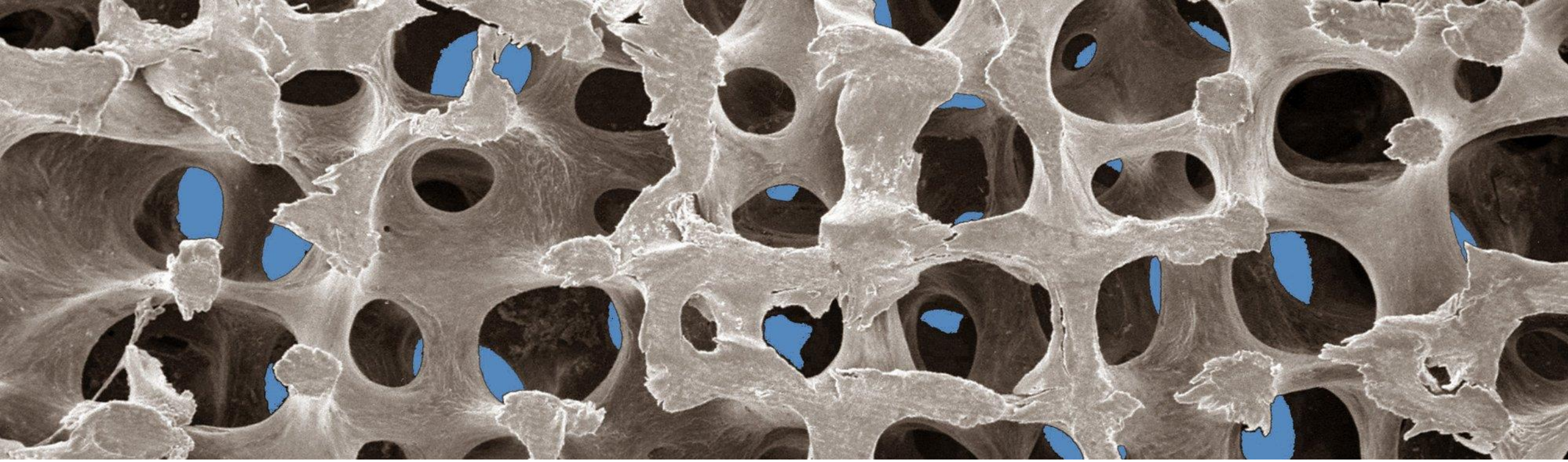

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Bone Remodeling

Biology – Theories – Models

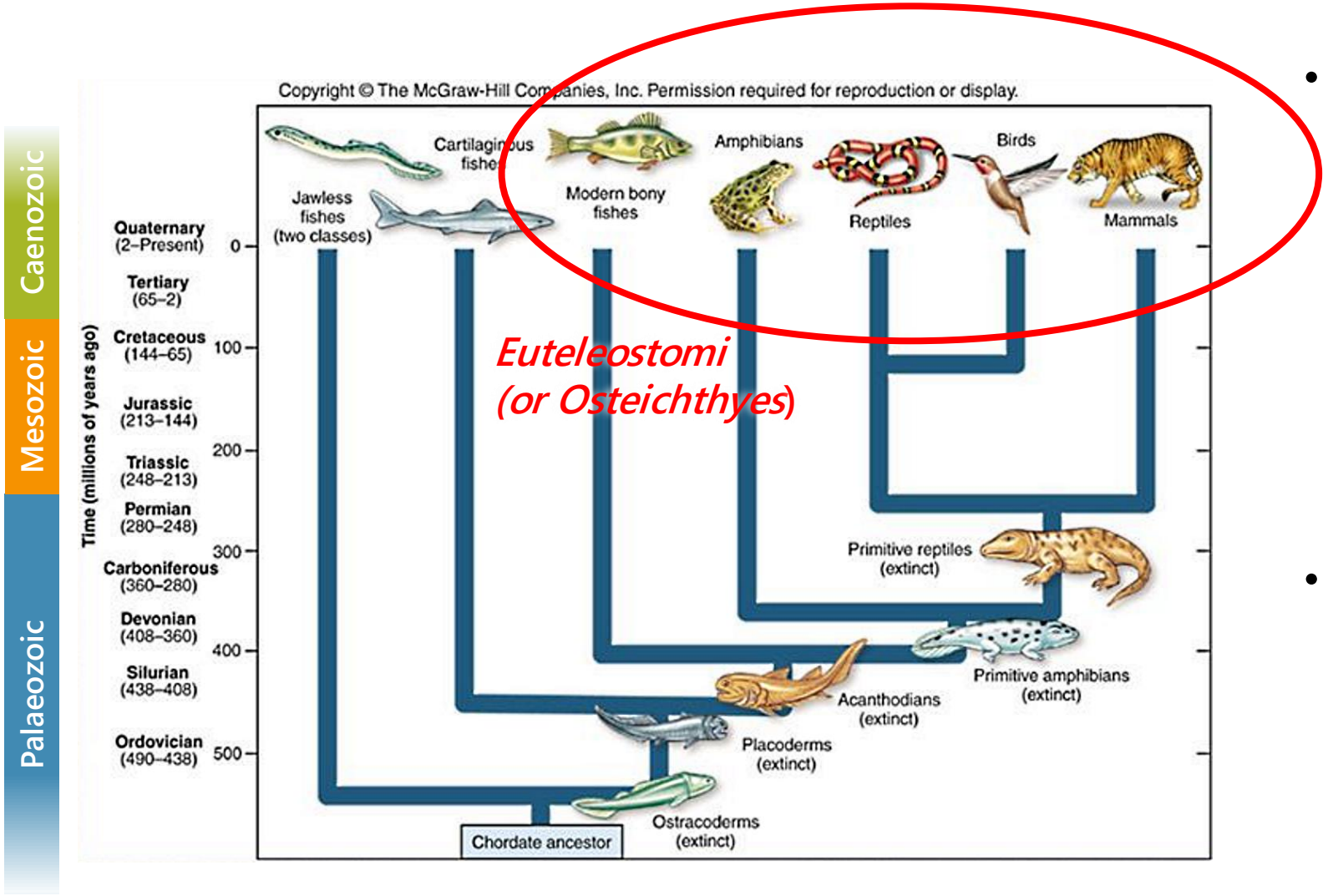
Computational Biomechanics

Summer Term 2016

Lecture 8/12

Frank Niemeyer

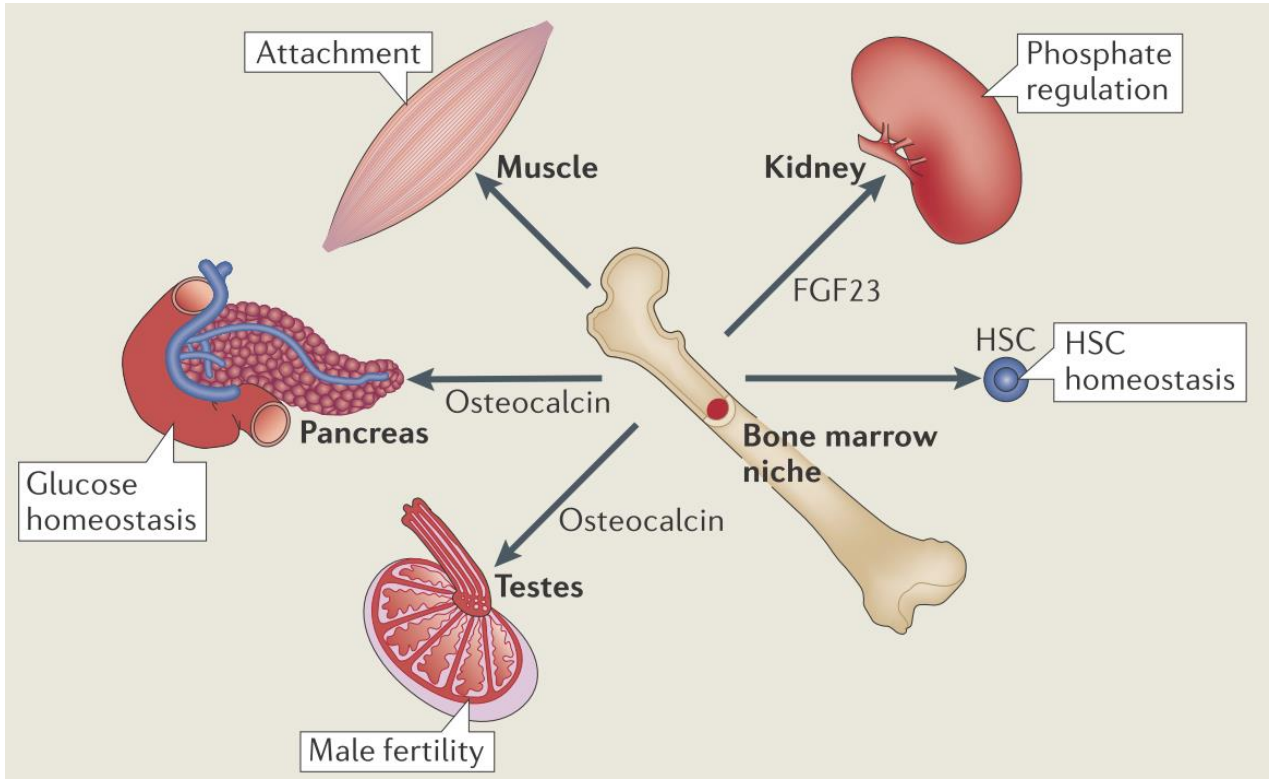
Skeletal Structures in Animals



Vertebrate family tree

- Exoskeltons (many invertebrates)
  - Calcium carbonate (molluscs, polychaetes)
  - Chitin (arthropods)
  - Silica (diatoms, radiozoa, sponges)
  - …
- Endoskeletons (vertebrates)
  - Cartilage
  - Bone (hydroxyapatite)

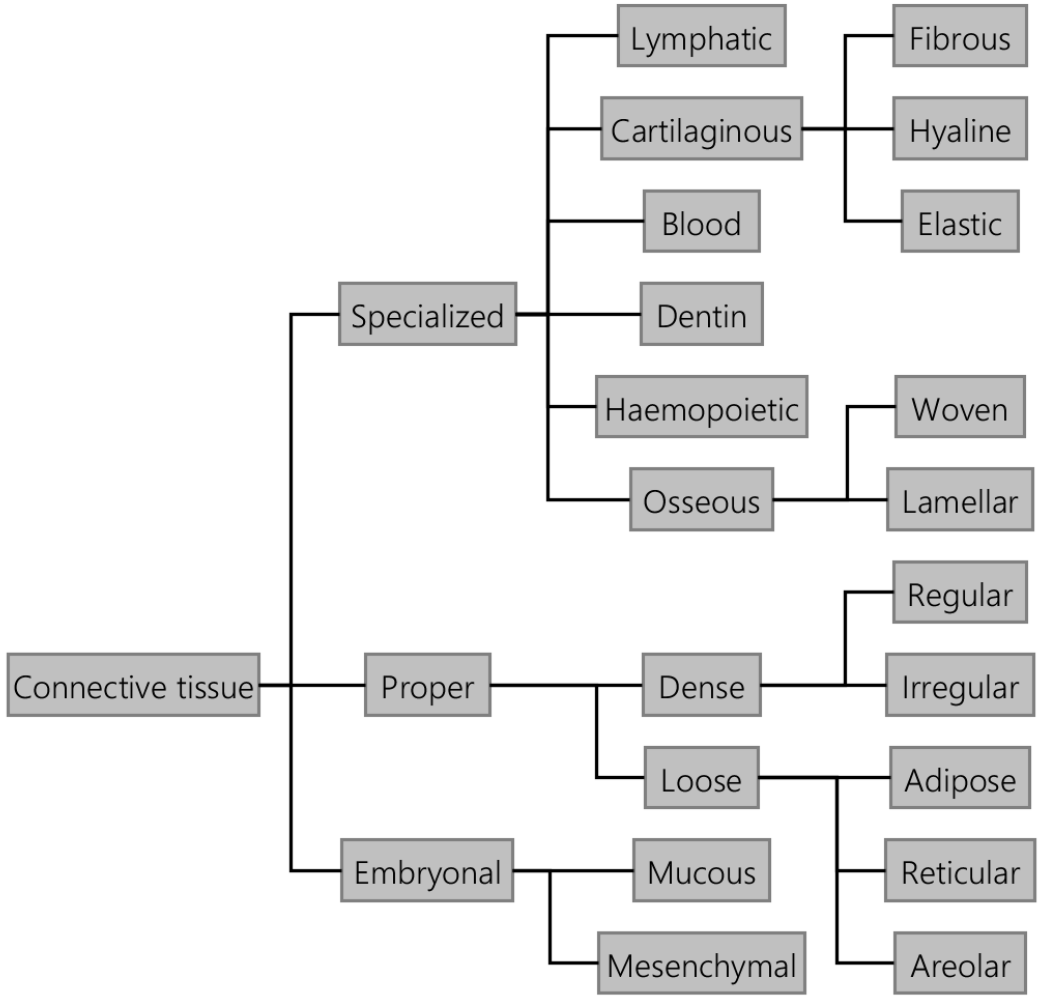
Bone in the Human Body



Long 2012

- Mechanical
  - Stability, support
  - Muscle/tendon/ligament attachment, joints, lever arms
  - Protection of vital inner organs
  - Sound transduction
- Metabolic
  - Mineral (Ca, P) reservoir, plasma calcium homeostasis
  - Acid-base balance (“buffer”)
  - Hematopoiesis (marrow)
  - Fat reservoir
- Endocrine
  - Phosphate level (FGF-23)
  - Glucose level, fat deposition (osteocalcin)

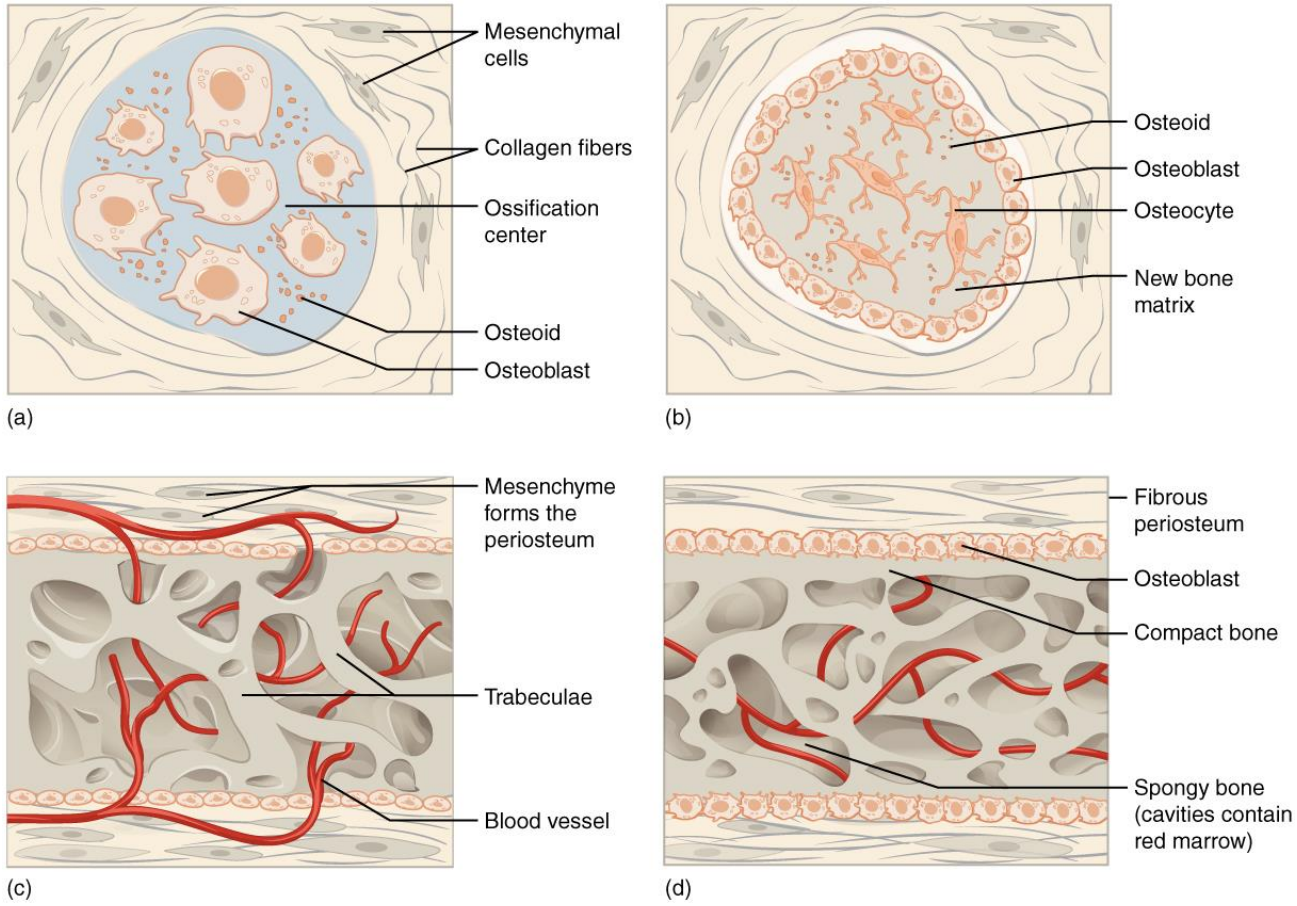
Connective Tissues



One possible classification of connective tissues

- Develops form mesoderm
- Mostly ECM
- Components
  - Fibers (collagen, elastin, fibrillin, fibrinogen)
  - Ground substance with GAGs, proteoglycans
  - Relatively sparsely populated with cells
- Tensile and compressive strength
- Bone tissue is one kind of *specialized connective tissue*

