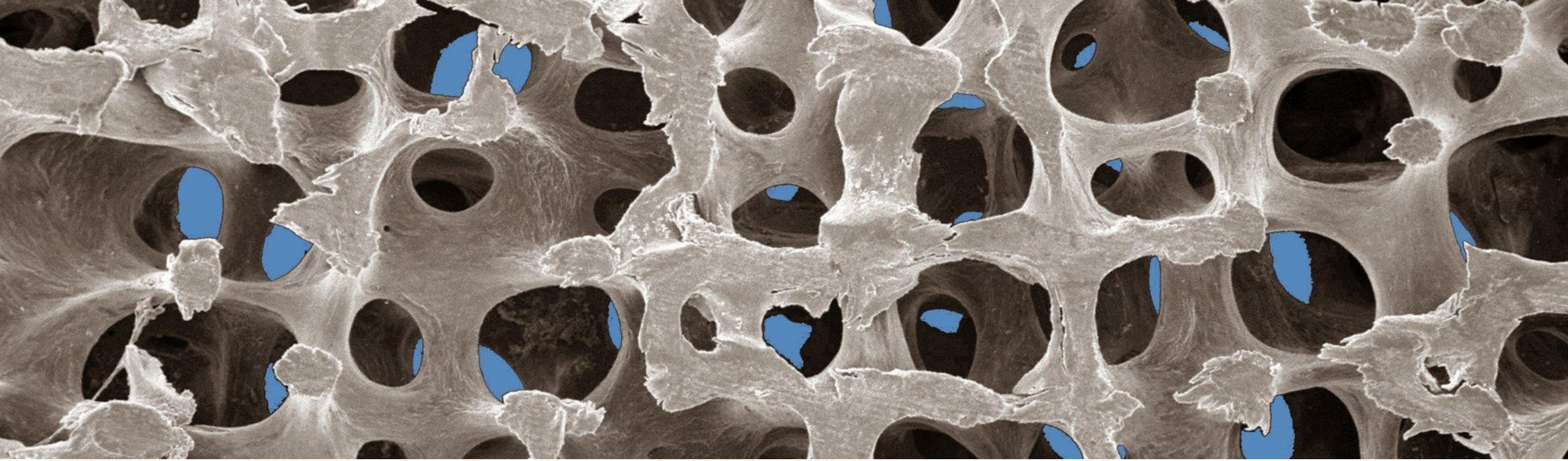

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Bone Remodeling & Fracture Healing

