Mechanobiology of the musculoskeletal system
Lecture I: Cell mechanics and bone mechanosensitivity

Mark S Thompson

C6: Engineering Science

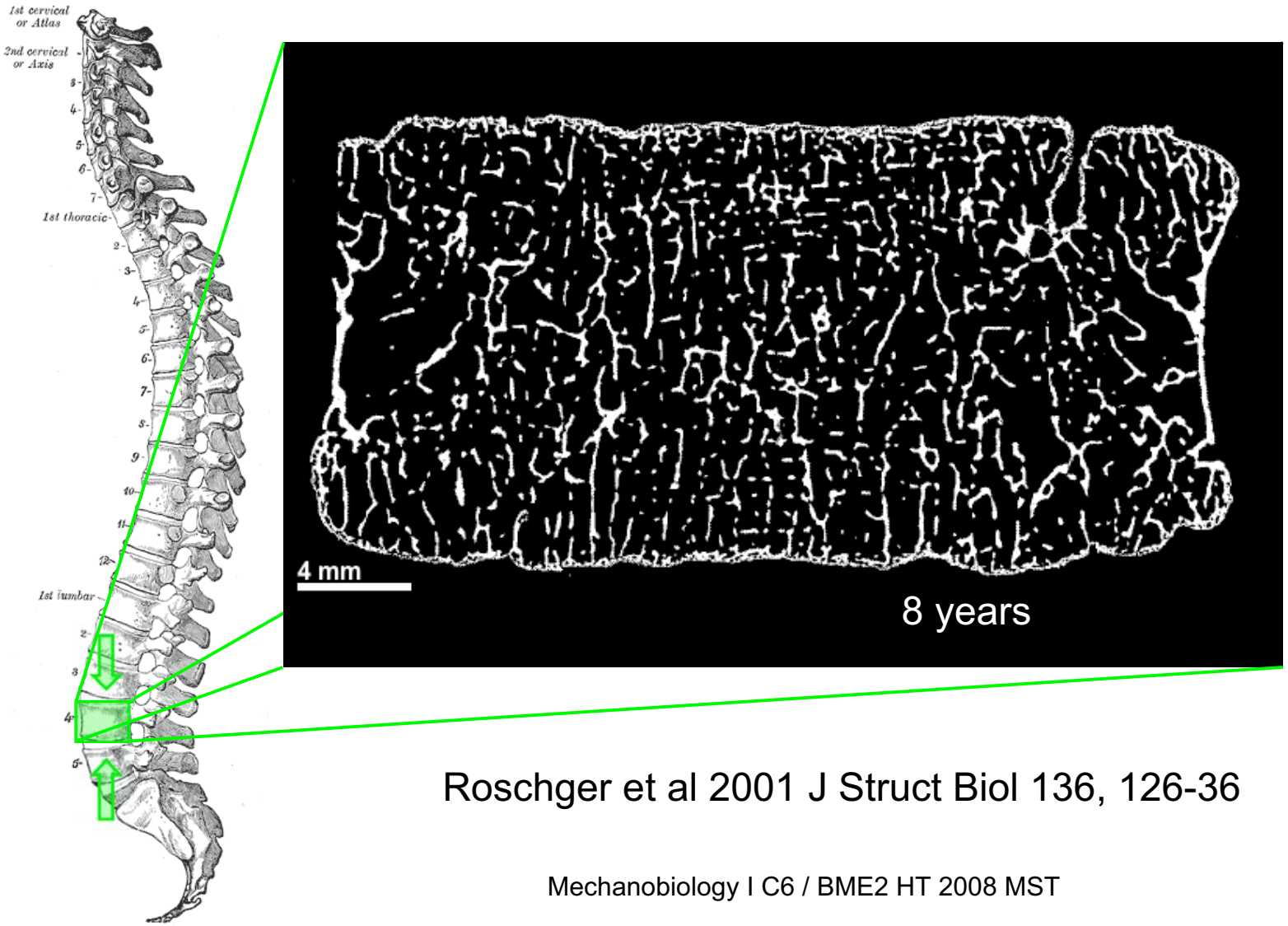
BME2: Biomedical Engineering

HT 2008

How to get the most out of these lectures

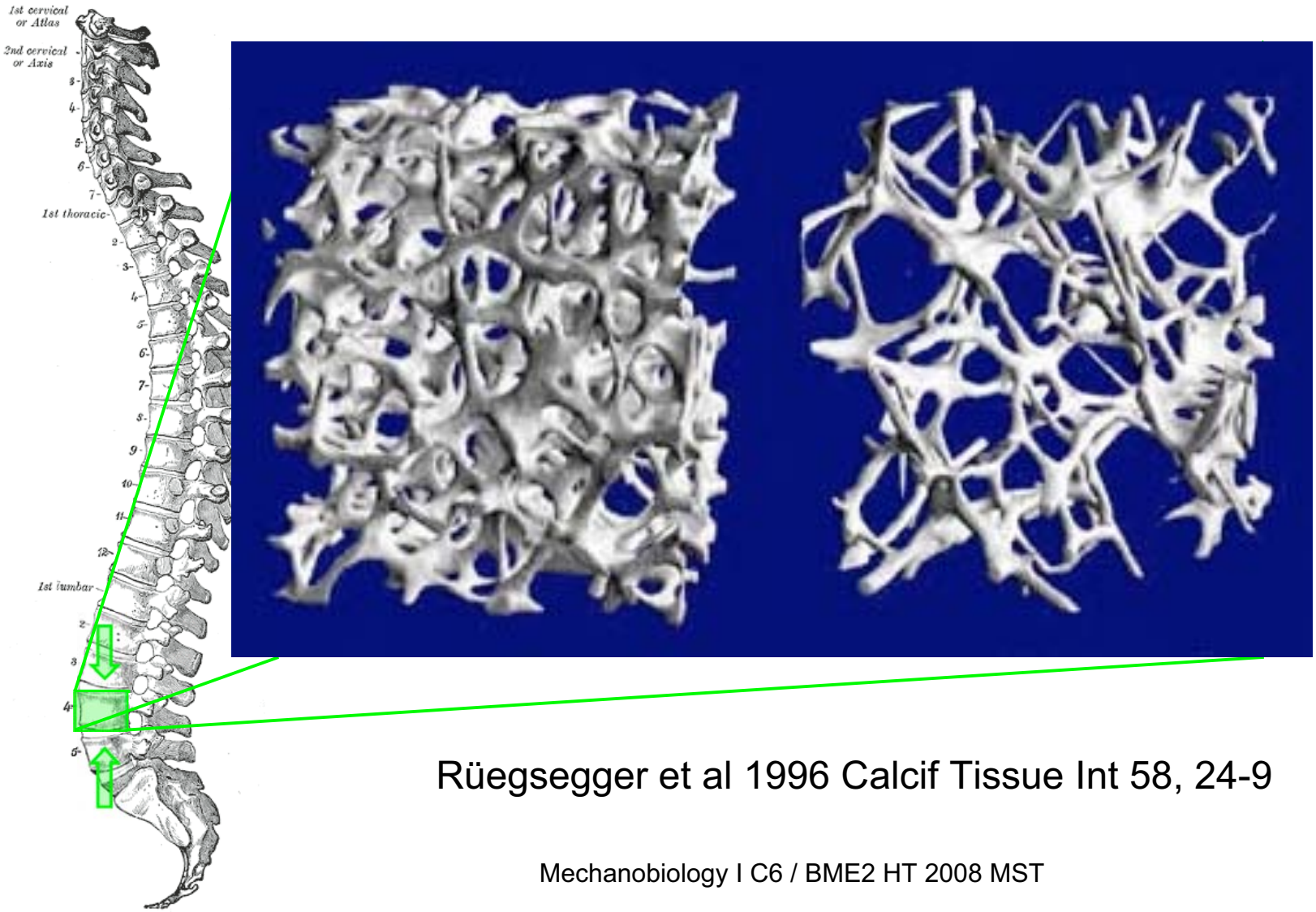
- Research topic
  - Independent thinking, evaluation
  - Source material
    - controversial
    - wrong
    - not yet in many books
  - Find papers to read:
    - http://www.pubmed.com/
    - http://newisiknowledge.com/

Why mechanobiology?



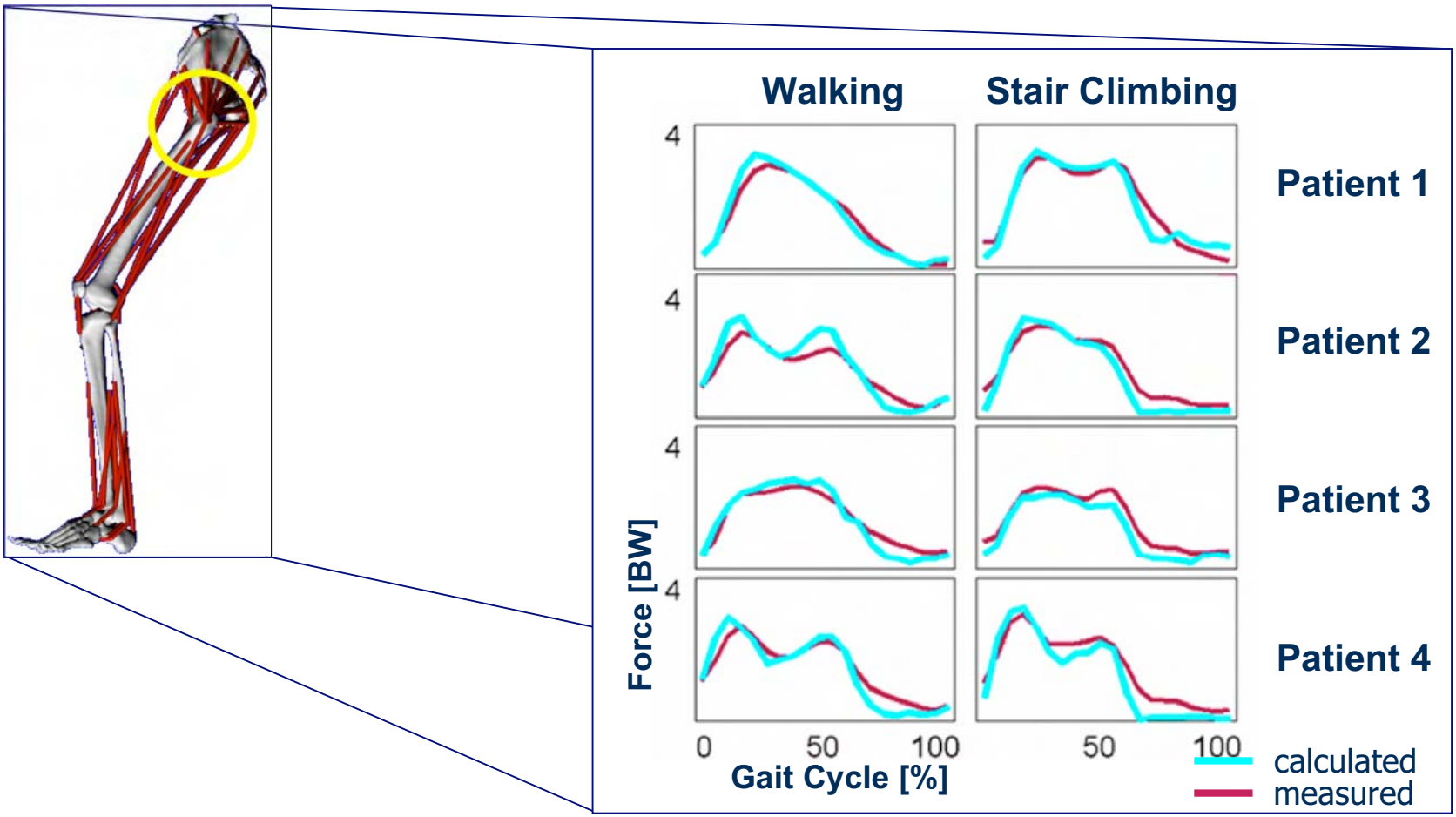
Roschger et al 2001 J Struct Biol 136, 126-36

Why mechanobiology?