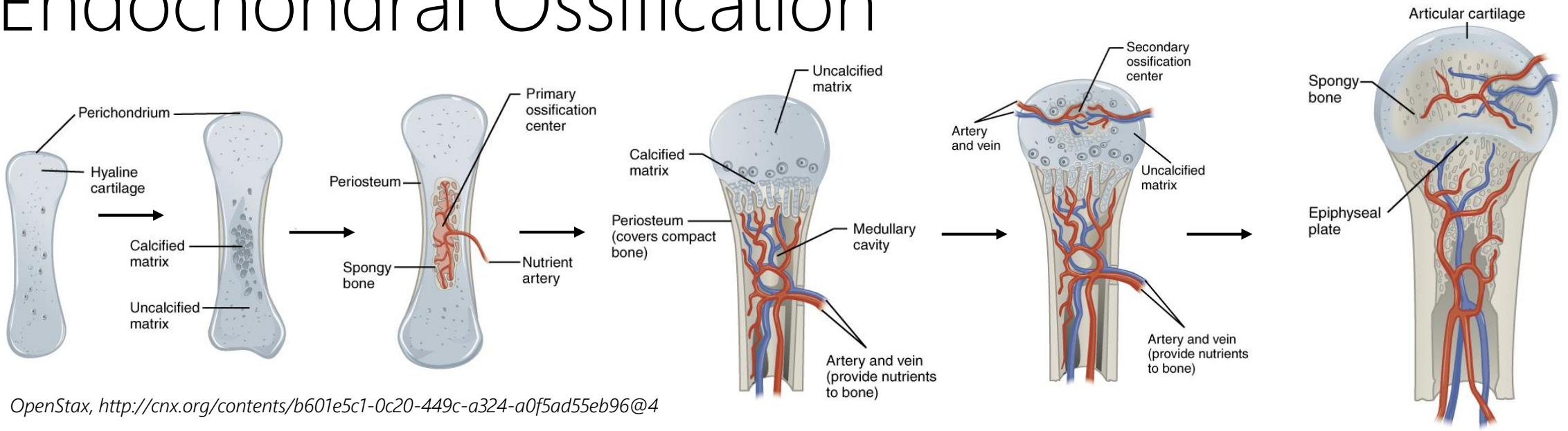
Intramembranous Ossification



OpenStax, http://cnx.org/contents/b601e5c1-0c20-449c-a324-a0f5ad55eb96@4

- Existing connective tissue, mesenchyme
- Initiated by MSCs
  - Replicate and differentiate (→ osteoprogenitor → osteoblasts)
- Osteoblasts deposit matrix
- Matrix mineralizes
- Formation of trabeculae
- Further appositional growth
- Flat bones (skull, mandible, maxilla, calvicles …)

Endochondral Ossification

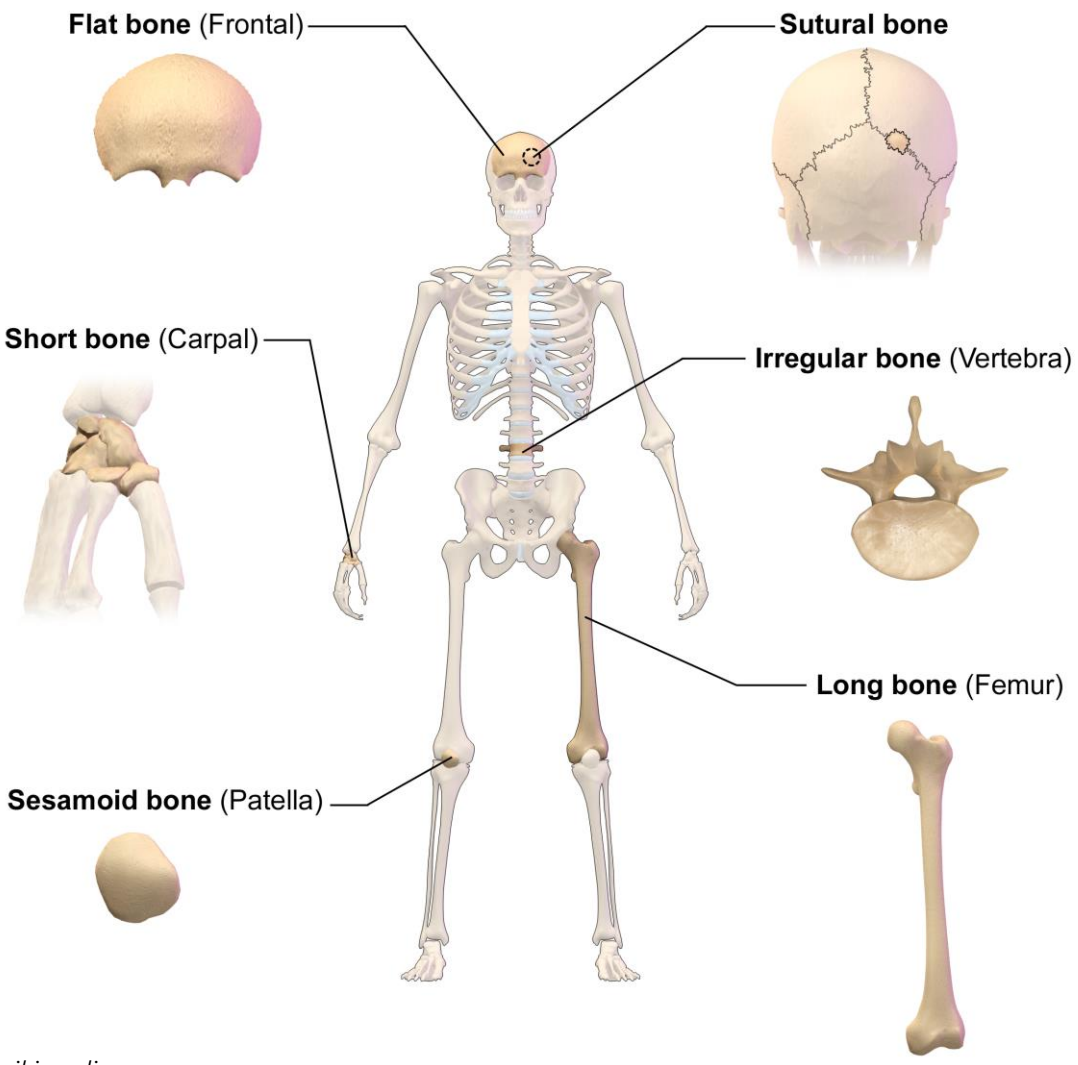


OpenStax, http://cnx.org/contents/b601e5c1-0c20-449c-a324-a0f5ad55eb96@4

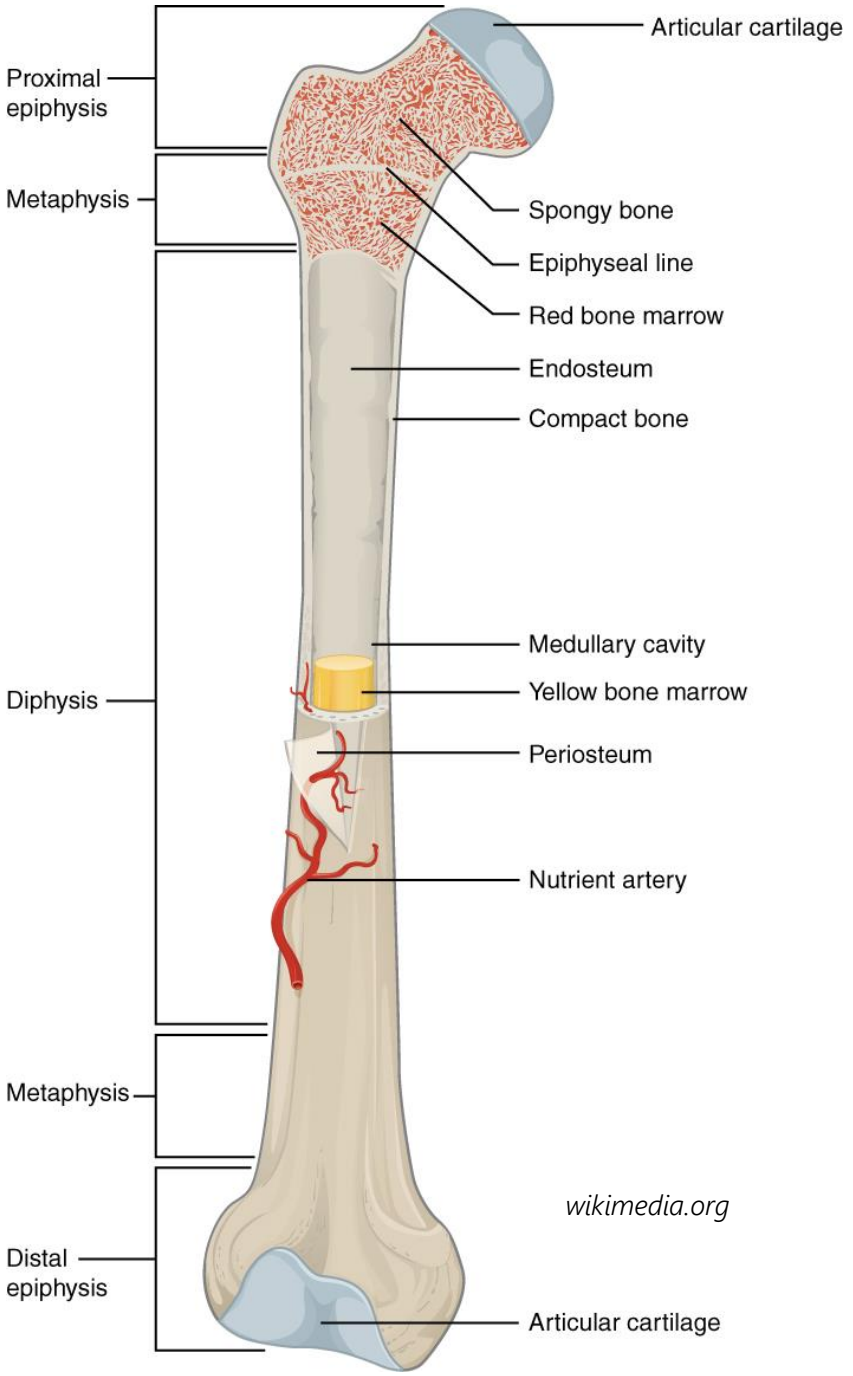
- Mesenchym transformed into cartilage model
- Hypertrophic chondrocytes secrete alkaline phosphatase
- Cartilage calcifies
- Formation of periosteum and invasion of blood vessels
- At “primary centers of ossification”: osteoblasts deposit collagenous matrix
- Hyaline cartilage remains on epiphyseal surfaces (and epiphyseal plate, for a while)
- Long bones, vertebrae, …

Types of Bone

Anatomic: Long Bones et al.



wikimedia.org



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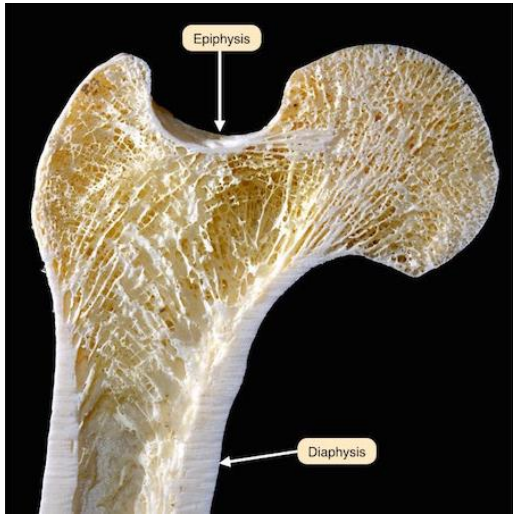
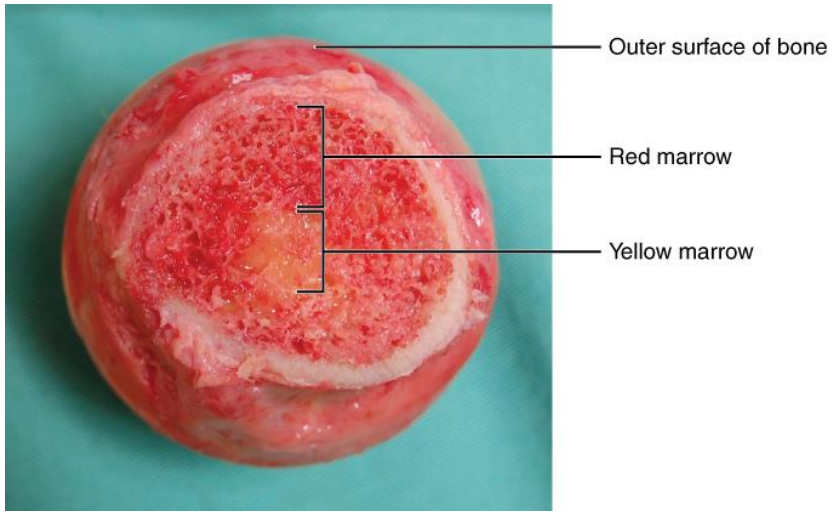
Macroscopic: Cortical vs. Trabecular Bone



http://medcell.med.yale.edu/systems_cell_biology/bone_lab.php



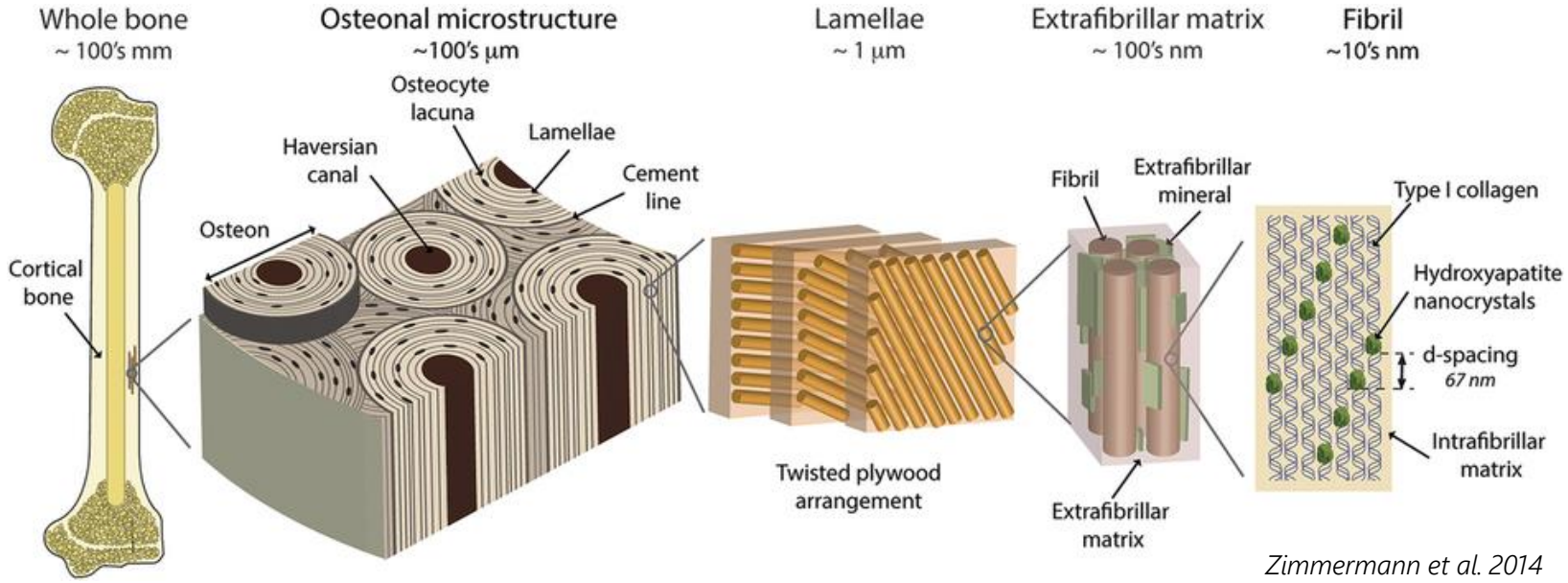
wikimedia.org

- Cortical bone a.k.a. compact bone a.k.a. *substantia compacta*
  - Hard outer layer (cortex)
  - Covered by periosteum and endosteum
  - ~ 80 % of bone-mass (adult human)
  - $\rho \approx 1.7\ \mathrm{g/cm^3}$, $E \approx 15\ \mathrm{GPa}$, $\Phi = 10\ \%$
- Trabecular bone a.k.a. spongy bone a.k.a. *substantia spongiosa*
  - Sponge-like morphology
  - Open-cell porous network of plates and rods, struts
  - Filled with marrow (myeloid tissue)
  - ~ 20 % of bone-mass (adult human)
  - $\rho \approx 0.5\ \mathrm{g/cm^3}$, $E \approx 500\ \mathrm{MPa}$, $\Phi = 50 - 90\ \%$