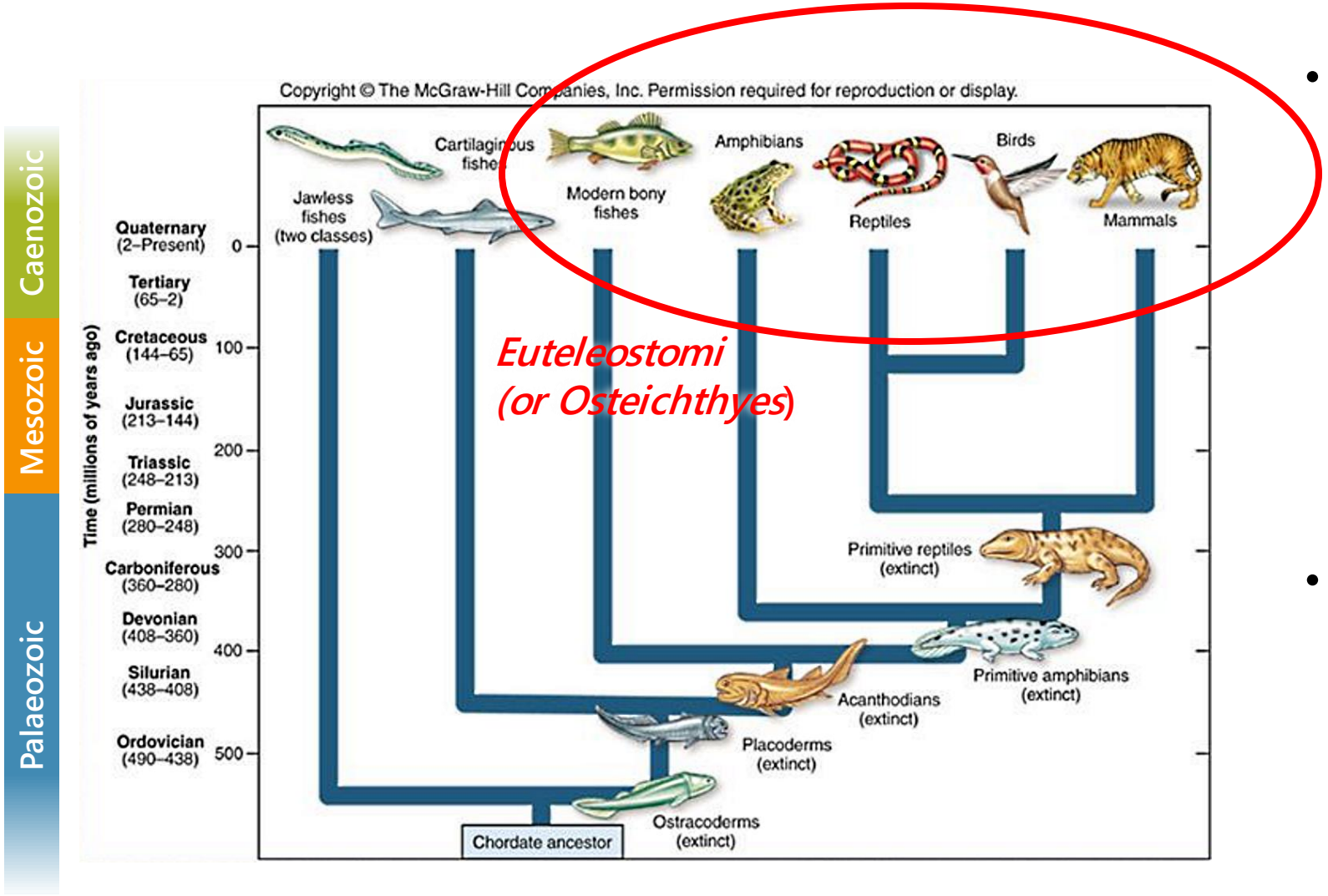
Biology – Theories – Models

Computational Biomechanics

Summer Term 2017

Martin Pietsch

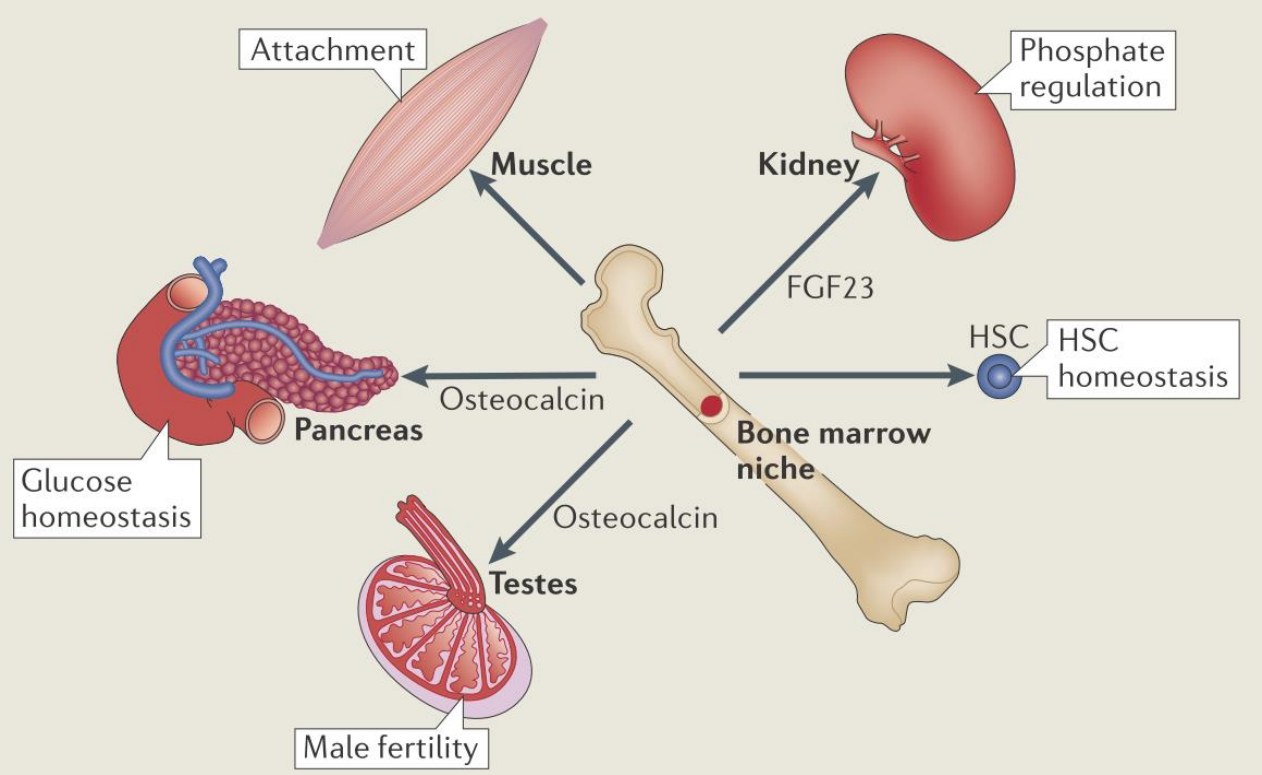
Skeletal Structures in Animals



Vertebrate family tree

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

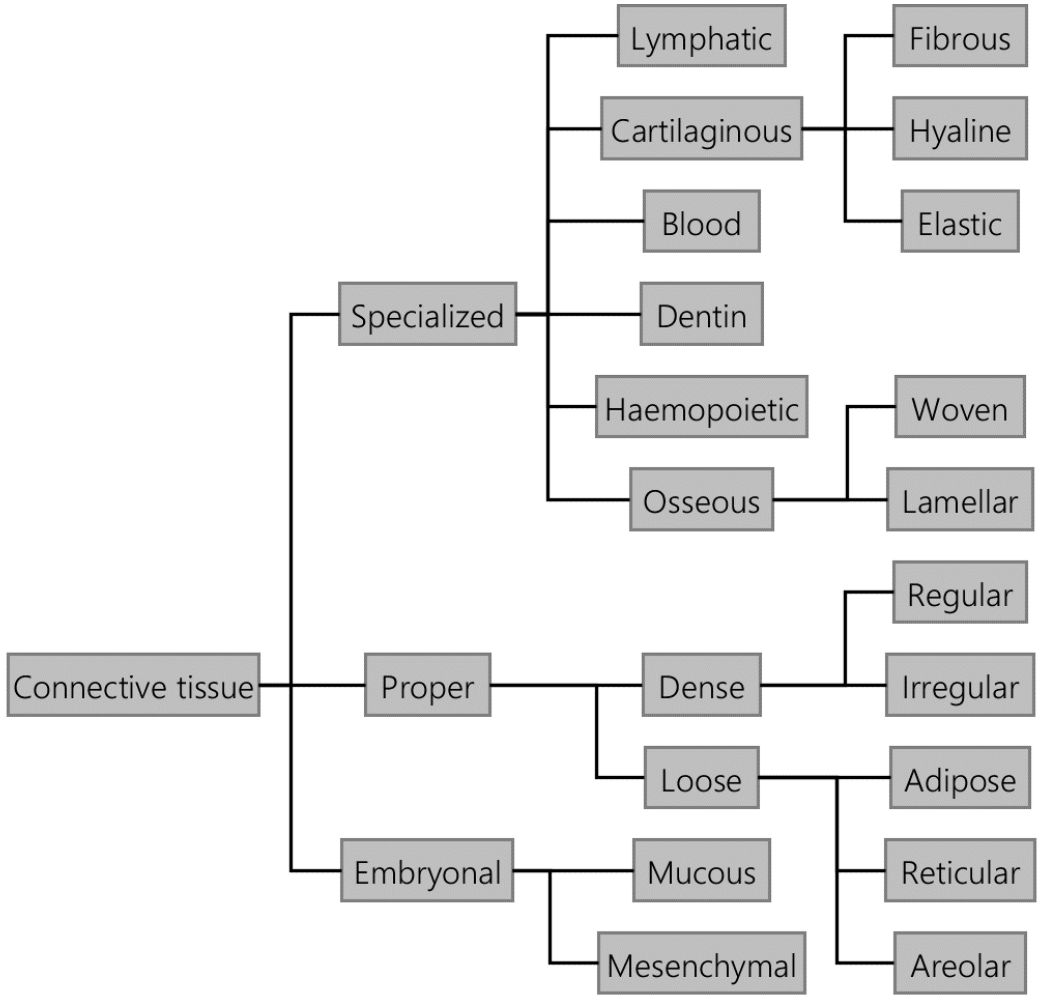
Bone in the Human Body



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

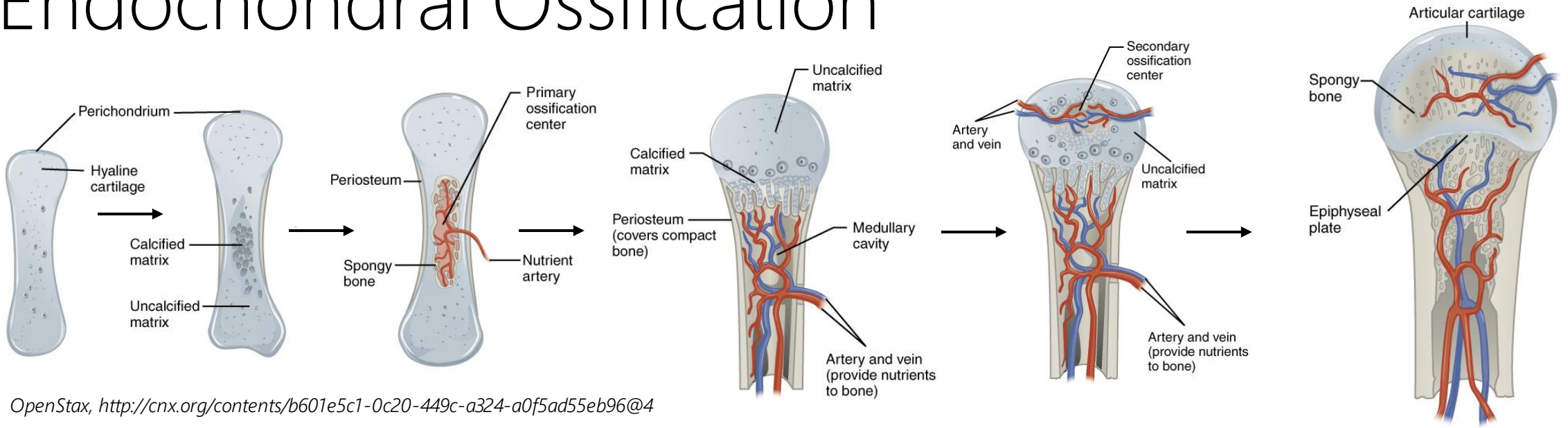
- Develops form mesoderm
- Mostly ECM
- Components
  - Fibers (collagen, elastin, fibrillin, fibrinogen)
  - Ground substance with GAGs, proteoglycans
  - Relatively sparsely populated with cells
- Tensile and compressive strength
- Bone tissue is one kind of *specialized connective tissue*

Intramembranous Ossification

- 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 …)

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

Endochondral Ossification

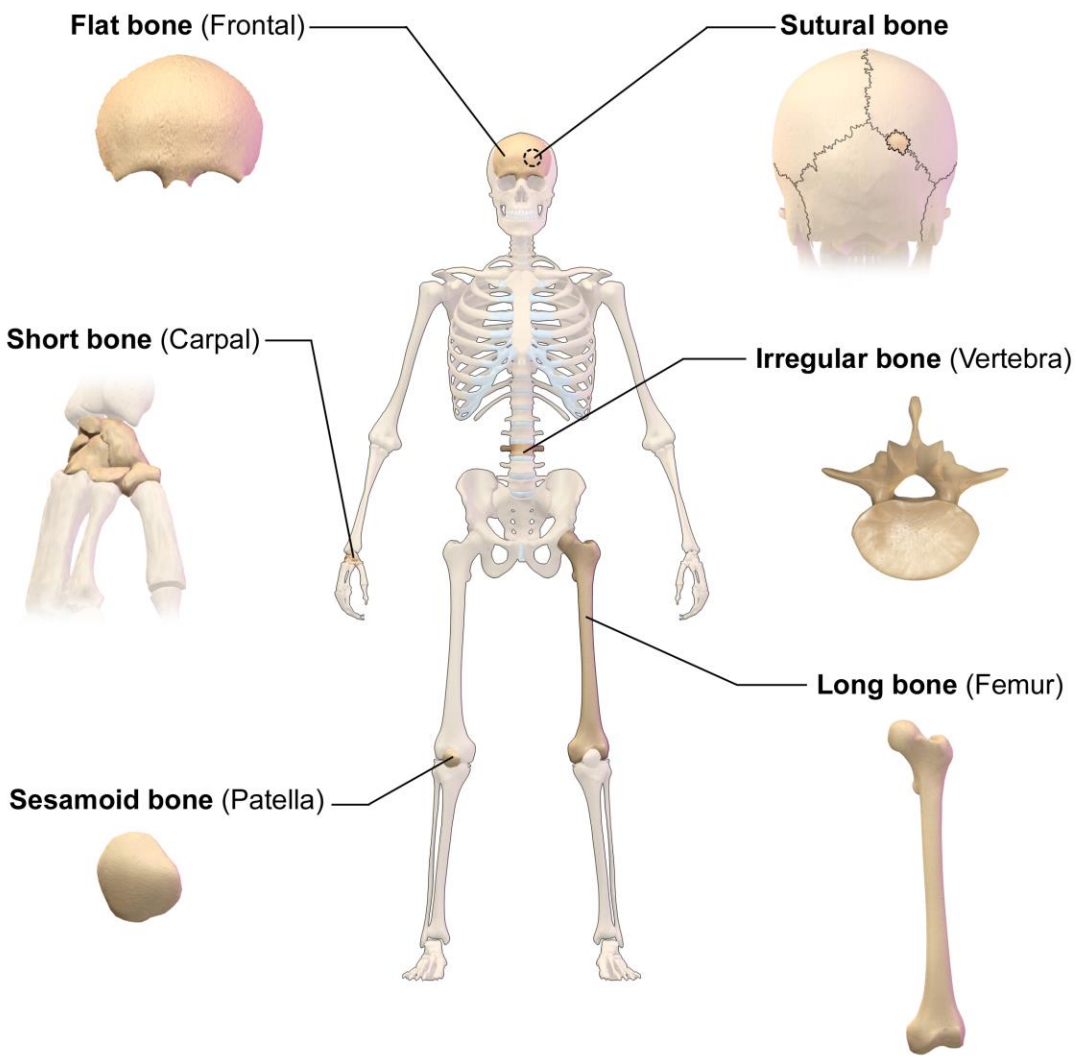


OpenStax, http://cnx.org/contents/b601e5c1-0c20-449c-a324-a0f5ad55eb96@4

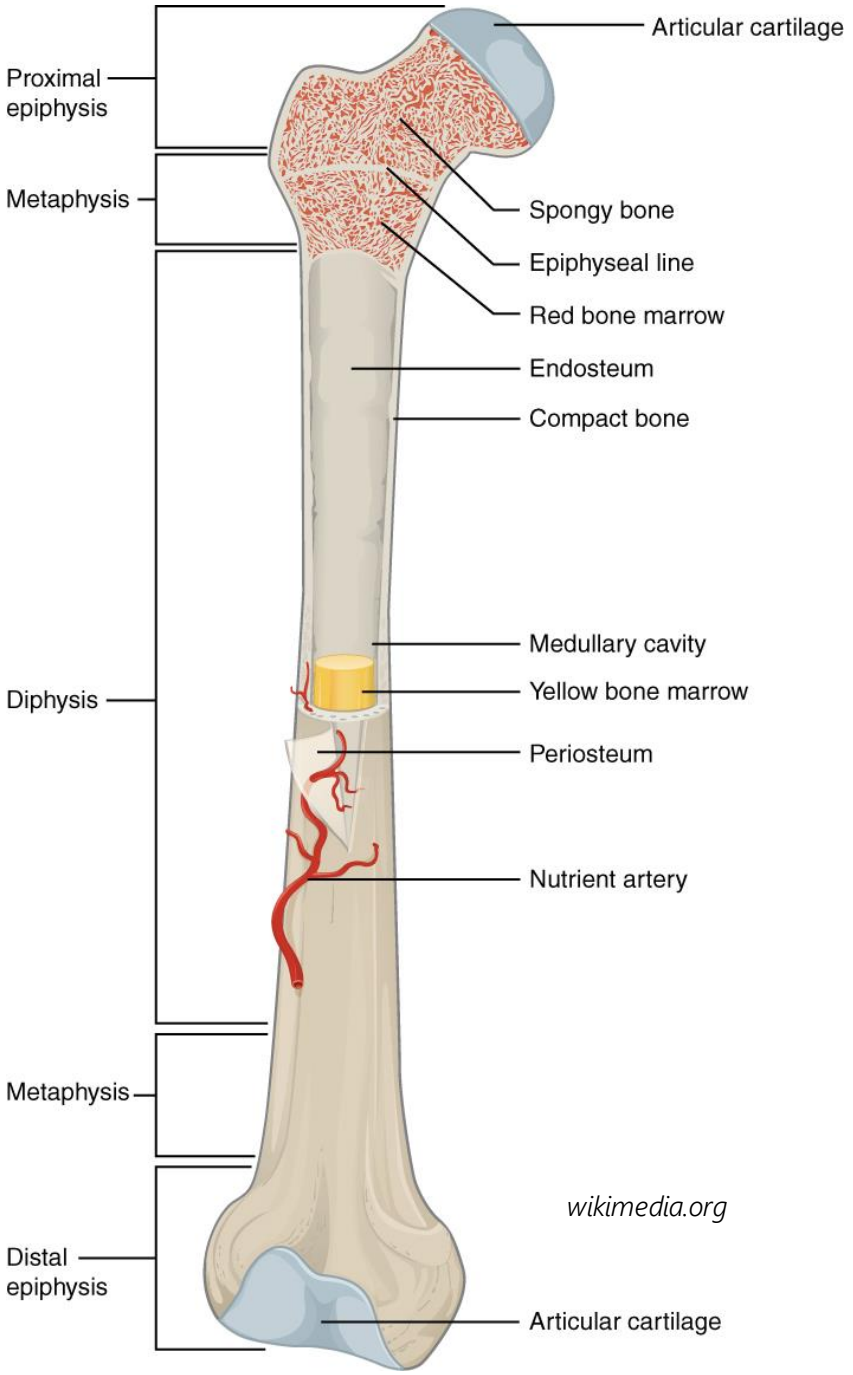
- Mesenchym transformed into cartilage model
- Hypertrophic chondrocytes secrete alkaline phosphatase
- Cartilage calcifies
- Formation of periosteum and invasion of blood vessels
- At "primary centers of ossification": osteoblasts deposit collagenous matrix
- Hyaline cartilage remains on epiphyseal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

Types of Bone

Anatomic: Long Bones et al.



wikimedia.org



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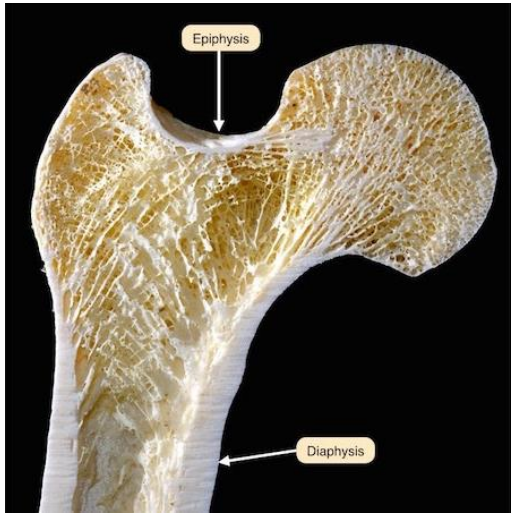
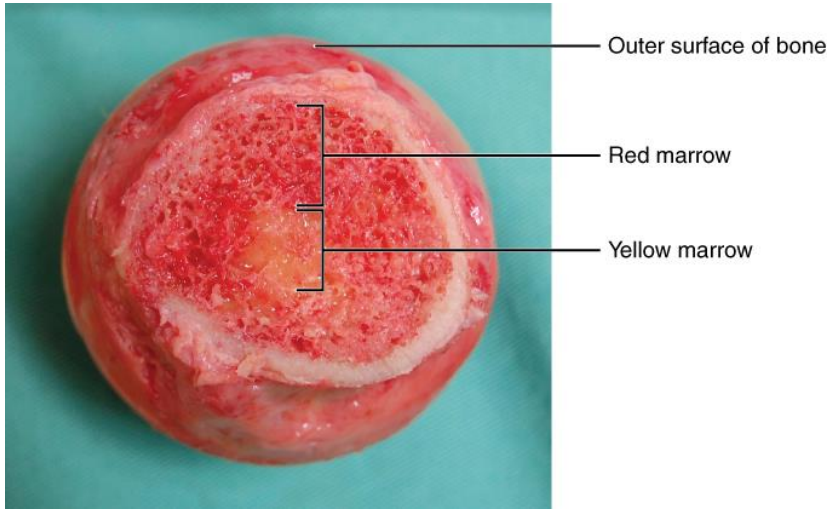
Macroscopic: Cortical vs. Trabecular Bone