Rüegsegger et al 1996 Calcif Tissue Int 58, 24-9

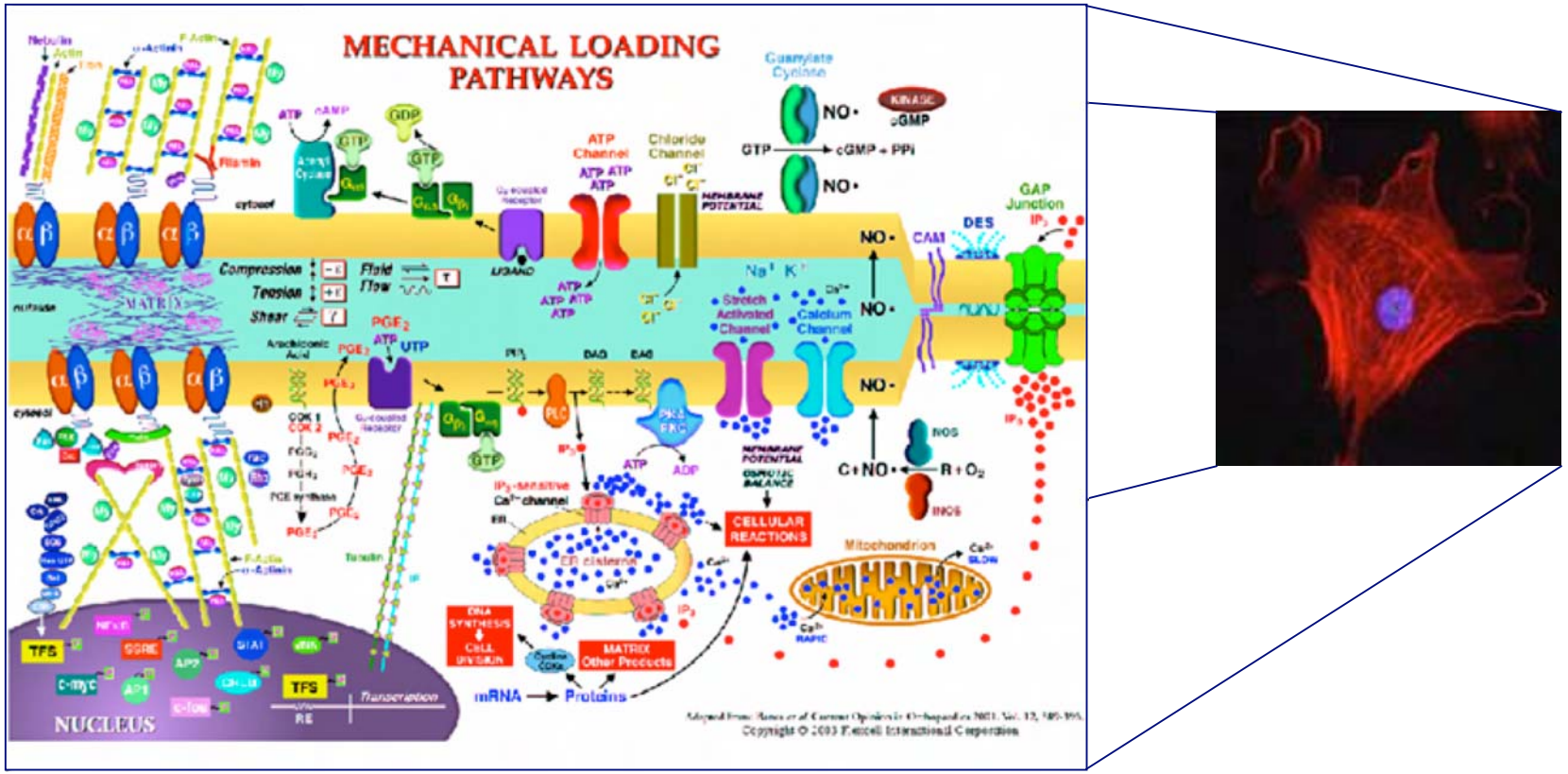
Definition



Heller et al 2001 J Biomech 34 883-93

Definition

http://www.eng.ox.ac.uk/obme



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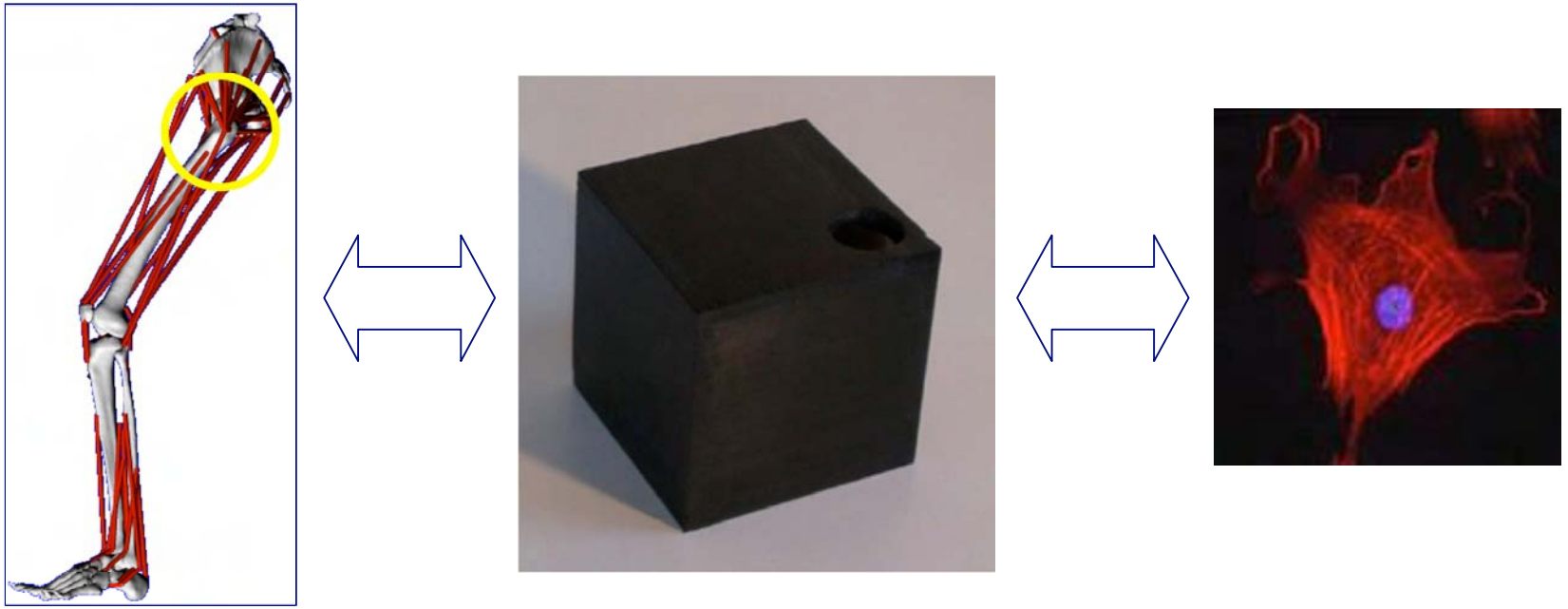


Mechanobiology

The study, at the cell and tissue level, of the mechanical and physical aspects of biological processes