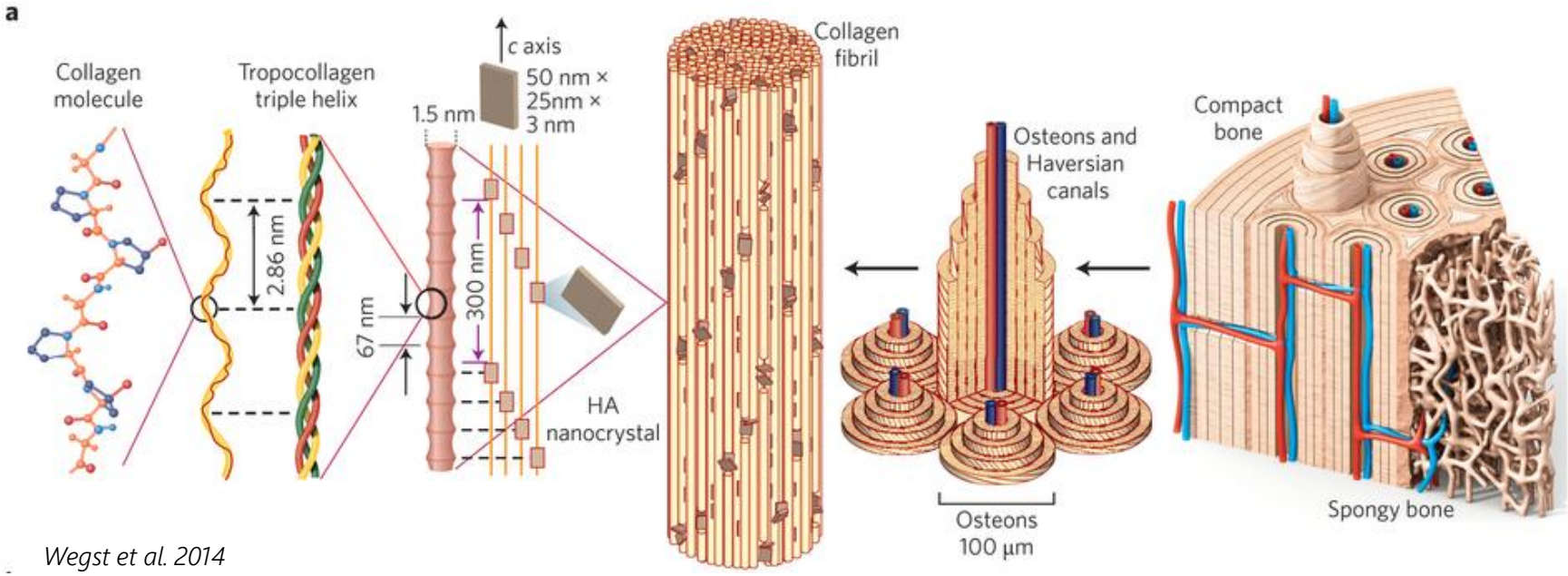
Microscopic: Woven vs. Lamellar Bone

- Woven bone a.k.a. primary bone
  - Small, randomly oriented collagen fibrils
  - Mechanically weak
  - Rich in osteocytes
  - Rapidly produced
  - Produced during fetal development and fracture healing (and Paget's disease)
- Lamellar bone a.k.a. secondary bone
  - Mature form of bone, replaces woven bone
  - Slow formation (1 - 2 μm/day)
  - Forms stacked or concentric "lamellae"
  - Thicker collagen fibers, aligned in parallel within each lamella

Hierarchical Structure of Bone

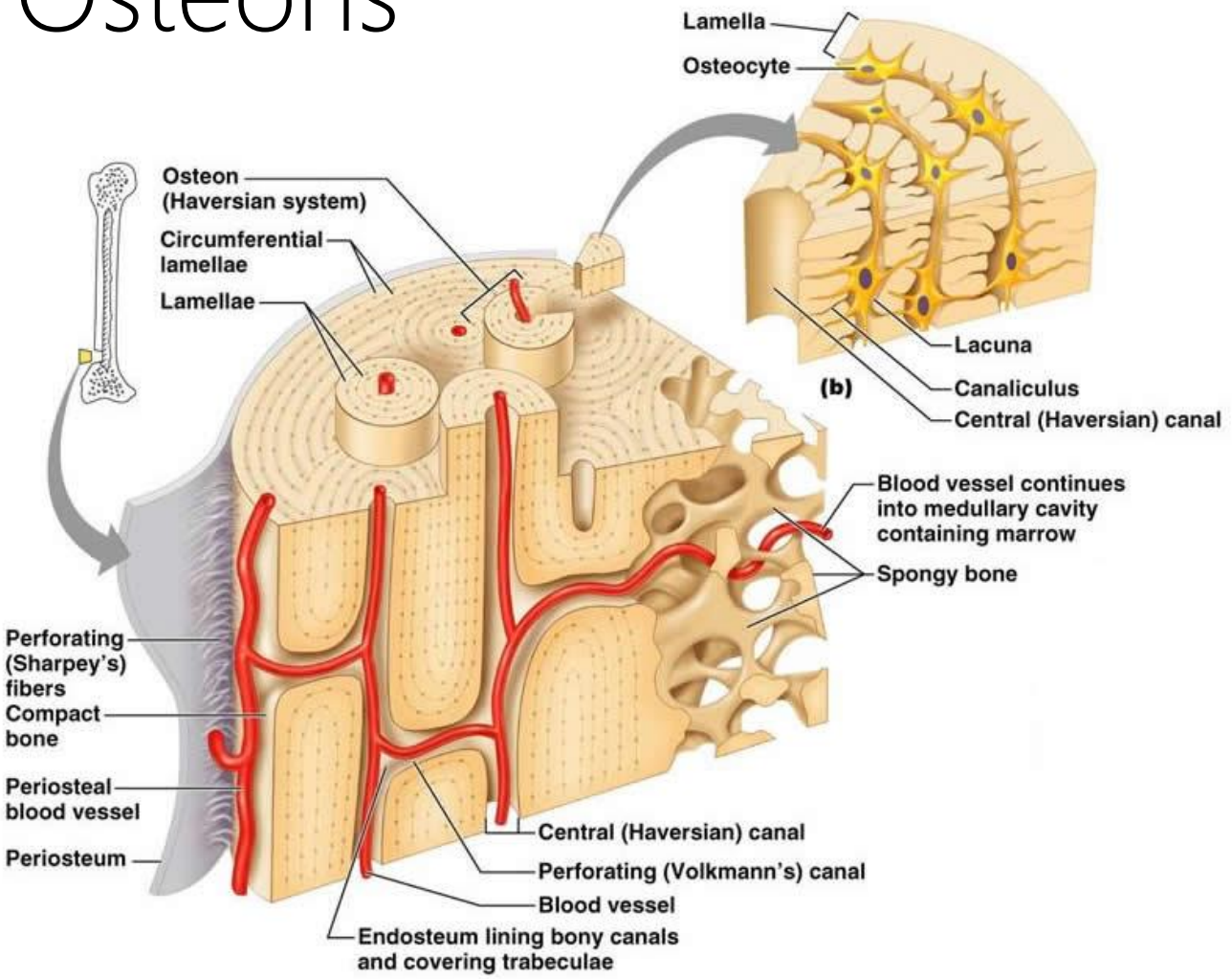


Zimmermann et al. 2014

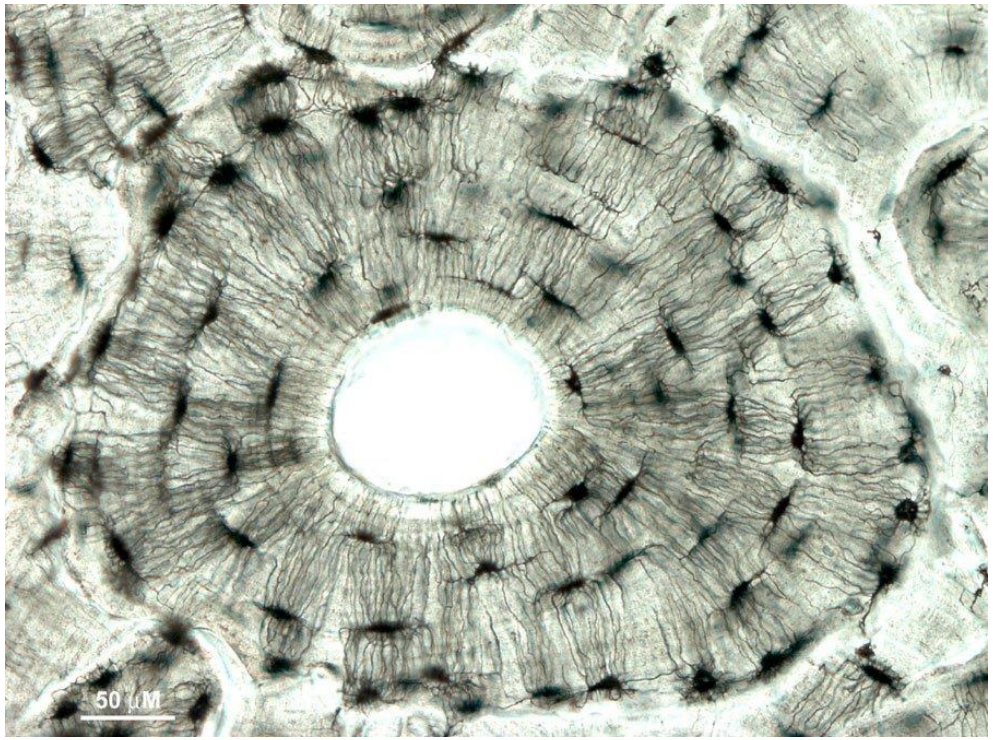


Wegst et al. 2014

Osteons



http://classes.midlandstech.edu/carterp/Courses/bio210/chap06/lecture1.html



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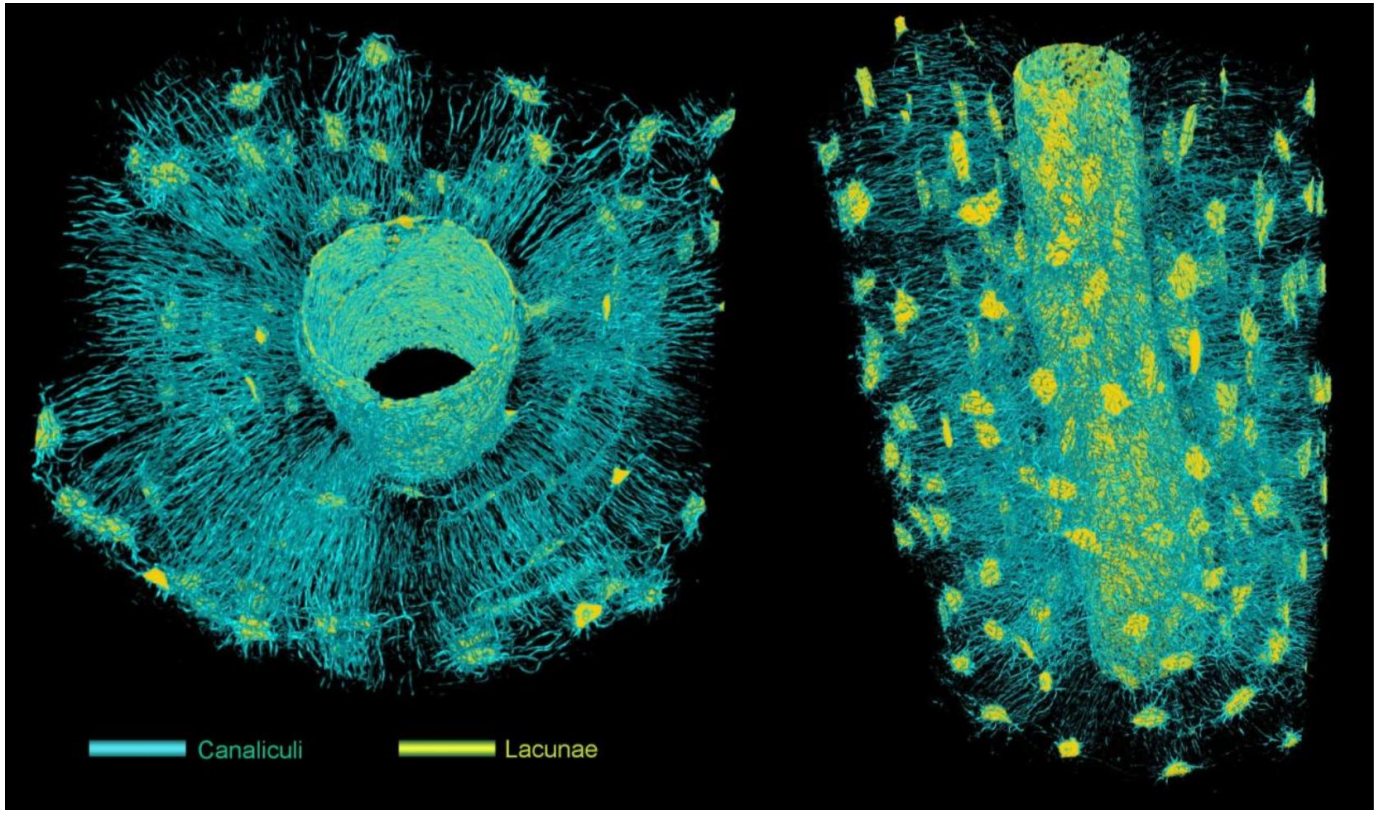
Canaliculi and the Osteocyte Network

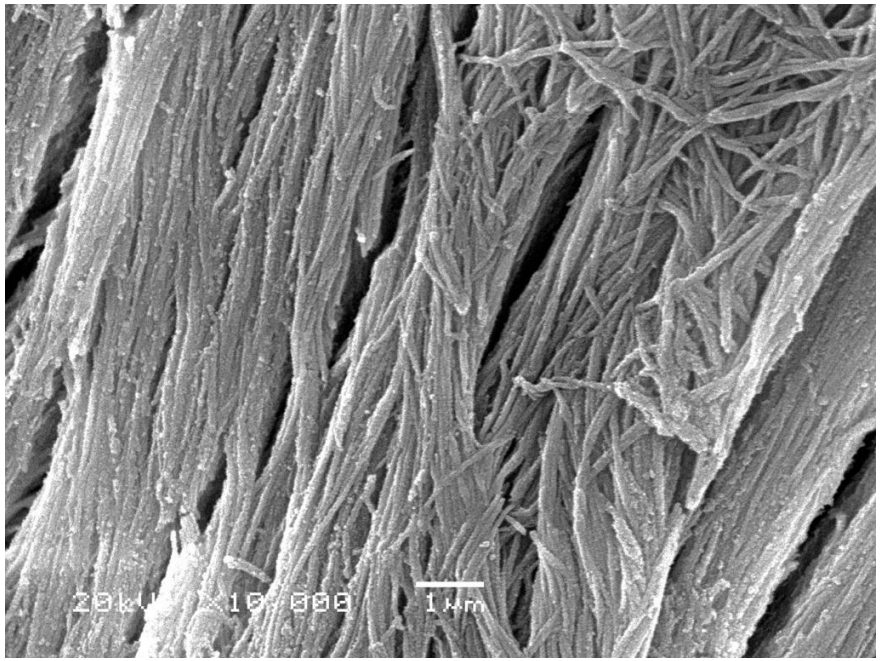
- Network of osteocytes, connected via cytoplasmatic extensions
- $\approx 42 \cdot 10^9$ osteocytes (brain: $\approx 86 \cdot 10^9$ neurons)
- $\approx 23.4 \cdot 10^{12}$ connections (brain: $\approx 150 \cdot 10^{12}$ connections)
- $\approx 175{,}000$ km of dendritic processes (brain: $\approx 165{,}000$ km)
- Turnover: 10^7 osteocytes/day (brain: 700 neurons/day)