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



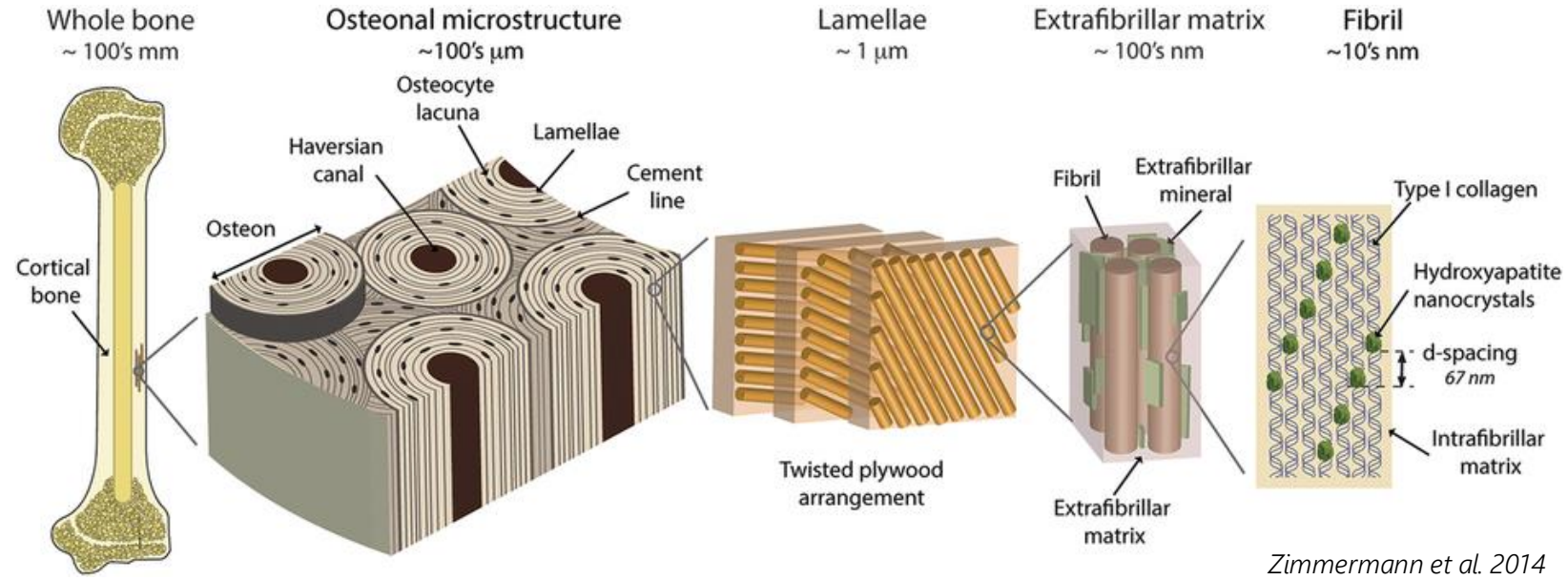
wikimedia.org

- 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)
  - $\rho \approx 0.5\ \mathrm{g/cm^3}$, $E \approx 500\ \mathrm{MPa}$, $\Phi = 50 - 90\ \%$

