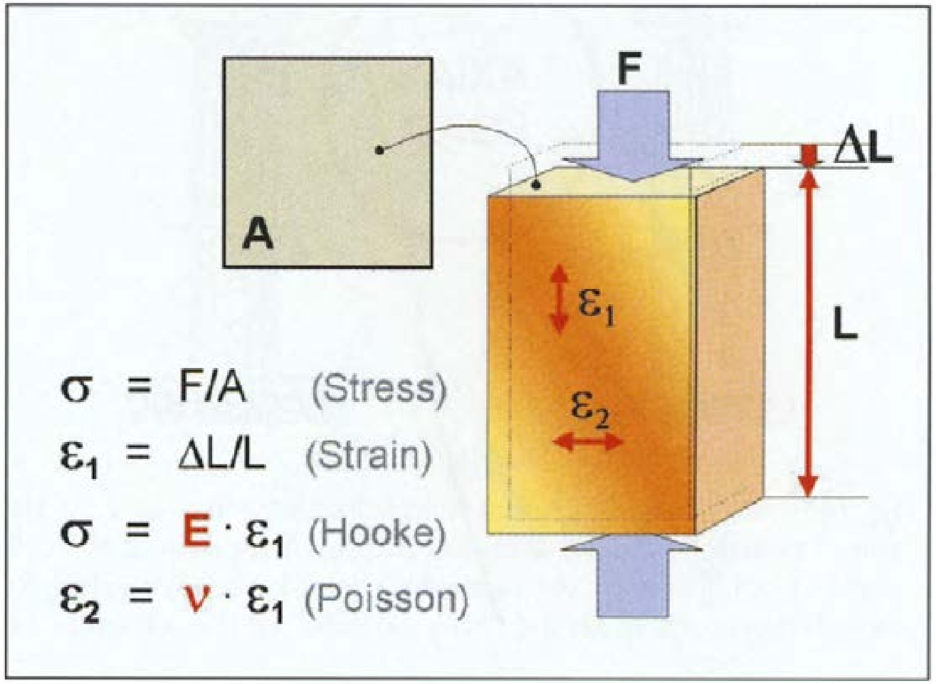


# Deformation $\varepsilon 2$ perpendicular to the leading axis is also produced.

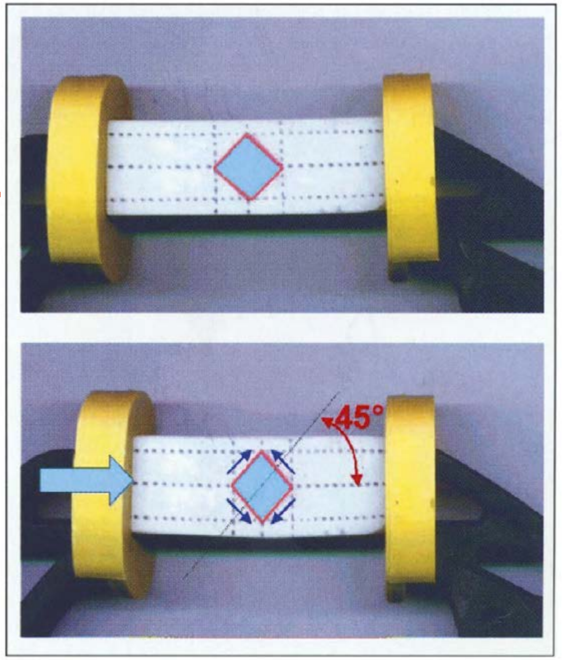
- Poisson's law:
  The relationship between $\varepsilon 1$ and $\varepsilon 2$

  $$\varepsilon 2 = \nu \cdot \varepsilon 1$$

  $$\nu = - \varepsilon 2 / \varepsilon 1$$




- The coefficient $\nu$ is the Poisson's ratio
- The value $\nu$ varies between 0 (cork) and 0.5 (pure rubber), and usually near 0.3 (metals & bone)




The square deformation to lozenge is caused by shear load.
Shear stress $\tau$ is the ratio of F/A

- Shear deformation: $\gamma$

The relationship between $\tau$ and $\gamma$ is given by:

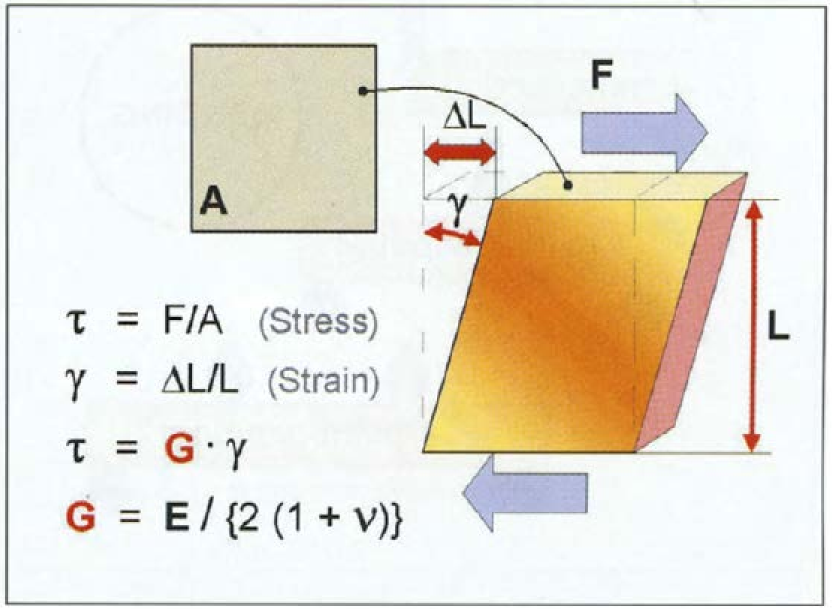
$$\tau = G \cdot \gamma$$

G: Modulus of rigidity, a third mechanical characteristics of a material

- Relationship between the three mechanical characteristics:

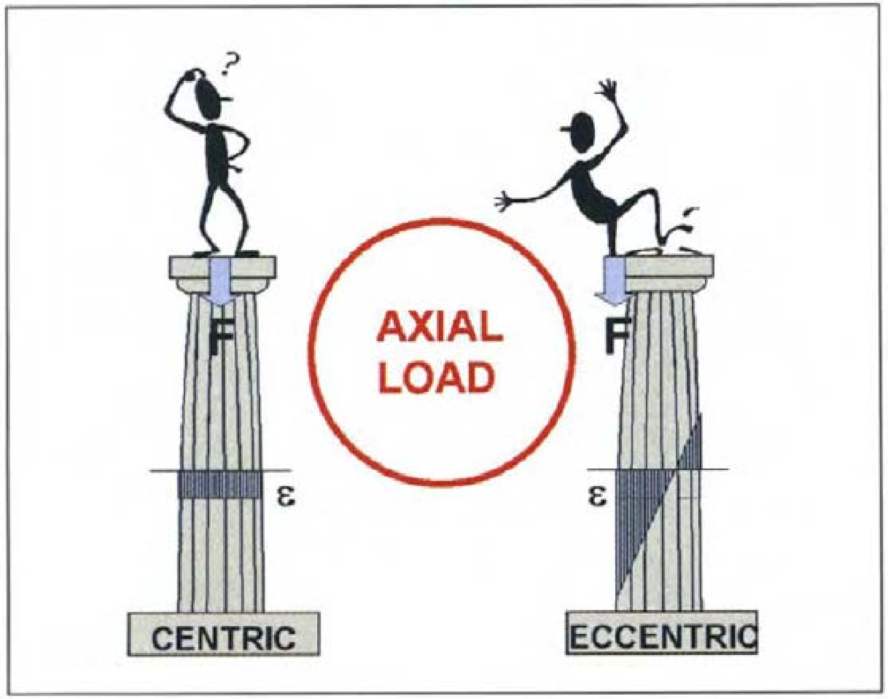
$$G = E / [2(1+\nu)]$$

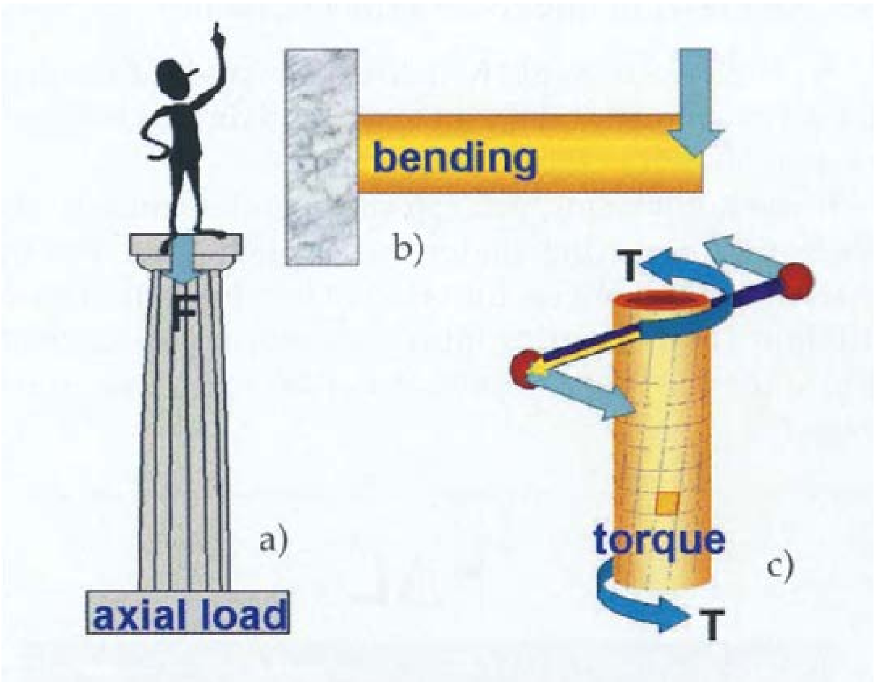
E: Young's modulus
0.2 GPa for plastics
2 GPa for cortical bone
0.1 ~ 1 GPa for cancellous bone
10 ~ 20 GPa for metals



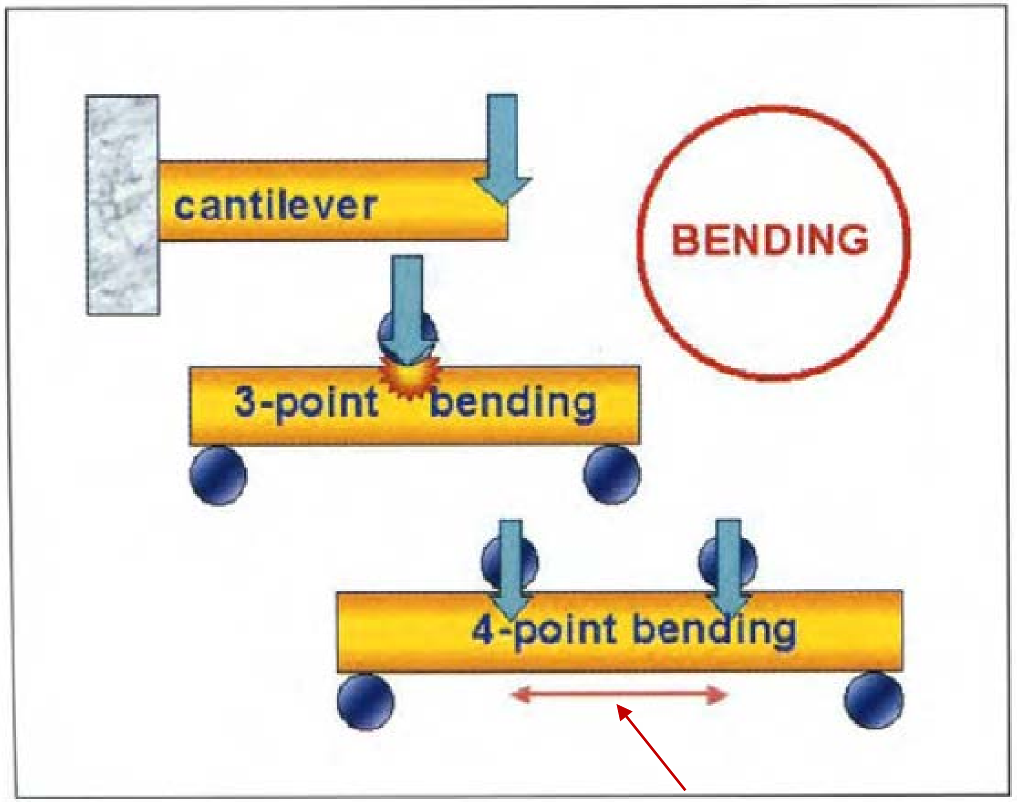
# Centric and Eccentric Axial Load

**When the compressing force is applied with a slight amount of eccentricity, this produces axial strains within the column which are in <u>tension</u>, not in <u>compression</u>.**
**This is due to the <u>bending moment</u>.**

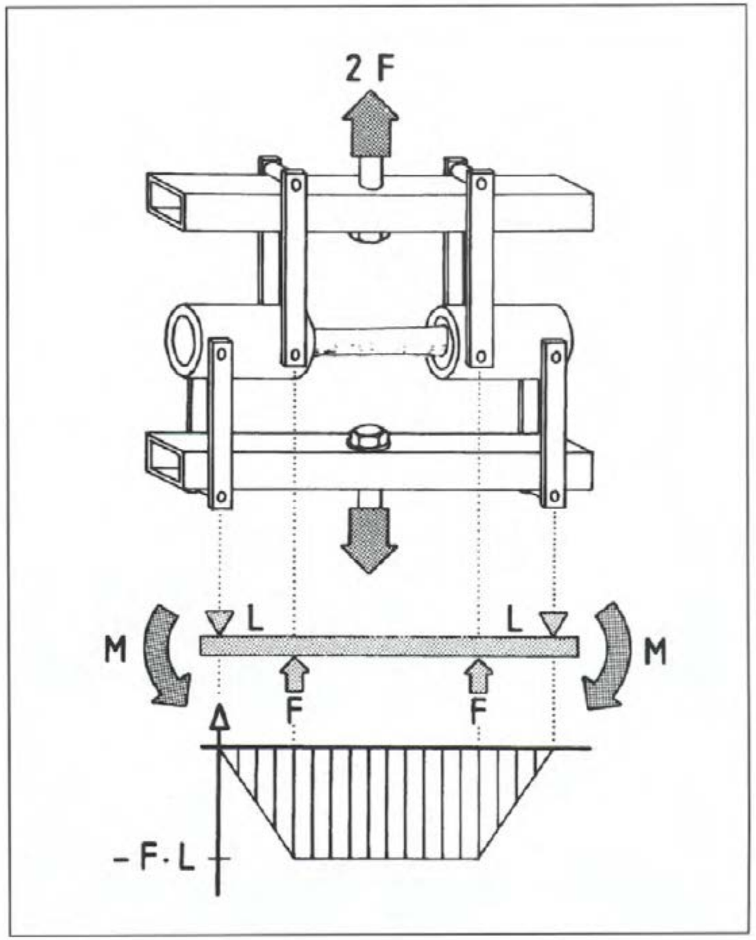


# Bending: the effect of a force applied perpendicularly to the axis of a beam

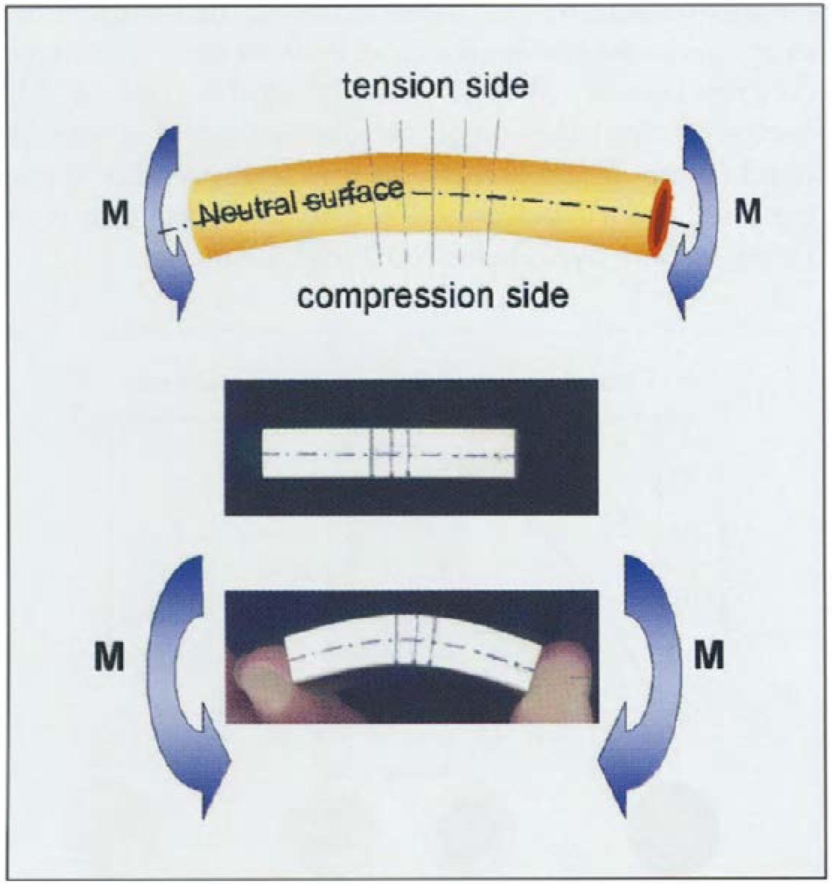



The bending moment is
constant within this zone.

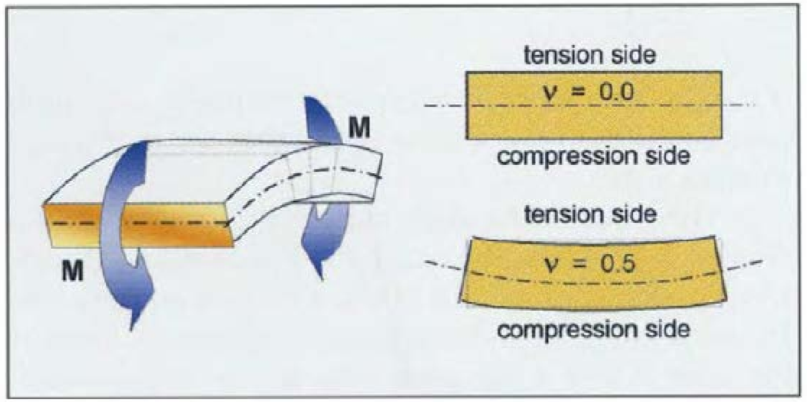


# The linear bending theory: A plane perpendicular to the beam axis remains a plane and remains perpendicular to this axis after bending

- Neutral surface: a surface located in the middle of the beam.
  - Not deformed in tension nor in compression when bending is applied.
  - In the cross-section of the beam, this neutral surface is the neutral axis.