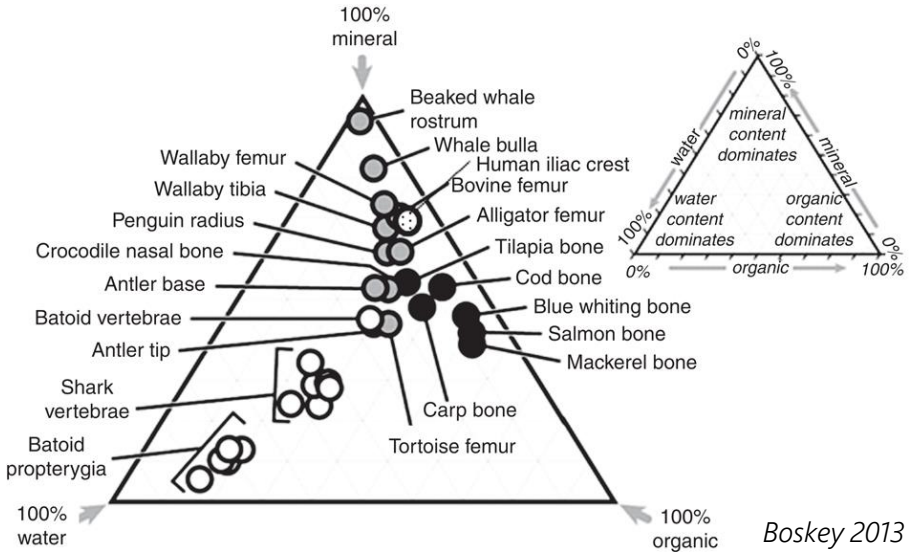
3D rendering of osteocyte network around haversian canal (Pacureanu et al. 2012)

Bone Matrix

- Organic part (~ 30 %)
  - Type-I collagen (90 – 95 %)
  - Non-collagenous proteins, e.g.
    - Proteoglycans
    - Osteocalcin, osteonectin, osteopontin
  - Lipids
  - Growth factors (IL-1/6, IGF, TGF-β, BMPs)
- Inorganic part (~ 45 %)
  - Primarily cristalized calcium-phosphates (hydroxyapatite, $Ca_5(PO_4)_3(OH)$)
  - Trace minerals (magnesium, fluoride, carbonate, …)
  - Distributed along collagen strands
- Water (~ 25 %)
- High tensile and compressive stiffness
- Composition varies with age, sex, site, ethnicity, health status

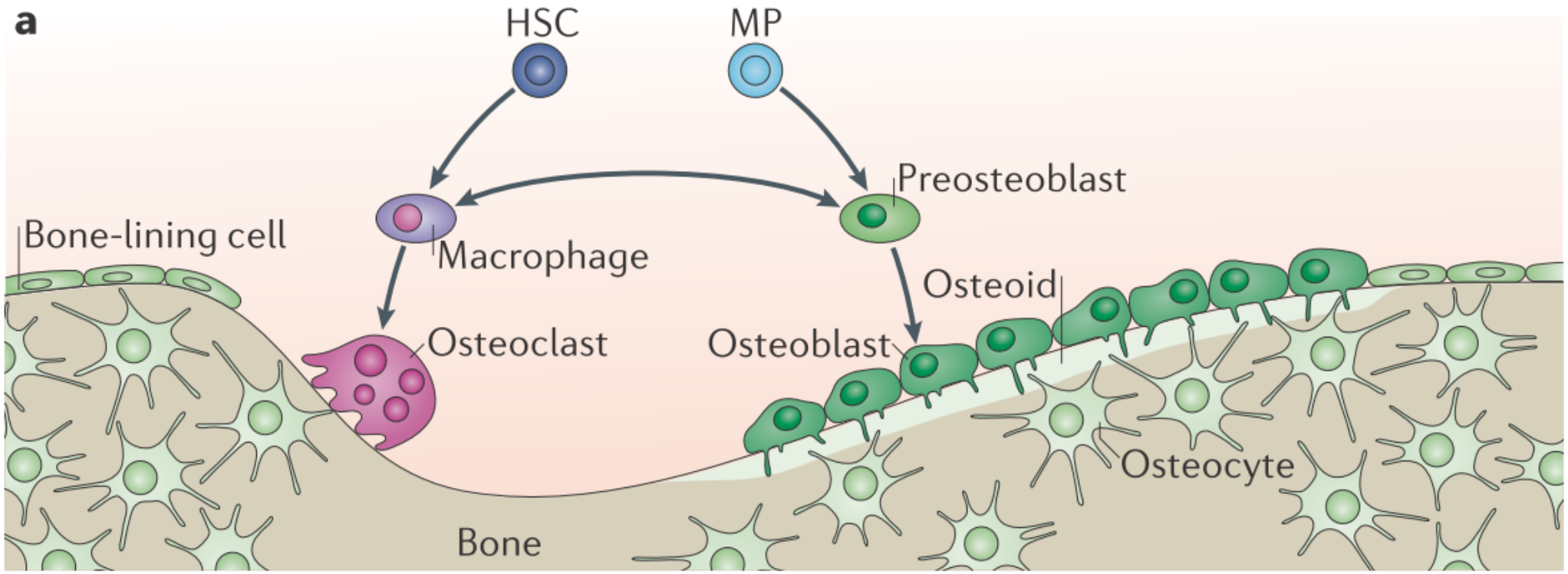


SEM of bone mineral (mineralized collagen fibers)
10000x magnification (wikimedia.org)



Boskey 2013

Bone Cells



Long 2012

Cell Lineages

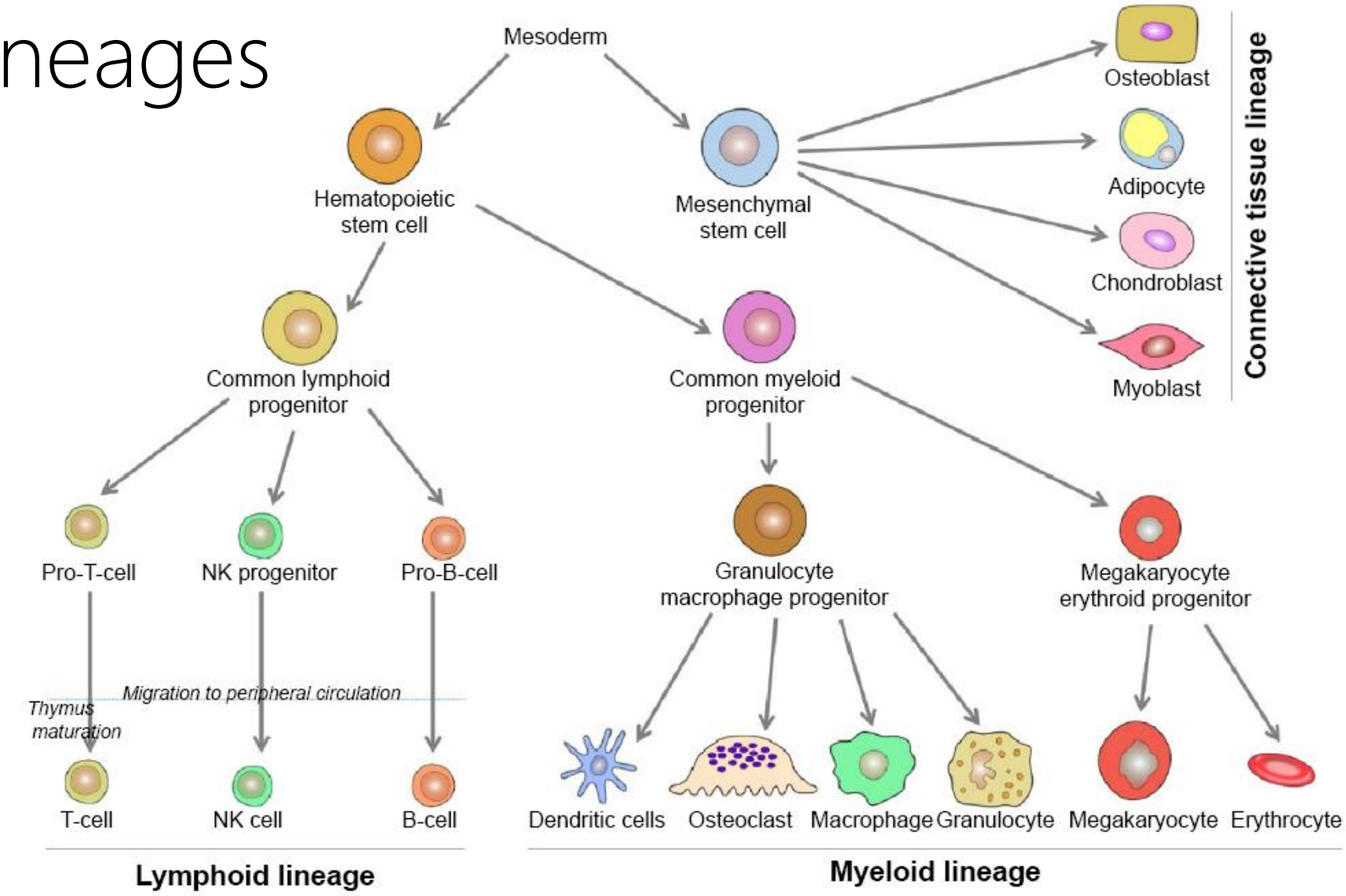
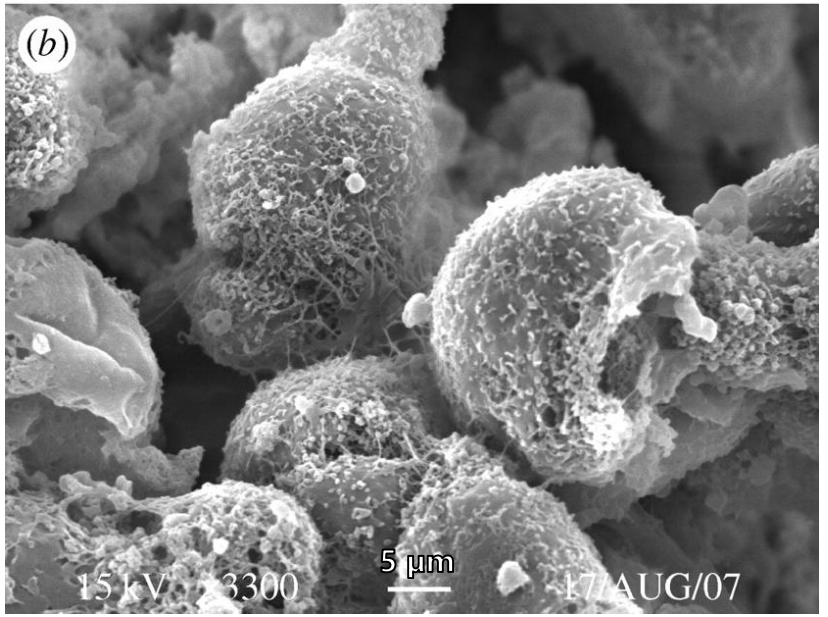


Figure 1 Main osteoimmunological cell differentiations and cell lineages.
Abbreviation: NK cell, natural killer cell.

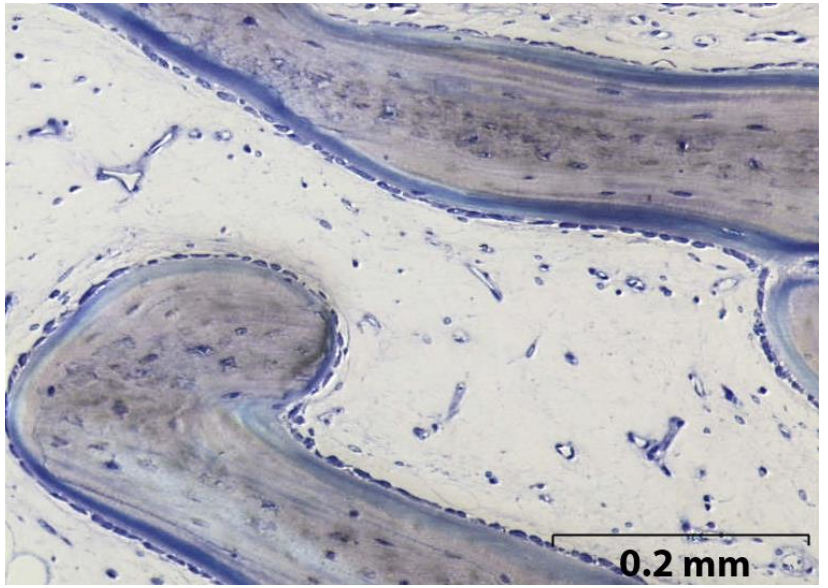
Numan et al. 2015

Osteoblasts & Lining Cells

- ~ 5 % of bone cells in adults
- Mononucleate bone forming cells
- Derived from MSCs → osteoprogenitors
- Connected to each other via gap and tight junctions and to osteocytes via processes
- Secrete osteoid and alkaline phosphatase for mineralization
- Extensive endoplasmatic reticulum, Golgi bodies and mitochondria
- After matrix production osteoblasts may either
  - … die (apoptosis; 50 – 70 %) or
  - … become inactive (flattened bone lining cells) or
  - … become embedded in bone (osteocytes; 10 – 20 %)



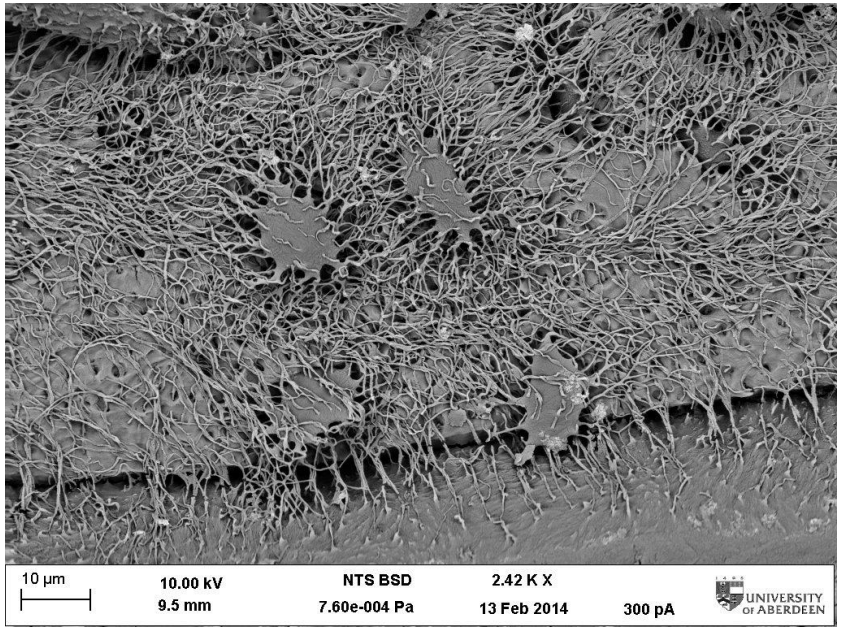
Osteoblasts grown on ChiPgAHAP20 scaffold (Verma et al. 2010)



Lining cells around trabeculae

Osteocytes

- ~ 90 – 95 % of all bone cells in adults
- ~ 30,000 cells/mm^3 of bone
  - Star-shaped, 7 µm × 15 µm
  - Inter-cell distance 20 – 30 µm
- 40 – 60 processes/cell ("filopodial ext.")
- Avg. half-life of 25 y
- Percentage of dead osteocytes: 1 % (birth) … 75 % (age > 80 y)