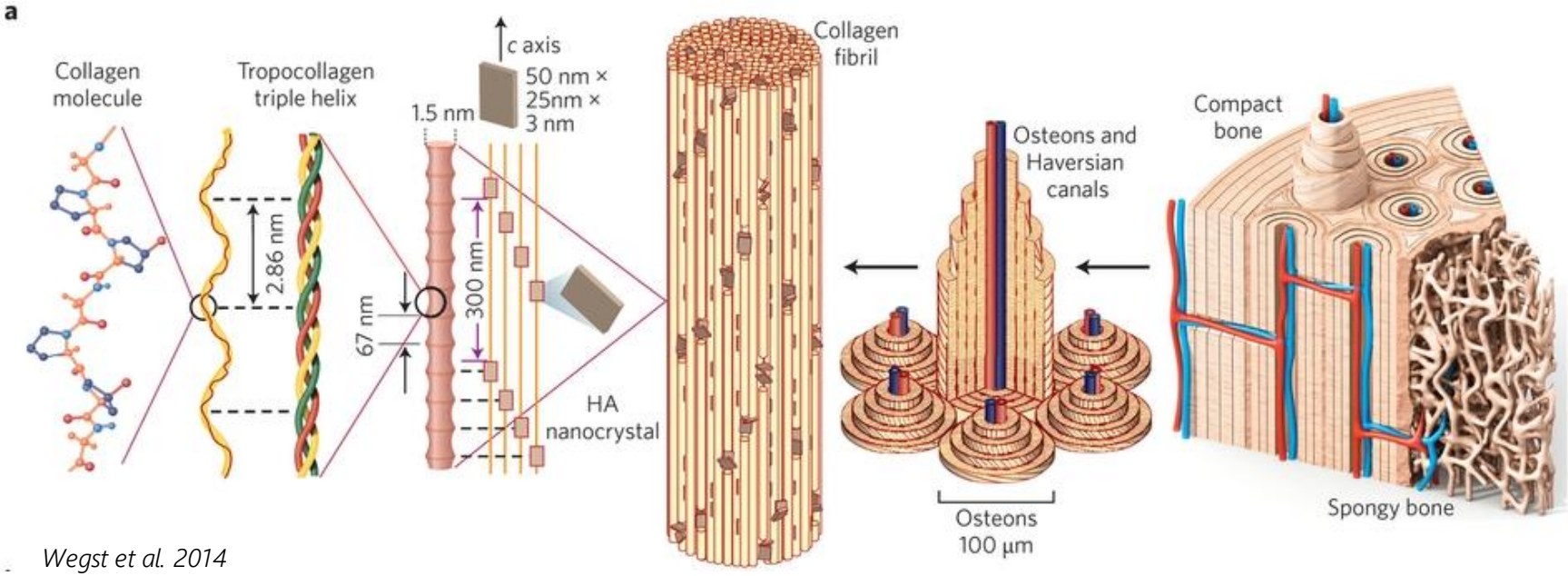
Microscopic: Woven vs. Lamellar Bone

- 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

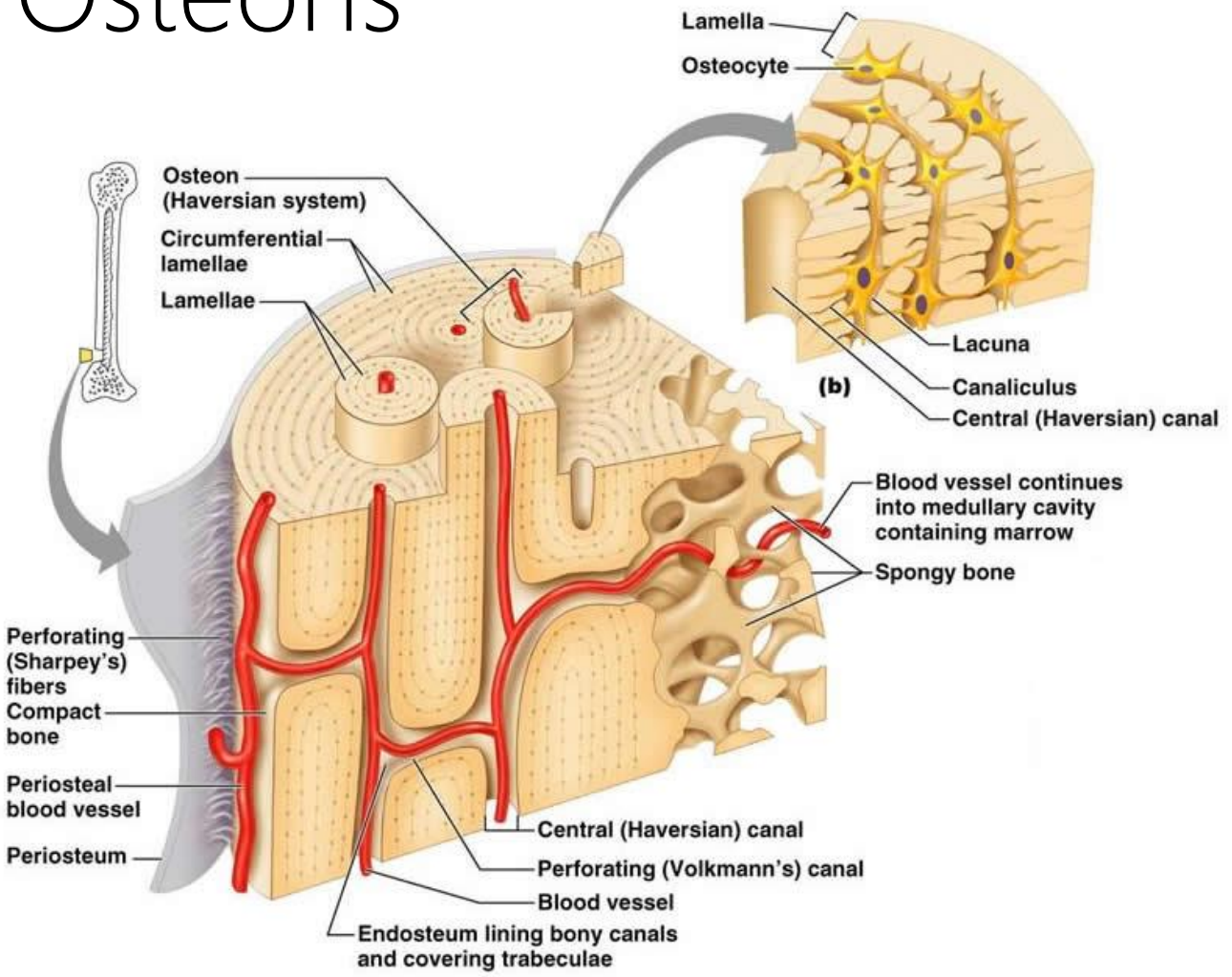


Zimmermann et al. 2014

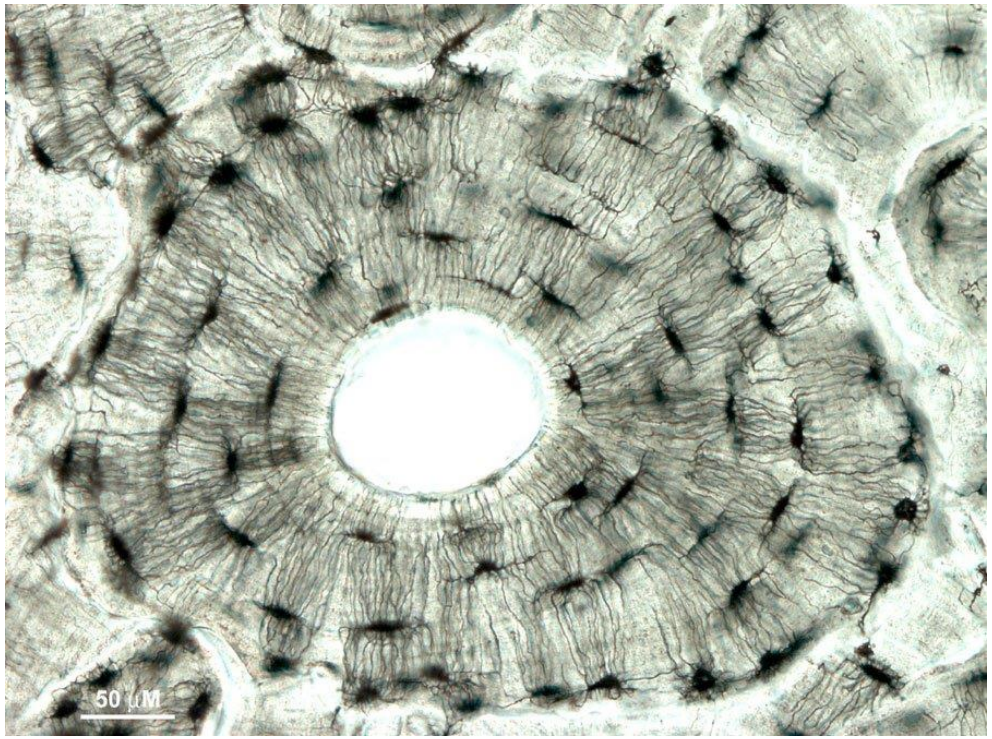


Wegst et al. 2014

Osteons



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



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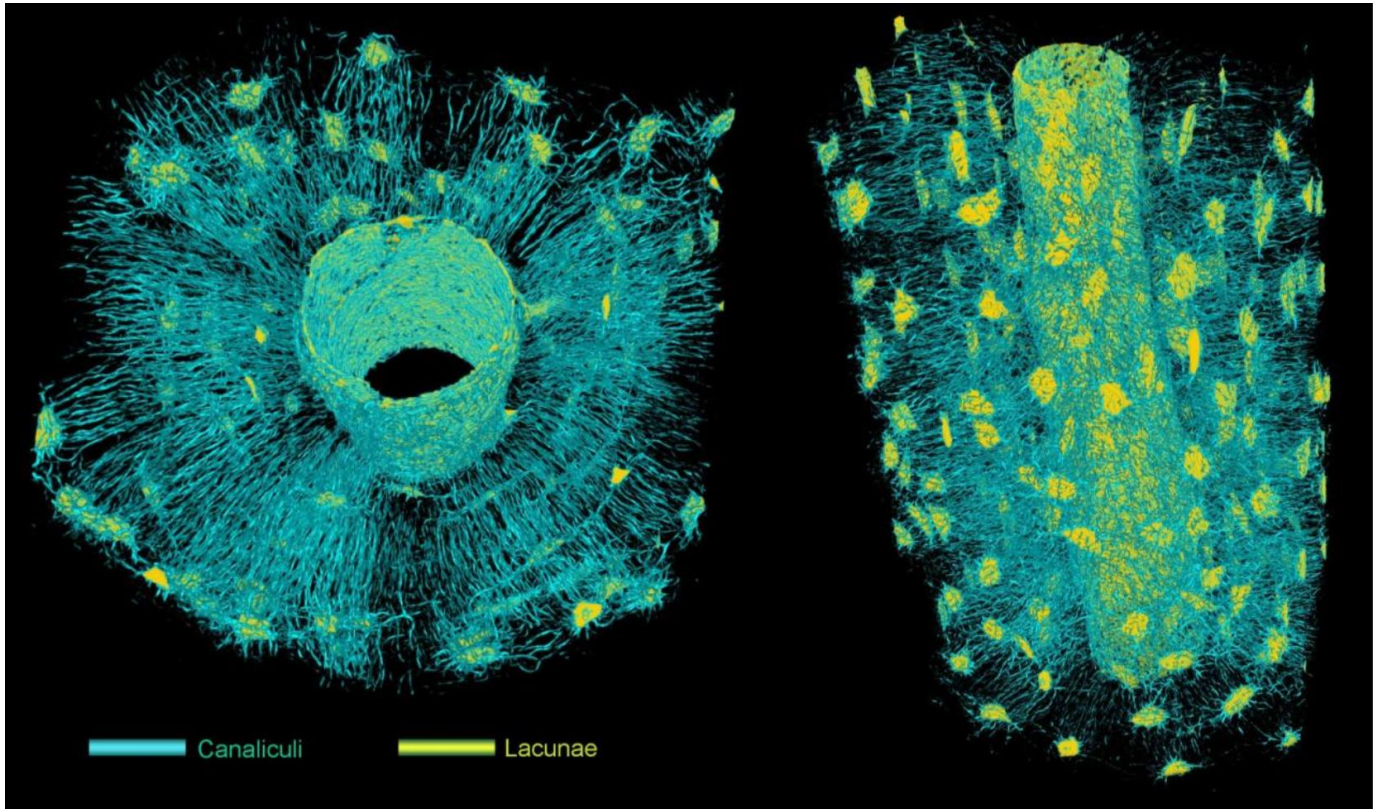
Canaliculi and the Osteocyte Network

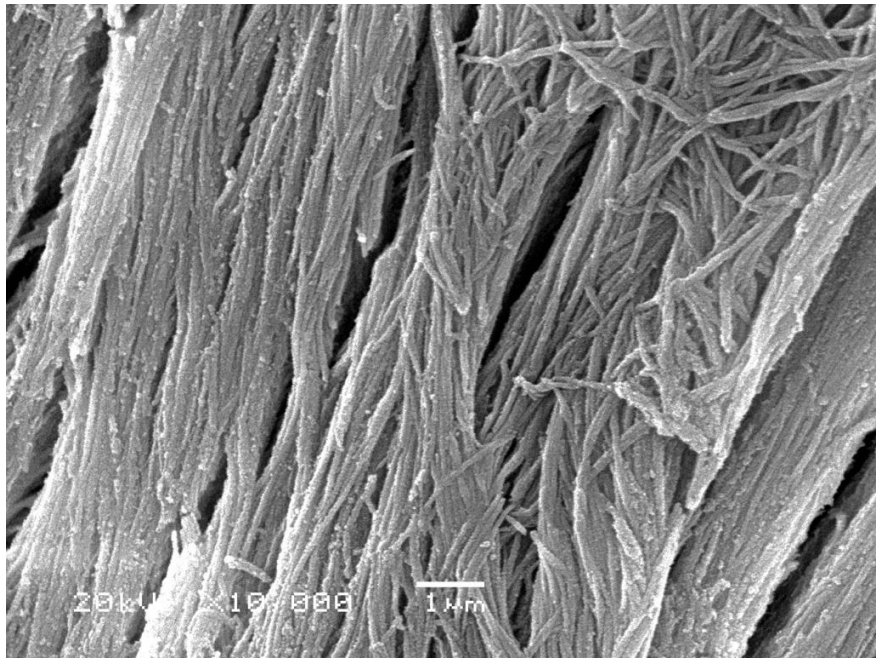
- Network of osteocytes, connected via cytoplasmatic extensions
- $\approx 42 \cdot 10^9$ osteocytes (brain: $\approx 86 \cdot 10^9$ neurons)
- $\approx 23.4 \cdot 10^{12}$ connections (brain: $\approx 150 \cdot 10^{12}$ connections)
- $\approx 175{,}000$ km of dendritic processes (brain: $\approx 165{,}000$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



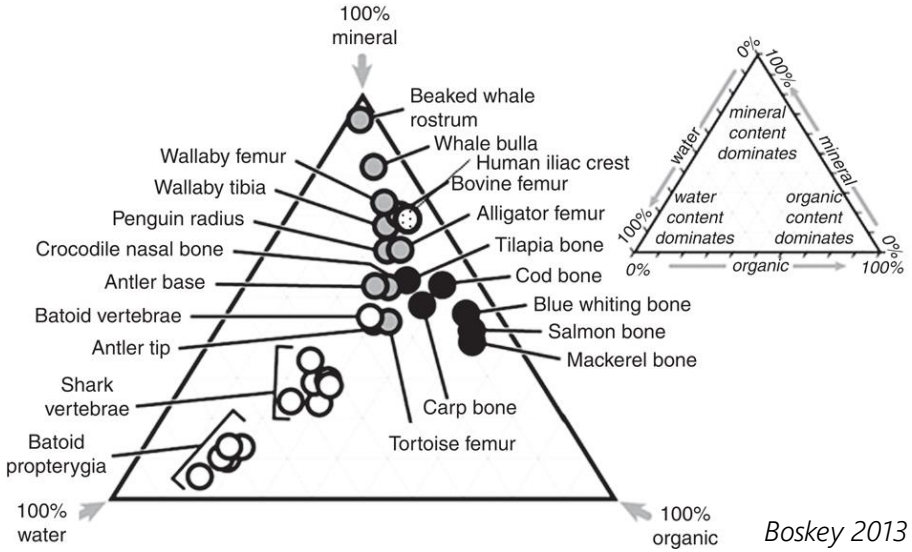
3D rendering of osteocyte network around haversian canal (Pacureanu et al. 2012)

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

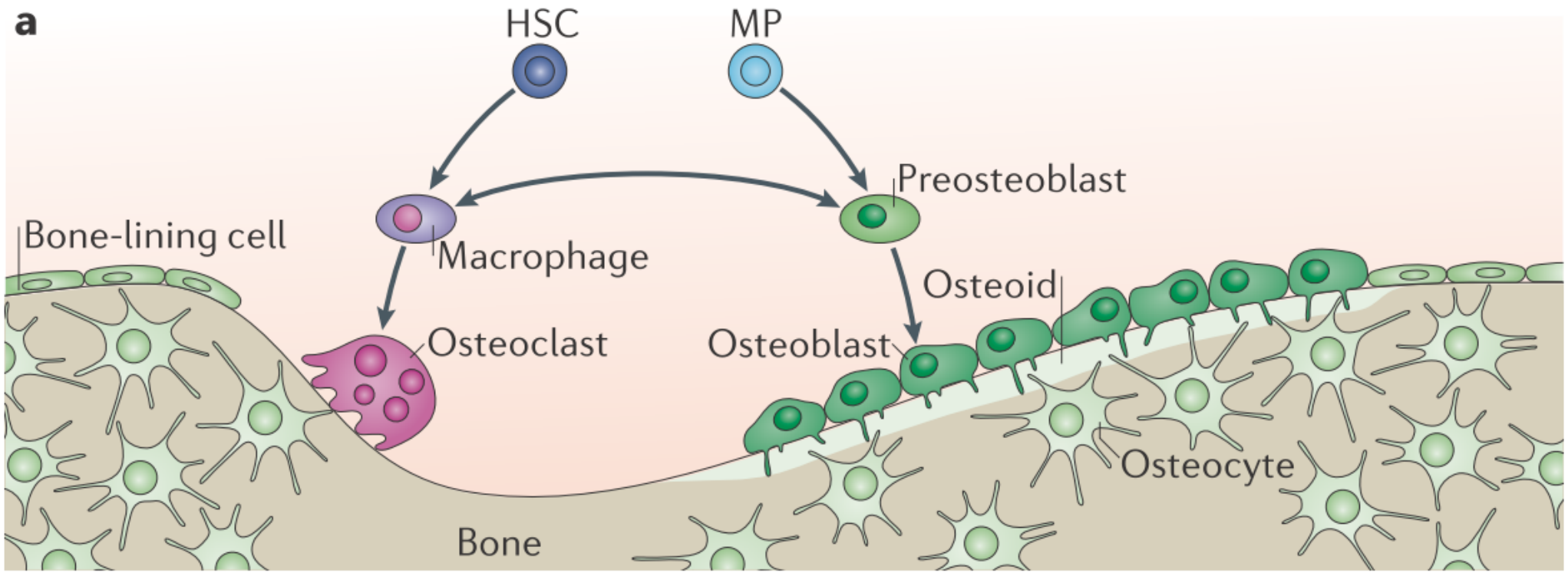


SEM of bone mineral (mineralized collagen fibers) 10000x magnification (wikimedia.org)