Musculoskeletal system - mechanical system - orthopaedics

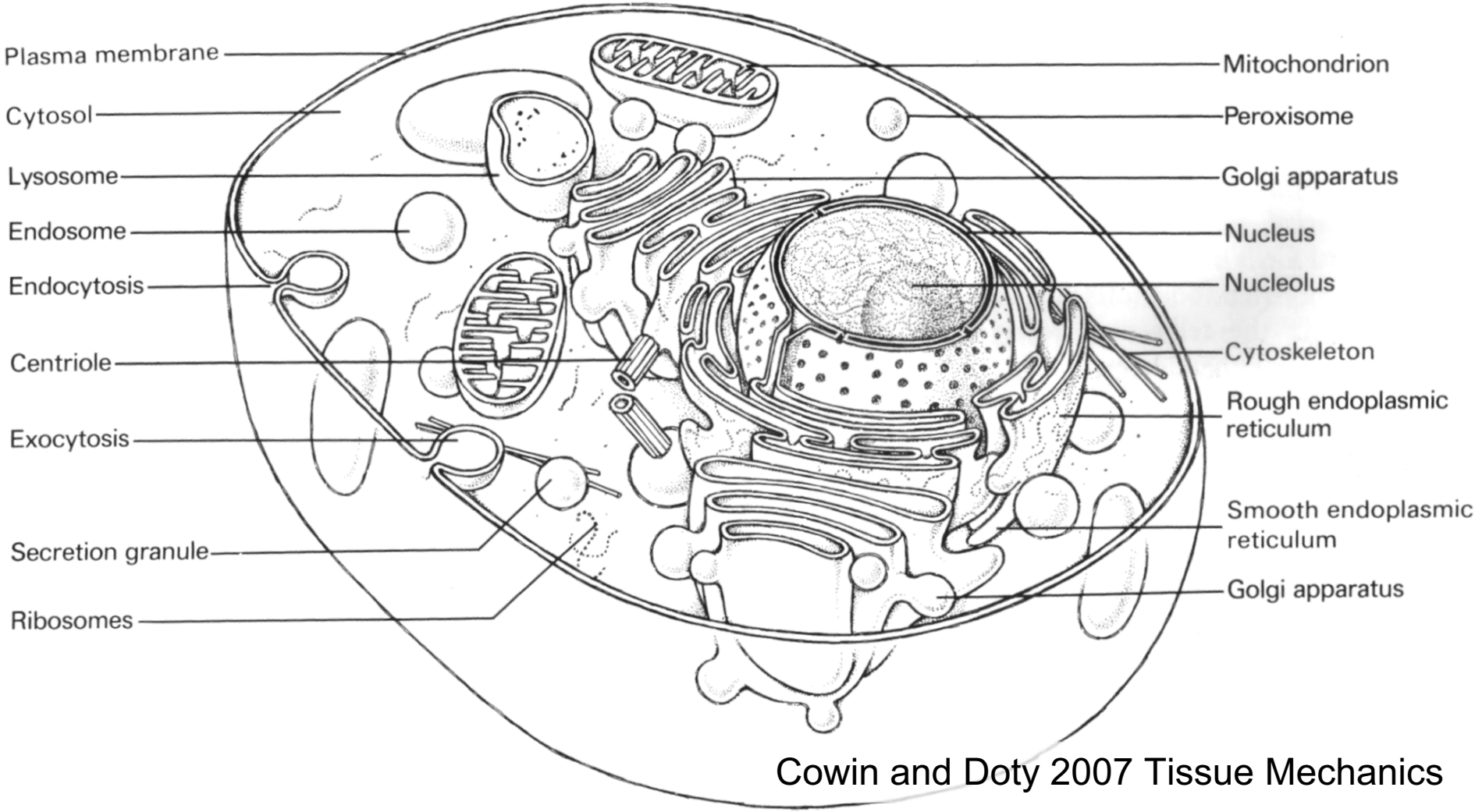
BME2 Mechanobiology

http://www.eng.ox.ac.uk/obme

- Lecture 1
  - Cell mechanics and bone mechanosensitivity
    [cells, local structure, bone mechano-regulation]
- Lecture 2
  - Fluid flow in musculoskeletal tissue
    [material models, focus on cartilage]
- Lecture 3
  - Tools for mechanobiology
    [digital image correlation, models]
- Lecture 4
  - Mechanobiology theories and applications
    [bone healing, tissue continuum models]

Cell structure (eukaryotic)

http://www.eng.ox.ac.uk/obme



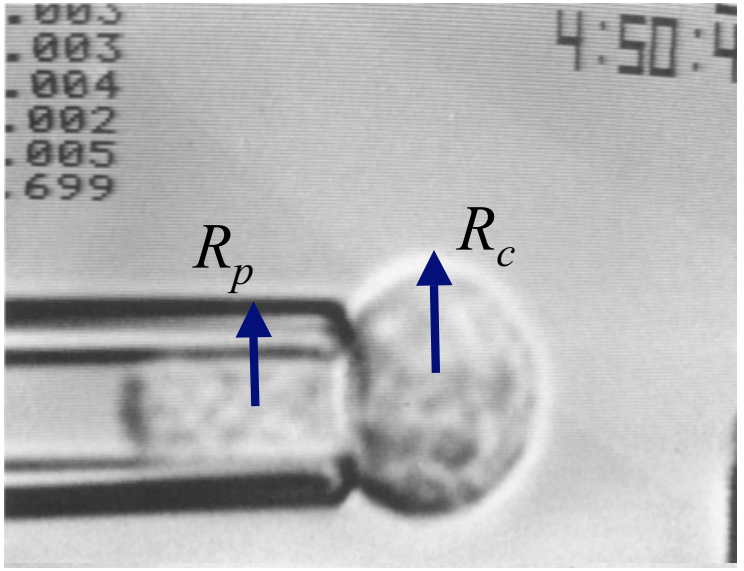
Cowin and Doty 2007 Tissue Mechanics

Cell structure

- Jelly bag model of cell mechanical behaviour
- Critical pressure to flow into pipette

$$P_{cr} = 2\tau_o(1/R_p - 1/R_c)$$

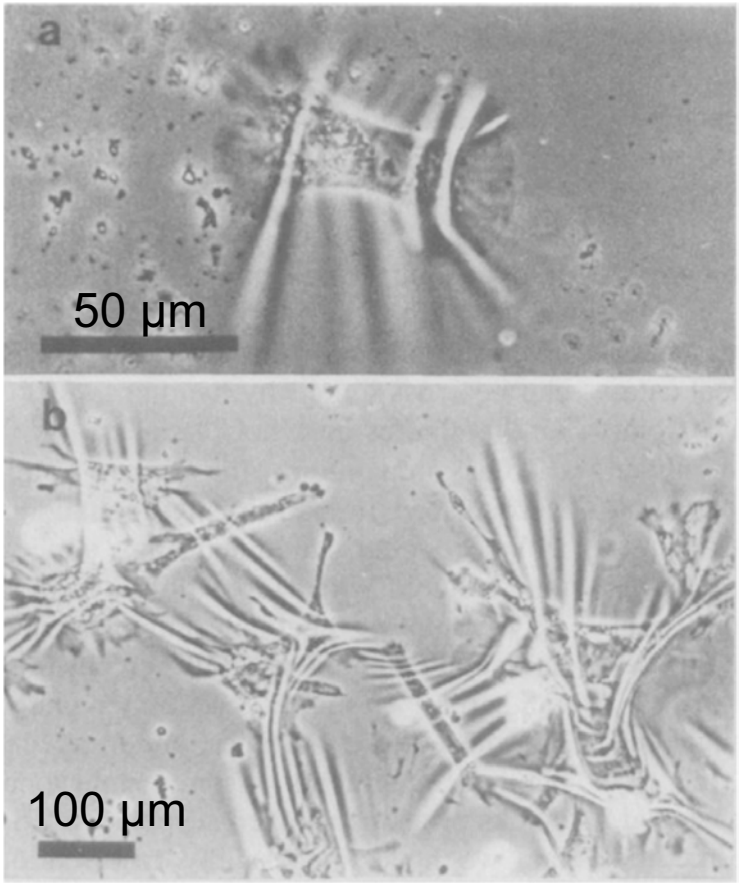
- "Surface tension"
  $\tau_o \approx 3.5 \times 10^{-5}$ Nm^{-1}
- Viscous flow
  for $P > P_{cr}$
- Membrane ruffled - can extend to c 2.1 x area



Evans and Yeung 1989 Biophys J 56, 151-60

Cell structure

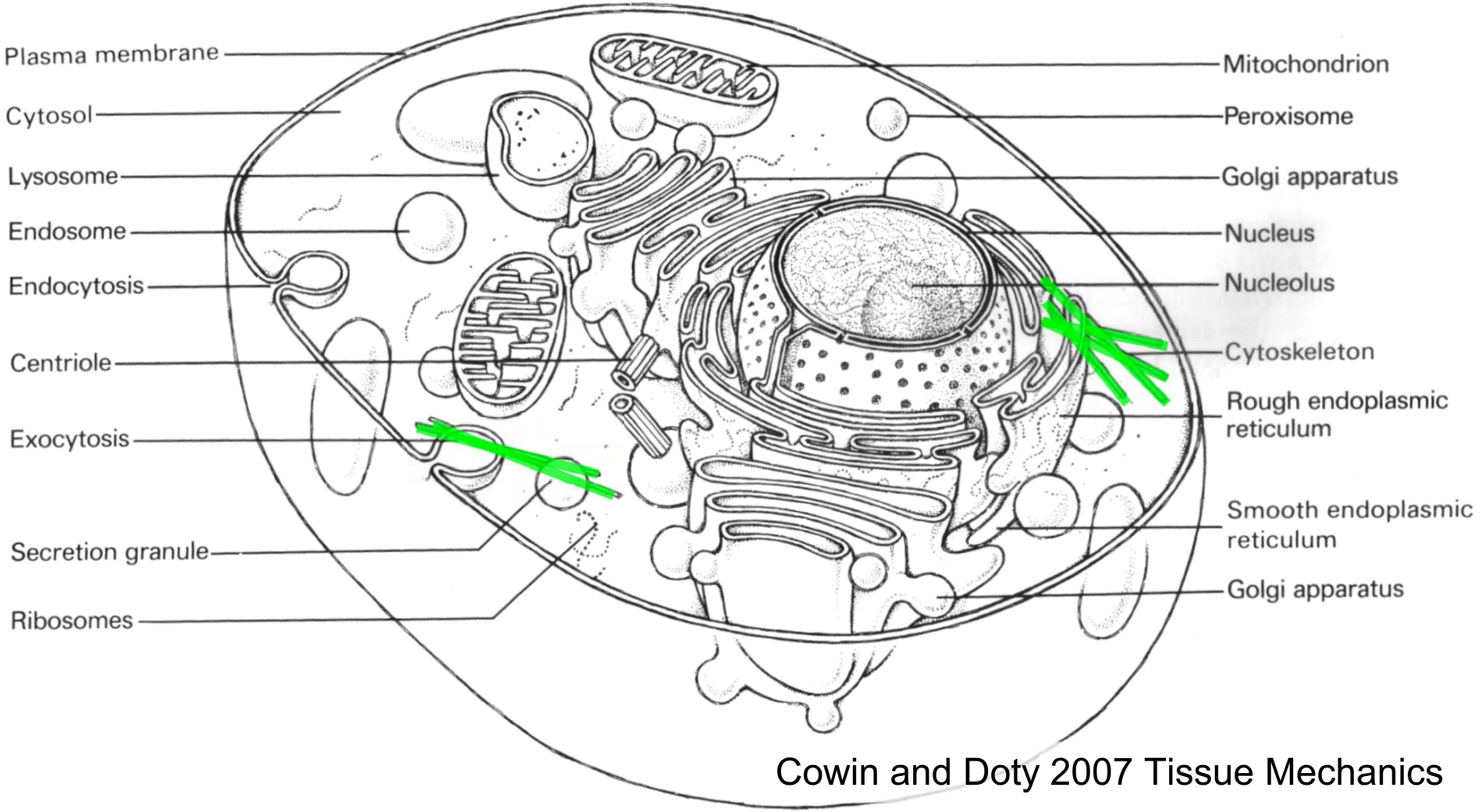
- Jelly bag model of cell mechanical behaviour
- Comparison with
  - Wrinkling of silicone