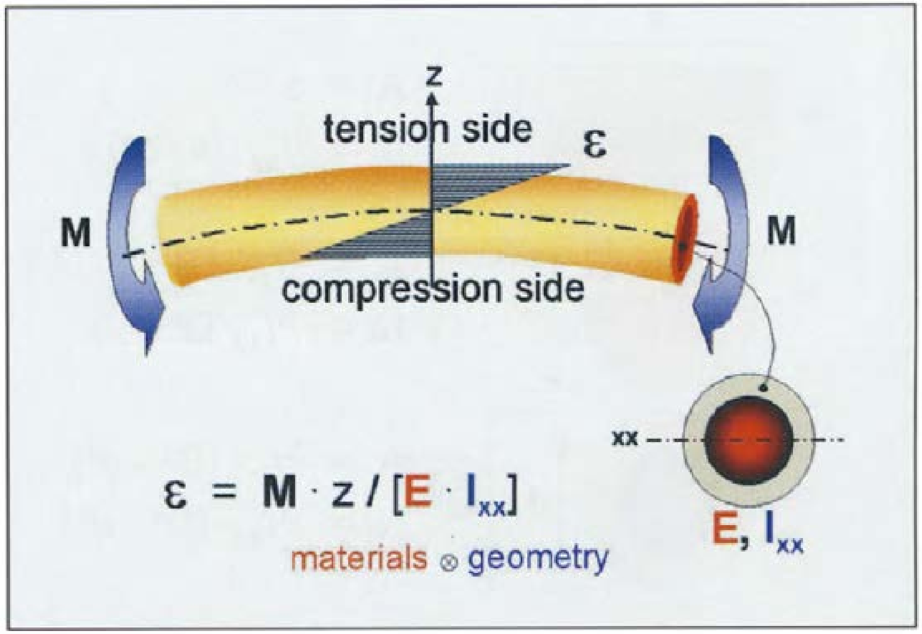


## Simplifying hypothesis




1. The cross-section of the beam is symmetric in regard to the plane of bending.
   - This hypothesis simplified the calculations.
2. The material of which the beam is made is elastic and follows Hooke′s law, and has the same modulus in tension and in compression.
   - There still is controversy.
   - Reilly & Berstein demonstrated a difference for the elastic modulus of cortical bone in tension and in compression, while Keaveny found the same modulus in tension and in compression.
3. Poisson′s ratio $\nu$ is neglected.
   - This hypothesis ($\nu \fallingdotseq 0$) is invalid by reality, but does not lead to too inaccurate results.

$$\varepsilon = \frac{M \cdot z}{[E \cdot I]}$$

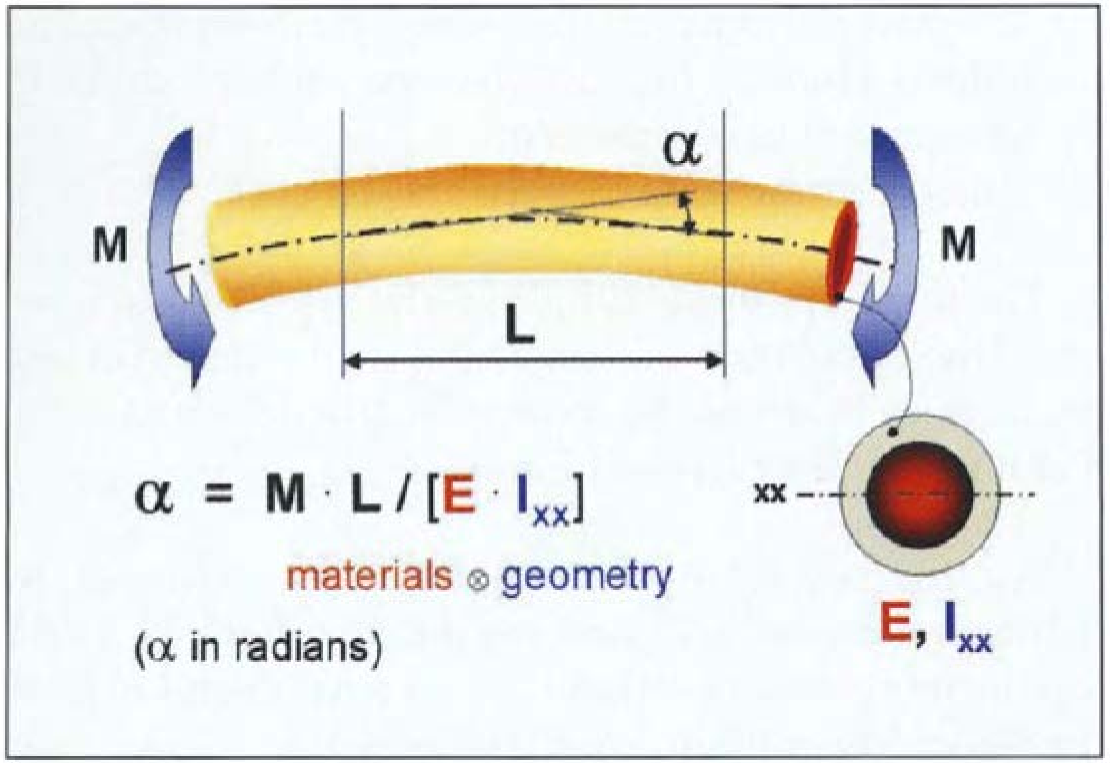



- Bone strain ε is…
  - Proportional to
    - The bending moment, M
    - The distance to the neutral axis, z
  - Inversely proportional to
    - The bending stiffness, [E · I]

E is the elastic modulus

$I_{xx}$ is the moment of inertia relatively to the neutral axis

**Bending produces global deformation (curvature)**
**This curvature can be measured by the angulation $\alpha$ produced on a segment of the length L of the beam.**

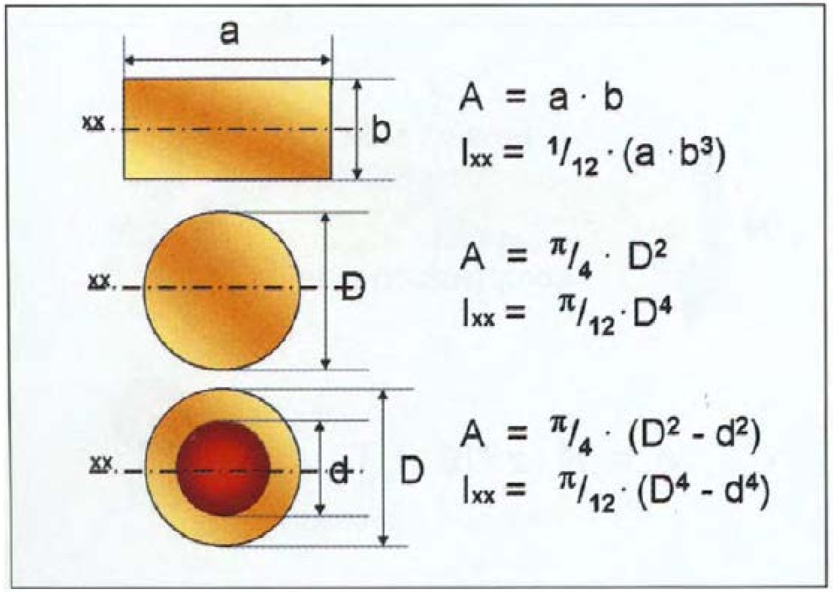


# Moment of inertia: important geometrical characteristics of the cross-section.

- For bending, it fulfils the same function as the cross-sectional area in axial load.

$$I_{xx} = \int_A x^2 \cdot dA$$

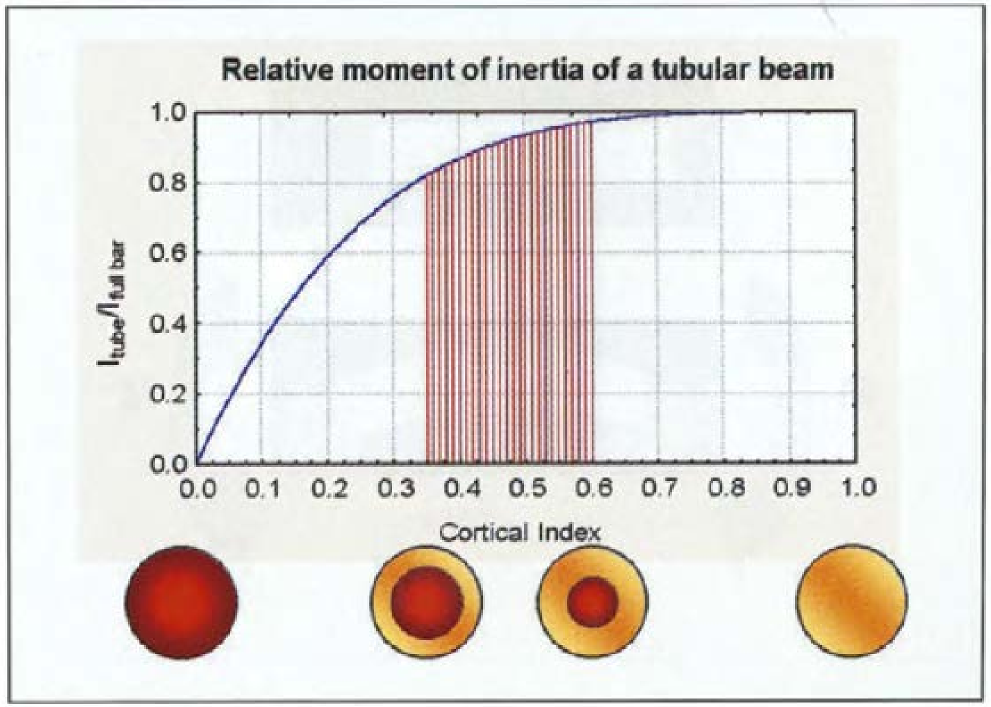
**<Calculation of moment of inertia for different cross-sections>**




- It is very unlinear in regard to the size
- It is proportional to the power 4 of the length – $[L^4]$
  (Area is only proportional to the square of the length – $[L^2]$)
  : this means that only the outer part of the cross-section plays a role in stiffness.

**For the diaphyseal bone, the cortical index is a coefficient comprised between 0 and 1, which displays the importance of the medullary cavity: 0 for an empty tube and 1 for a full bar.**
**For diaphyseal bones, it varies between 0.35 and 0.6.**

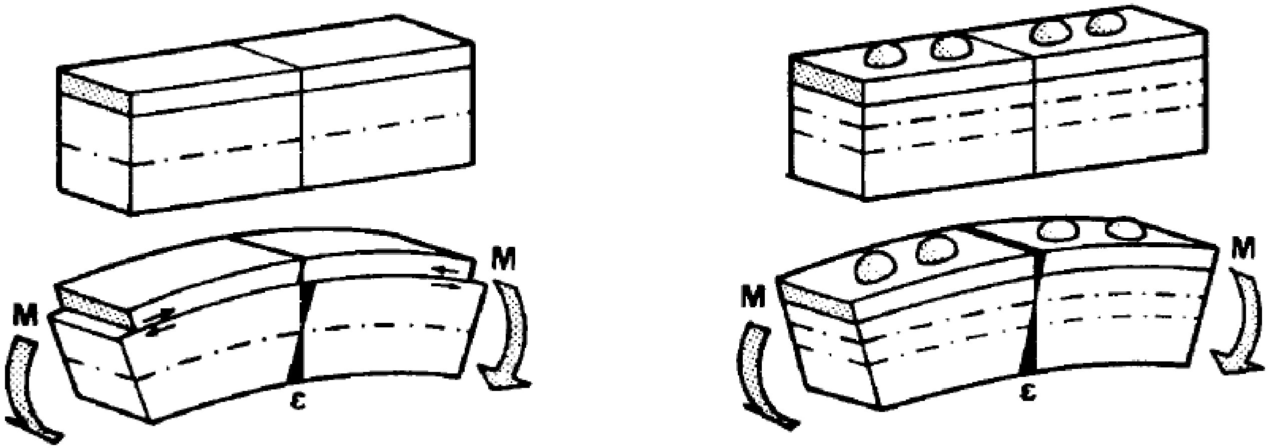
- **With regard to the moment of inertia of a full bar, the reduction is less than 5% for a thick tube (normal bone), and nearly 20% for a thin tube (osteoporotic bone).**



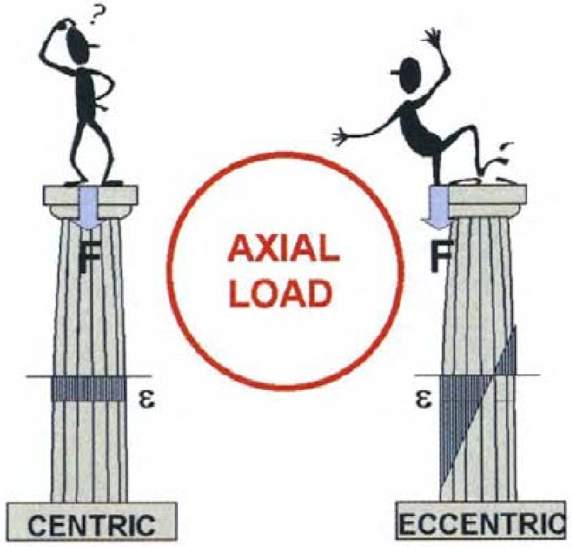
## The composite beam theory

- *Linear bending theory* is generalized to *composite beam theory* in case where the beam is made of more than one different materials.
  - For instance, reinforced concrete consists of iron bars embedded in concrete.
- A new hypothesis must then be made:

  4. No slippage occurs between the different materials.



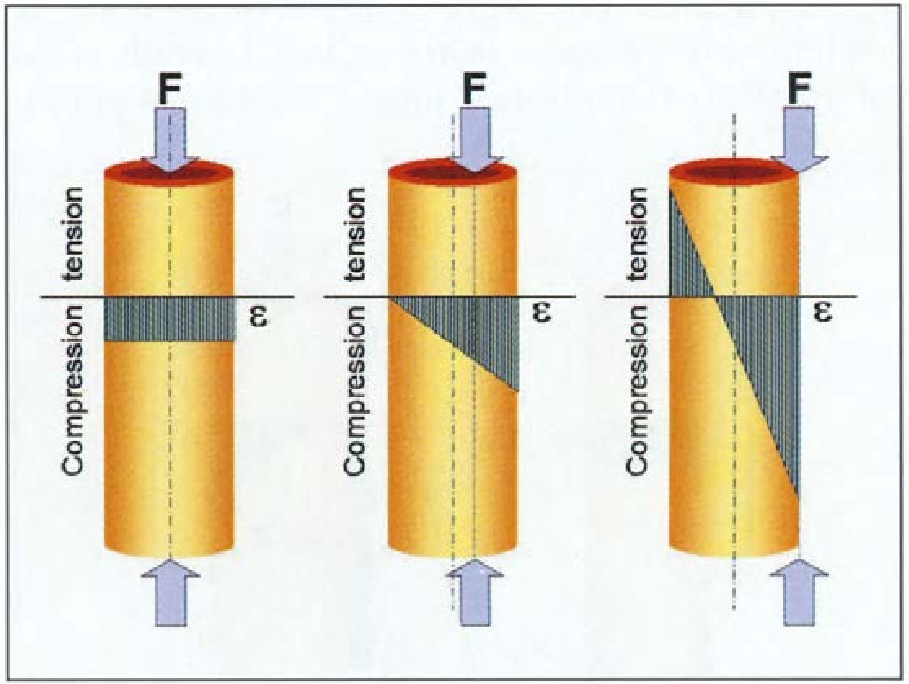
# Eccentric Axial Load




**Eccentric axial load produce inhomogenous strain within the column due to the bending moment relative to the neutral axis.**

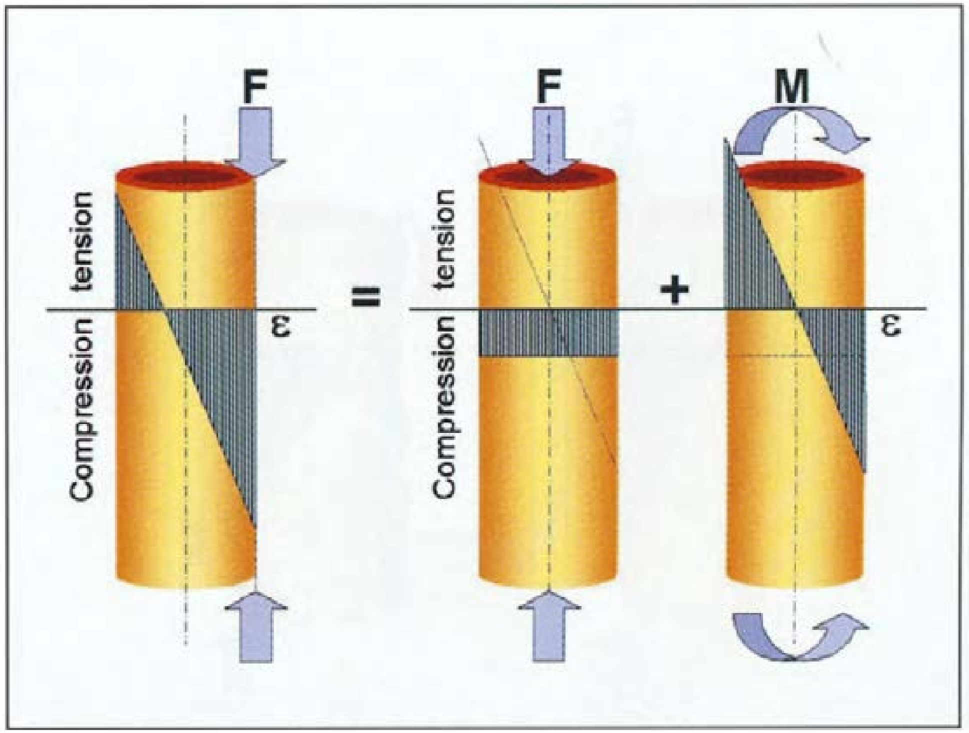
- **using the linear bending theory, we are able to calculate the effect of this bending.**

- **The strain within the beam is homogenous only if the load is applied at the centroid (i.e. the center of gravity of the cross-section).**



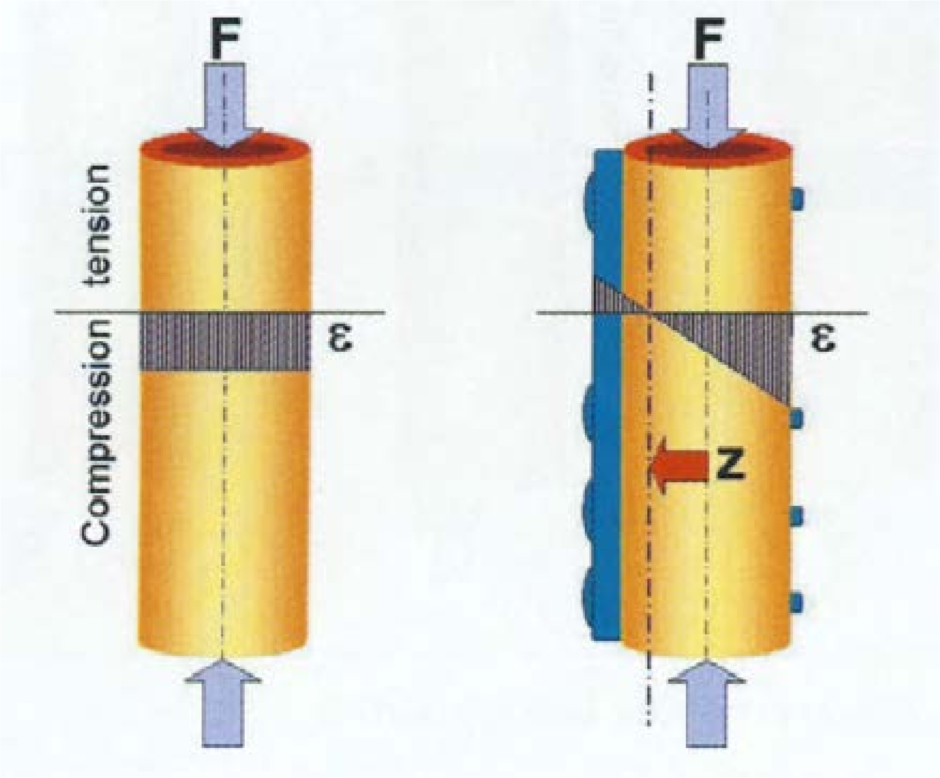
# Eccentric load application = superimposition of centric load application and bending moment

- The bending moment being the product of the axial force by the distance of the line of application of this force to the neutral axis.