Boskey 2013

Bone Cells



Long 2012

Cell Lineages

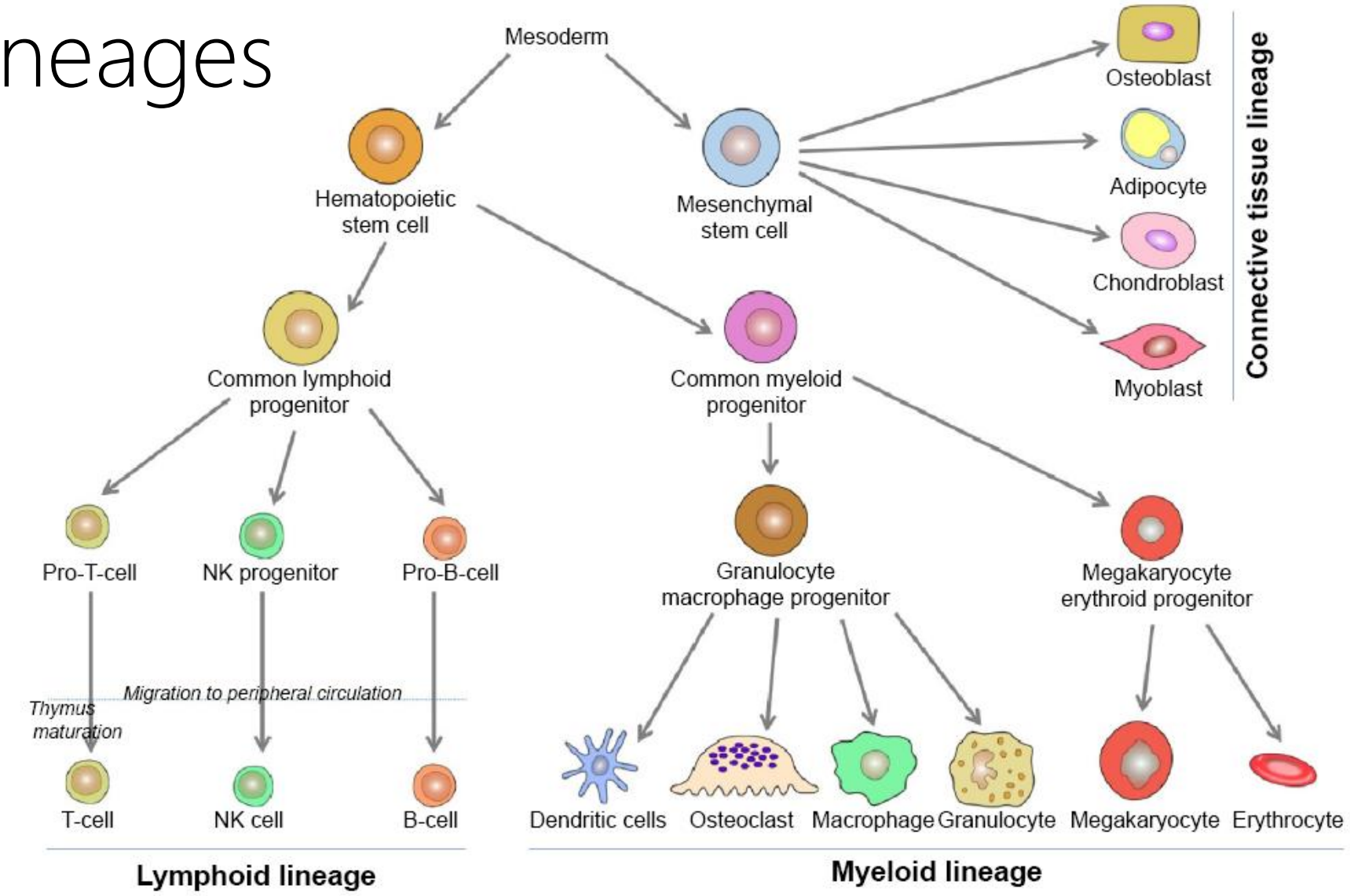
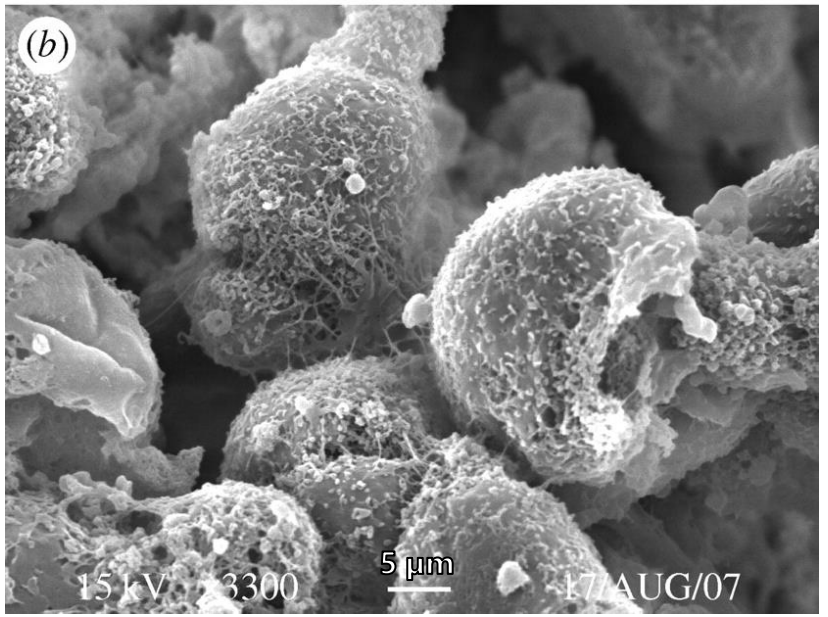


Figure 1 Main osteoimmunological cell differentiations and cell lineages.
Abbreviation: NK cell, natural killer cell.

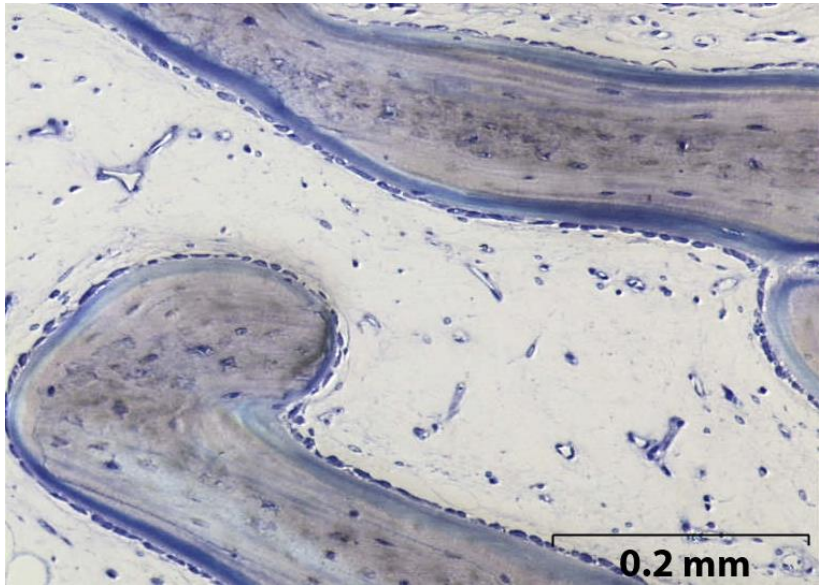
Numan et al. 2015

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
- 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 %)



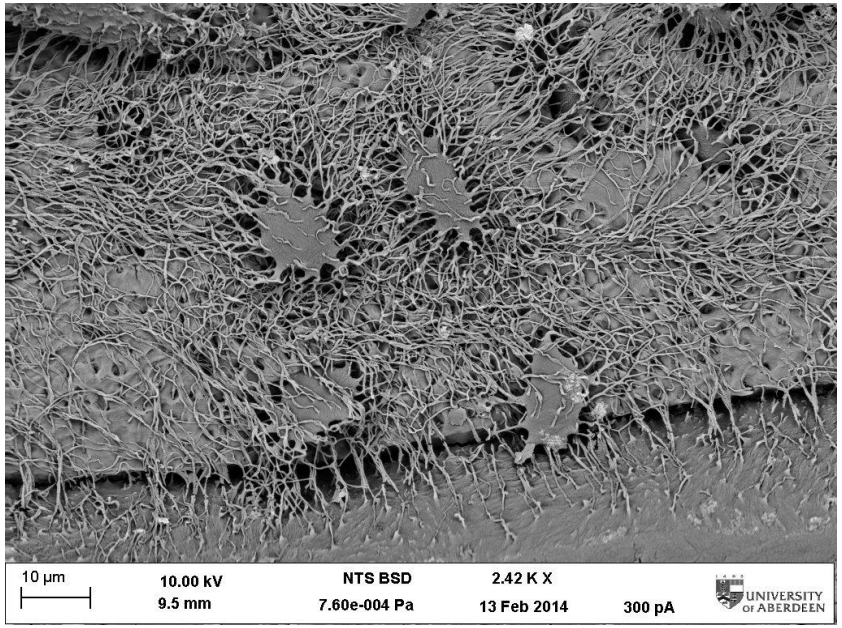
Osteoblasts grown on ChiPgAHAP20 scaffold (Verma et al. 2010)



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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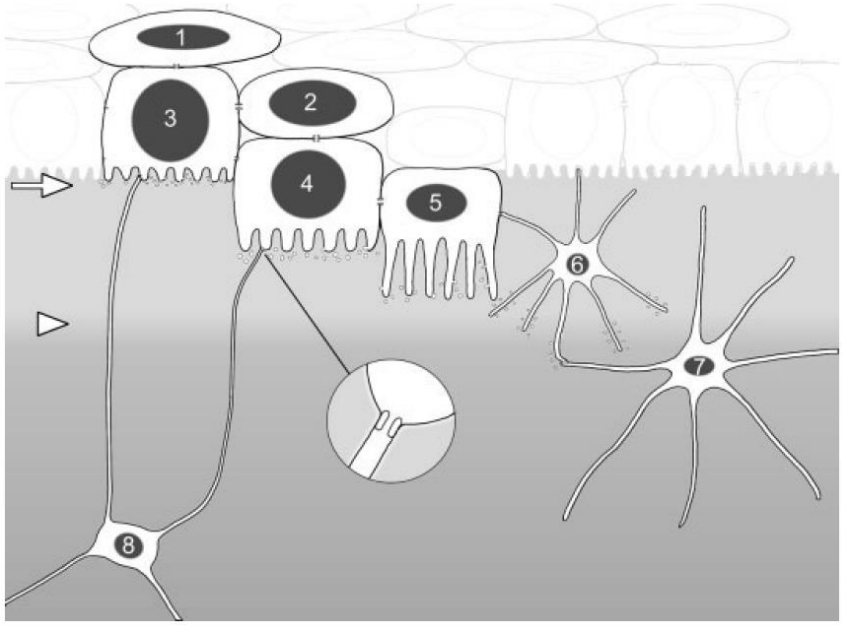
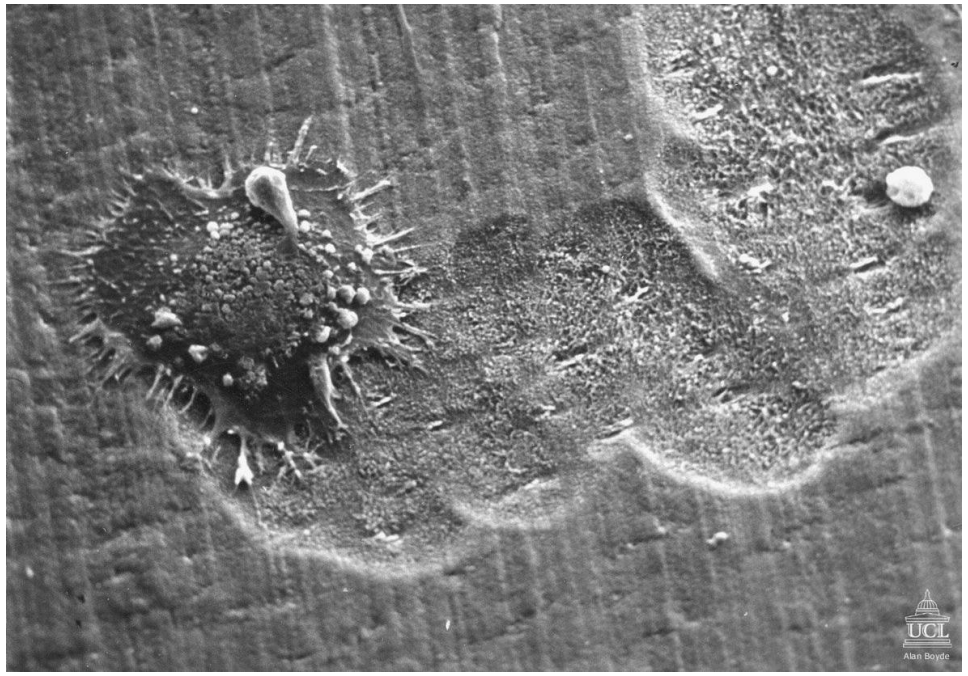


Franz-Odendaal et al. 2006

- 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



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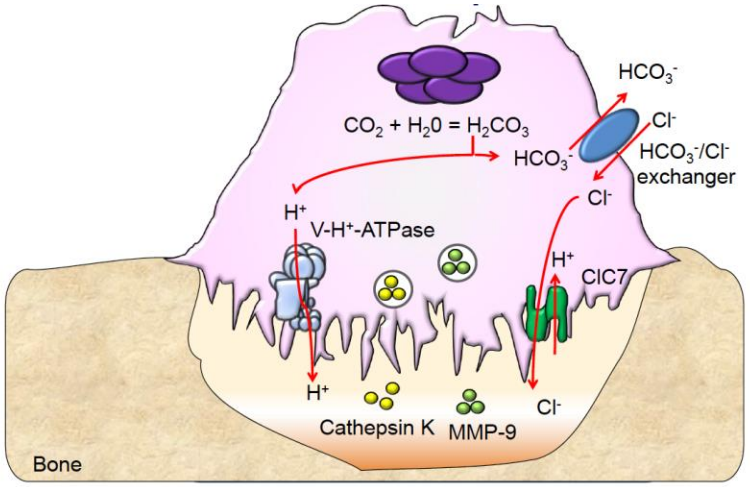


Fattore et al. 2012

Remodeling

- Observation: bone
  loading ("Wolff's la
- Roux: remodeling a
  regulated by cells i
- Bone is constantly
  turnover) in respons
  - 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.