Harris et al 1980 Science 208, 177-9

Cell structure

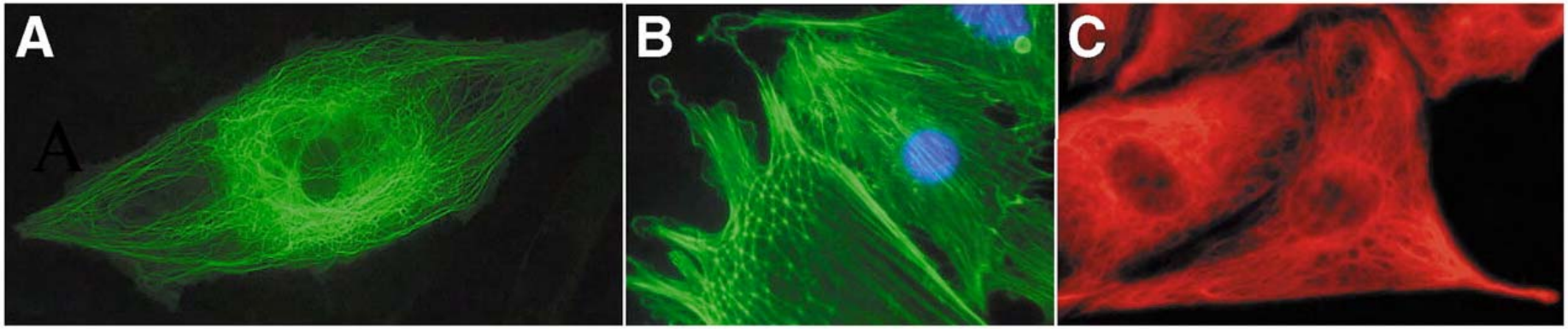
http://www.eng.ox.ac.uk/obme



Cowin and Doty 2007 Tissue Mechanics

Cell structure

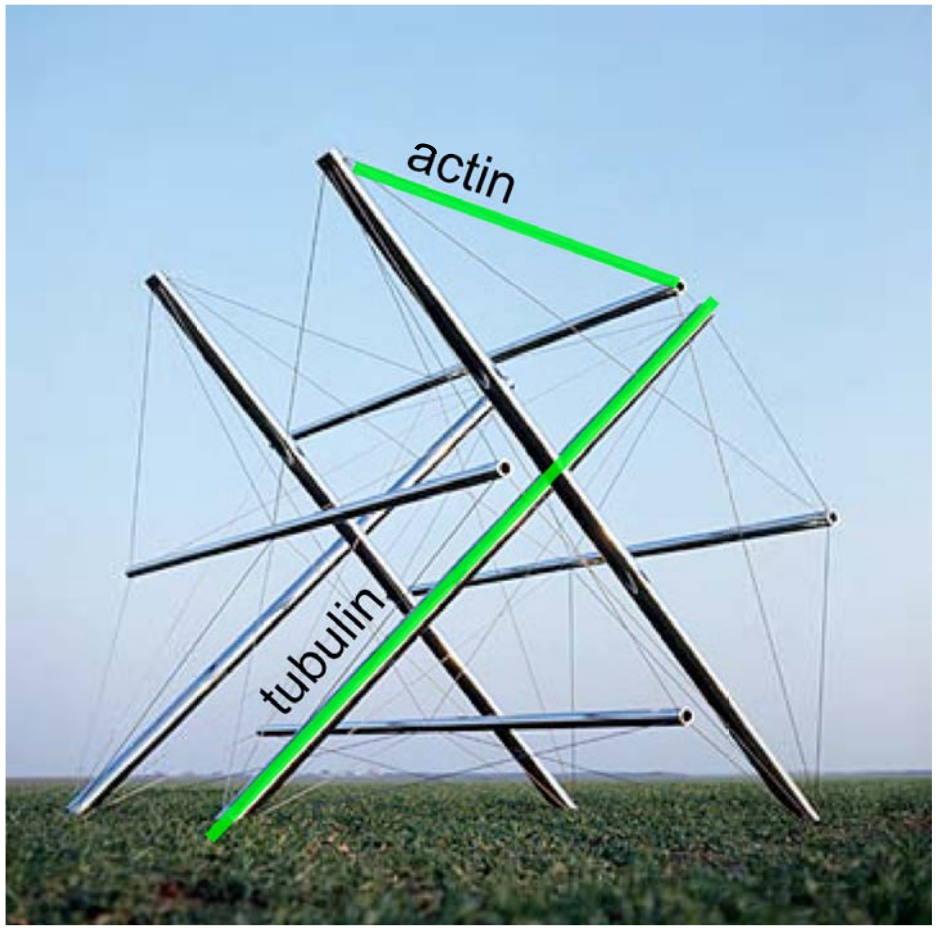
- Endothelial cell cytoskeleton
  - A. Microtubules (GFP-tubulin),
  - B. Microfilaments (rhodaminated-phalloidin for actin)
  - C. Intermediate filaments (antibodies to vimentin)



Ingber 2003 J Cell Sci 116, 1157-73

Cell structure

- Tensegrity
- Actin filaments
  ∅ 7 nm (isolated 1945)
  - “ties”
- Tubulin ∅ 25 nm,
  length µm - mm
  - “struts”
- Vimentin filaments
  ∅ 8 - 11 nm
  - “intermediate filaments - bundling”



Kenneth Snelson “City Boots” 1968

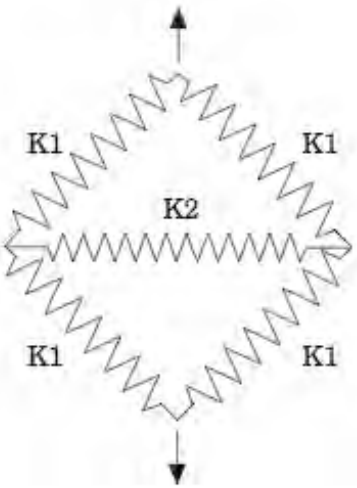
Ingber 1993 JCellSci 104: 613-627

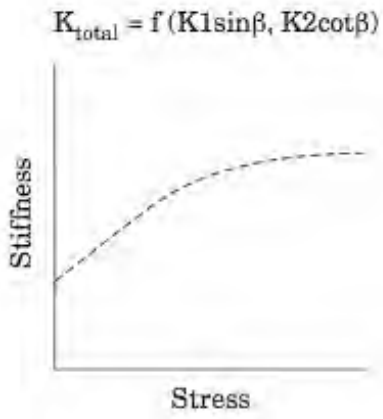
Cell structure

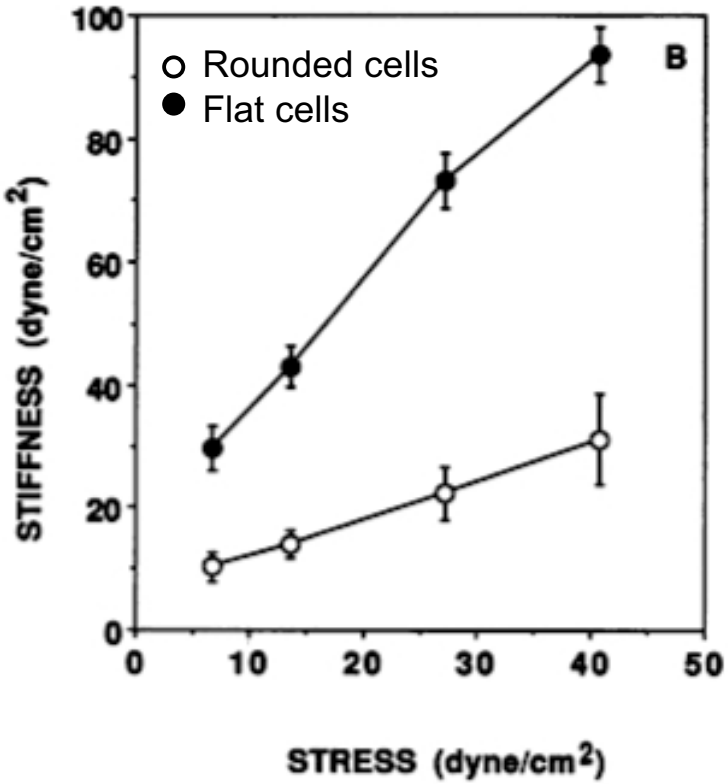
- Loading experiments

  e.g. ferromagnetic bead
  ∅ 5.5 µm
  peptide coated

- Stiffening effect
  - Alignment







Wang and Ingber 1994 Biophys J 66, 2181-9

Cell structure

- Microtubule buckling
- Euler:

  $Py = M = -EI\, d^2y/dx^2$

  $y = A \sin ax + B \cos ax$

  $a^2 = P / EI;$

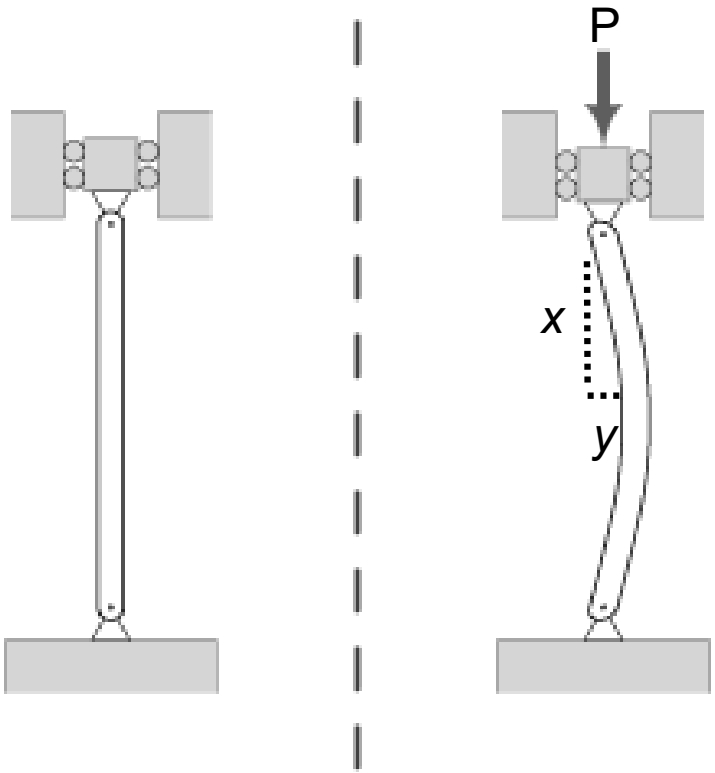
  $B = 0;$

  $A \sin aL = 0;$ so $aL = \pi, 2\pi, \ldots$

  $P_{\text{critical}} = \pi^2 EI / L^2$
- Microtubule properties:

  EI = 2.15 x 10^{-23} Nm^2

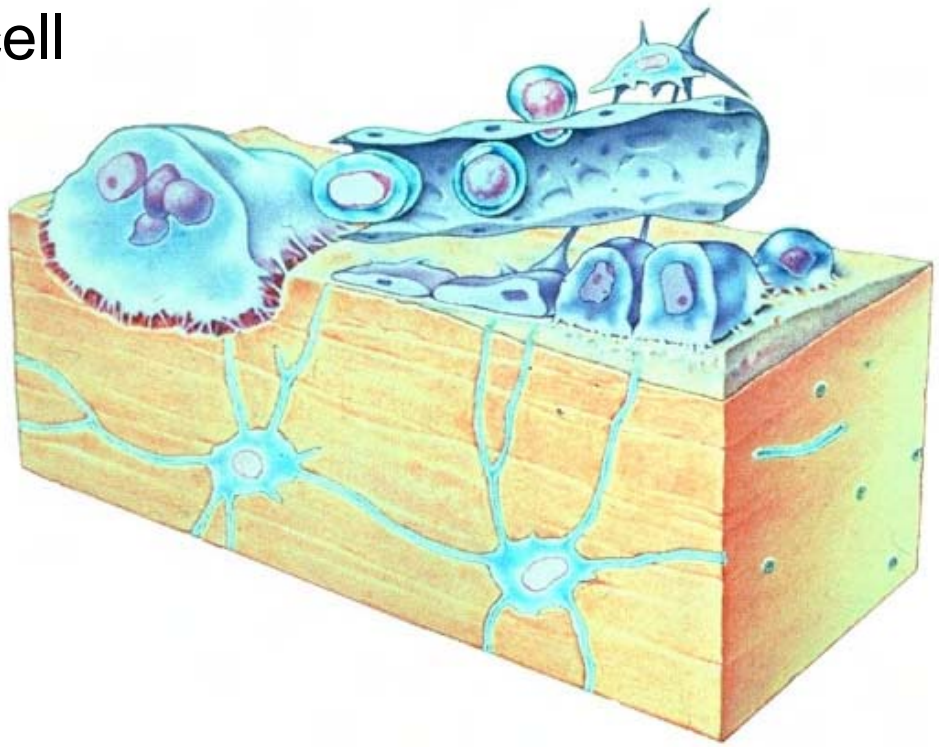
  L = 10 μm so P_{critical} ~ 2 pN
- In reality vimentin constrains
  - P_{critical} ~ 100 pN