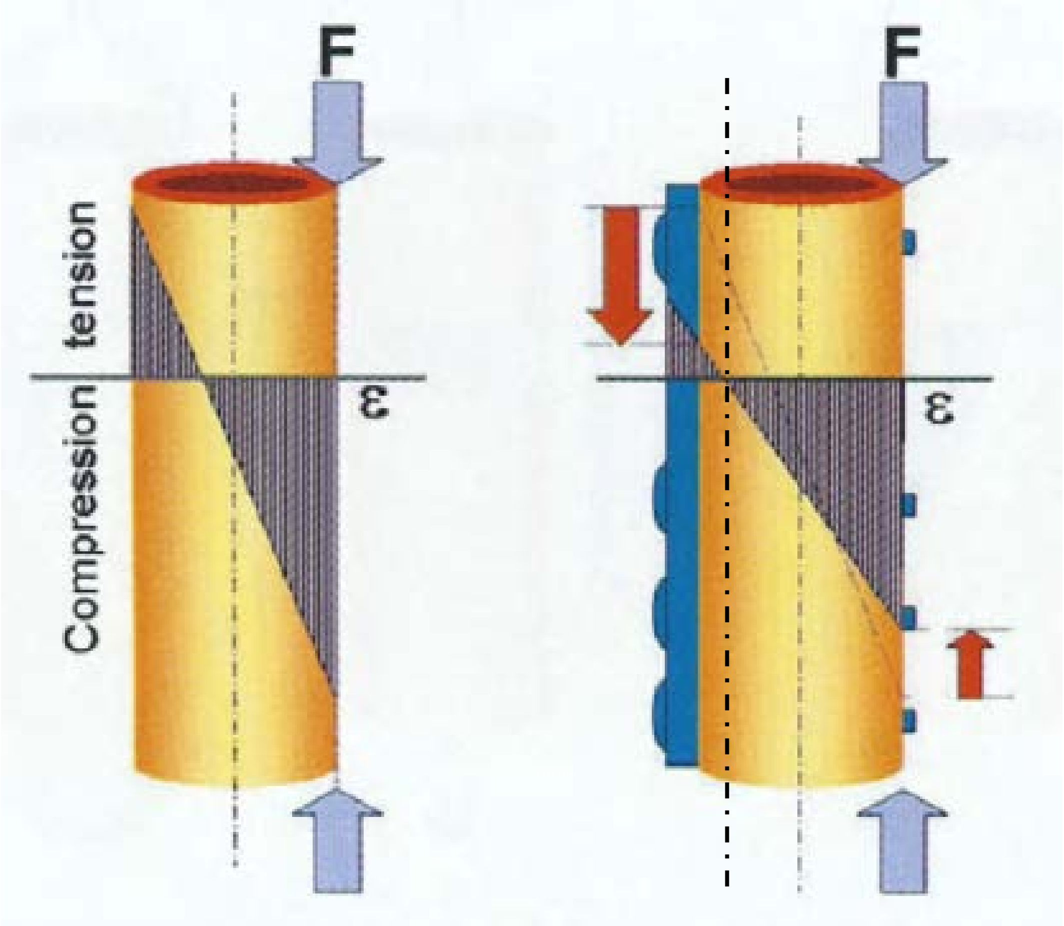
## Typical application of these principles:
# Plate in a bone subjected to centric axial load

- The application of a plate produces apparent eccentricity due to the shift Z of the neutral axis from the center of the bone to the plate
  - The strain is markedly reduced under the plate and might be transformed from compressing strain to tensile strain
  - At the opposite cortex, the strain is increased.



## Stress protection by the plate in a bone subjected to eccentric axial load

**When the plate is affixed to the tensile aspect of the bone, in application of the tension band principle, the effect is similar but less pronounced.**



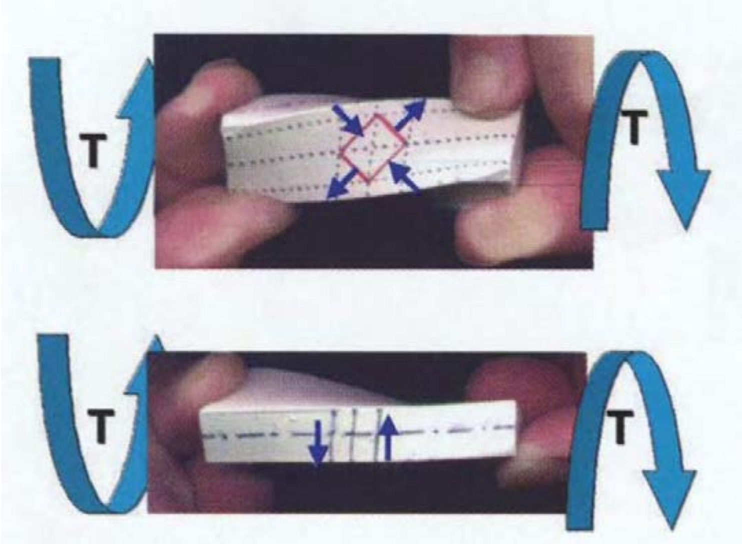

- **Marked reduction of the strain under the plate and less pronounced reduction of strain at the opposite cortex.**

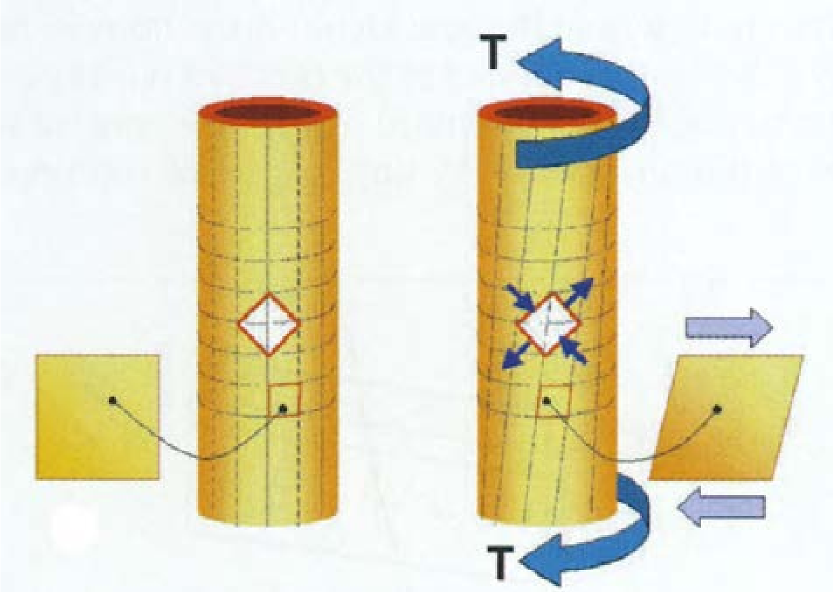
# Torque

**Angular deformation** γ in torque is proportional to the torque and inversely proportional to the torsional stiffness [G.Ip]

$\gamma = T / [G.Ip]$

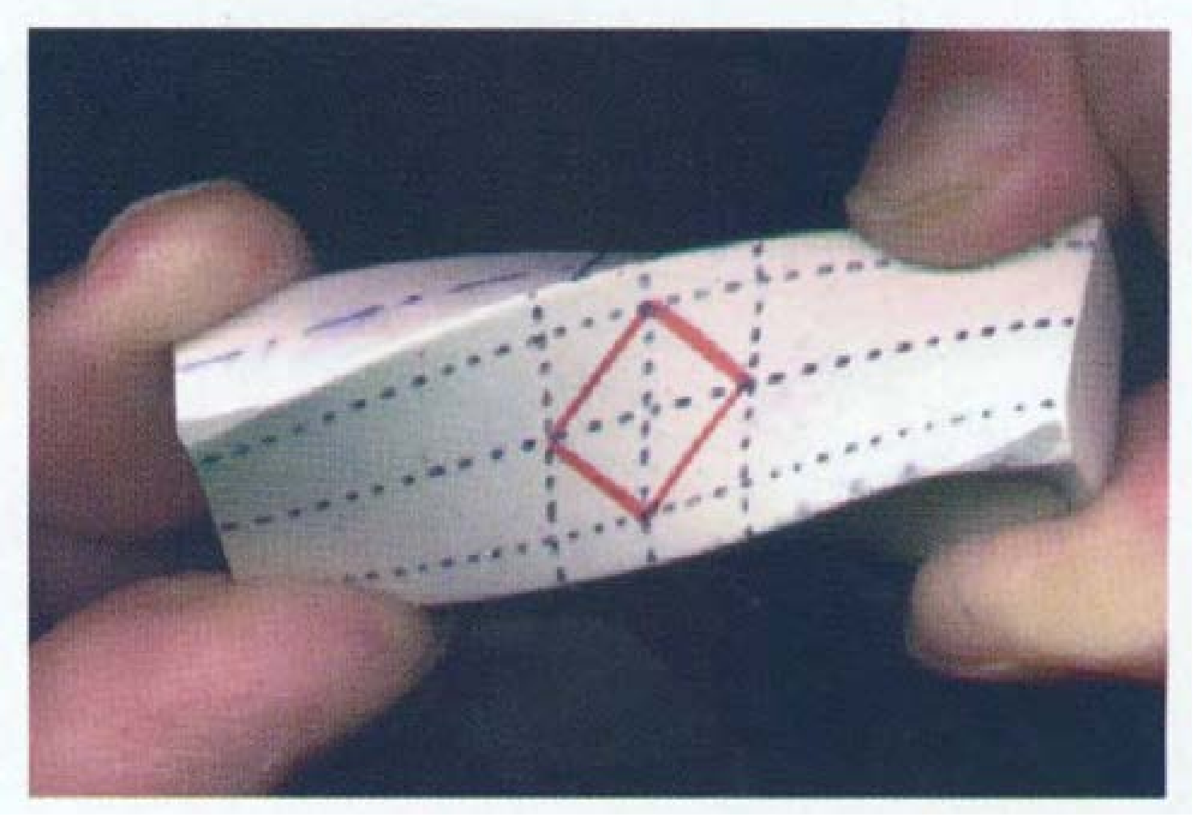
G is the modulus of rigidity
**Ip is the polar moment of inertia of the cross-section.**
**Ip = $I_{xx} + I_{yy}$ (sum of the two principal axial moments of inertia**



# Torque is not as simple as bending.

- Deformation of eraser in torque indicate that a plane does not remain planar after deformation, but makes more of an S shape.



# No linear torsion theory

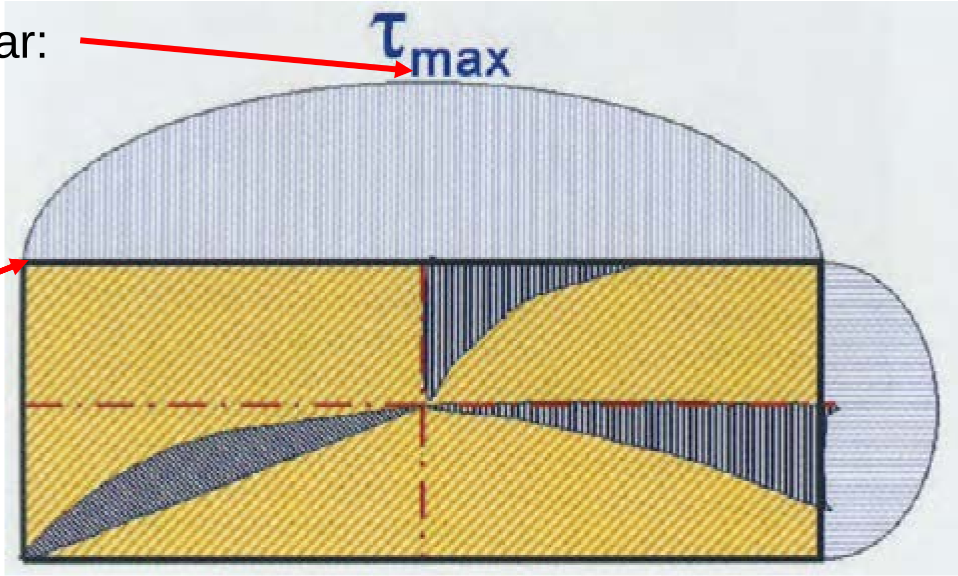
**Linear bending theory allows a simple calculation of the relationship between the strain and bending moments applied.**

**The equation $\gamma = T / [G.Ip]$ suggest that linear torsion theory can be stated. However, this would lead to wrong results.**

- **For a non-circular beam, the strain and the stress are not linearly proportional to the distance to the centroid of the cross-section.**
- **The complex relationship between the stress and its distance to the centroid:**

Maximal stress measured hear:

Stress is equal to zero here:



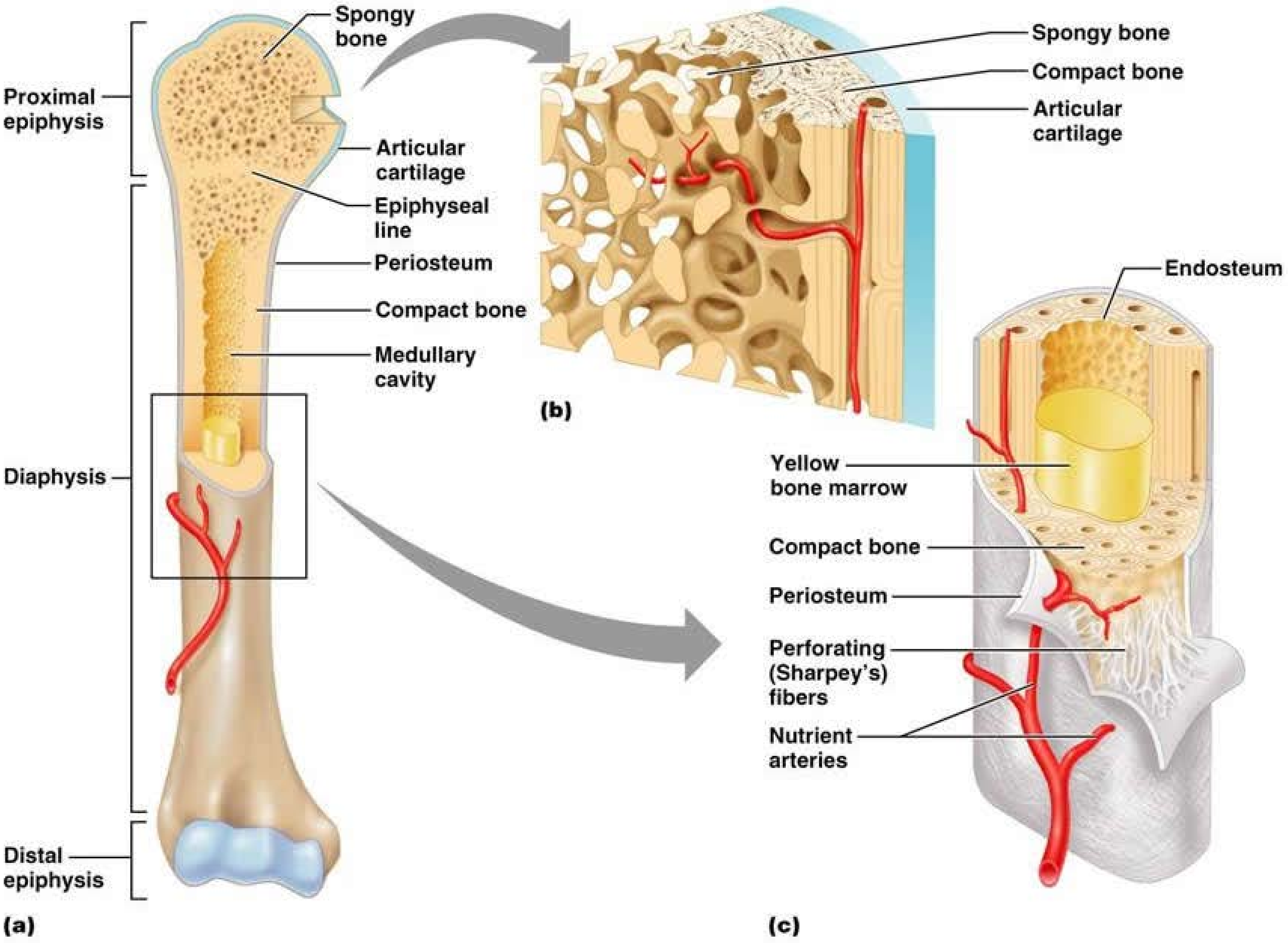
# Bone

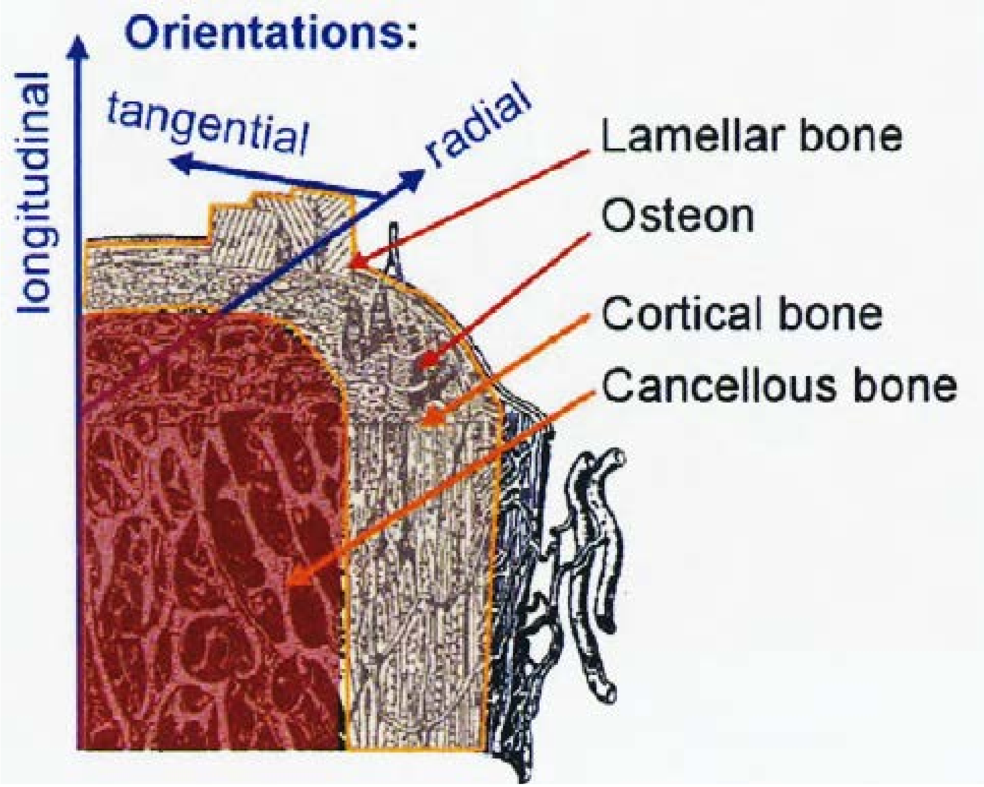
## Cortical Bone

- Dense and homogenous
- Anisotropic: mechanical properties are not equal in all directions.
  - Stronger about the longitudinal orientation than in tangential or the radial direction
  - This is due to the fact that osteons are oriented along the long axis of the bone and they are glued to the neighboring osteons.
- The orientation of the anisotropic directions relate to its adaptation to physiological loads: *Wolff's Law*
  - *The shape of the bone being given, the amount and the structure of bone adapts itself to the (dynamic) physiological loads applied to it.*