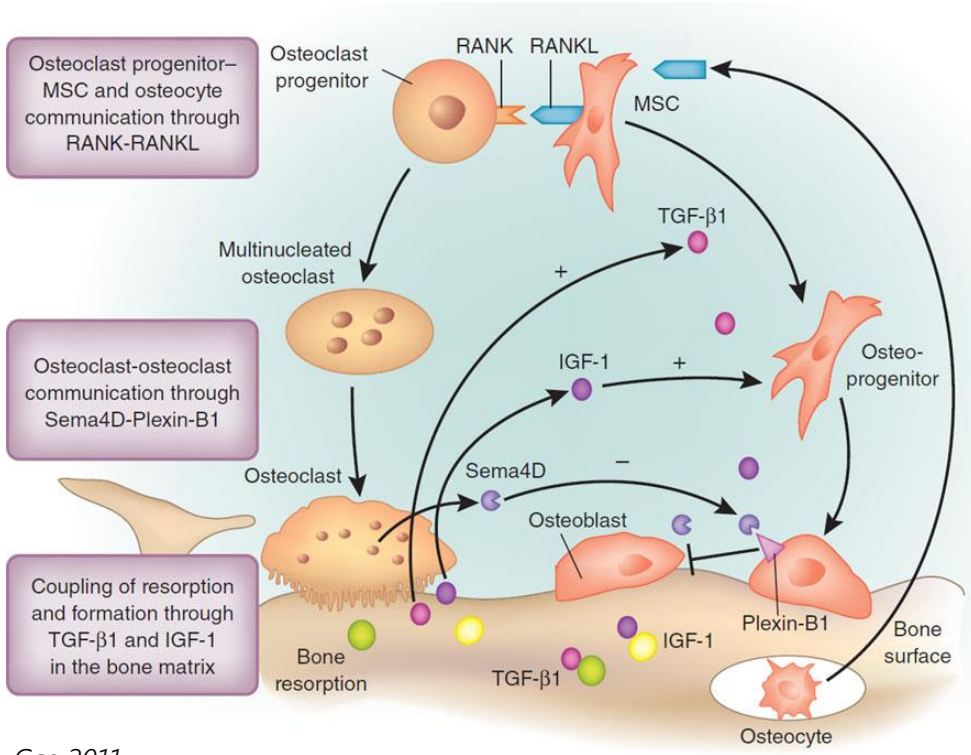
Wilhelm Roux, 1881

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
- 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

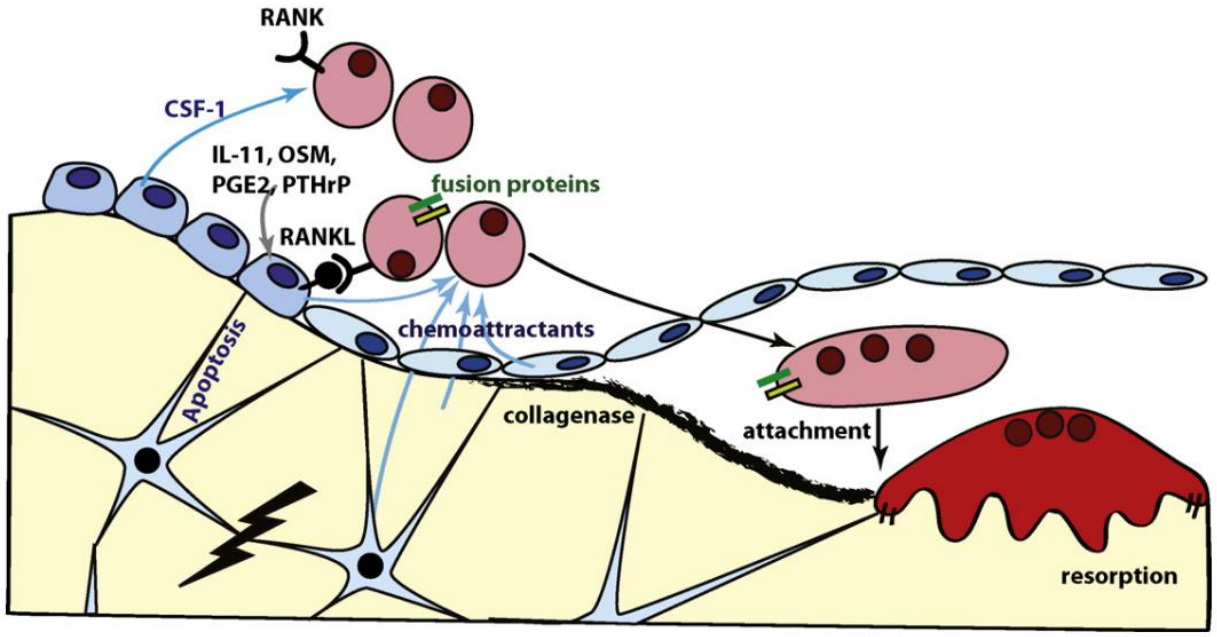


Cao 2011

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
- …

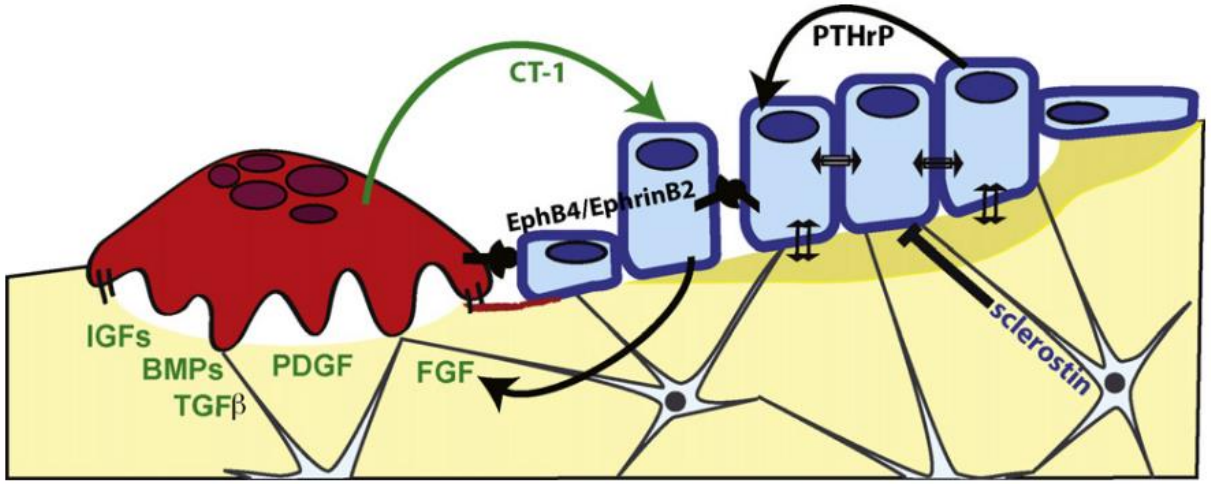
Activation & Resorption



Sims & Gooi 2008

- Osteoclast differentiation regulated by cells of osteoblast lineage
- 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

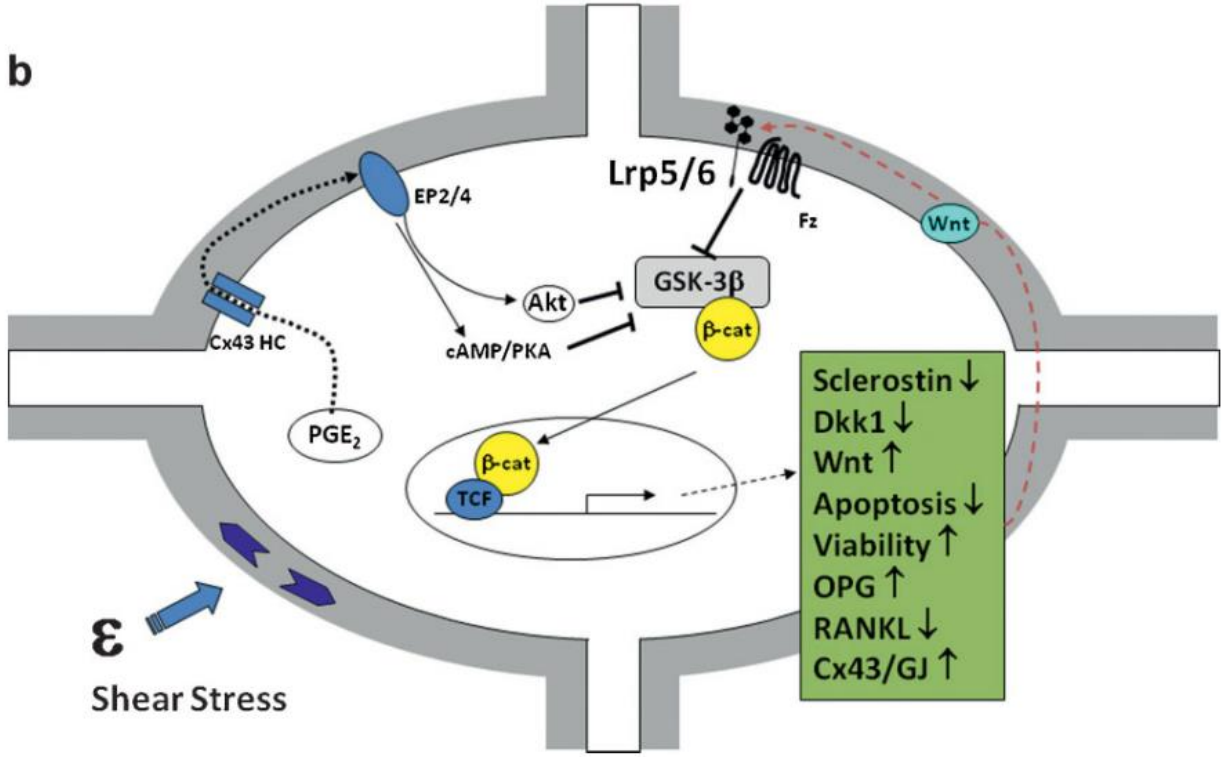


Sims & Gooi 2008

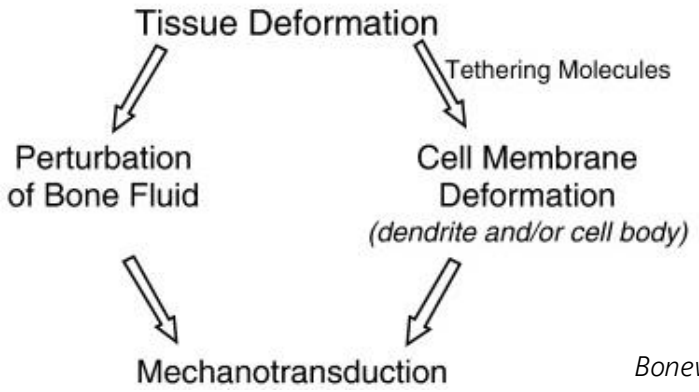
- Osteoblast differentiation and bone formation regulated by
  - Growth factors released from resorbed matrix (IGFs, BMPs, TGF-β, PDGF, FGF)
  - Growth factors secreted by osteoclasts (CT-1)
  - Growth factors secreted by osteocytes (sclerostin, TGF-β)
- Apoptosis of osteoclasts (induced by Bim/caspase-3, 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