Volokh et al 2000 J Biomech 33, 1543-9
Brangwynne et al 2006 J Cell Biol 173, 733-41

Bone cells - introduction

- Osteocyte
- Osteoblast
- Osteoclast
- Mesenchymal stem cell

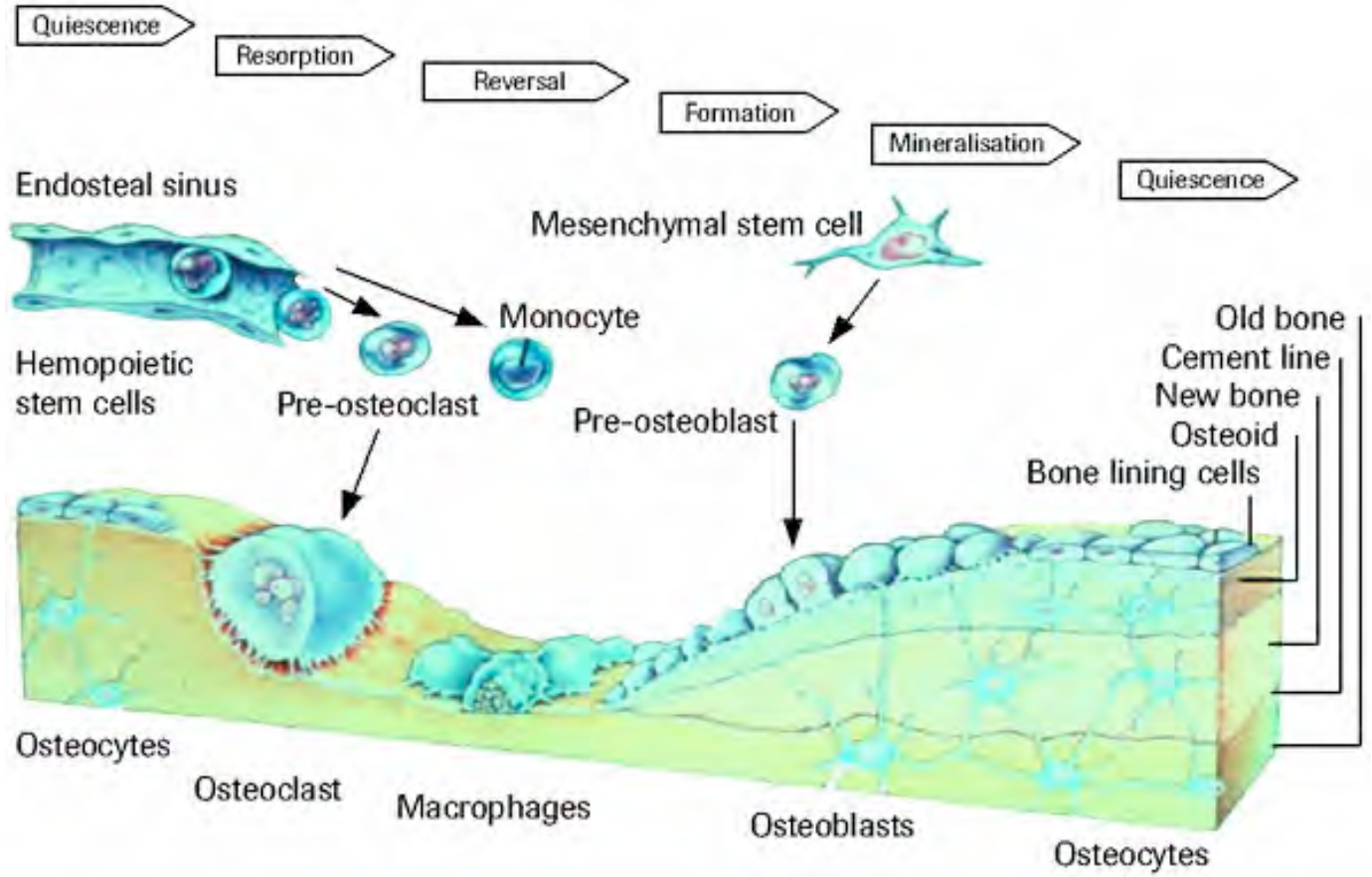


Bone cells - introduction

- Healthy bone cell activity

Bone marrow:

Source of blood cells & bone cells

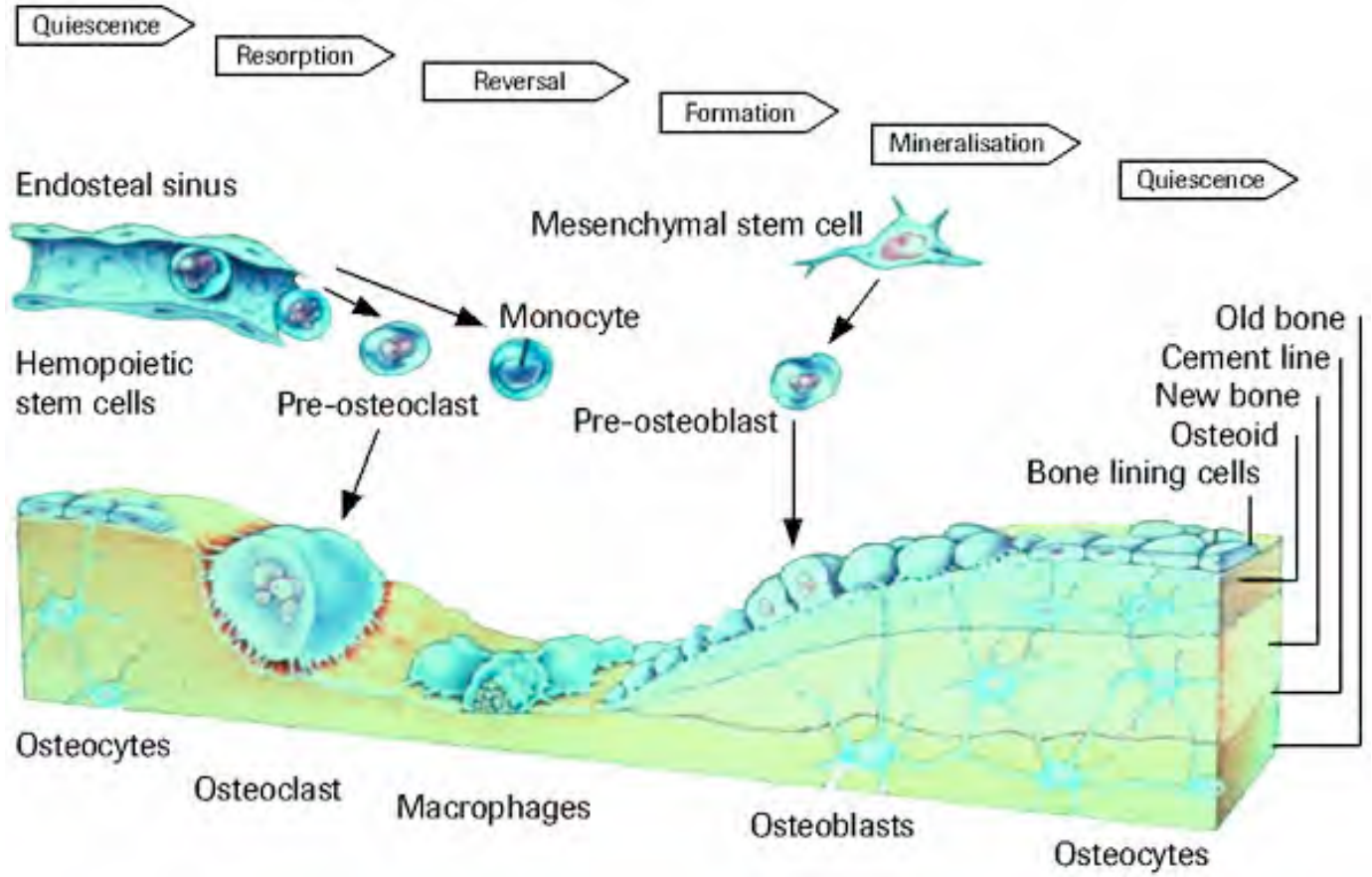


Bone cells - introduction

- Healthy bone cell activity

Osteoclasts resorb bone material (also macrophages)