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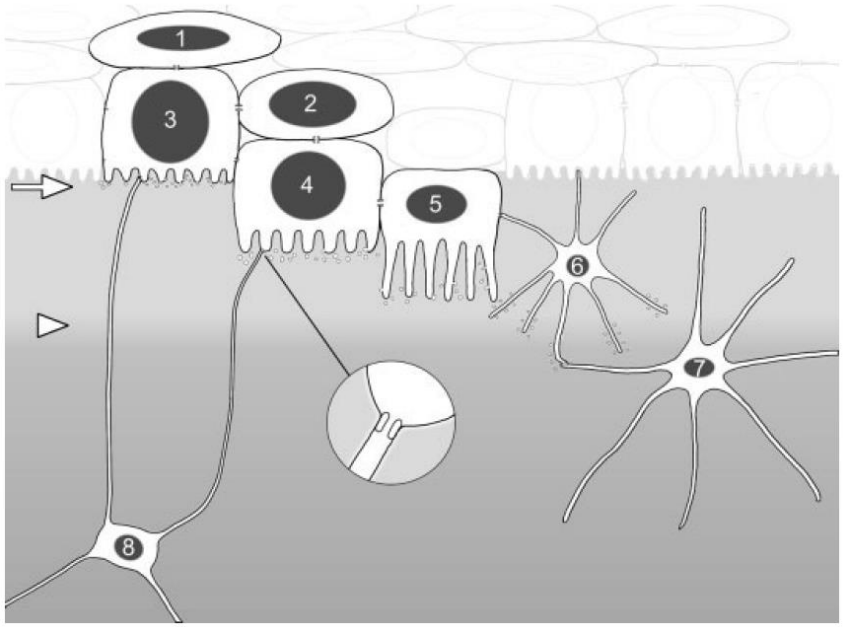
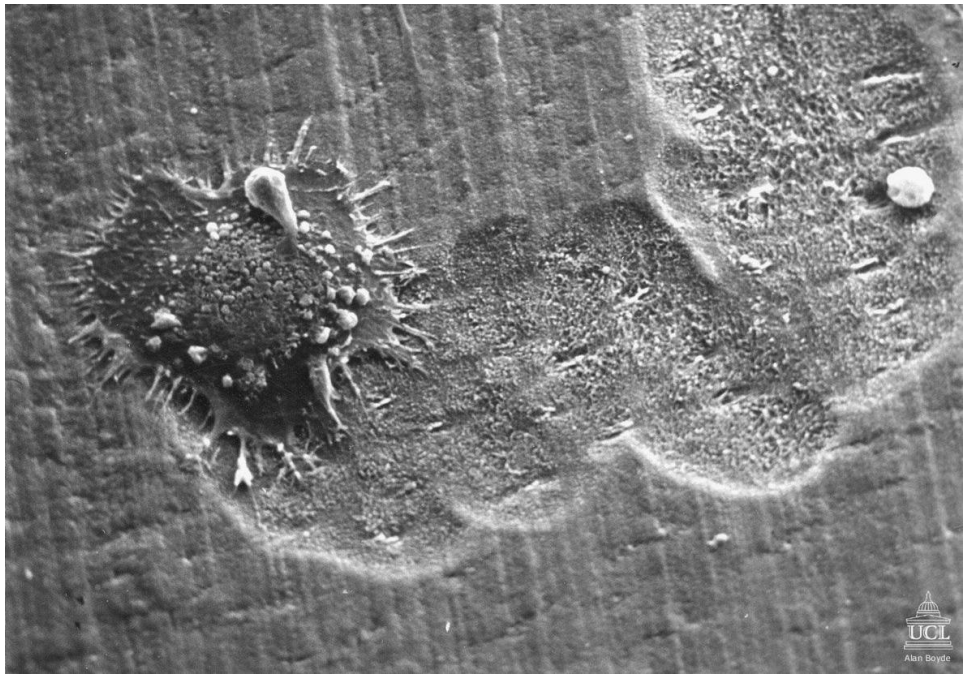


Franz-Odendaal et al. 2006

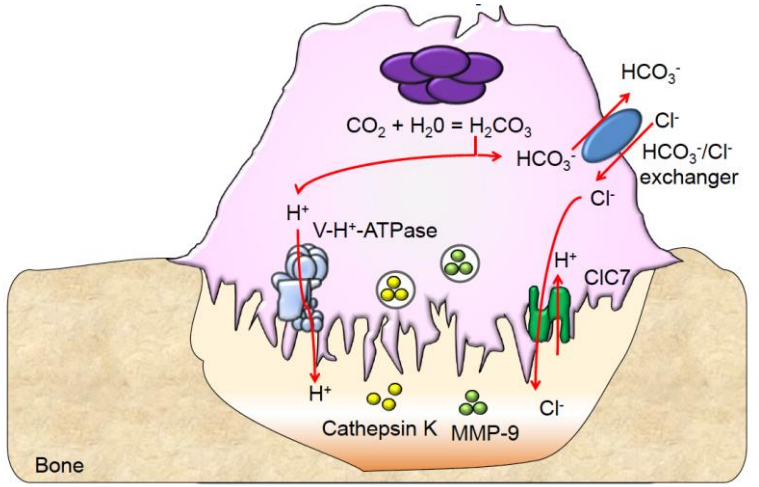
- Terminally differentiated osteoblasts, incorporated in mineralized matrix, reside in lacunae
- Osteoblasts becoming osteocytes slow down matrix production and are "buried" by neighbors
- Transformation takes ~ 3 days
- Involved in bone turnover (sensors, osteolysis)
- Secrete hormons and other signaling proteins (FGF-23, sclerostin, …)

Osteoclasts

- Bone resorbing cells
- Large, multinucleated cells
  - ~ 5 nuclei, 100 – 200 µm diameter
  - Derived from monocytes
- Located on bone surface in Howship's lacunae (resorption pit)
- Move via chemotaxis to remodeling sites
- Phagocytic-like mechanism
  - Release HCl for dissolution of hydroxyapatite
  - Enzymes digest organic components



boneresearchsociety.org, © Alan Boyde



Fattore et al. 2012

Remodeling

- Observation: bone
  loading ("Wolff's la
- Roux: remodeling a
  regulated by cells i
- Bone is constantly
  turnover) in response to
  - Mechanical loading (local)
  - Metabolic influences (systemic)
- Both control loops interact at a cellular level
- Purpose:
  - Repair damage (micro-fractures)
  - Adapt to load
  - Adapt to growth
  - Maintain Ca/P homoeostasis
- Remodeling rate: ~ 10 % per year
  - Complete bone mass replaced within 7 – 10 years

„[Im] Gefolge primärer Abänderungen der Form und Inanspruchnahme oder auch bloß der Inanspruchnahme der Knochen, [vollziehen sich] bestimmte, nach mathematischen Regeln eintretende Umwandlungen der inneren Arch
Regeln folge
betreffender

Der züchtende Kampf der Theile oder die Theilauslese im Organismus. Zugleich eine Theorie der functionellen Anpassung. Ein Beitrag zur Vervollständigung der Lehre von der mechanischen Entstehung des sogenannten Zweckmäßigen.

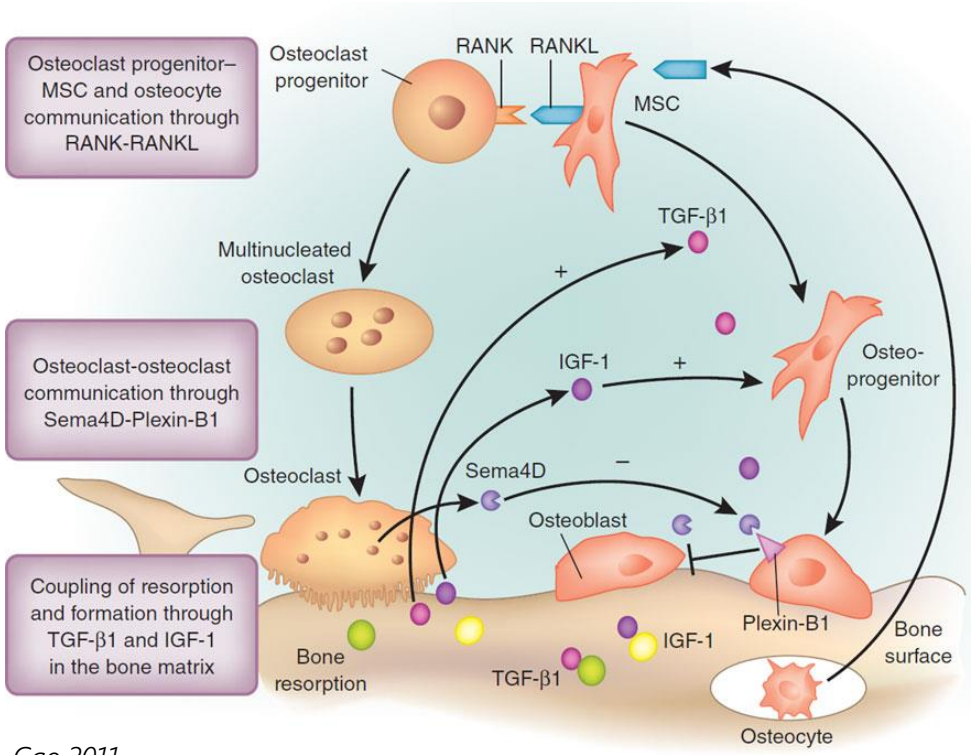
Wilhelm Roux, 1881

Julius Wolff (1836-1902), © Charité

Wilhelm Roux (1850-1924)
© Martin-Luther Universität Halle-Wittenberg

Mechanobiology

- Remodeling: resorption by osteoclasts followed by formation by osteoblasts (“coupling phen.”)
- Takes in place in BMUs
- Osteonal vs. hemi-osteonal
- Complex coordinated action, requiring tight regulation
  - Direct contact
  - Gap junctions
  - Paracrine signaling
- May also be influenced by
  - Sympathic nervous system
  - Hematopoietic stem cells
  - Immune system
  - Vasculature
  - Articular cartilage
- Not yet completely understood

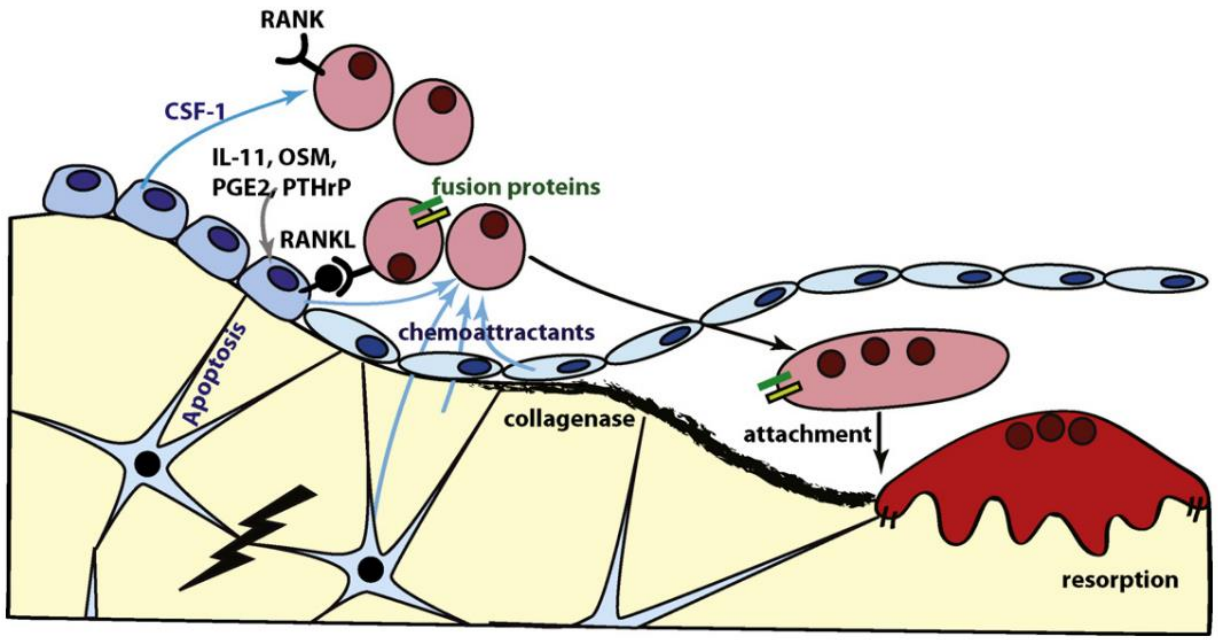


Cao 2011

Molcules that influence bone formation:

- Osteocalcin
- Osteonectin
- Alkaline phosphatase
- Fibronectin
- Thrombospondin
- Proteoglycans I and II
- Osteopontin
- Bone sialoprotein
- Bone morphogenic proteins (BMP)
- Fibroblast growth factors (FGF)
- Insulin-like growth factors (IGF)
- Platelet-derived growth factor (PDGF)
- Transforming growth factor β (TGF-β)
- Epidermal growth factor (EGF)
- Parathyroid hormone (PTH)
- Estrogene
- Dexamethasome
- Thyroxin
- Calcitonin
- Prostaglandins
- Interleukin-I
- Vitamin D
- …

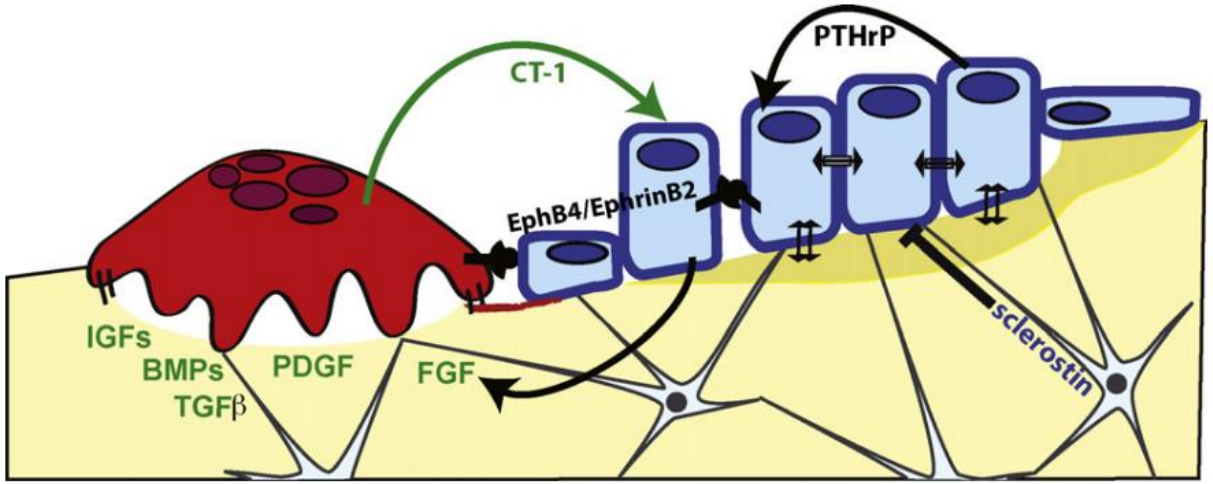
Activation & Resorption



Sims & Gooi 2008

- Osteoclast differentiation regulated by cells of osteoblast lineage
  - CSF-1 stimulates osteoclast precursor proliferation
  - Chemoattractants attract osteoclast precursors
  - RANKL promotes osteoclast differentiation (fusion)
  - OPG inhibits osteoclast formation
- Osteocyte apoptosis → ↑ RANKL
- Osteoblast lining cells prepare surface for osteoclast
- Attachment to bone surface
- Resorption (~ 3 weeks, ~ 60 – 100 µm cavity depth)