## Cancellous Bone

- Consists of bone trabeculae


Spongy bone
Proximal epiphysis
Articular cartilage
Epiphyseal line
Periosteum
Compact bone
Medullary cavity
Diaphysis
Distal epiphysis
(a)
Spongy bone
Compact bone
Articular cartilage
(b)
Endosteum
Yellow bone marrow
Compact bone
Periosteum
Perforating (Sharpey's) fibers
Nutrient arteries
(c)

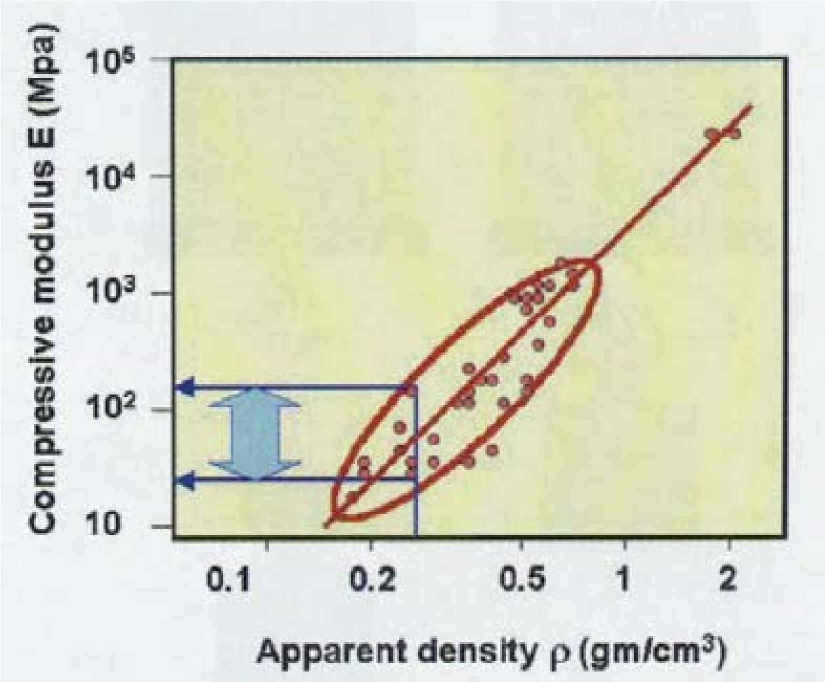


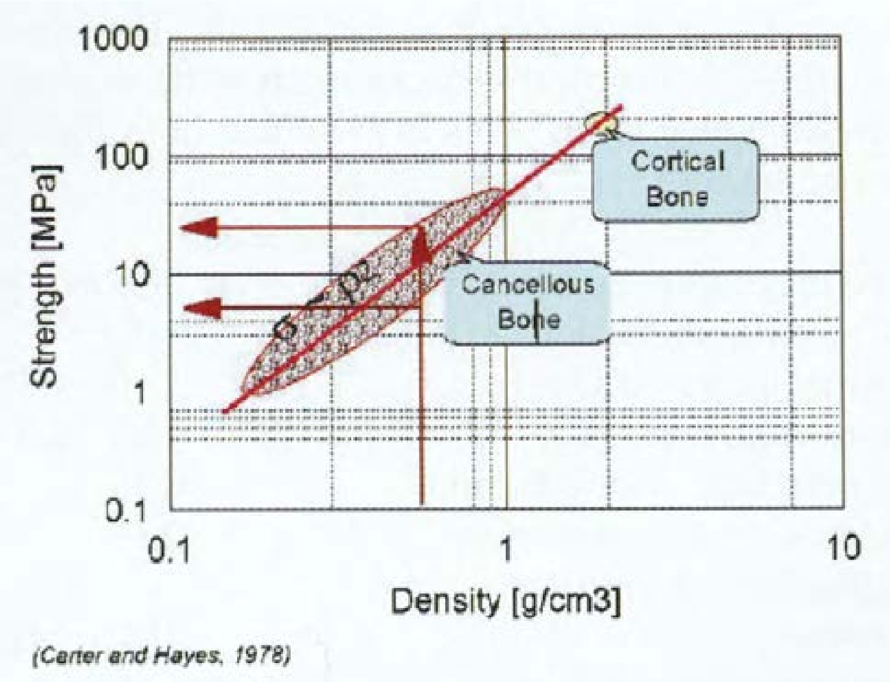

# The mechanical properties of the cortical bone given by Reilly and Burstein:

| | | |
|---|---|---|
| Longitudinal modulus E | 17.0 | GPa |
| Transverse modulus E | 11.5 | GPa |
| Poisson's ratio ν longitudinal | 0.46 | |
| Poisson's ratio ν transverse | 0.58 | |
| Longitudinal strength σ tension | 133 | MPa |
| Longitudinal strength σ compression | 193 | MPa |
| Longitudinal shear strength τ (/bone axis) | 68 | MPa |
| Transverse strength σ tension | 51 | MPa |
| Transverse strength σ compression | 133 | MPa |

# Mechanical properties of bone

- **CARTER & HAYES: Mechanical properties (elastic modulus E and stress σ) are related to the cube and the square of the density $\rho$ relatively.**



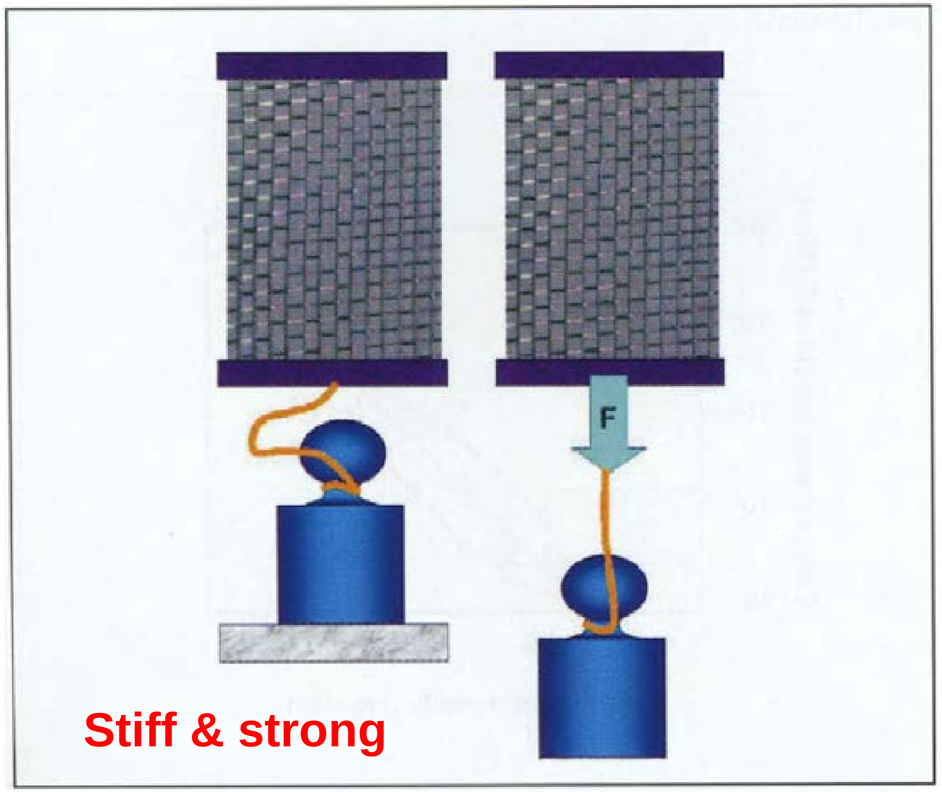
# Mechanical strength of bone in relation to the direction of load

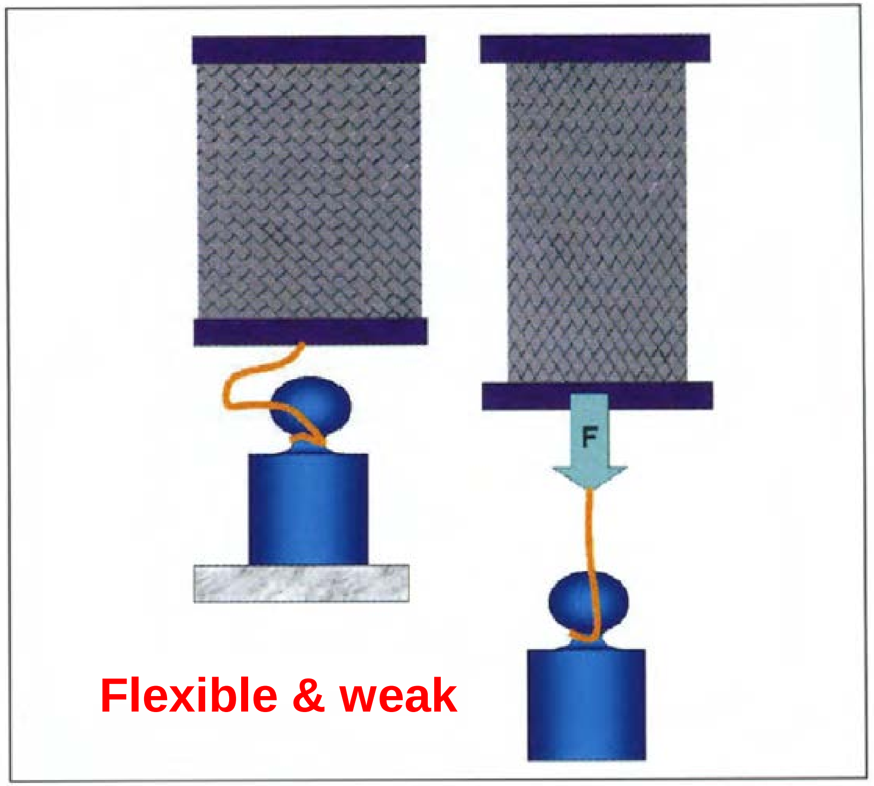
- Sound prediction of the mechanical properties from the density is very imprecise because it pretty much depends on the orientation of the load in regard to the bone structure

**<u>Load applied…</u>**

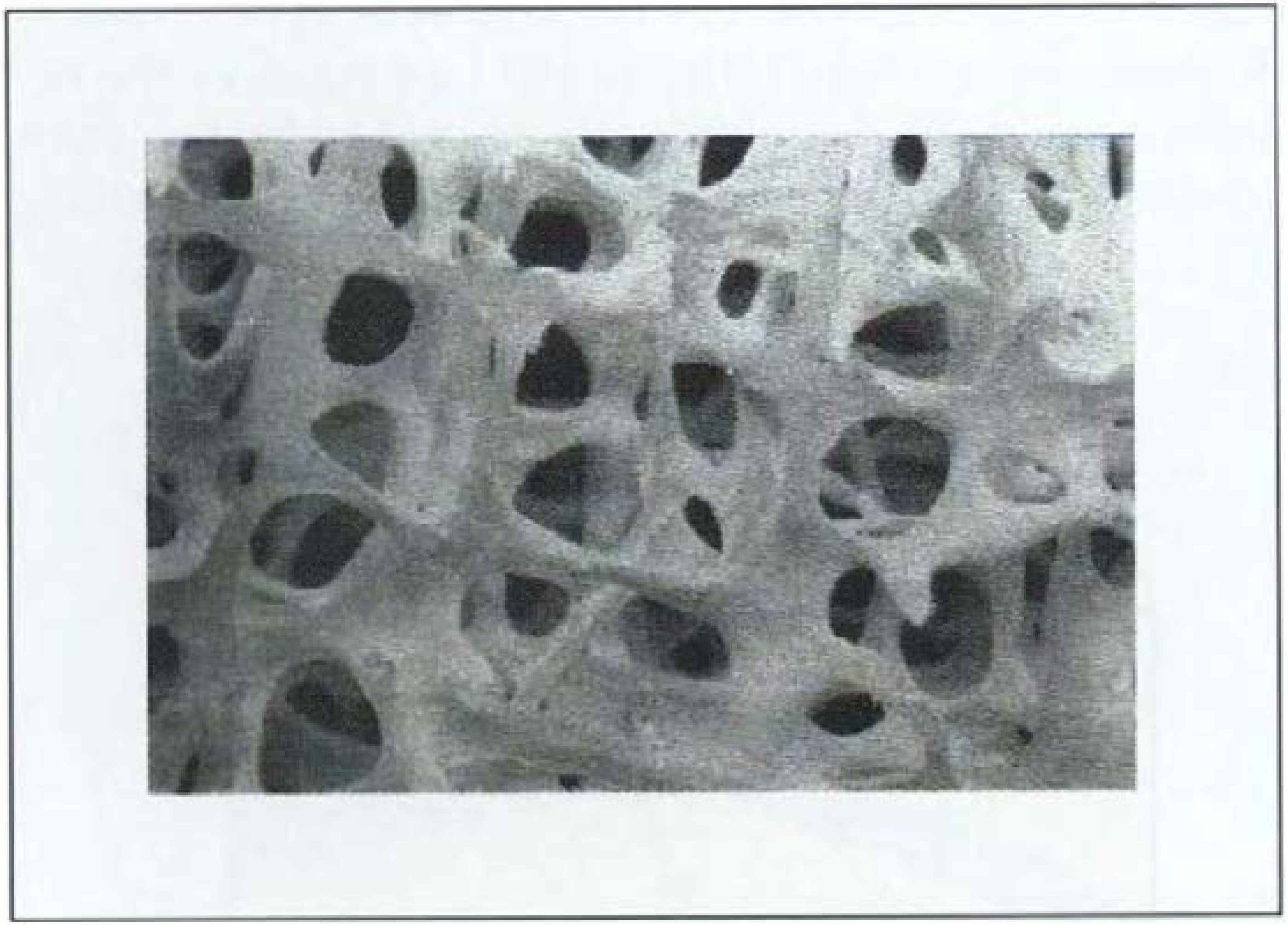
**In the same direction as the trabeculae**

**At 45° to trabeculae**



# Bone is orthogonal: the trabeculae are connected at 90 º each other.

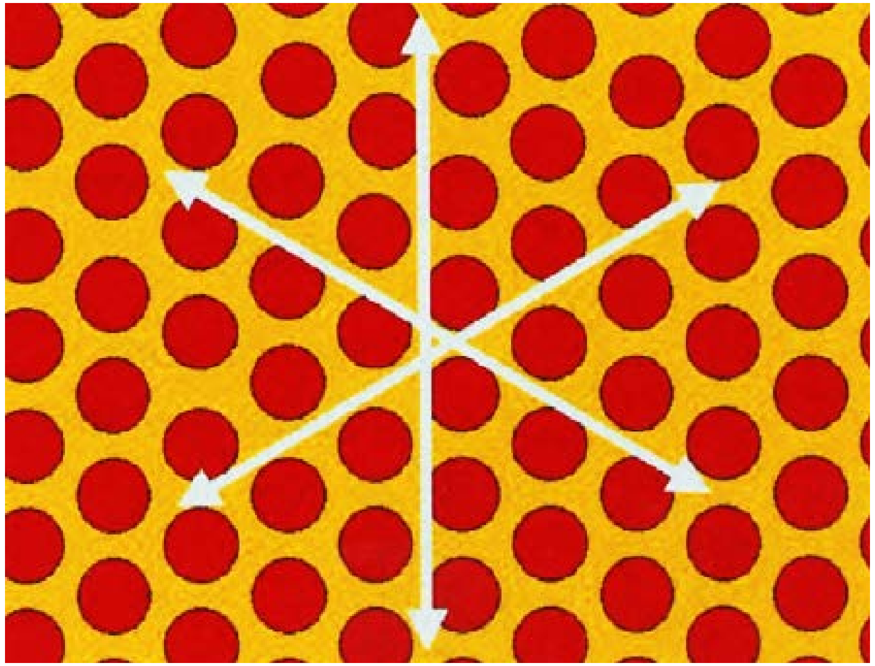


# Simulation of Bone

**Engineers prefer to use a standard material, such as plastic foam (e.g. Polyurethane).**

**Using foam is not relevant because of the structural difference**

- **Foam: a set of bubbles of gas in a solid or liquid material**
  1. **The structure of foam is not orthogonal, rather has a hexagonal structure.**
  2. **It is not trabecular because the holes are not interconnected.**

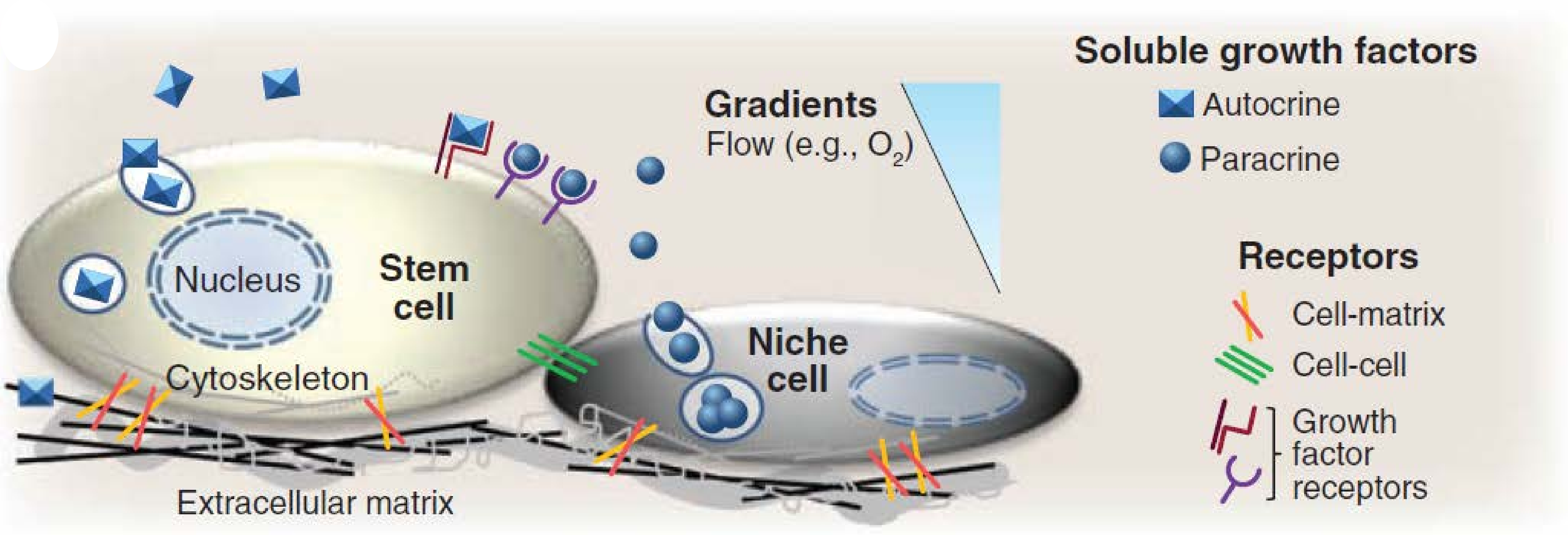


# Mechanotransduction

[DE Discher, 2009 Science; Orr AW 2006 Dev Cell]

# Niche

- In vivo microenvironment that regulates stem cell survival, self-renewal, and differentiation
- Key niche component include GFs, cell-cell contacts, cell-matrix adhesions



# Forces

Cells generate force and are often exposed to force
The very first stages of cell differentiation in embryogenesis are indeed blocked after knockout of ubiquitous force-generating myosins
Fluid forces typical of blood flow have been found to initiate an endothelial program in isolated ESCs
Imposing substrate strains of just 5% can induce MSC differentiation toward smooth muscle.