Increase in $[Ca^{2+}]$



Bone cells - introduction

- Healthy bone cell activity

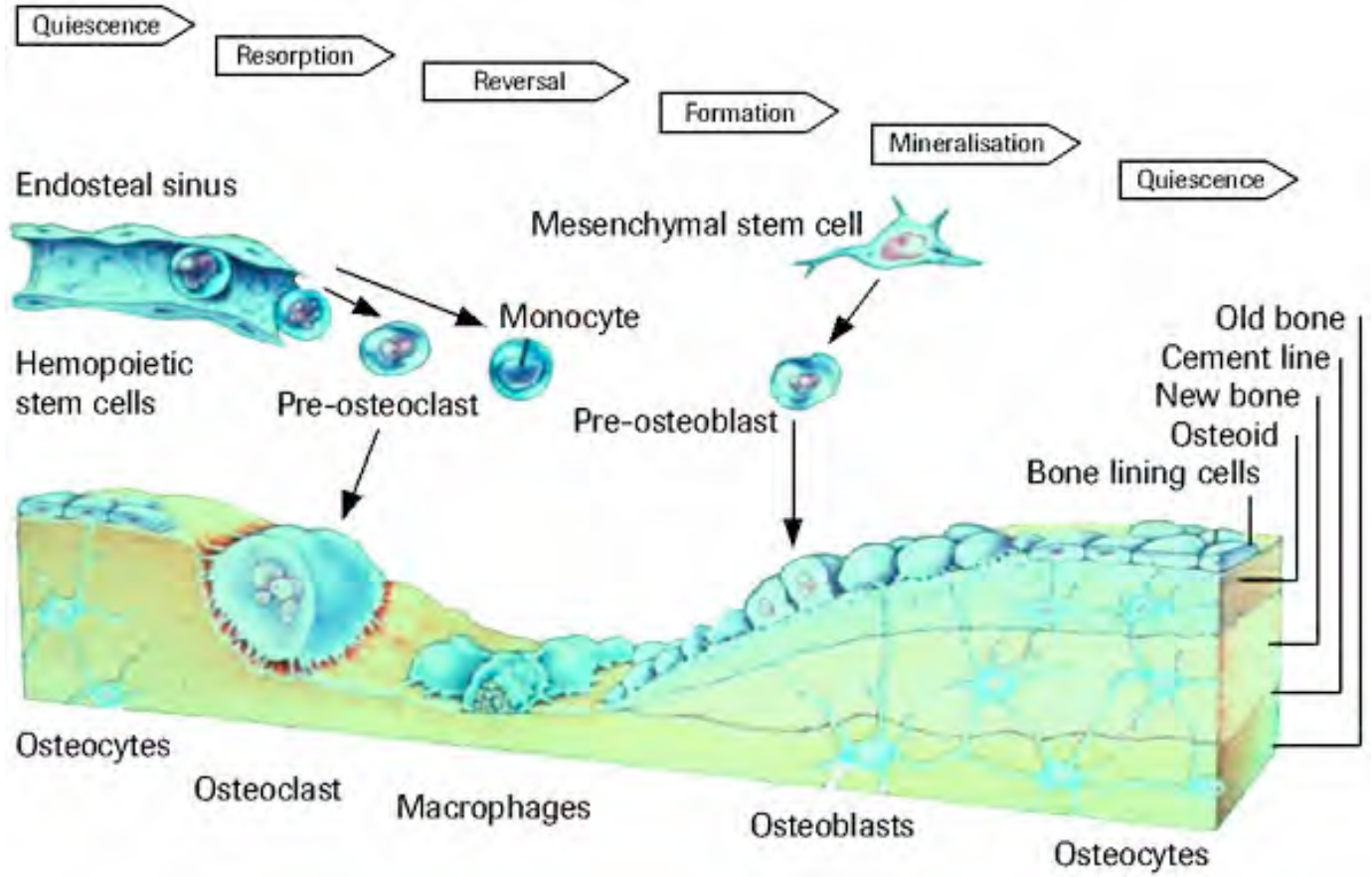
Osteoblasts deposit bone material

Trapped osteoblasts become osteocytes

Decrease in $[Ca^{2+}]$

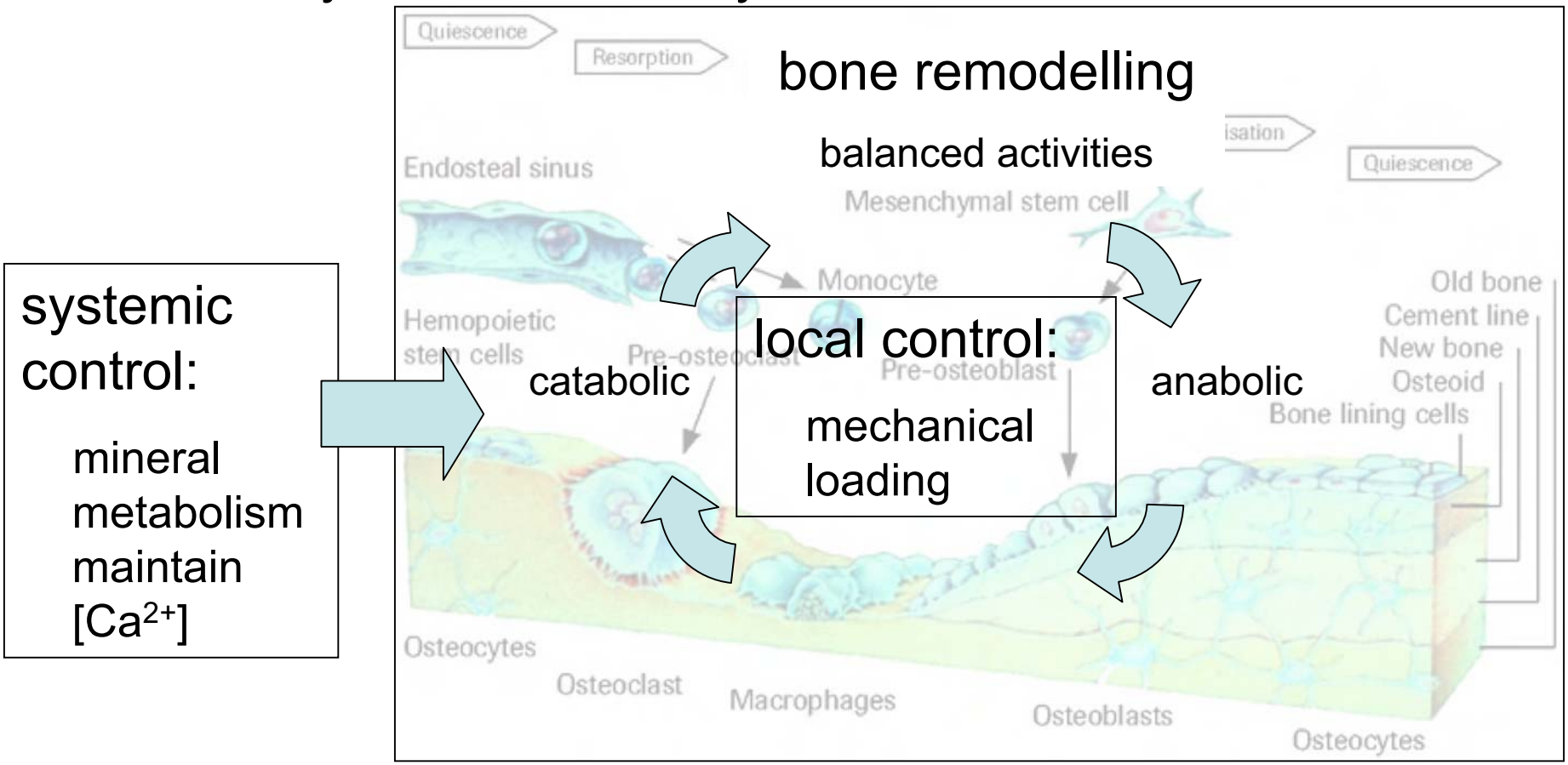
Osteocytes

Sensor network?



Bone cells - introduction

- Healthy bone cell activity



Pogoda et al 2005 Osteoporos Int 16, suppl. 2: S18-24

Bone cells - introduction

- Disuse osteoporosis
  - Spinal cord injury: increased fracture risk
  - Bed rest: resorption increased, formation reduced
  - Astronauts: bone mass reduced after space flight
  - Hibernation: hamsters, squirrels, bats lose bone
  - Serum hormone levels disturbed:
    - Parathyroid, Leptin, Insulin-like growth factor I
- Imbalance: resorption vs formation
  - Increased activity

Rittweger et al 2005 Bone 36, 1019-29

Bone cells - introduction

- Black bear (ursus americanus)
  - ~ 6 mths hibernation p.a.
    - Bone strength increases with age!
  - Blood serum samples
    - Before, during, after hibernation
  - Increased markers for both:
    - Resorption & formation
  - Bone formation maintained
    - Parathyroid hormone key?

Donahue et al 2006 J Exp Biol 209, 1630-8

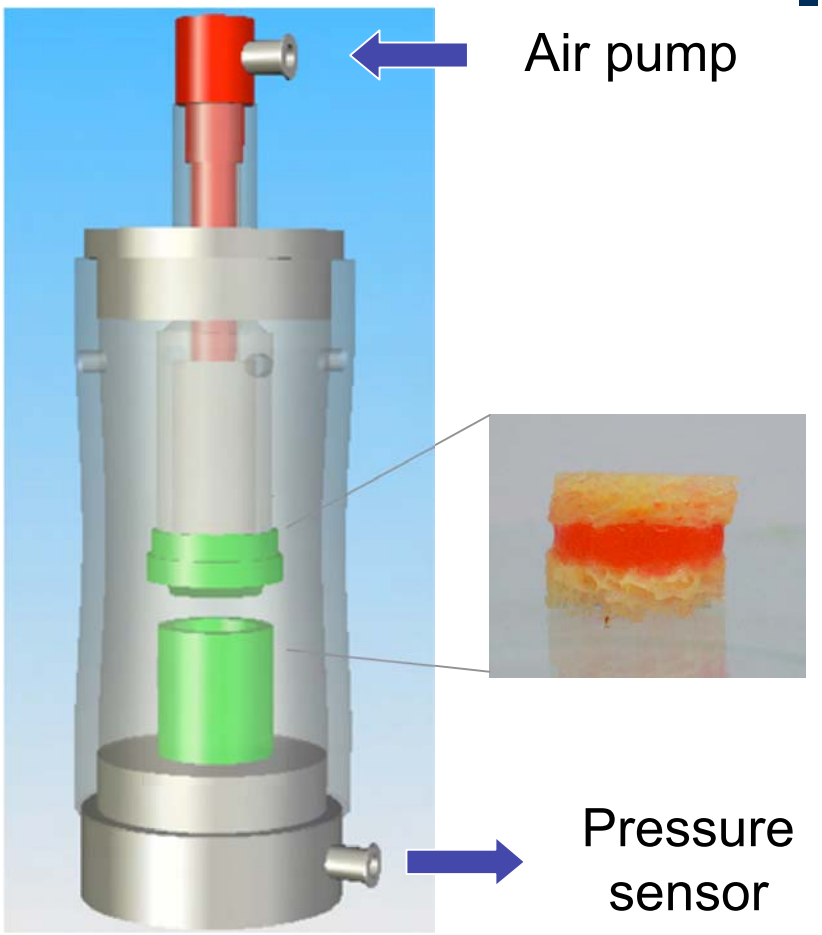
Bone cells - mechanosensitivity

- Mechanosensitivity of individual bone cells
- in vitro cell culture experiments:
  - Fluid flow (membrane shear stress)
  - Single cell stretching, squashing
  - Substrate stretching (2D & 3D)
- Biological response
  - Short term (changes in mRNA)
  - Longer term (changes in protein synthesis)
  - Differentiation

Bone cells - mechanosensitivity

- Cell differentiation
- Mesenchymal stem cells: (niche in bone marrow)
  - “Multipotent” - key in healing response (recruited to injury site)
  - Cartilage, skeletal muscle, osteoblast, osteocyte, *cardiac muscle, nerve, liver, pancreas, adipose*
  - Differentiated cells lay down extracellular matrix - tissue
- Differentiation
  - Process of choice of cell specialism
  - Factors influencing: mechanical & biological