Reversal, Transition, Termination

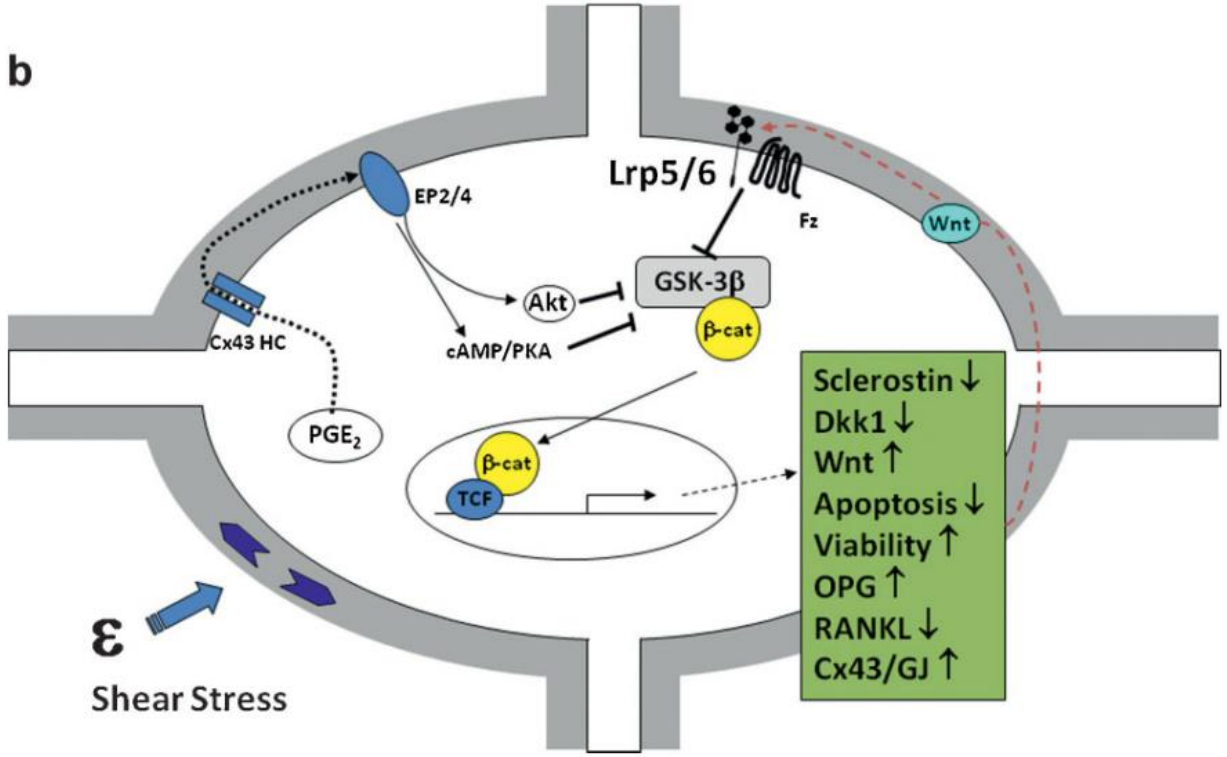


Sims & Gooi 2008

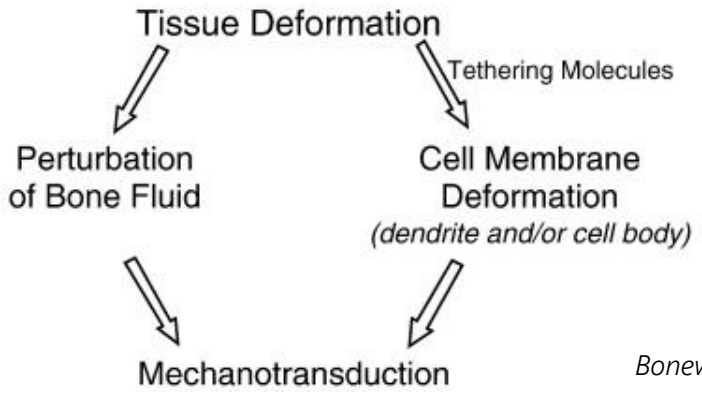
- Osteoblast differentiation and bone formation regulated by
  - Growth factors released from resorbed matrix (IGFs, BMPs, TGF-β, PDGF, FGF)
  - Growth factors secreted by osteoclasts (CT-1)
  - Osteoclast's membrane-bound molecules
  - Local topography
  - Growth factors secreted by osteocytes (sclerostin, TGF-β)
- Apoptosis of osteoclasts (induced by Bim/caspase-3, estrogen, released Ca)
- Osteoid deposition (~ 3 months)
- Mineralization

Mechanotransduction

- Classical hypothesis: osteocytes as mechano-sensors
  - Physiological loading induces fluid-flow in canaliculi
  - Sensed by integrins on cell surface
  - Activation of mechanotransduction pathways
  - Overactivation: less sclerostin → bone gain
  - Underactivation: less OPG → bone loss
- Regulation by osteocyte apoptosis
  - Mechanical loading enhances solute transport in canaliculi → inhibited bone resorption
  - Mechanical unloading: hypoxia → osteocyte apoptosis → bone resorption
  - Micro-damage: also induces osteocyte apoptosis → bone resorption

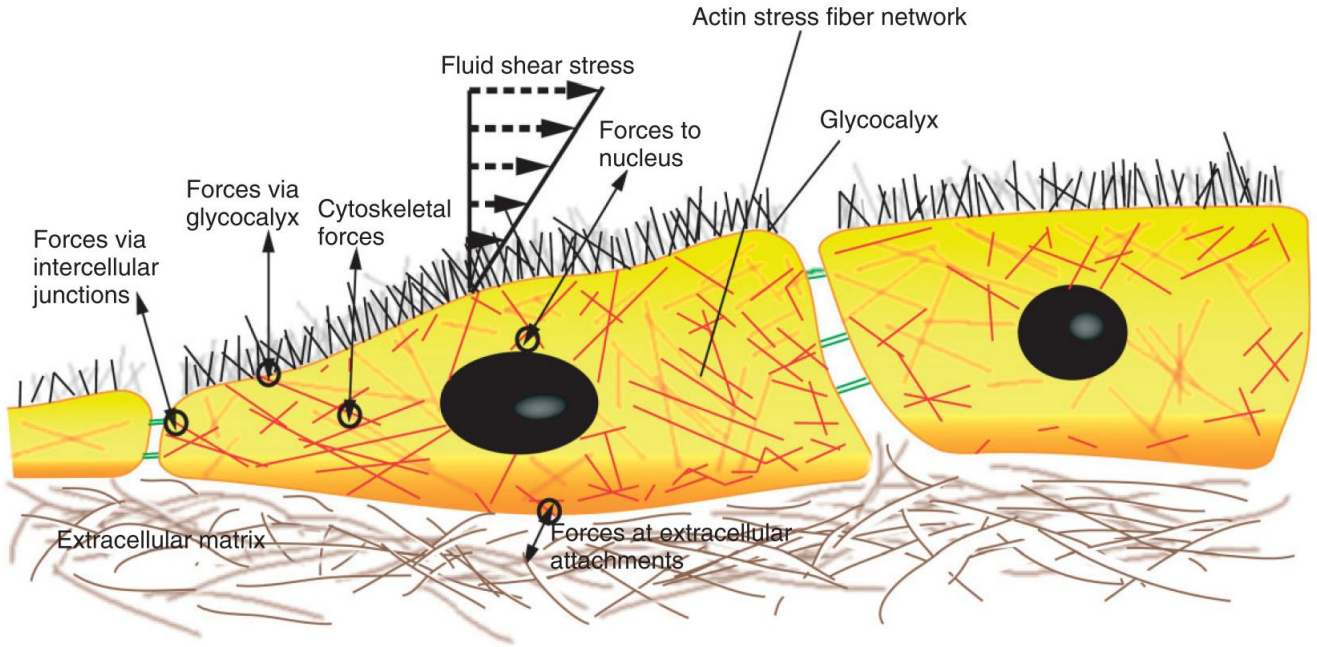


Wnt/β-catenin pathway, *Bonewald 2011*



Bonewald et al. 2008

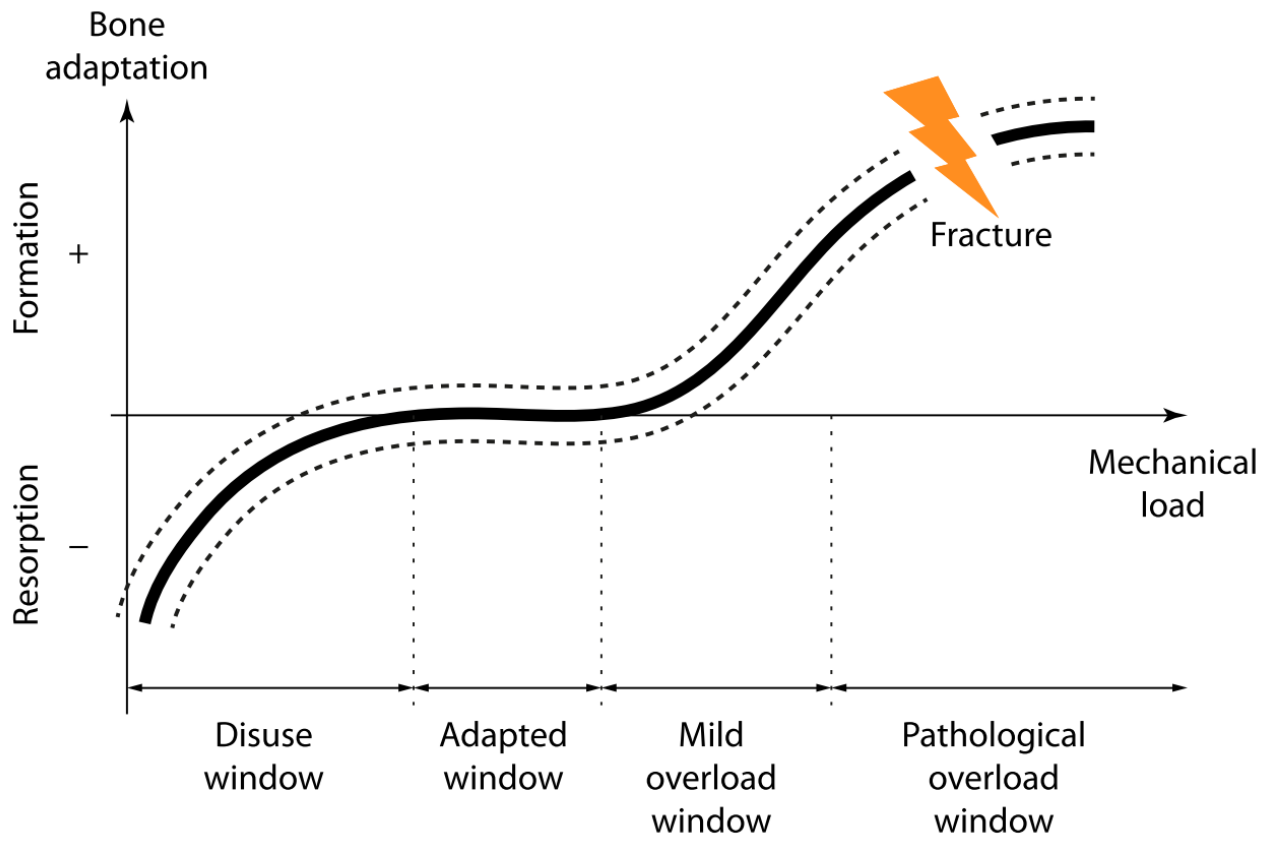
Mechanotransduction



Kolahi & Mofrad 2010

- Possible stimuli
  - Fluid-flow induced shear stress
  - Fluid pressure
  - Electric fields due to piezo-electric properties of bone/minerals
  - Direct cell strain (deformation of cytoskeleton)
  - Bending of cilia
- Issues
  - Bone cell behavior strongly dependent on micro-environment
  - Difficult to observe osteocytes in situ
- MSCs & osteoblasts also react to mechanical stimuli

The "Mechanostat" Model

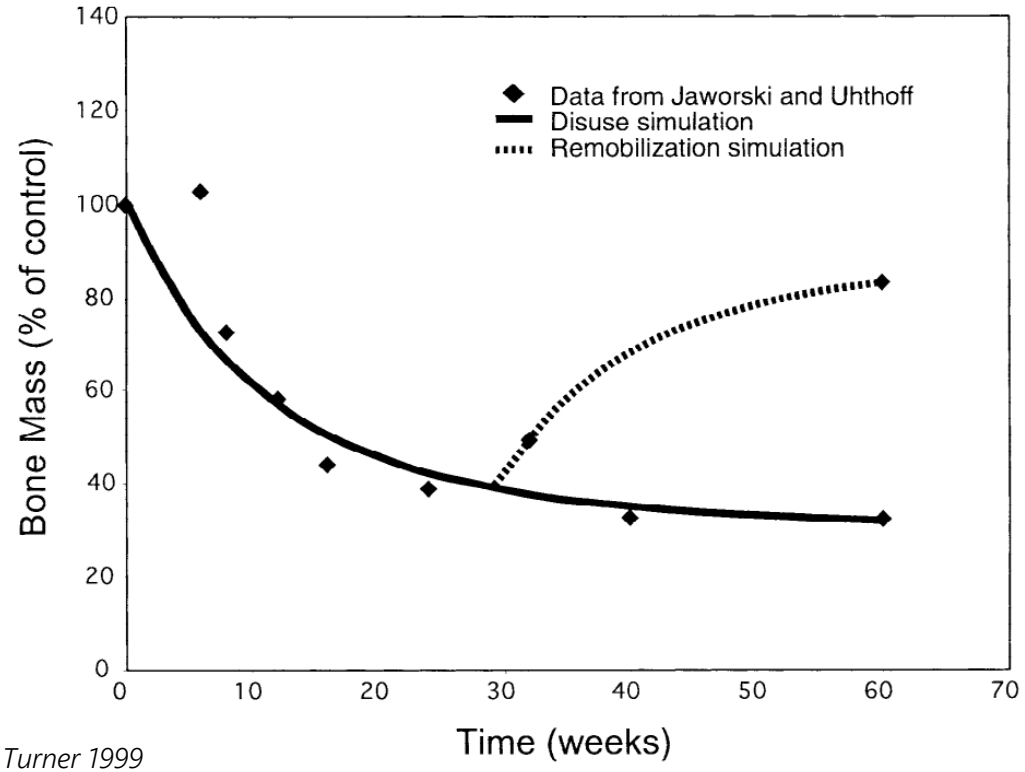


Niemeyer 2013

- A.k.a. "Utah paradigm of skeletal physiology", developed in 1960s by Harold M. Frost
- Semi-quantitative refinement of Wolff's law
- Bone adaption is regulated such that 'peak strain' stays within some physiological interval
  - Human tibia: 800 – 1500 με*
- Thermostat analogue: negative feedback loop → dynamic equilibrium
- Purely mechanical, quasi-static, neglecting
  - Load cycle number, frequency, duration, pauses, deformation speed …
  - Non-mechanical influences

*$1\ \mu\varepsilon = 1\ \mu = 10^{-6} = 0.0001\ \%$

The Principle of Cellular Accommodation



Turner 1999

- (Linearized) mechanostat equation:

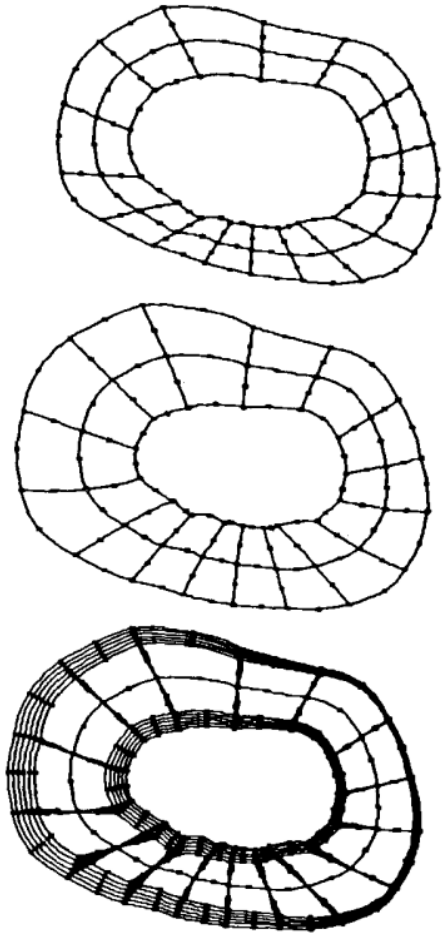
$$\dot{\rho} = k(S - S_0)$$

  - E: elastic modulus, S: peak stimulus, S_0: set-point
  - "Disuse fallacy", "constant set-point fallacy"
- Cells accommodating to strain environment:

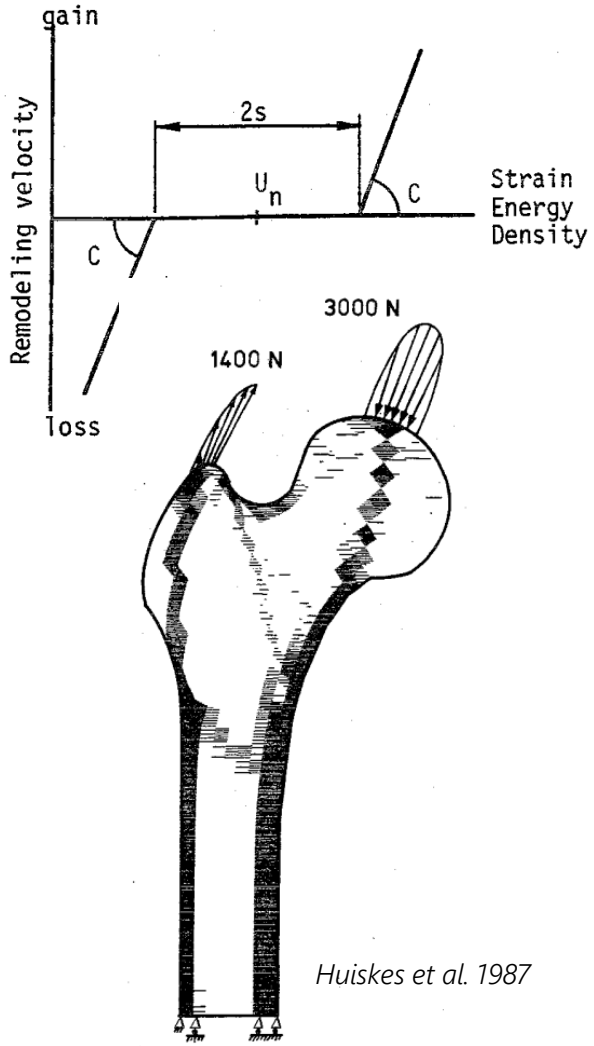
$$\dot{\rho} = k\big(S - F(S,t)\big)$$

  - F: relaxation function (non-constant set-point)
  - E.g. $F(S,t) = S_0 + (S - S_0)\big(1 - e^{-t/\tau}\big)$
- Disuse: $\dot{\rho} = -kS_0e^{-t/\tau}$
- Set-point varies from site to site, depending on what cells have accommodated to
- Load-path dependence

The Adaptive Elasticity Theory



Hart et al. 1984



Huiskes et al. 1987

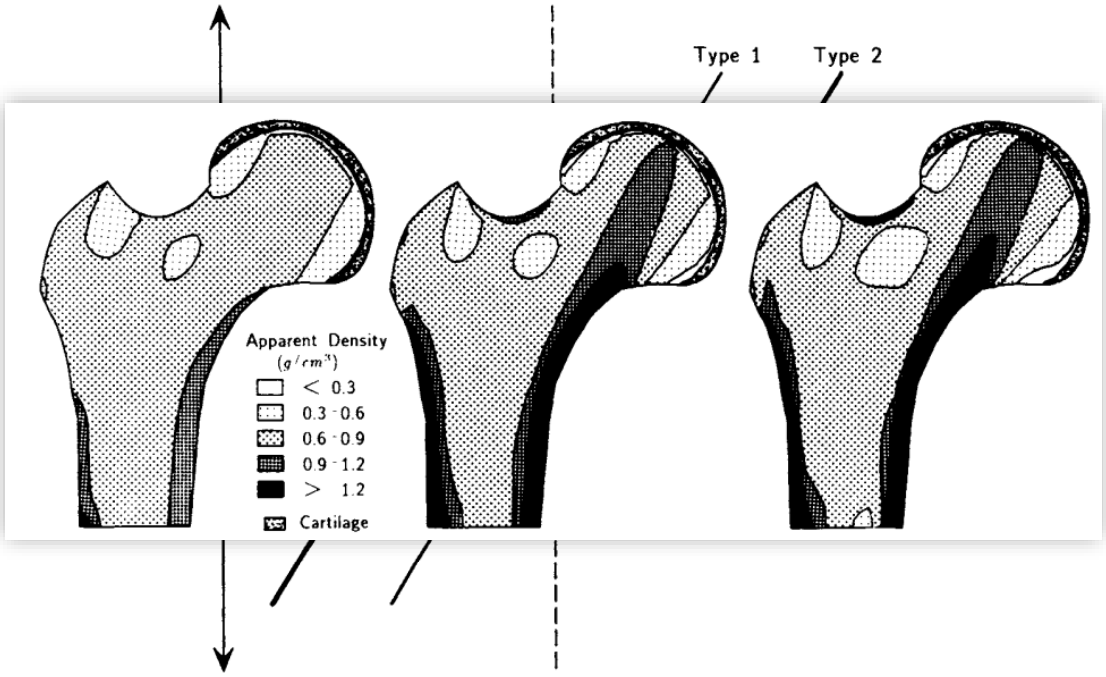
- Bone as porous material, strain as remodeling stimulus (Cowin and Hedegus 1976)
  - Internal remodeling: $\dot{E} = A_{ij}\left(\varepsilon_{ij} - \varepsilon_{ij}^{0}\right)$
  - External remodeling: $\dot{X} = B_{ij}\left(\varepsilon_{ij} - \varepsilon_{ij}^{0}\right)$
  - E: elastic modulus, A, B: matrices of remodeling coefficients, ε: strain tensor, ε^{0}: equilibr. strain
- "Representative" SED amplitude as driver stimulus (Huiskes et al. 1987)
  - Non-linear, "lazy zone"
  - Internal remodeling:

$$U = \frac{1}{2}\sum_{i}\sum_{j}\varepsilon_{ij}\sigma_{ij}$$

$$\dot{E} = \begin{cases} C(U - (1+s)U_n) & \text{if } U > (1+s)U_n \\ C(U - (1-s)U_n) & \text{if } U < (1-s)U_n \\ 0 & \text{otherwise} \end{cases}$$

  - External remodeling: analogous

Bone Maintenance and Self-Optimization



$$\dot{r} = \begin{cases} c \cdot (\psi_b - \psi_{b_{AS}}) + c \cdot w & (\psi_b - \psi_{b_{AS}} < -w) \\ 0 & (-w \leq \psi_b - \psi_{b_{AS}} \geq +w) \\ c \cdot (\psi_b - \psi_{b_{AS}}) - c \cdot w & (\psi_b - \psi_{b_{AS}} > +w) \end{cases}$$

Beaupré and Carter 1990b

- Bone as self-optimizing material ('bone maintenance theory' by Fythrie and Carter 1986)
  - Optimization objective: change e.g. density ρ, such that

$$\left(\frac{\rho_c}{\rho}\right)^2 \left(\psi - \left(\frac{\rho}{\rho_{AS}}\right)^2 \psi_{AS}\right) \rightarrow 0$$

- Time-dependent extension (Beaupré and Carter 1990)
  - Daily continuum-level stimulus

$$\psi = \sqrt[m]{\sum_i n_i \bar{\sigma}_i{}^m}$$

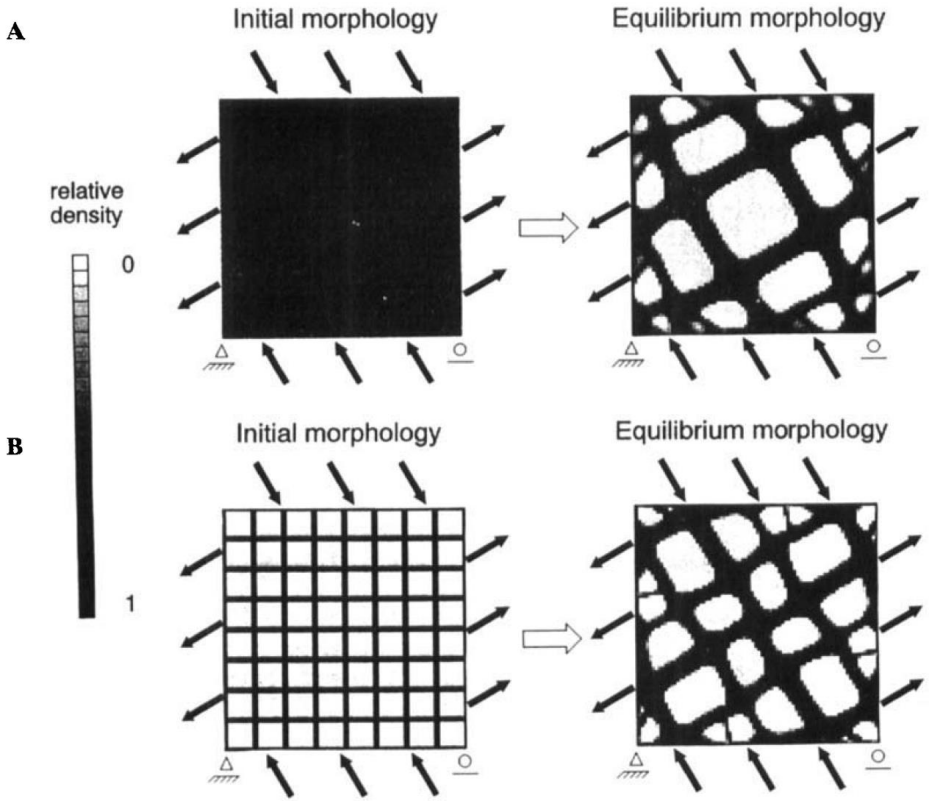
    - $\bar{\sigma}_i = \sqrt{2EU}$ continuum-level effective stress; E: apparent elastic modulus; U: apparent SED; i: load case; n_i: number of load cycles; m: constant
  - Surface density-dependent remodeling rate

$$\dot{\rho} = \dot{r} S_v(\rho) \rho_c$$

S_v: apparent density → surface area density

Weinans et al. 1992, Mullender et al. 1994, Mullender & Huiskes 1995, Mullender & Huiskes 1997

Semi-Mechanistic Tissue-Level Remodeling 1/2

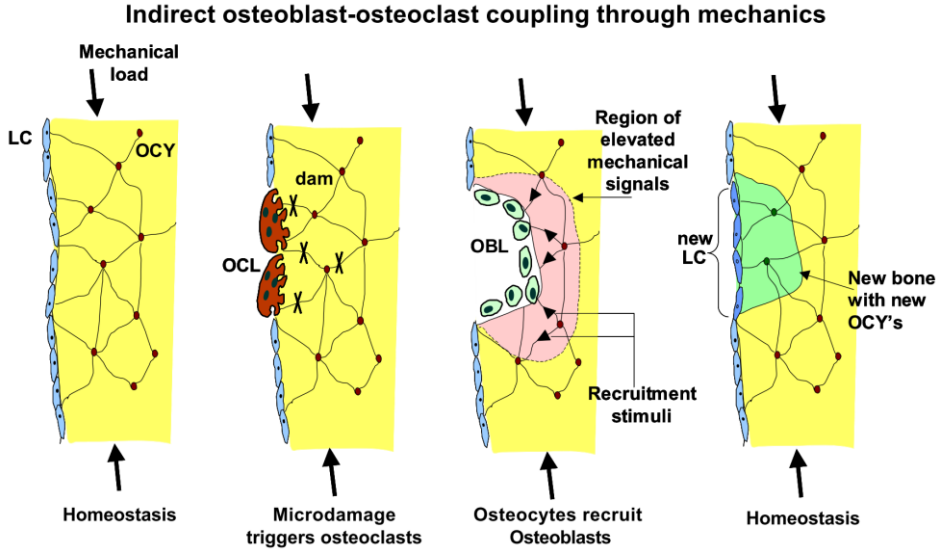


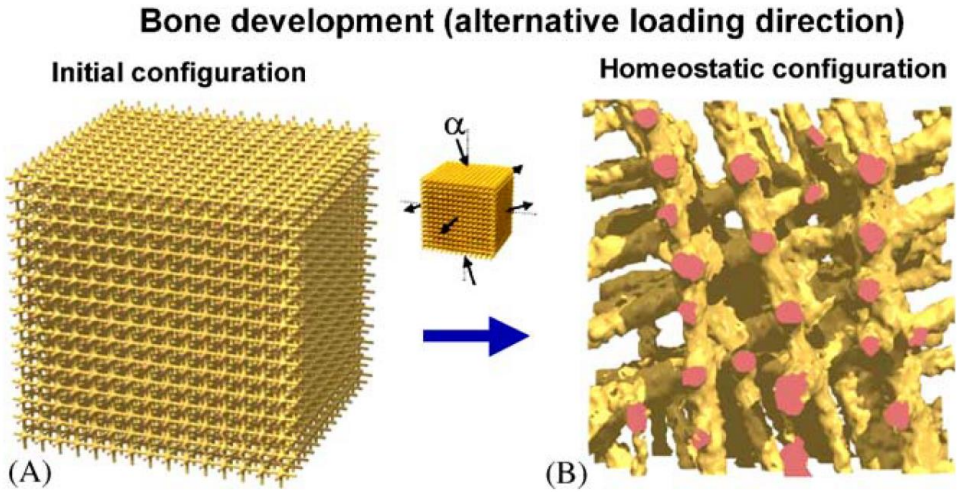
Mullender & Huiskes 1995

- Basic idea: Modify relative density $m \in [0.01, 1]$ depending on accumulated, distance-weighted stimulus Φ measured by uniformly distributed sensor cells → $\dot{m} = \tau\Phi$
- Stimulus $\Phi(\boldsymbol{x}, t) = \sum_i w_i(\boldsymbol{x})(S_i(t) - k)$ where
  - Weight for sensor i: $w_i(\boldsymbol{x}) = \exp(-\|\boldsymbol{x} - \boldsymbol{x}_i\|/D)$
  - Sensor i @ $\boldsymbol{x}_i$ measures SED $S_i(t) = \frac{1}{2}\boldsymbol{\sigma}(\boldsymbol{x}_i, t) : \boldsymbol{\varepsilon}(\boldsymbol{x}_i, t)$
  - Young's modulus $E = E_{\text{bone}} m^3$ (isotropic, linear elastic)
- Simulations
  - Osteocyte density 1600/mm², range D = 0.025 mm, 2×2 mm² × 20 µm square domain
  - Φ evaluated per element centroid
  - MOL (FEM + explicit Euler integration)
- Observations, issues
  - Trabeculae appear from uniform initial state → self-organization
  - No influence of strain rate, frequency, etc. …
  - Osteocyte density independent of m
  - Remodeling can happen *anywhere* (not only on surfaces)

Huiskes et al. 2000, Ruimerman et al. 2001, Ruimerman et al. 2005

Semi-Mechanistic Tissue-Level Remodeling 2/2





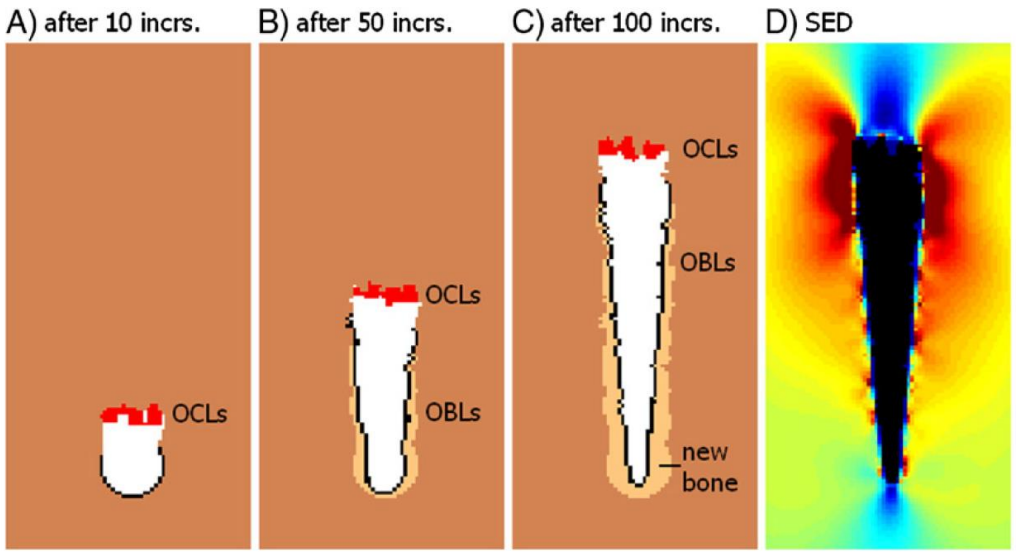
Ruimerman et al. 2005

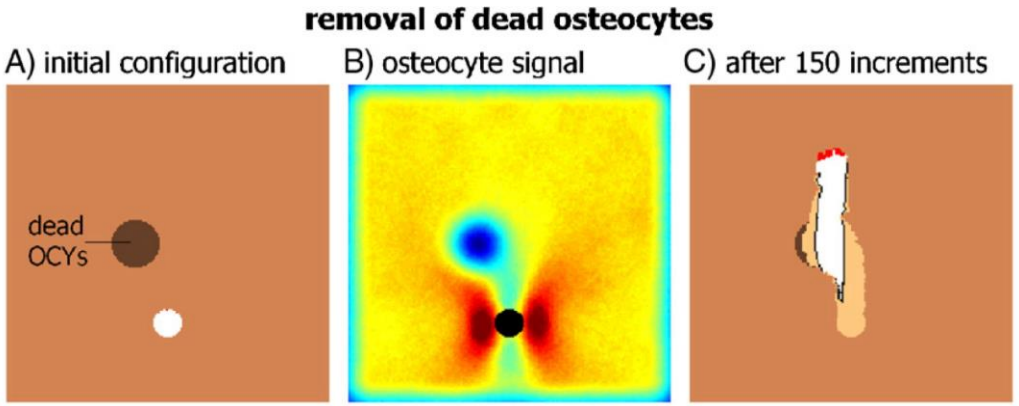
- Instead of net change: distinguish resorption and formation
  - Osteoclasts recruited by osteocyte apoptosis or disuse
  - Osteoclast activity causes strain perturbations
  - Osteoblasts stimulated by osteocytes (SED)
- Density rate of change

$$\dot{m} = \begin{cases} \tau \cdot (\Phi(x,t) - k_{\mathrm{tr}}) - r_{\mathrm{oc}} & \text{if } \Phi > k_{tr} \\ -r_{\mathrm{oc}} & \text{otherwise} \end{cases}$$