# Stem cells may well have more than the typical ensemble of force-coupled signaling pathways as a means to sensitize themselves to micro-environments that range-physically-from flowing fluids and strained tissues to solid tissues of varied elasticity

## MSCs

- When MSCs are grown on firm gels that mimic the elasticity of muscle and that are coated with collagen I, myogenic markers are up-regulated, whereas when MSCs are grown on rigid gels that mimic pre-calcified bone, the cells appear osteogenic
- Cell lines that are already committed to muscle or bone appear less responsive to the same cues

## NSCs

- Neuron differentiation is favored on soft matrices that mimic normal brain, whereas differentiation into glia is promoted on harder matrices that typify glial scars.

## Not only physical contributions to differentiation but also that carefully made materials can help prime the expansion of specific progenitors

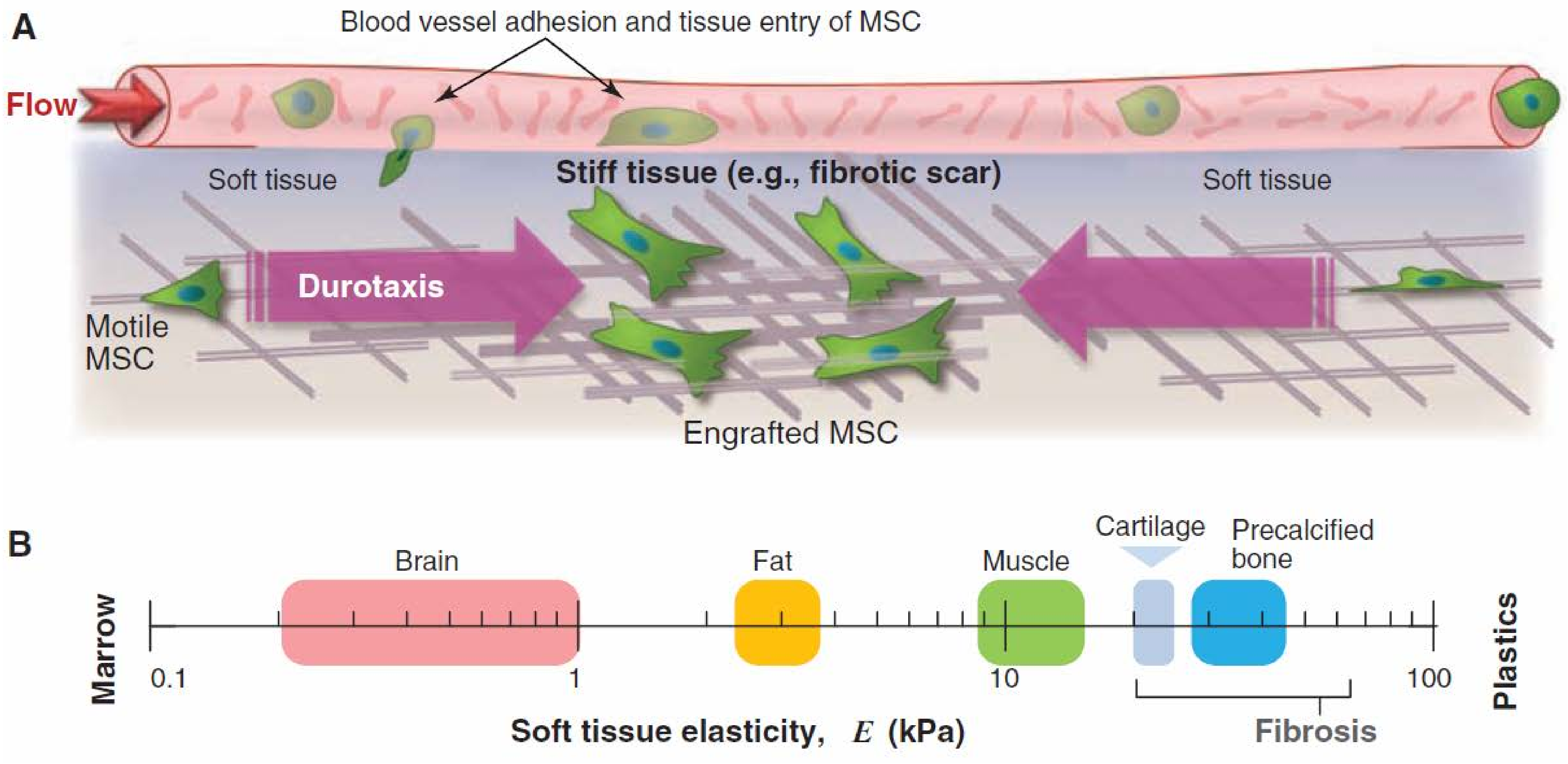
# IV (intravenous) injection of stem cells

- Fluid forces push and drag the cells
- Fluid forces oppose adhesion to the vessel wall
- Similar process to those widely studied with leukocytes and metastatic cancer cells

# Metastatic "capture" depends strongly on at least one matrix fibrosis factor

- Lysyl oxidase (cross-linker of collagen)

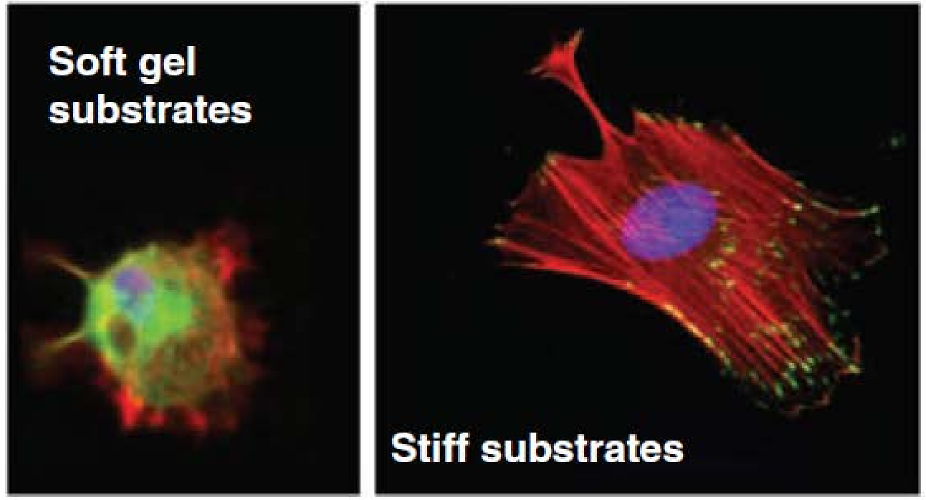
- The fibrotic tissue is locally rigidified by at least seven-fold compared with normal tissue
- AFM probing gives cell-scale elasticities of E = 20~60 kPa for fibrotic wounds
- Rigid fibrotic tissue can contribute a homing signal
- Durotaxis: most cells are found to adhere, to spread, to assemble their cytoskeleton and to anchor more strongly to stiff substrates.

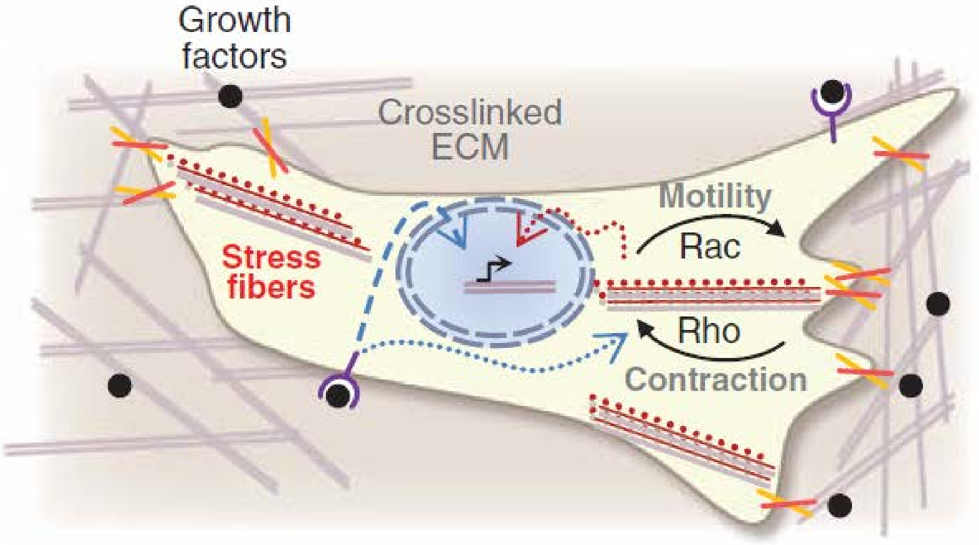


**In a gradient of elasticity, cells therefore accumulate on stiffer substrates, which might constitute a biophysical basis for why MSCs home to sites of injury and fibrosis**

**Matrix can also be a more potent differentiation cue for MSCs than standard induction cocktails.**

**Well-studied differentiation of fibroblasts to myofibroblasts requires both a stiff matrix and TGF $\beta$, with GF release from the ECM dependent on cell contraction-driven unfolding of the ECM complex that sequesters the TGF $\beta$.**



# Effect of matrix rigidity:

- Cardiogenesis involves a complex interplay of mechanochemical factors
- Embryo-derived cardiomyocytes maintain their spontaneous beating on substrates with elasticity less than or equal to that of normal heart tissue.
- But the cells stop beating on rigid matrices that mechanically mimic a fibrotic scar
- ROCK (Rho kinase effector) inhibition selectively blocks cell dysfunction on rigid substrates

# Effect of area:

- If the area of MSC contact is controlled with adhesive patterns, it is found that mixed induction cocktails that induce both fat and bone lead to <u>adipogenesis on small islands</u> (which minimized matrix contact) and <u>osteogenesis on large islands</u> (which maximize contractile anchorage)

# Mechano-transduction

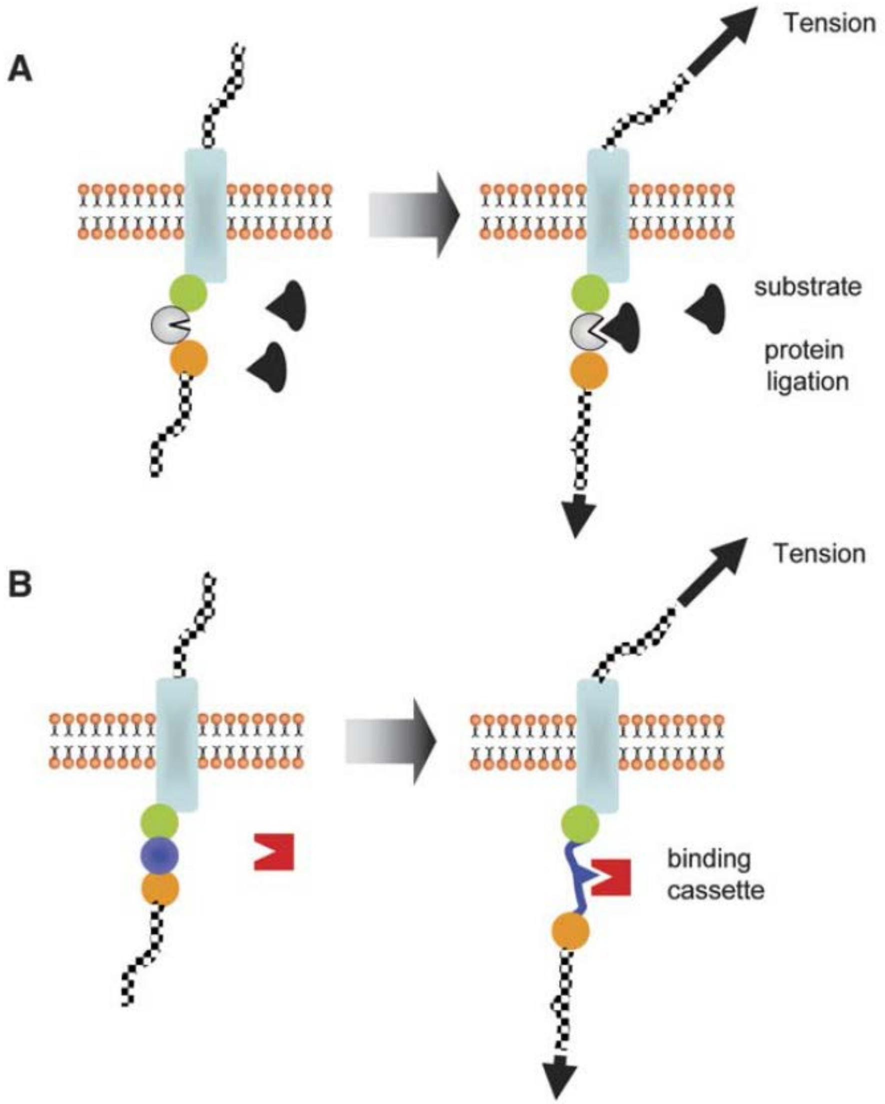
**The conversion of physical force into biochemical information**
**Examples:**

- Pressure and shear stress of pumping blood in vascular system
- Force-driven bone remodeling
- Neural responses to pressure organize sensing to hearing and touch
- Inflation and deflation of lung

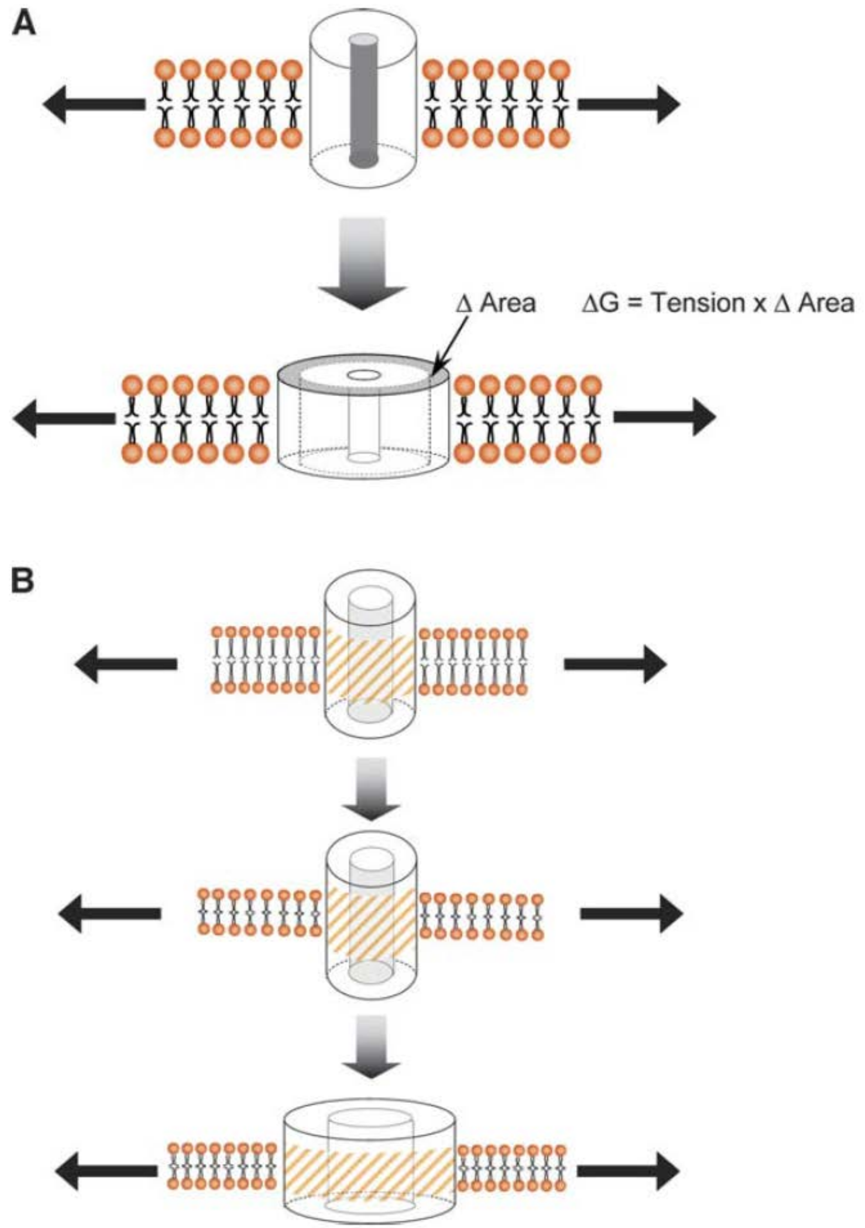
**Remarkable breadth of mechano-sensitive events makes it unlikely that one or even a few mechano-transducers can account for all of these events.**

# Force-Induced Change in Protein Conformation

- Protein folding in general favors the formation that yields the lowest free energy
- Physical forces that modify the energy landscape will therefore directly alter protein folding
- Just as protein phosphorylation or other posttranslational modifications mediate signal transduction in large part through changes in protein conformation, force-induced effects on conformation represent a general mechanism by which enzymatic activity or protein interactions can be modified to mediate signaling



- Stretch-sensitive channels provide the best-studied example.
- Increasing tension within the lipid bilayer from 10-12 to 20 dyn/cm increases the channel-opening probability
- If the open state occupies a greater area within the bilayer, membrane tension will result directly in lower free energy.
- Protein unfolding under tension represents a one-dimensional instance of the same principle where unfolding lowers the free energy.