Bone cells - mechanosensitivity

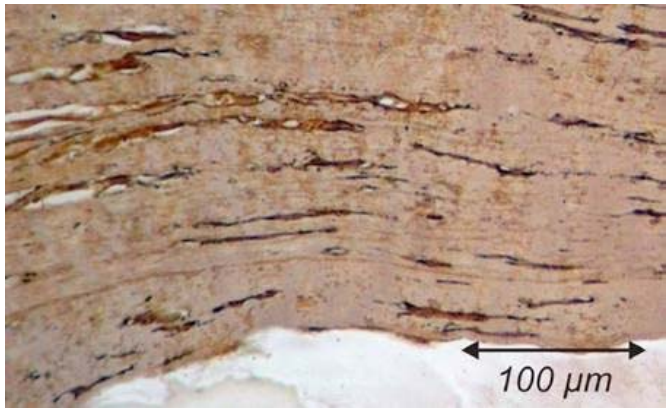


- Bioreactor
  - Pneumatically driven
    - Silicone membranes
  - Fibrin / bone sandwich
  - Mesenchymal stem cells
  - Air filters
  - Medium with nutrients
  - 10 kPa, 0.05 – 1 Hz, up to 3 weeks

Matziolis et al 2006 Tissue Eng 12, 201-8

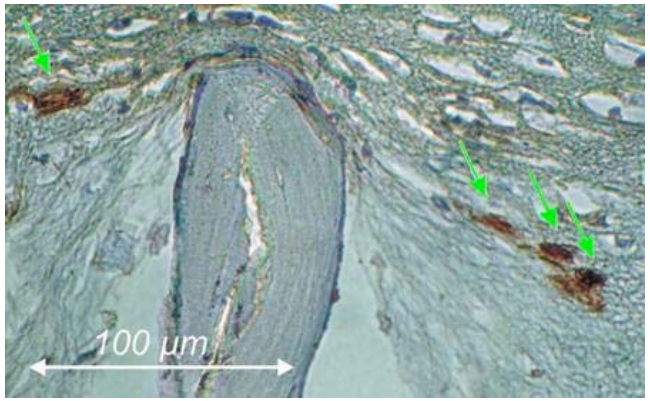
Bone cells - mechanosensitivity

- Stimulated



von Kossa (silver nitrate)

- von Kossa indicates matrix calcification



Collagen X (antibody)

- Collagen X indicates endochondral ossification
- Neither present in unstimulated constructs

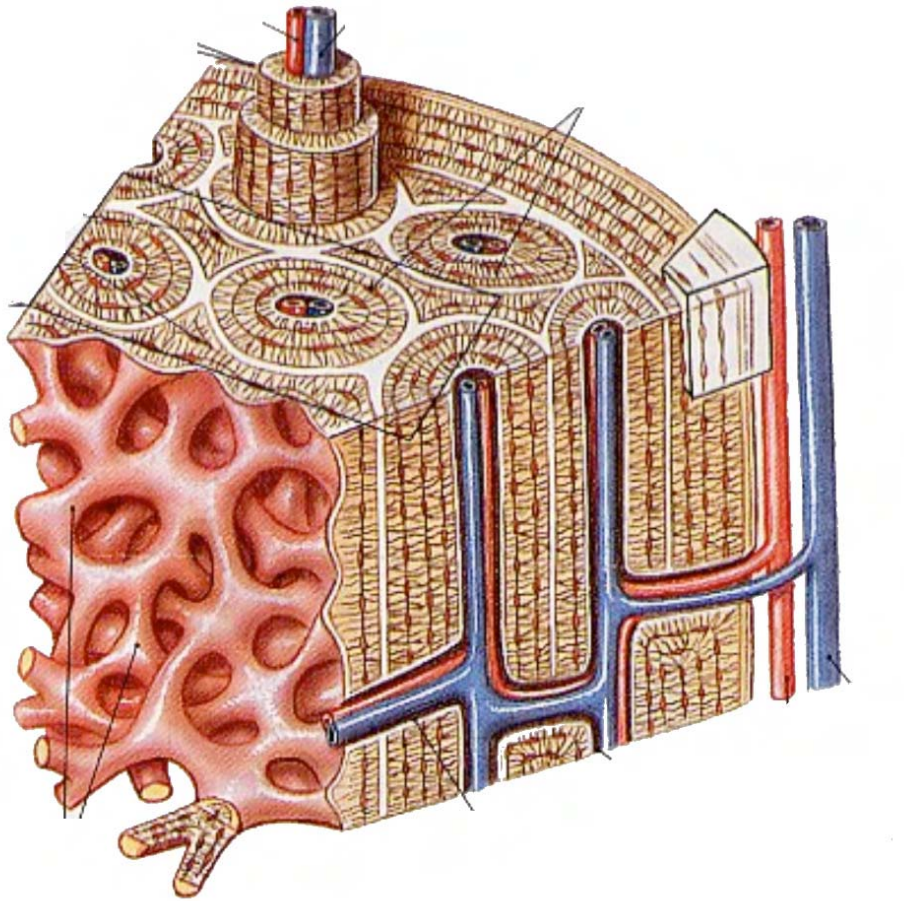
Matziolis et al 2006 Tissue Eng 12, 201-8

Bone cells - mechanosensitivity

- Bone mechanotransduction paradox:
  - In vitro strains for activation:
    - 2 - 10%
  - Measured in vivo strains
    - ~ 0.2%
  - Bone failure strain
    - ~ 0.8%
  - What are the processes for sensing mechanical loading?
  - Which cells are involved?

Bone cells in context

- Tissue microstructure
  - Cortical bone
  - Trabecular bone
  - Osteon
  - Osteocyte
  - Osteo-blasts, -clasts
- Canaliculi
  - strain concentrators
  - fluid flow
  - Which cells?
  - Osteocyte network?



Martini 1997 “Fundamentals of anatomy and physiology”

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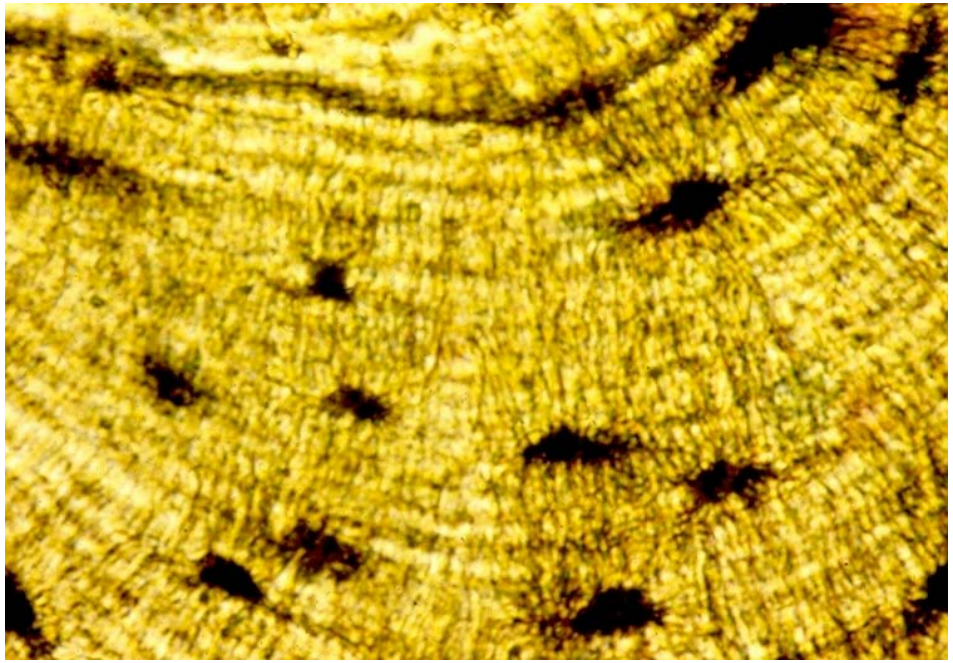
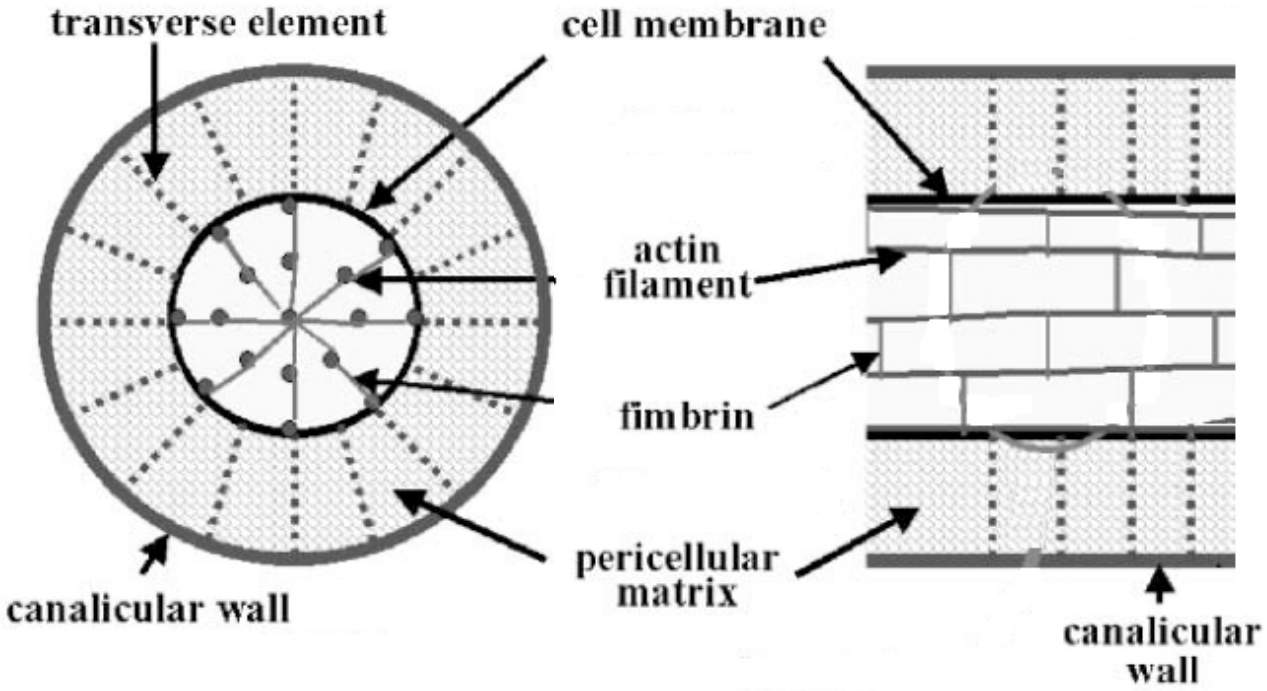