  - where $\Phi(x,t) = \sum_i w_i(x)\mu_i S_i(t)$
  - k_{tr}: bone formation threshold; r_{oc}: resorption rate; μ_i: mechanosensitivity of osteocyte i
- Probability of resorption
  - Micro-cracks → osteocyte apoptosis → random
  - Disuse: proportional to mechanical stimulus

Agent-Based (CPM) Osteoclast Simulation 1/2

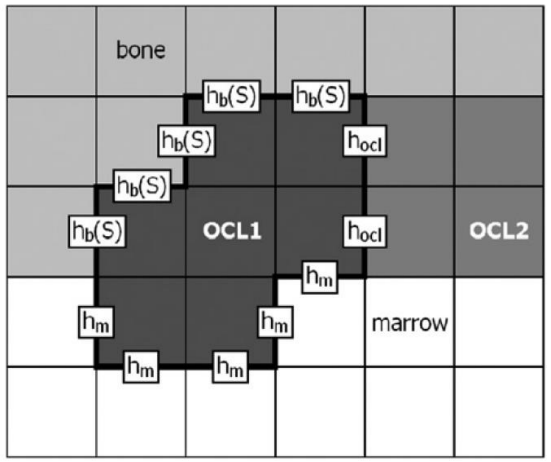


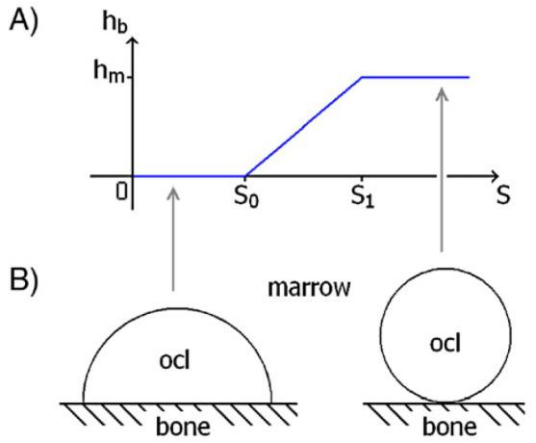


van Oers et al. 2008

- Builds on previous models; changes:
- Explicit osteoclast simulation via CPM
  - Osteoclasts attach to surfaces where osteocyte signal is weak
  - resorb until strong signal causes detachment
  - removed if detached for a certain amount of time
- Osteoblasts recruited to surfaces, where signal > $S_{\mathbf{obl}}$ for a period of time
- Osteoclasts are either placed …
  - Manually (osteonal remodeling)
  - Randomly: appear with certain probability on exposed surfaces with weak signal

Agent-Based (CPM) Osteoclast Simulation 2/2





van Oers et al. 2008

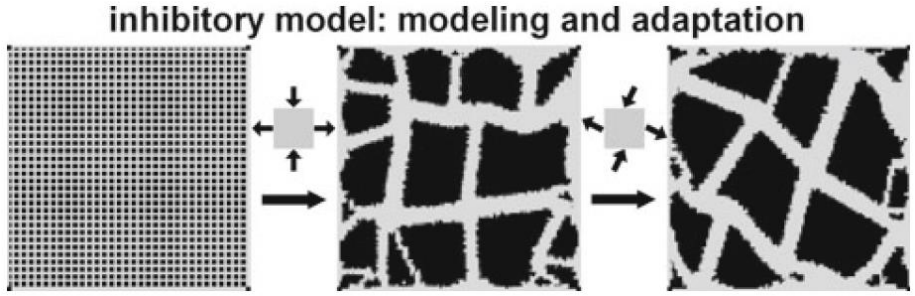
- Cellular Potts model (CPM): cellular automaton approach to simulate cell motion
  - "Agents" (cells) occupy multiple lattice sites
  - Cells have internal state, move according to rules, depending on internal state and neighbors
- Cells as 'fluid droplets' of ~ constant volume, adhesion as surface tension → minimize Hamiltonian, in this case

$$H = \lambda(V - V_0)^2 + \int_{\text{surf}} h(A)\, dA$$

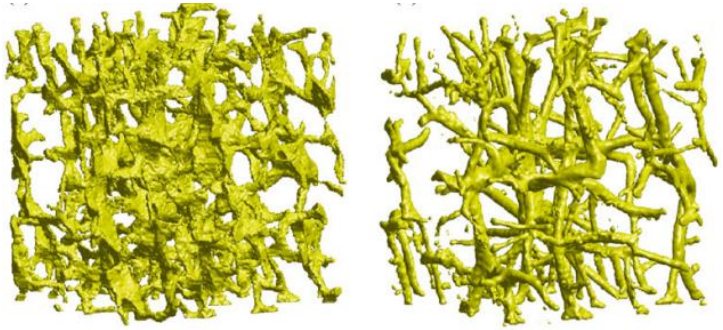
- Contact energies differ for different substrates; here: depends on osteocyte signal:
  - Below S_0: low contact energy → strong adhesion
  - Above S_1: high contact energy → no adhesion

Further Approaches

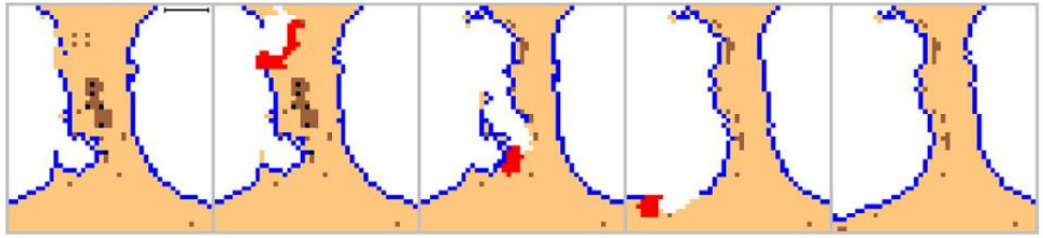
- Sclerostin-based “inhibition inhibition” [sic]
- Osteocyte-viability-based remodeling
- Fluid-shear stress regulated remodeling
- Microdamage-targeted resorption



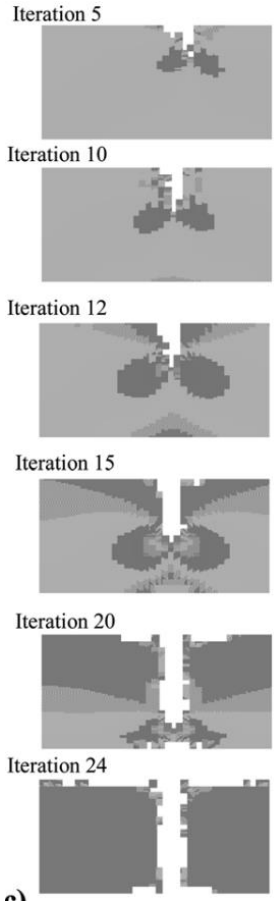
van Oers et al. 2010



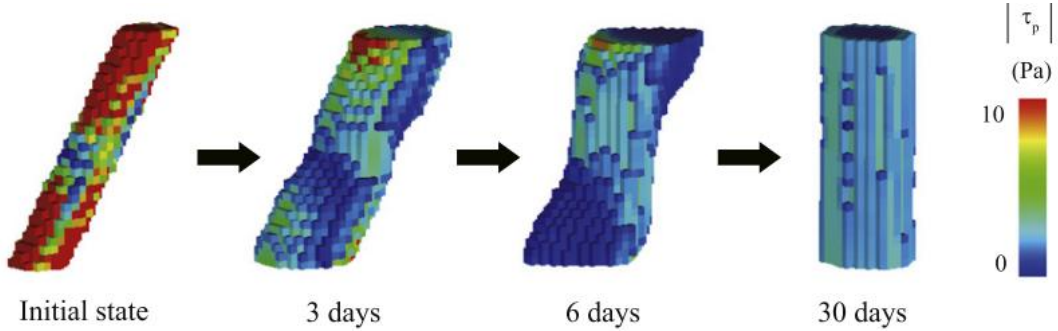
Wang et al. 2013



van Oers et al. 2011

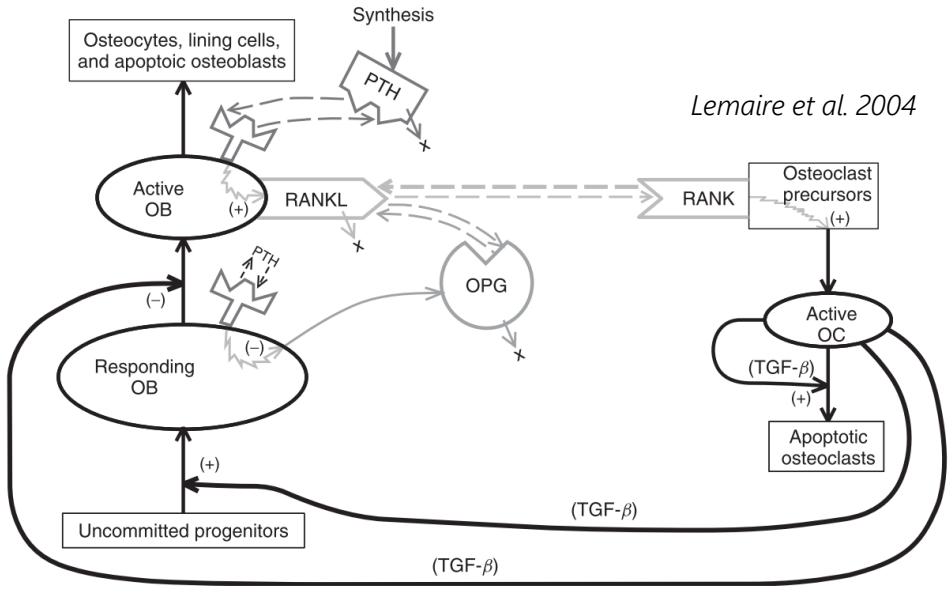


McNamara & Prendergast 2005



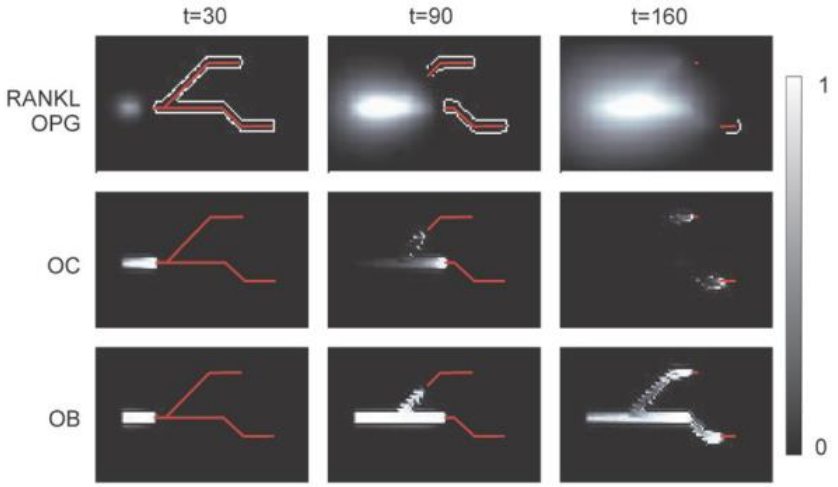
Kameo et al. 2011

Cell- and Sub-Cell-Level Models



Lemaire et al. 2004

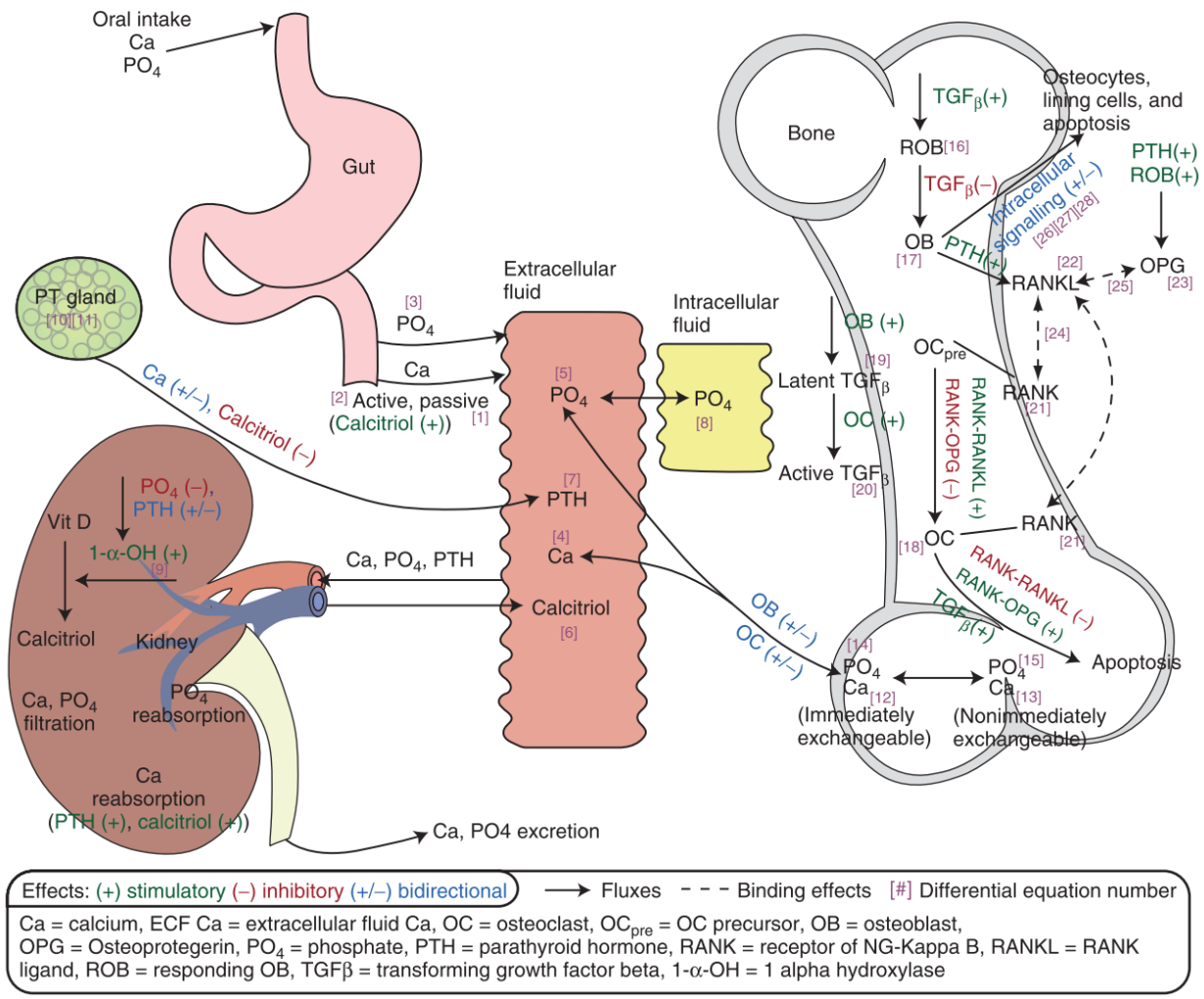
- Lemaire et al. 2004:
  - Model of molecular interactions (kinetics) between osteoblasts and osteoclasts
  - Space-less
  - No solid or fluid mechanics



Ryser et al. 2009

- Ryser et al. 2009:
  - Model of a single BMU and how it reacts to signaling molecules
  - No solid or fluid mechanics
  - *Many* parameters …

Systems-Level Models



Peterson & Riggs 2010

- Peterson & Riggs 2010:
  - Simulate interactions calcium homeostasis ↔ bone metabolism
  - Space-less
  - No solid or fluid mechanics
  - *Even more* parameters …