# Mechano-Transduction in Focal Adhesions or Cytoskeletal Structures

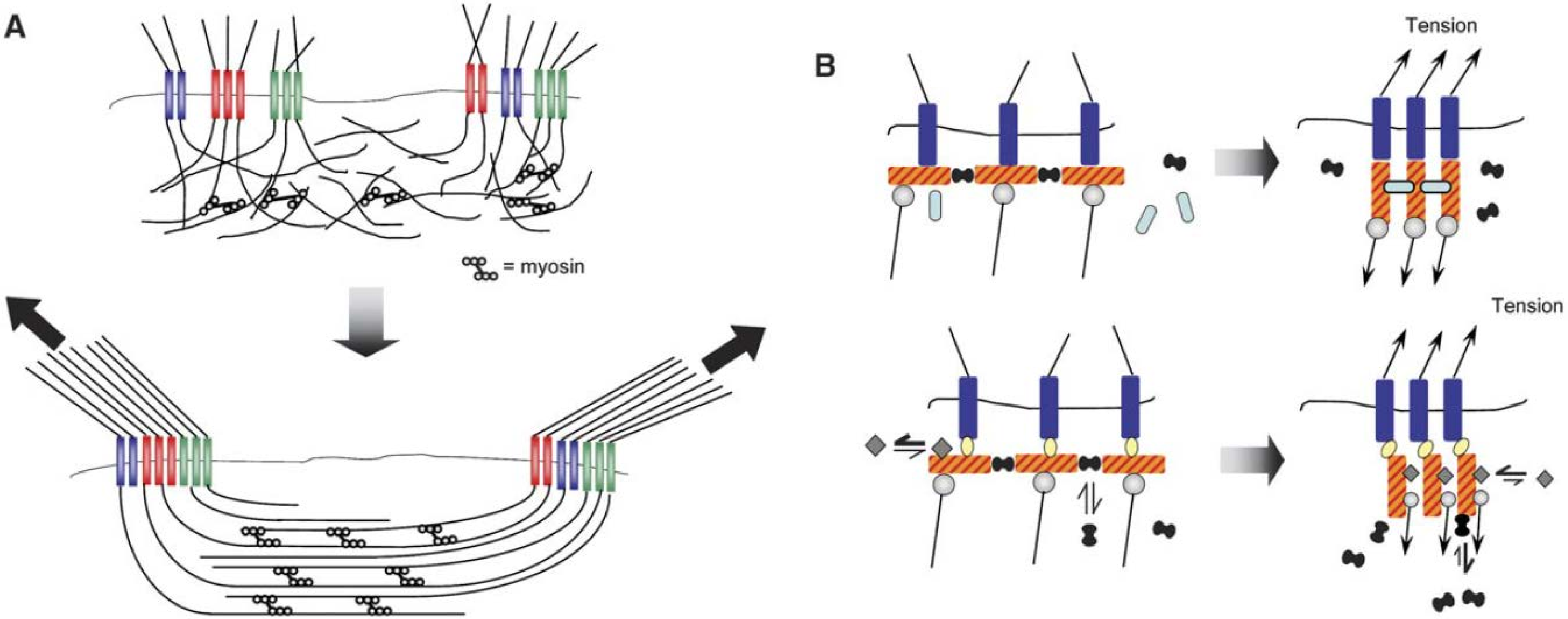
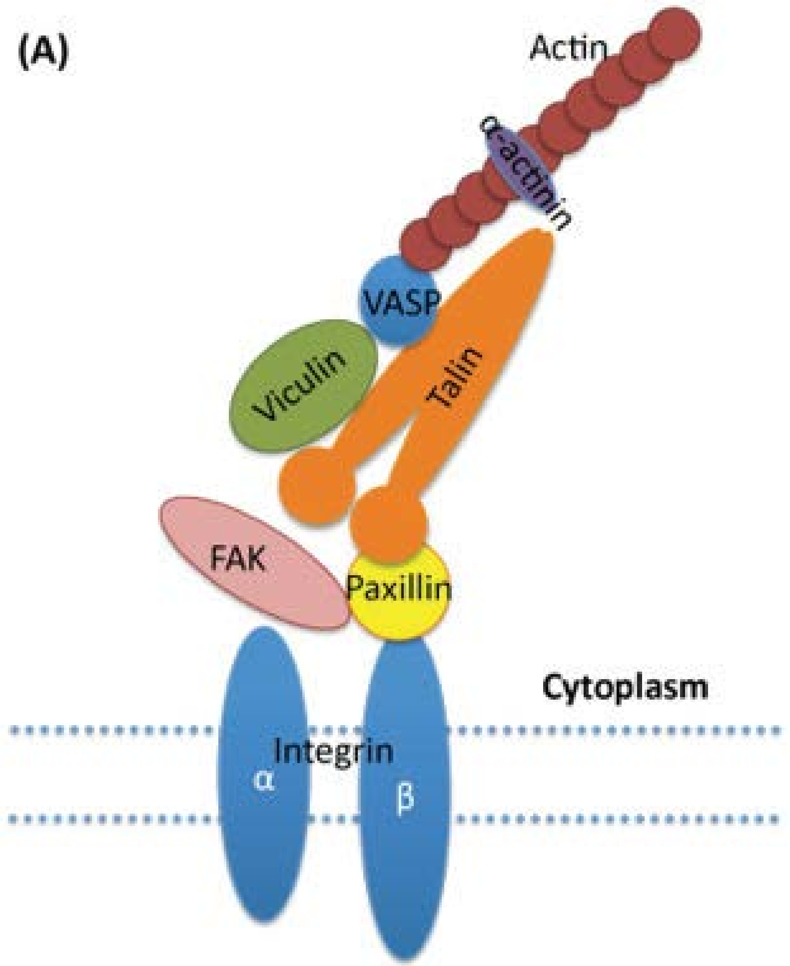
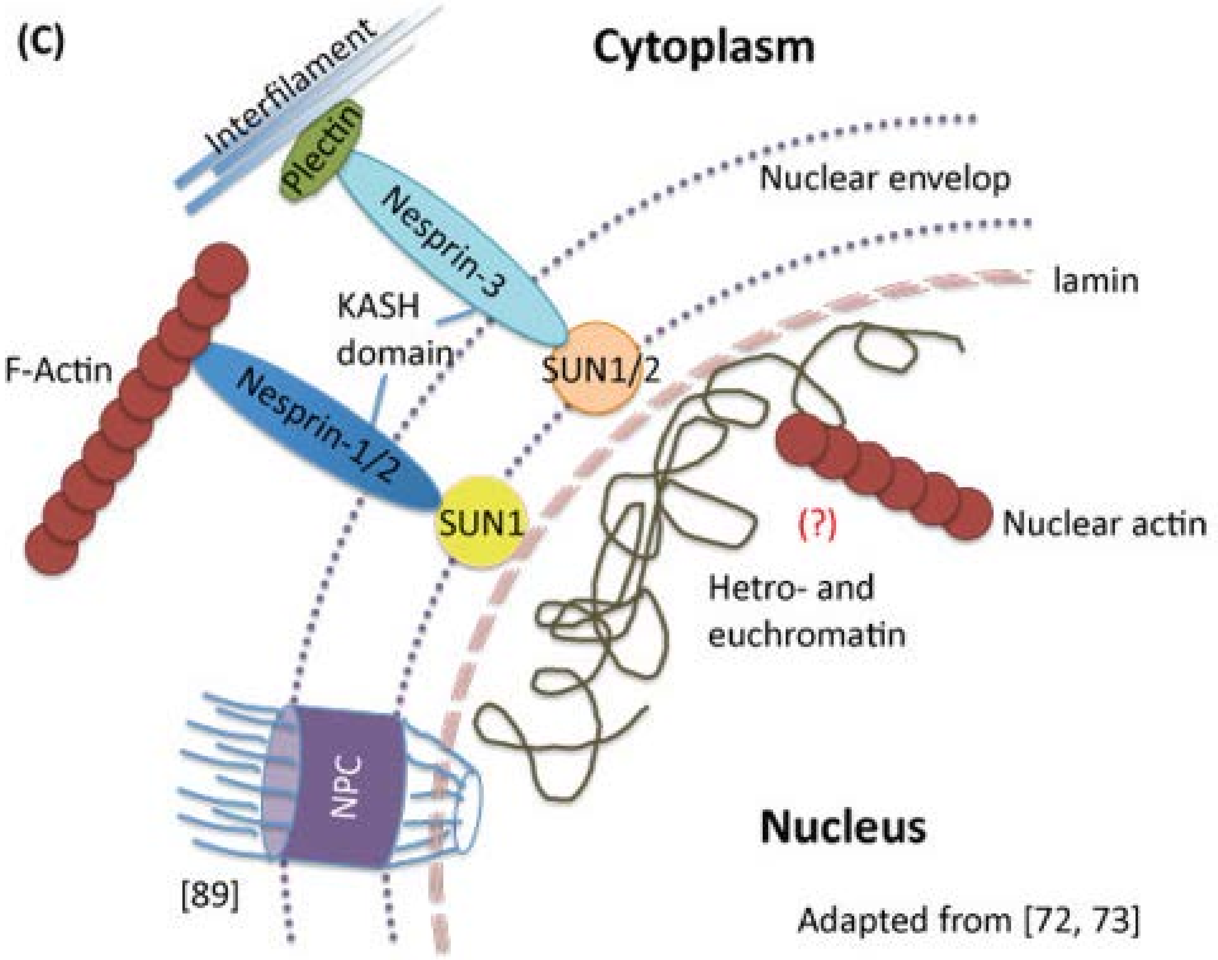


Table 1. Estimated In Vivo Magnitudes of Mechanical Stimuli on Cells

| Physical Profile | Mechanical Stimulus | Typical Values |
|---|---|---|
| Arterial blood flow | Fluid shear stress | 1–3 $N/m^2$ |
| Cell migration | Traction stress | 3.0–5.5 $kN/m^2$ (normal); 1 $kN/m^2$ (cancer) |
| Proximal tubule flow | Fluid shear stress | 0.3 $N/m^2$ |
| | Fluid drag force | 0.0074 pN/microvillus |
| | Bending torque | 0.016 pN-μm/microvillus |
| Stretch-activated channels | Membrane tension | 0.012 N/m |
| Outer hair cell stereocilia | Compression stiffness | 0.001 N/m |
| | Force/Δ membrane potential | 0.1–20 pN/mV |
| Osteocyte processes (bone canaliculi) | Fluid shear stress | 0.8–3.0 $N/m^2$ |
| | Fluid drag force | 20 × shear force |
| | Tissue strain | 0.03%–0.1% |



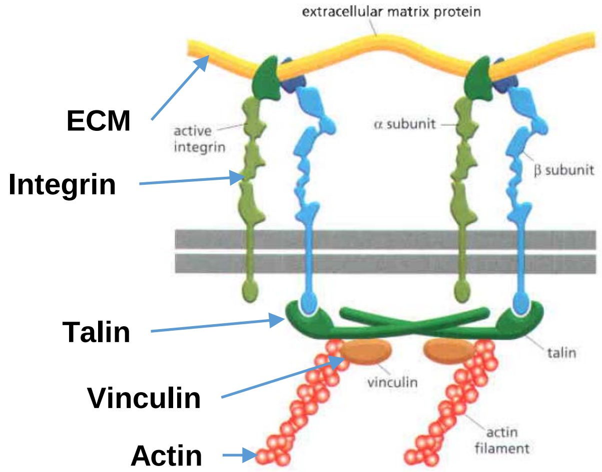



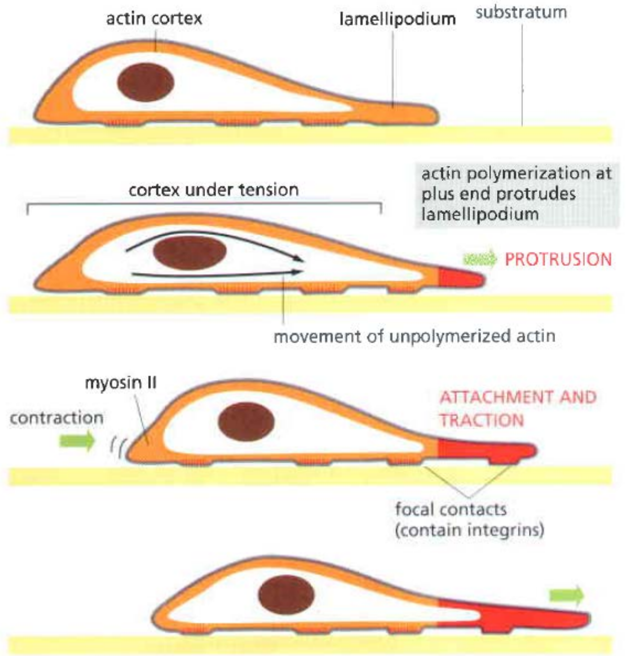

Adapted from [72, 73]

# Assembly & Remodeling of Integrin Adhesions

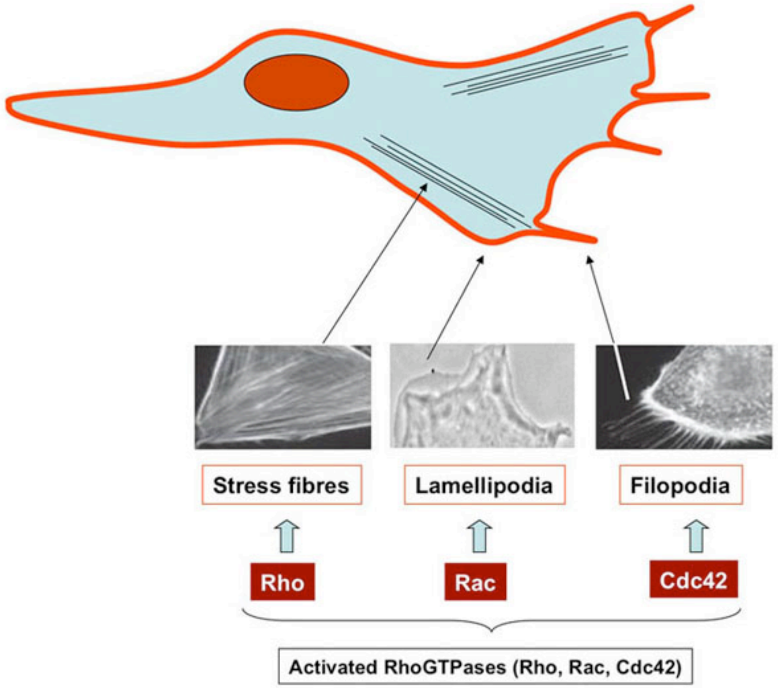
## Lamellipodia
## Local complexes

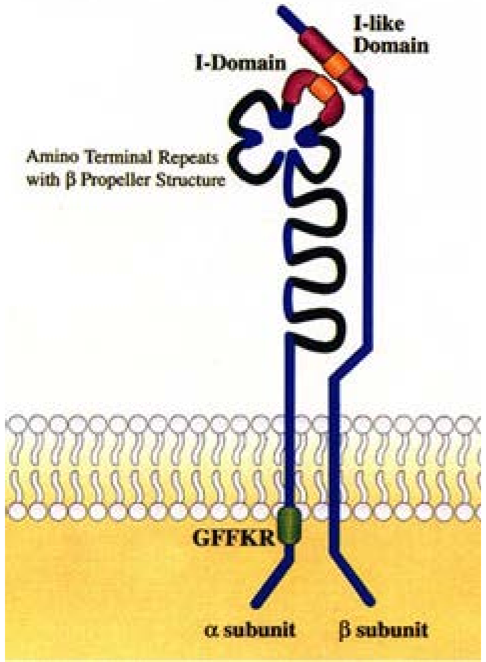
- Earliest integrin-containing structures
- 100nm in diameter
- Including integrin, talin, paxillin
- Binding of vinculin -> talin
    - Triggers clustering of activated integrin
    - Associate with actin through the vinculin tail
    - Strengthening actin-integrin link
    - Drive growth into larger focal complexes



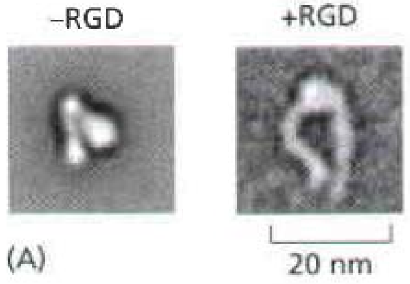



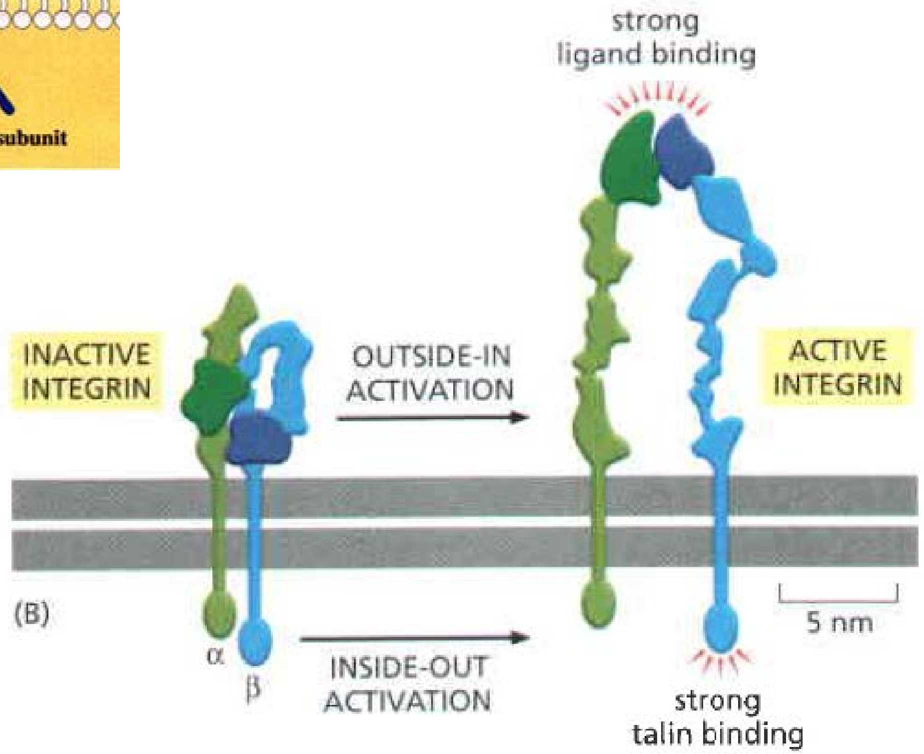

## Lamella
## Tropomyosin & myosin II are prominent
## Located ~2-4um from the leading edge



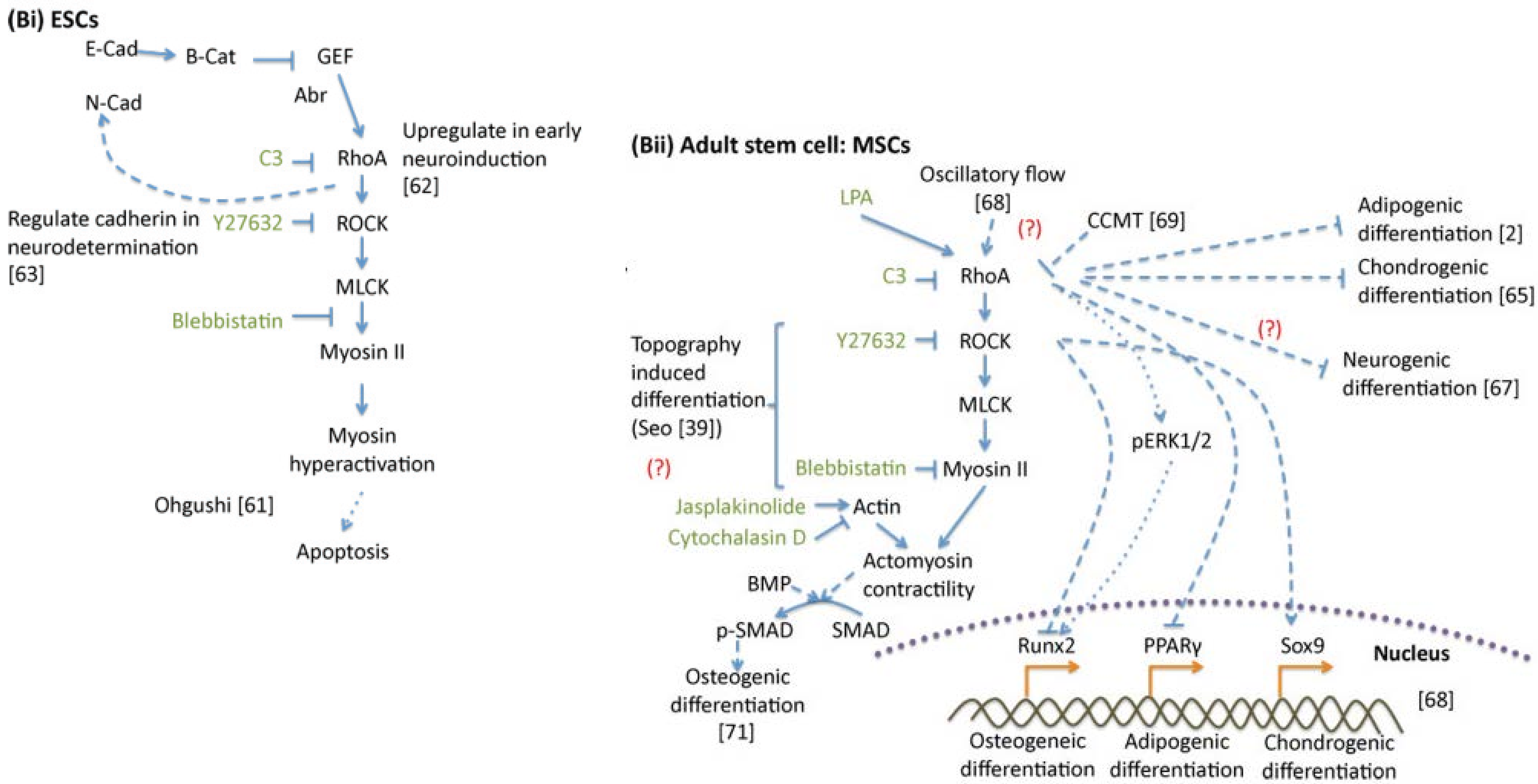




If the traction force is increased, the attached adhesion grows: relaxation of tension leads to its dissociation.