http://facstaff.bloomu.edu/jhranitz/Courses/APHNT/Lab_Pictures/

Bone cells in context

- Osteocyte process



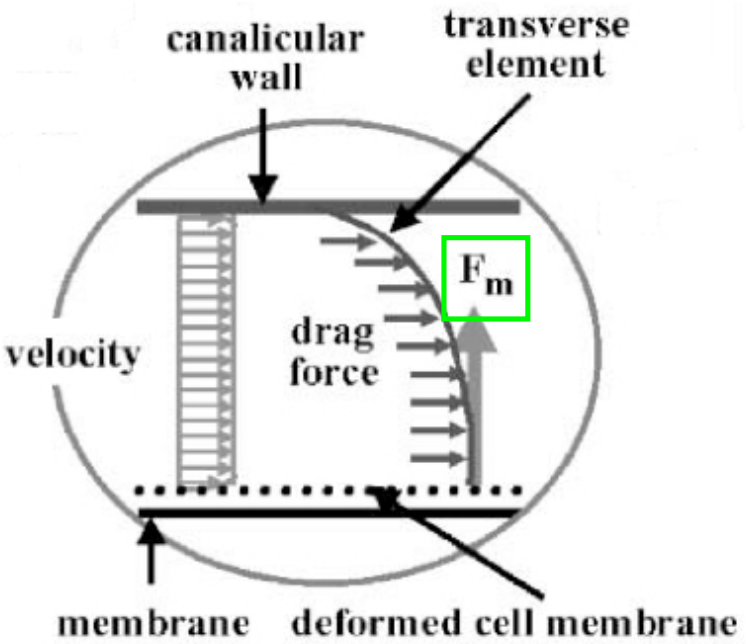
Transverse section Longitudinal section

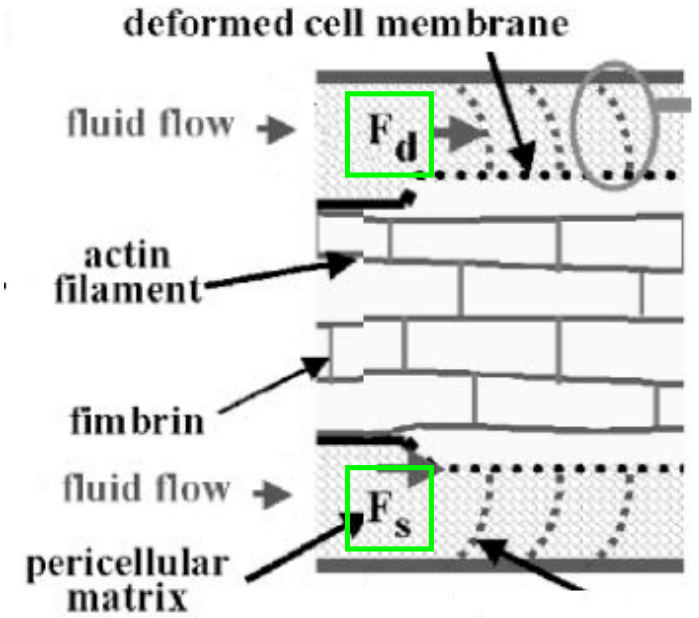
You et al 2001 J Biomech 34, 1375-1386

Han et al 2004 PNAS 101, 16689-94

Bone cells in context

- Osteocyte process





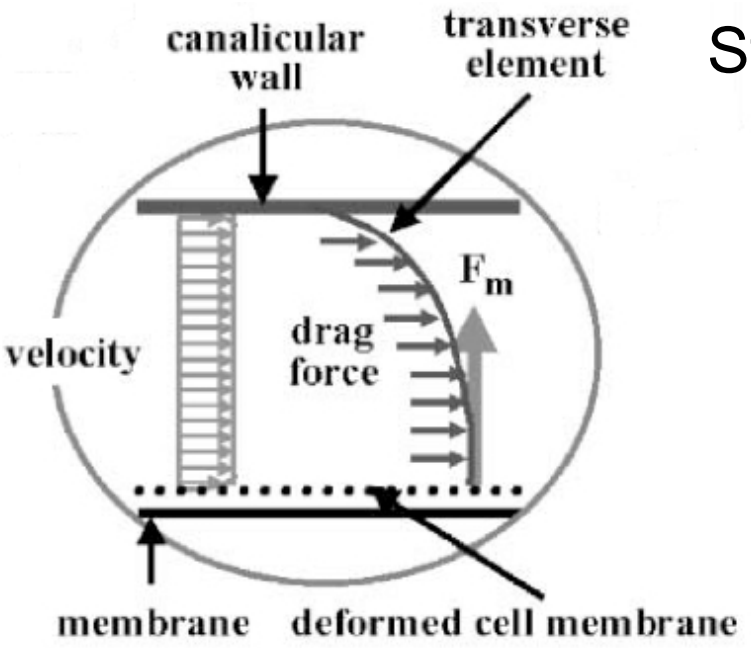
$$F_d \sim 20\, F_s$$

You et al 2001 J Biomech 34, 1375-1386

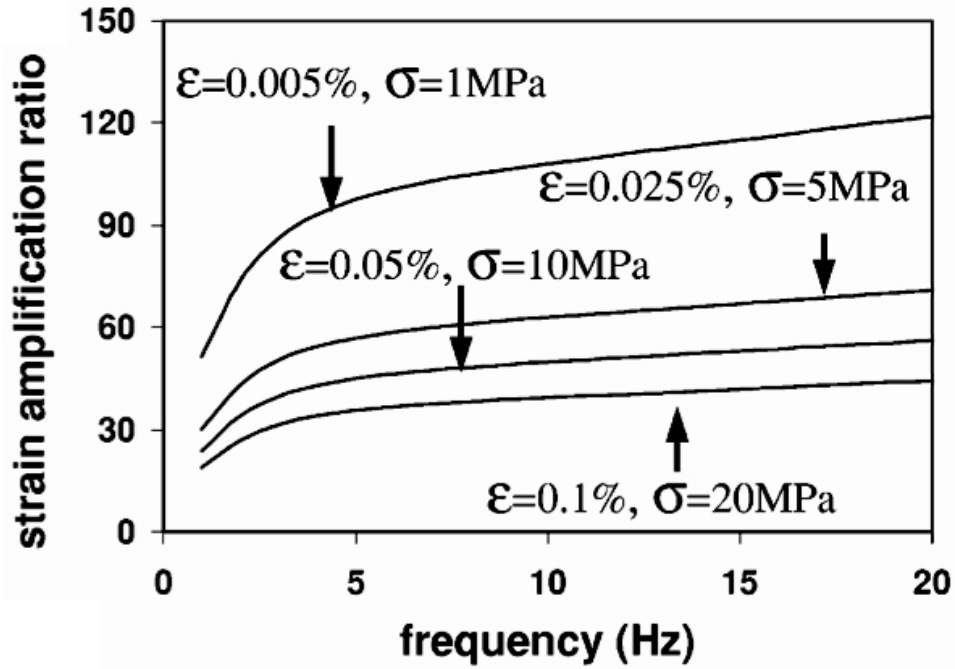
Han et al 2004 PNAS 101, 16689-94

Bone cells in context

- Osteocyte process



Strain amplification vs frequency



You et al 2001 J Biomech 34, 1375-1386

Han et al 2004 PNAS 101, 16689-94

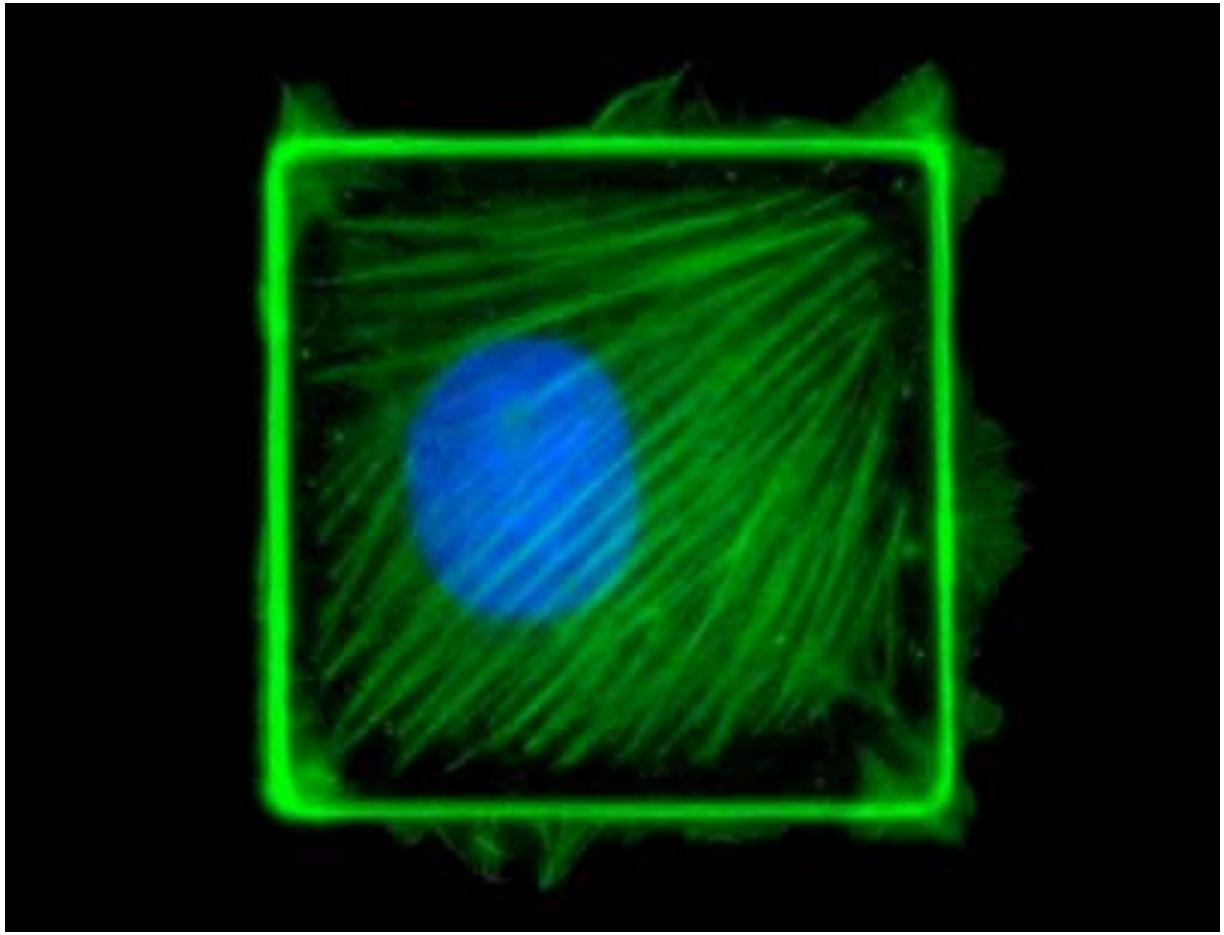
Bone cells in context

- Low level strains do have a bone stimulatory effect

  Rubin et al 2001 Nature 412, 603-4
- How is trabecular bone stimulated?
- Other competing hypotheses
  - Damage - osteocyte processes
  - Matrix damage - remodelling as repair

Summary

- Cells have a mechanical architecture
  - Cytoskeleton; tensegrity
- Roles of bone cells in maintaining healthy bone
  - Importance of mechanical sensitivity
- Differentiation of stem cells and mechanical sensitivity
  - Tissue staining (histology)
- Bone mechanosensitivity paradox
  - Possible mechanisms



Ingber, D.E. 2006

Website: http://www.childrenshospital.org/research/Site2029/mainpageS2029P23sublevel24.html