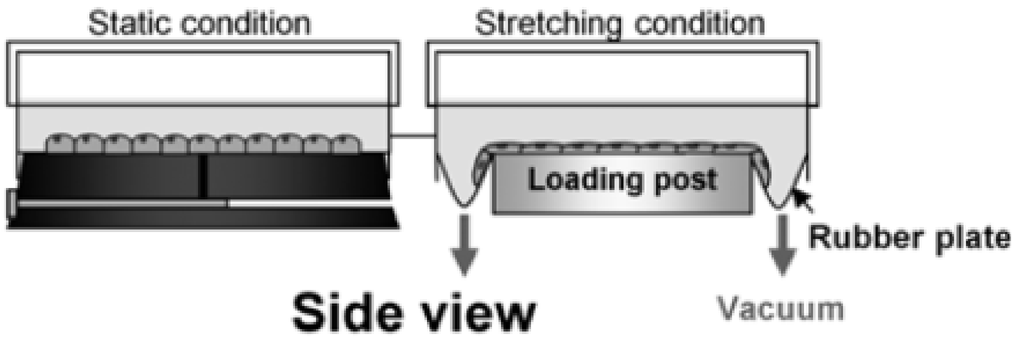
# Signal Transduction in Mechano-Sensing

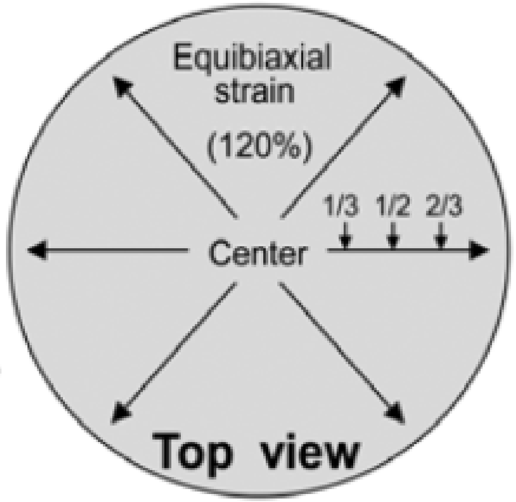


# Bioreactor

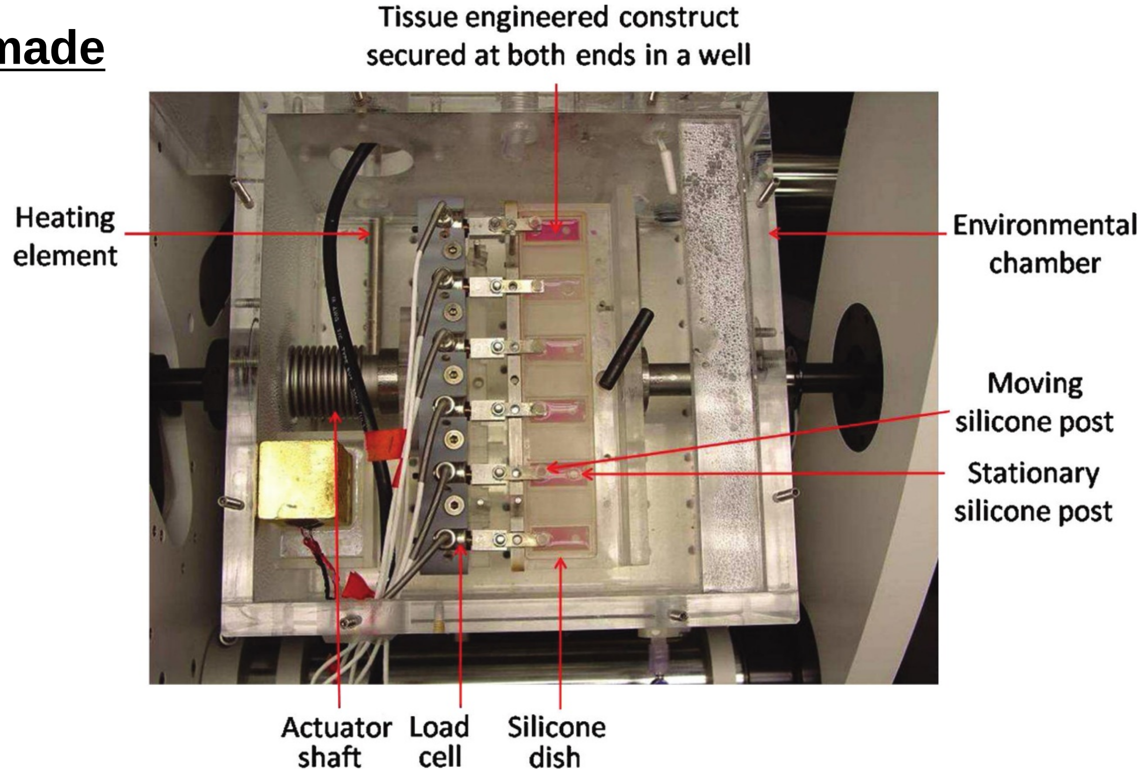
# Bioreactor for tensile stimulus

**FlexCell FX4000**

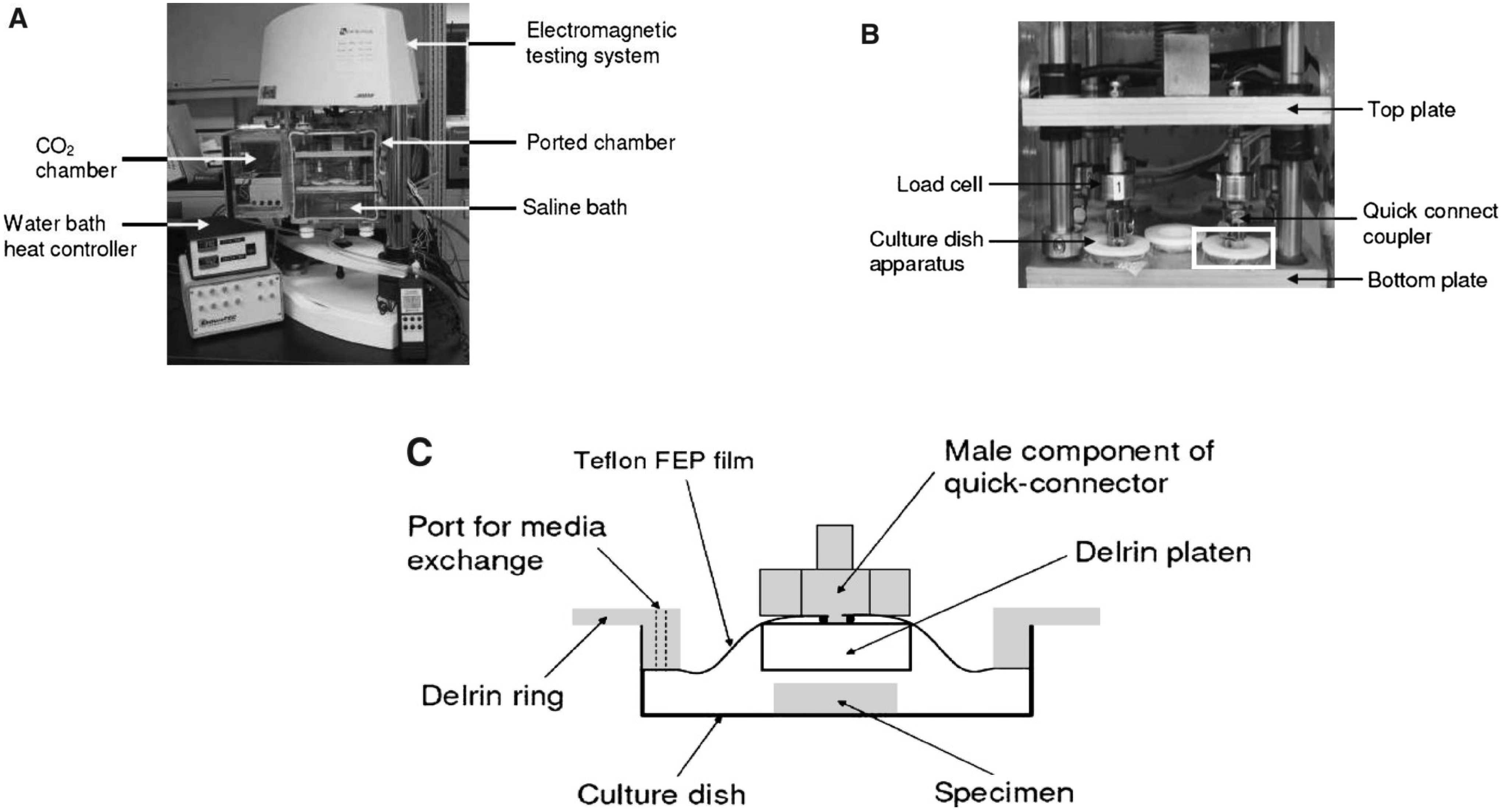


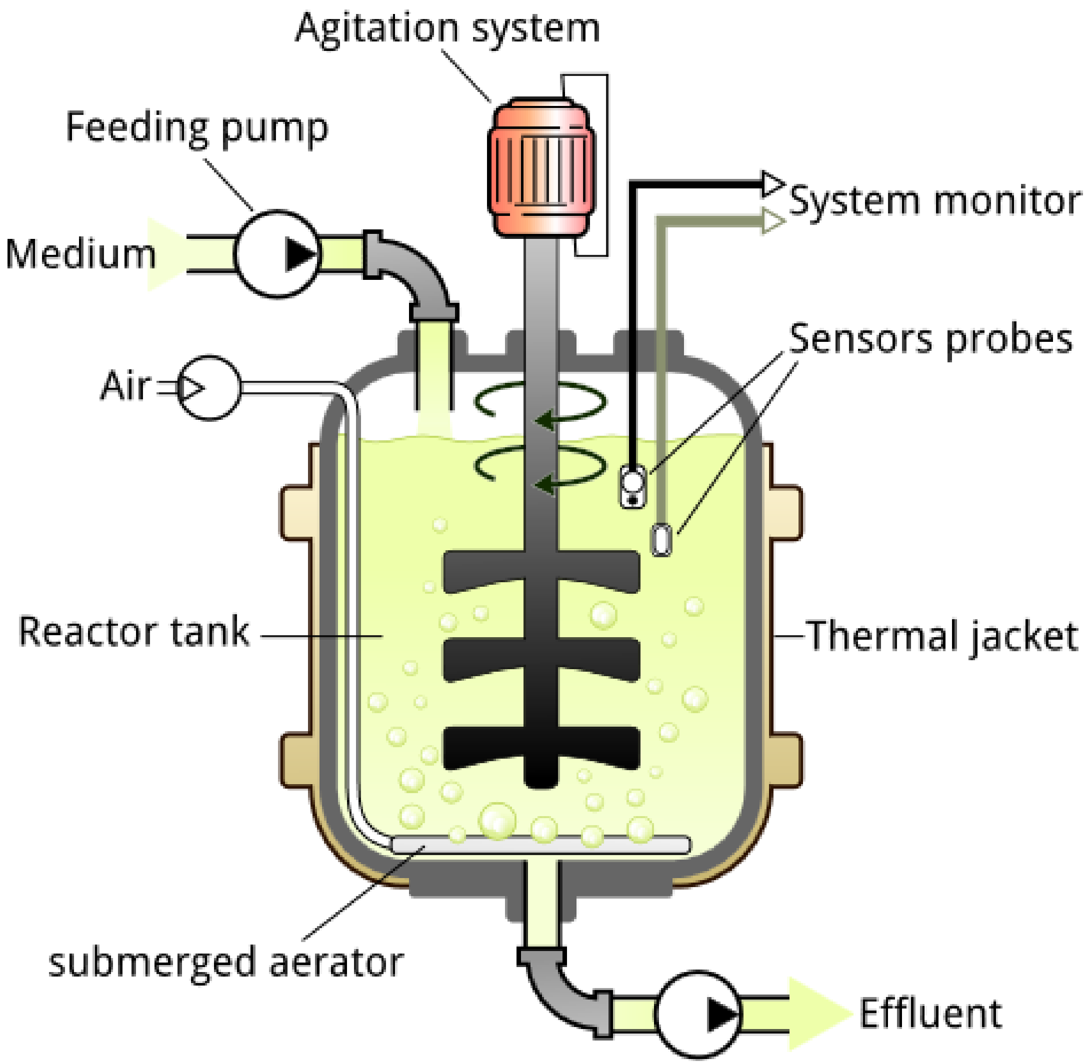

**Custom-made**



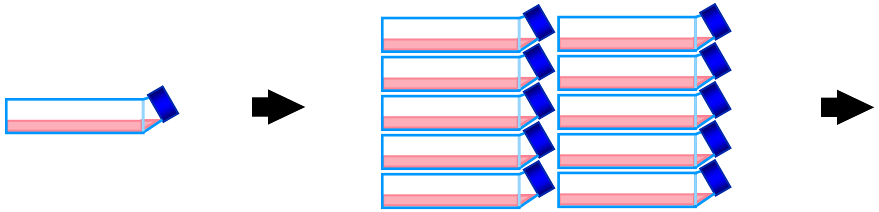
# Compressive Stimulus Bioreactor

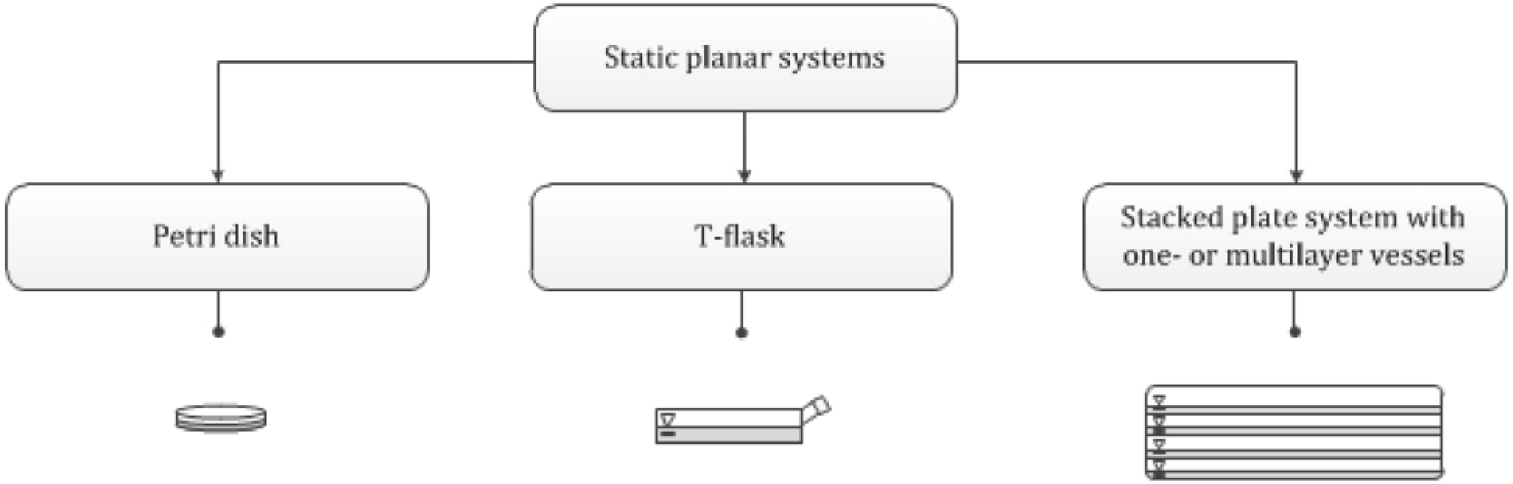


# Mass Culture

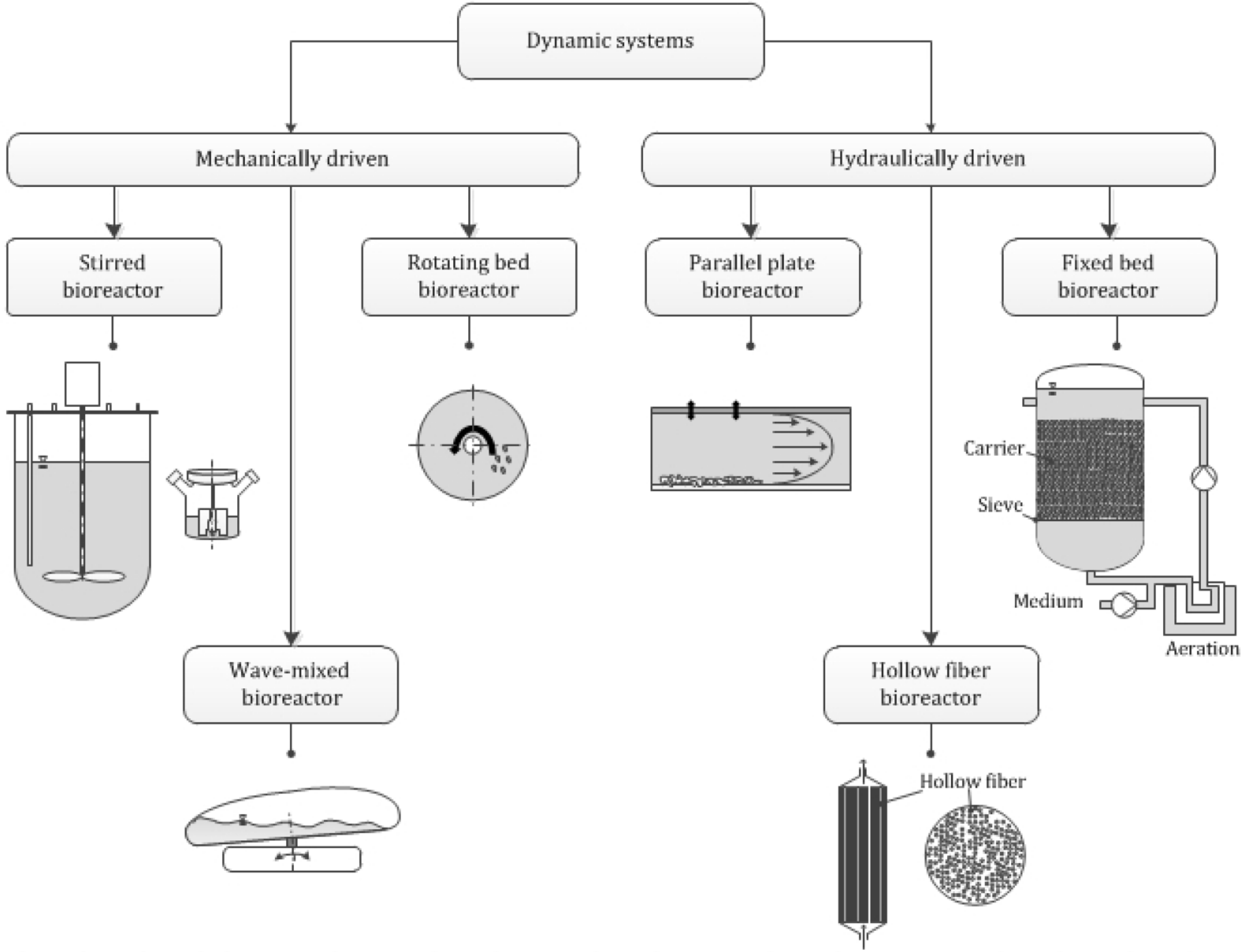


# Scaling up of Stem Cell Culture

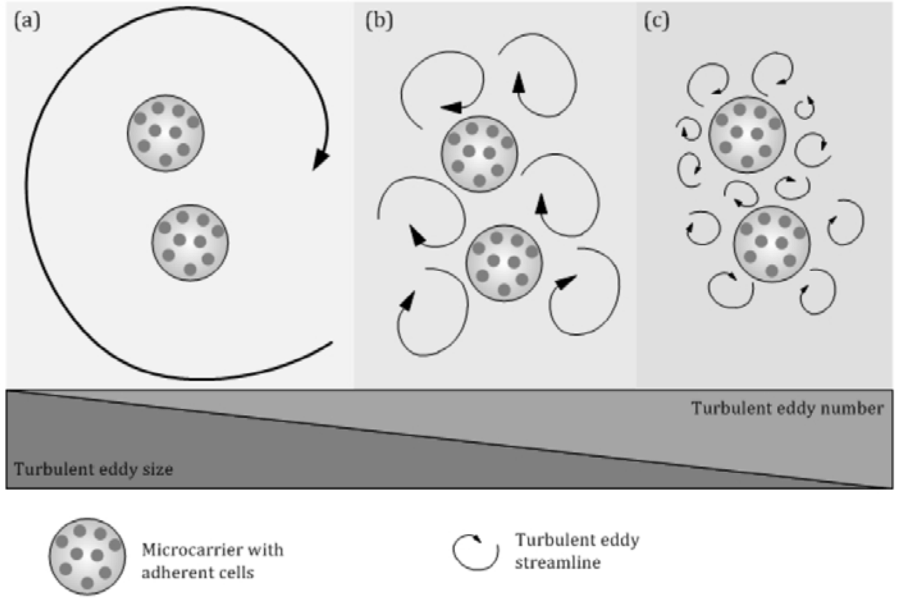


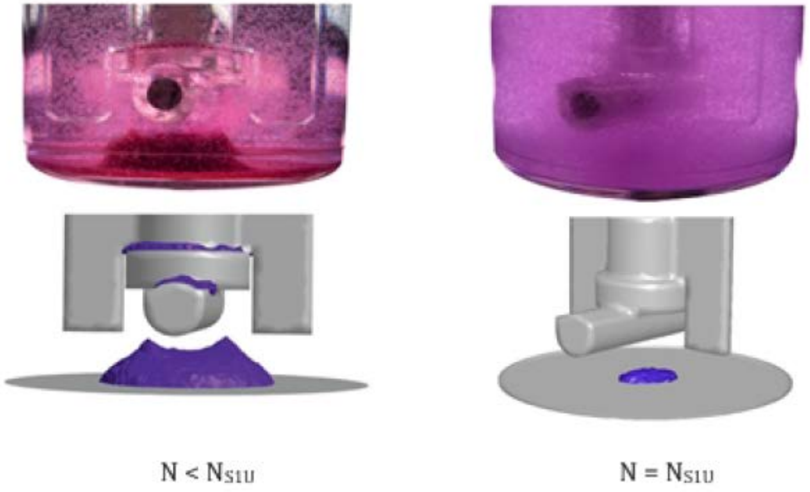


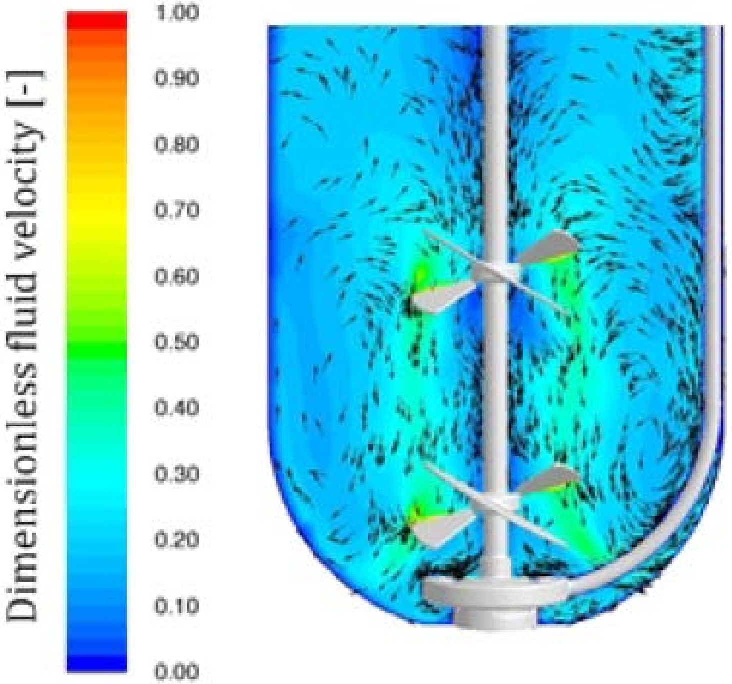
# Dynamic Mass Culture



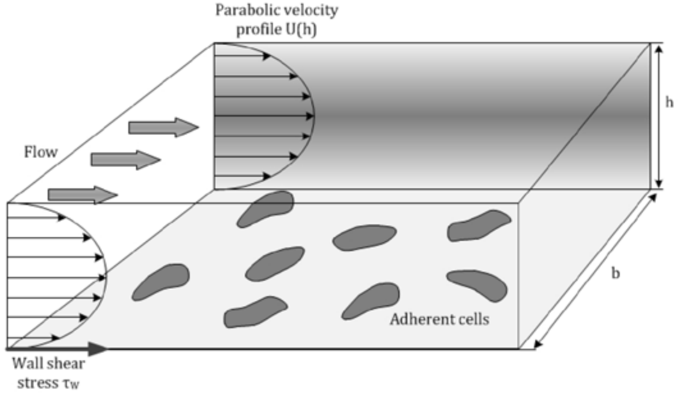
# Stirred Bioreactor



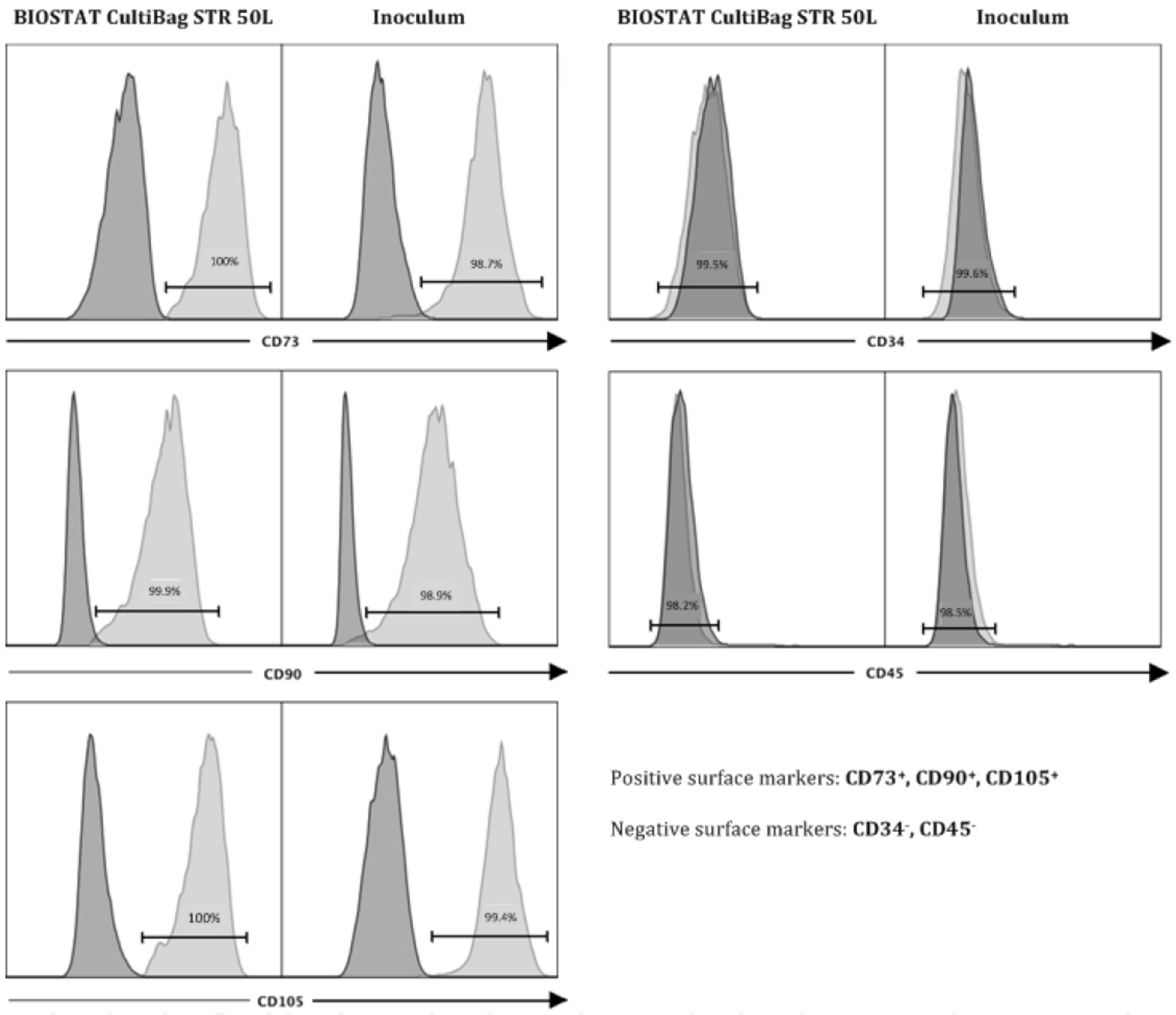



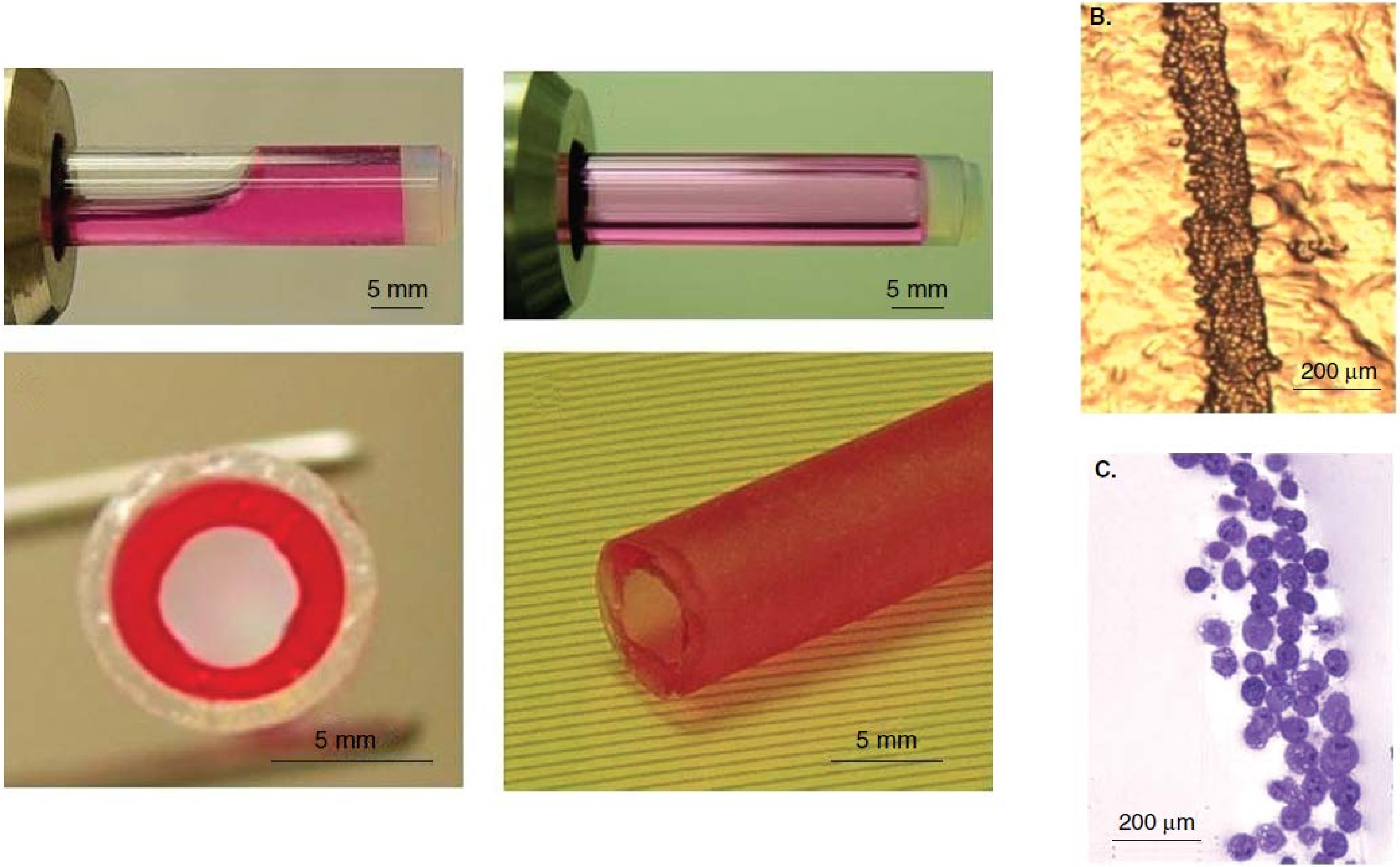
# Parallel Plate Bioreactor

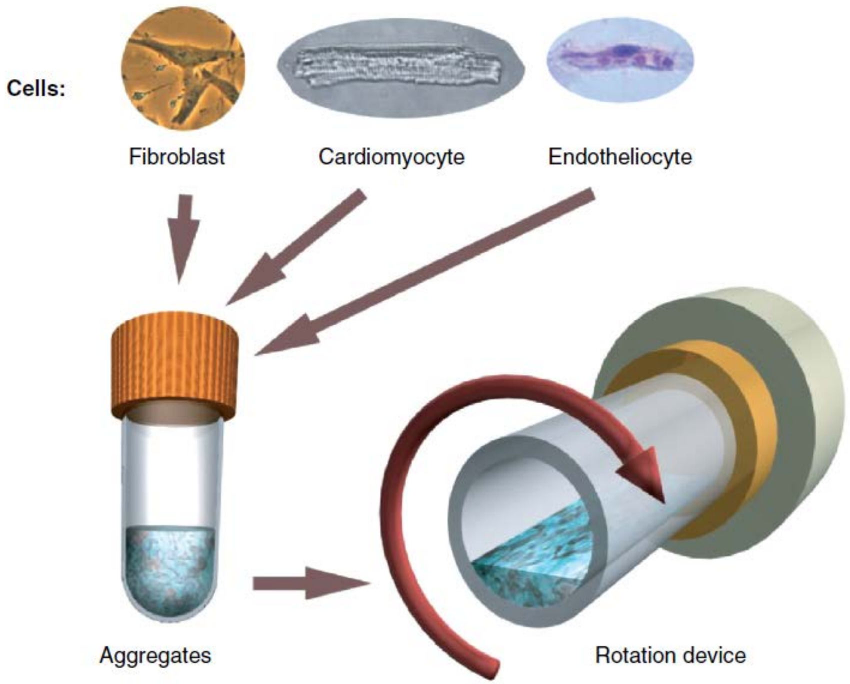



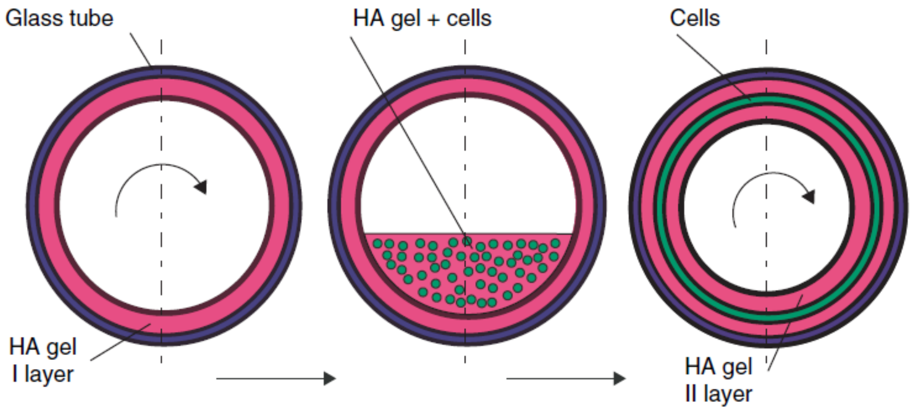
# Quality Control after Mass Culture

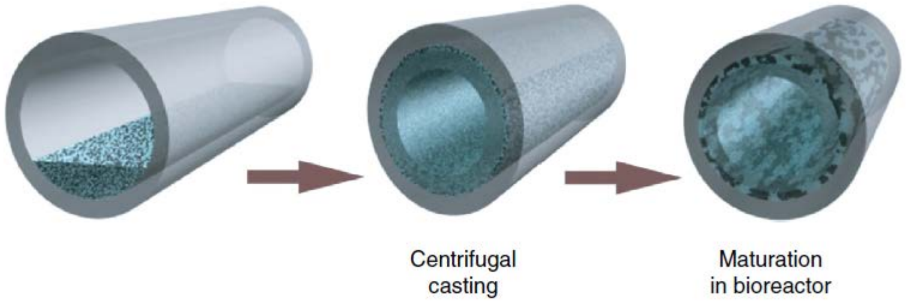


# Bioreactor-Free TE




Cells:
Fibroblast
Cardiomyocyte
Endotheliocyte
Aggregates
Rotation device


Glass tube
HA gel + cells
Cells
HA gel
I layer
HA gel
II layer


Centrifugal
casting
Maturation
in bioreactor