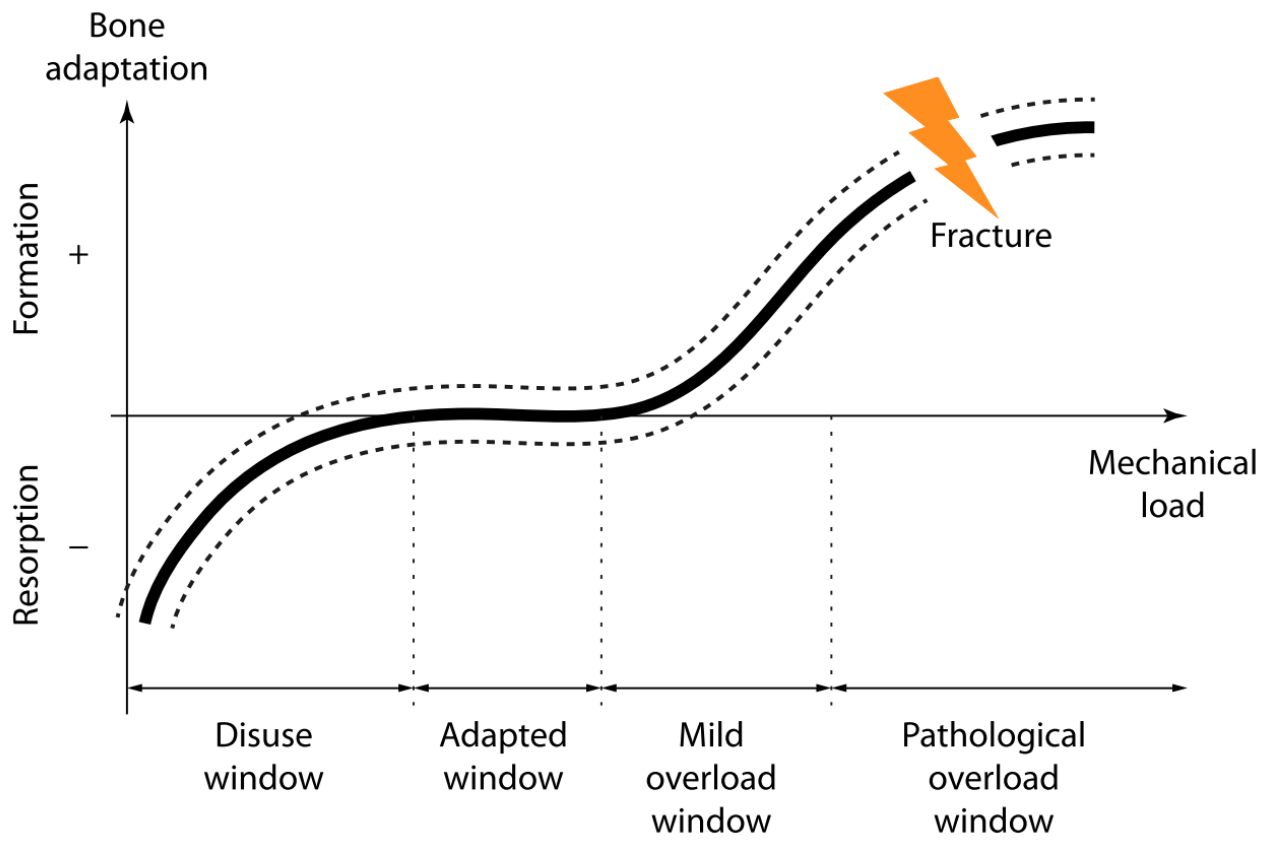


Wnt/β-catenin pathway, *Bonewald 2011*



Bonewald et al. 2008

The "Mechanostat" Model

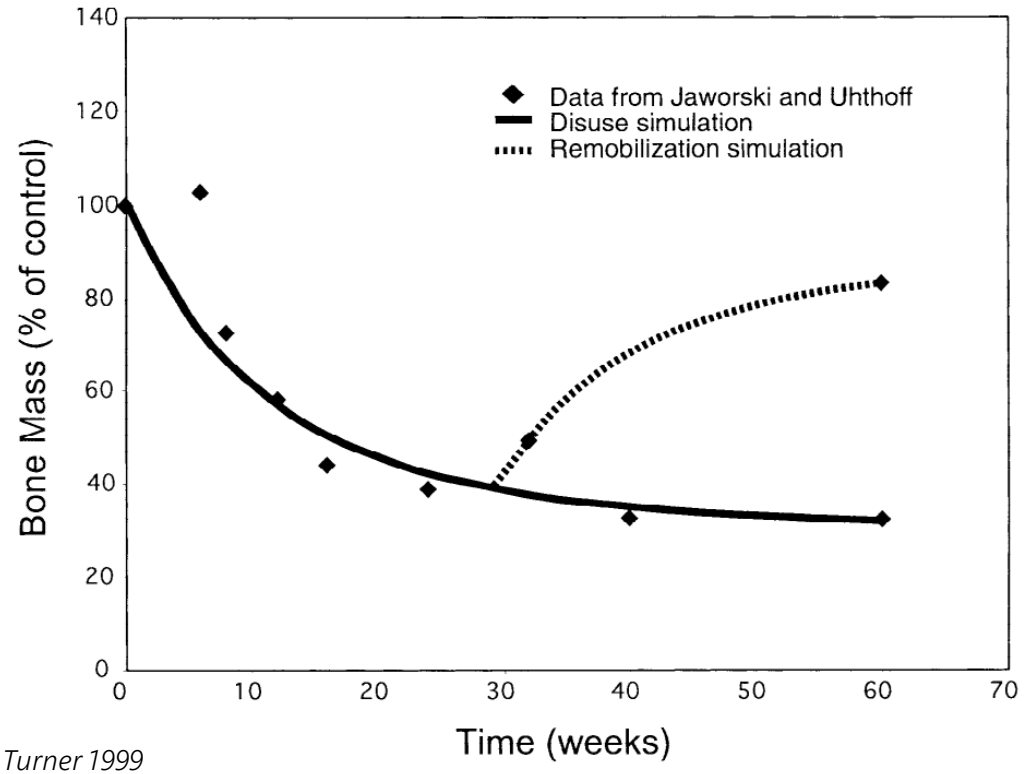


Niemeyer 2013

- 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:

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

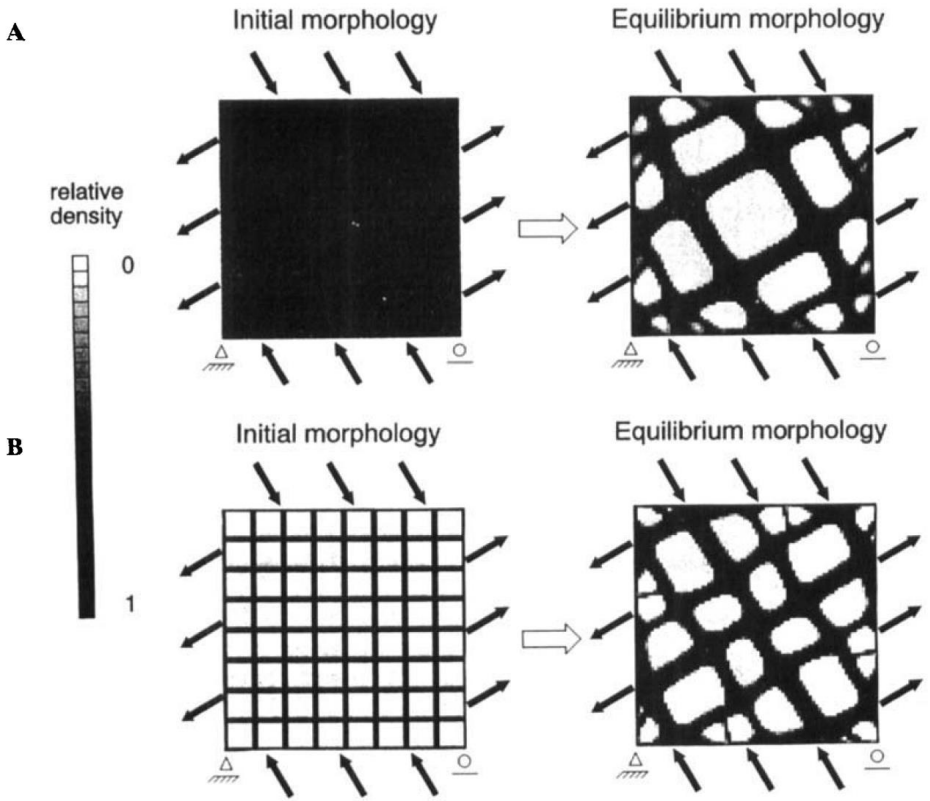
  - E: elastic modulus, S: peak stimulus, S_0: set-point
  - "Disuse fallacy", "constant set-point fallacy"
- Cells accommodating to strain environment:

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

  - 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

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

Semi-Mechanistic Tissue-Level Remodeling 1/2

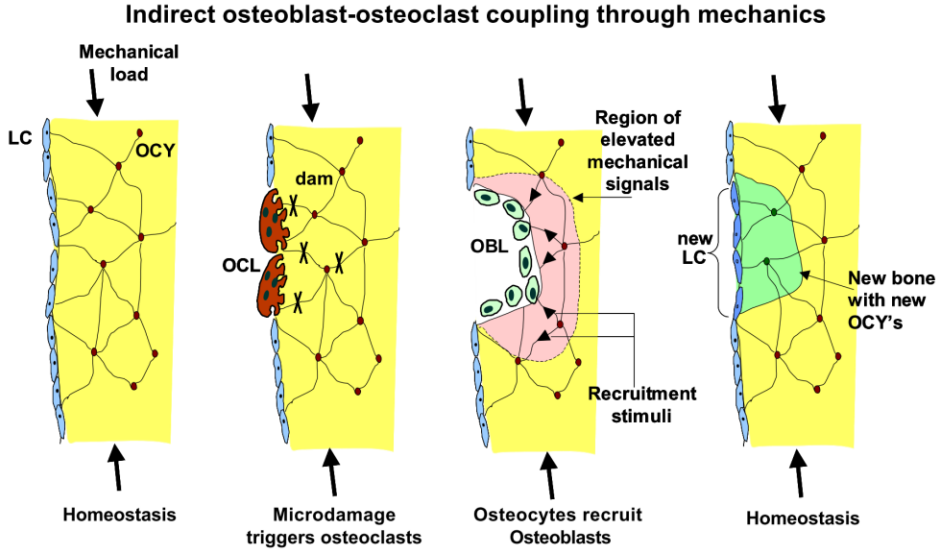


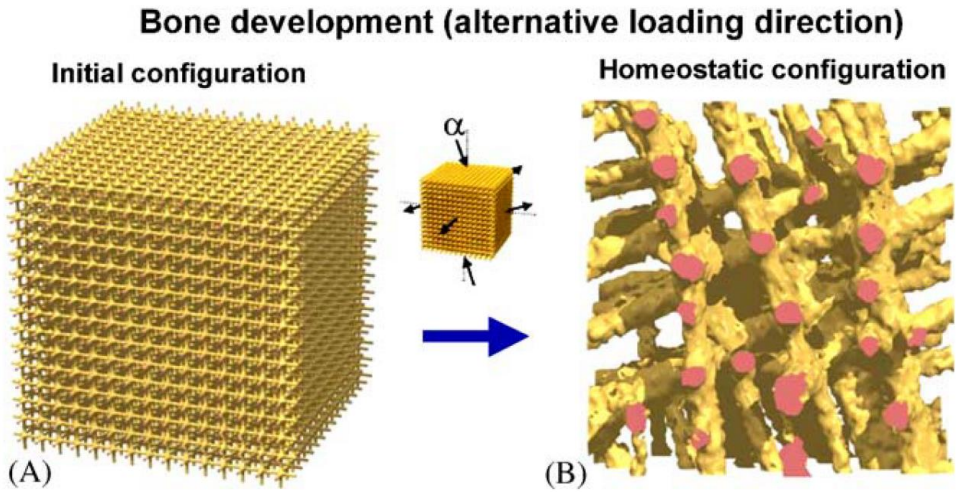
Mullender & Huiskes 1995

- 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



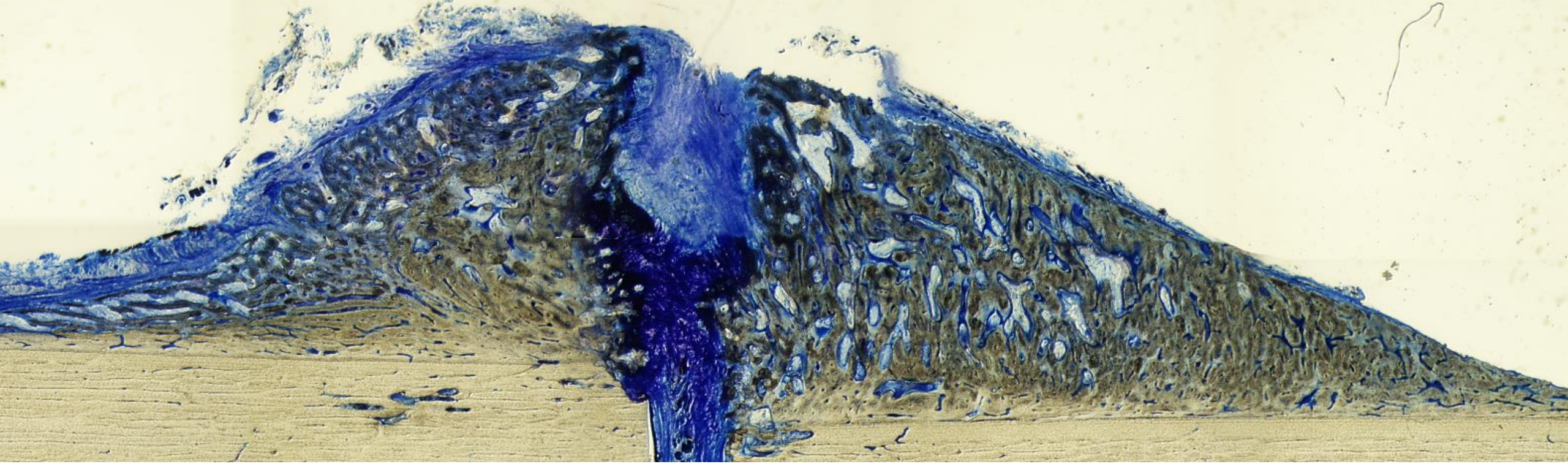


Ruimerman et al. 2005

- 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

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

  - where $\Phi(\boldsymbol{x}, t) = \sum_i w_i(\boldsymbol{x}) \mu_i S_i(t)$
  - 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

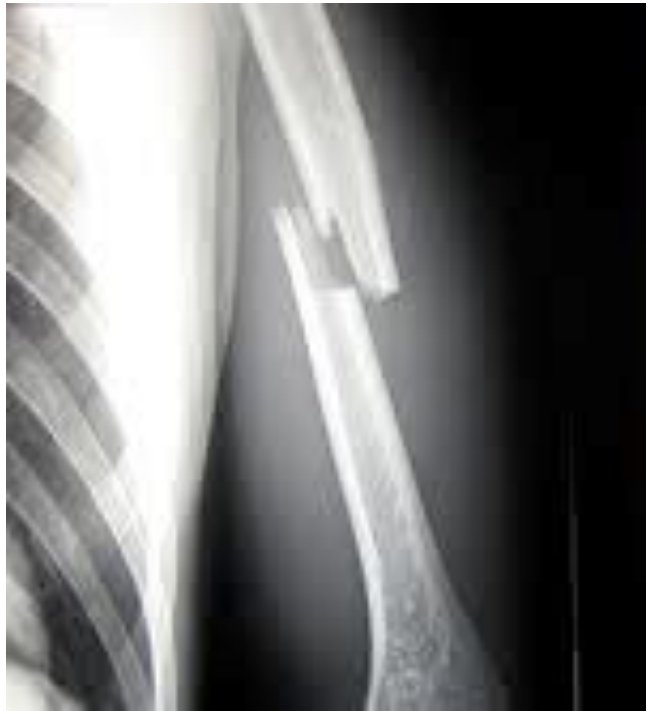


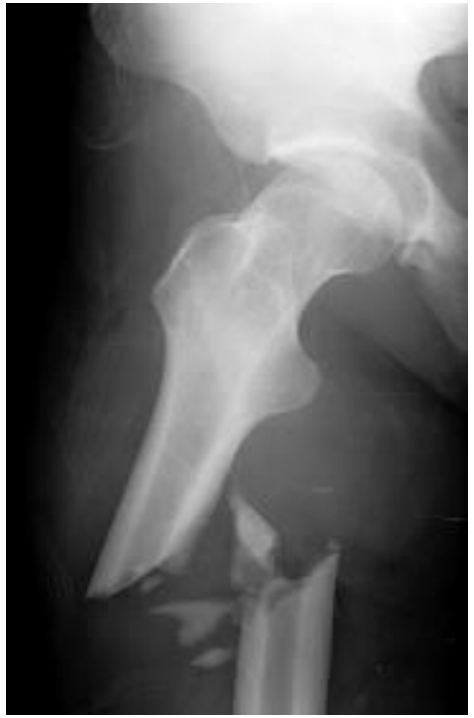
(Bone) Fracture Healing

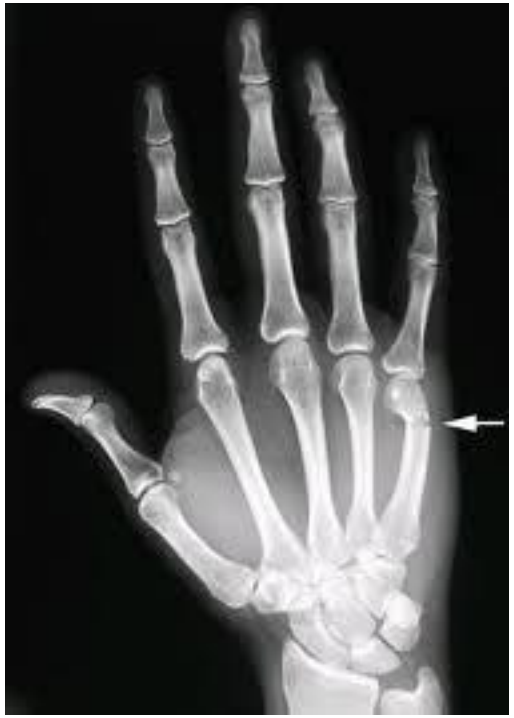
Part 1/2

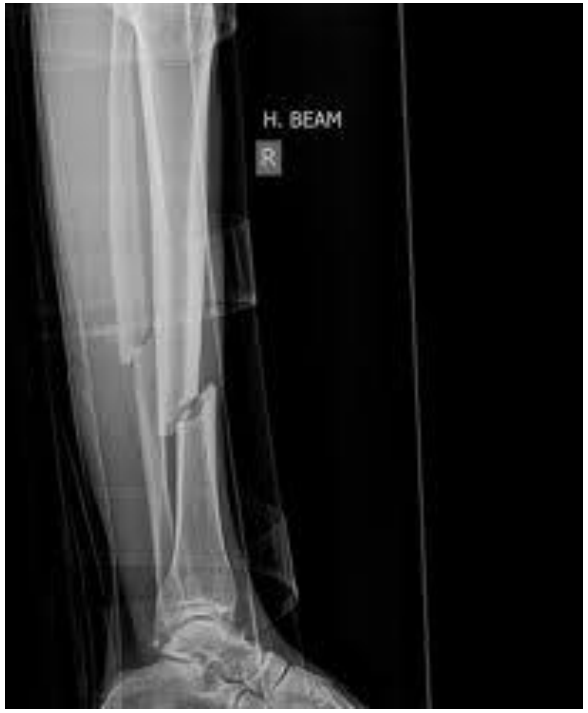
Clinical View

Fracture Sites



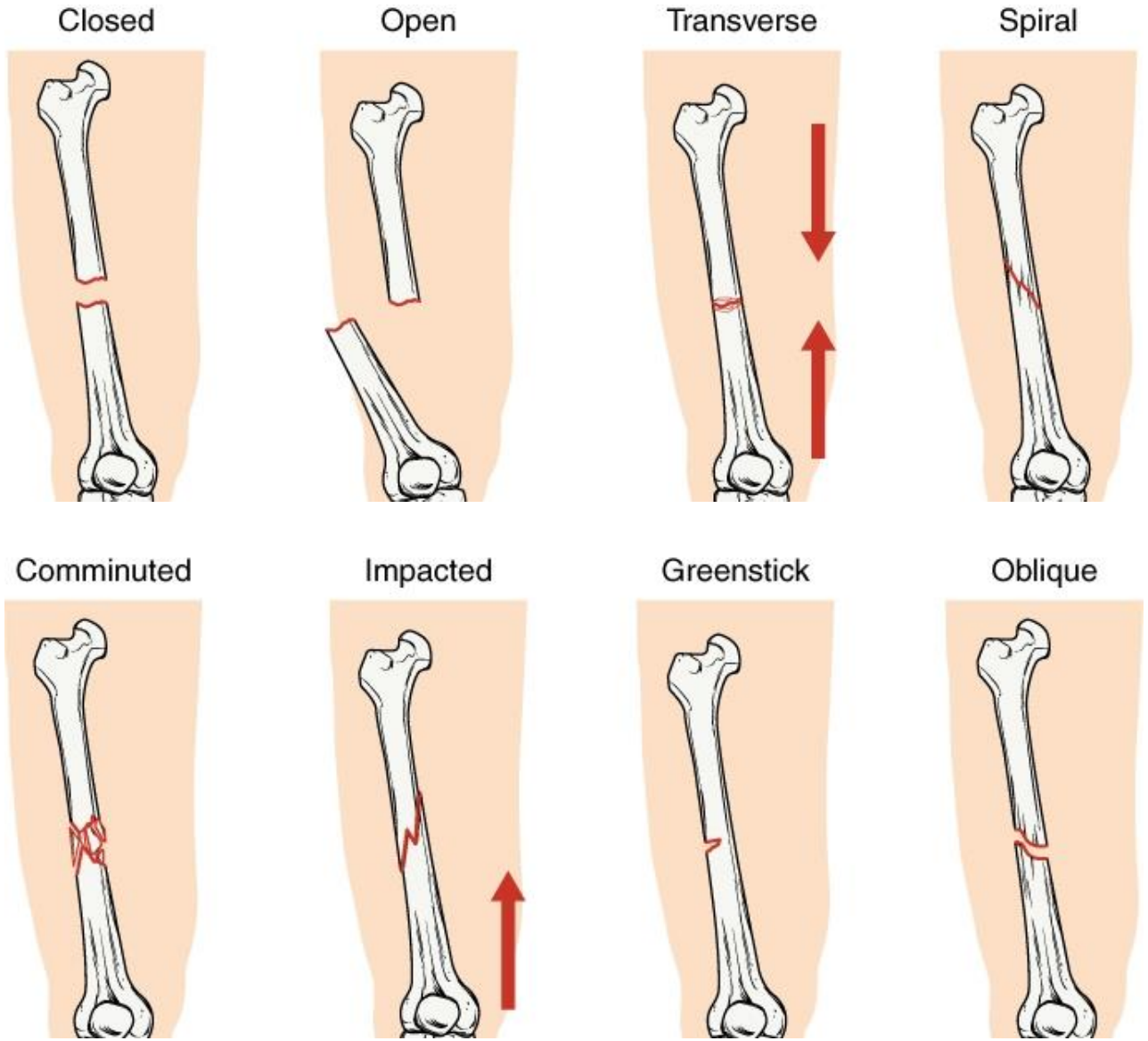






Fracture Types

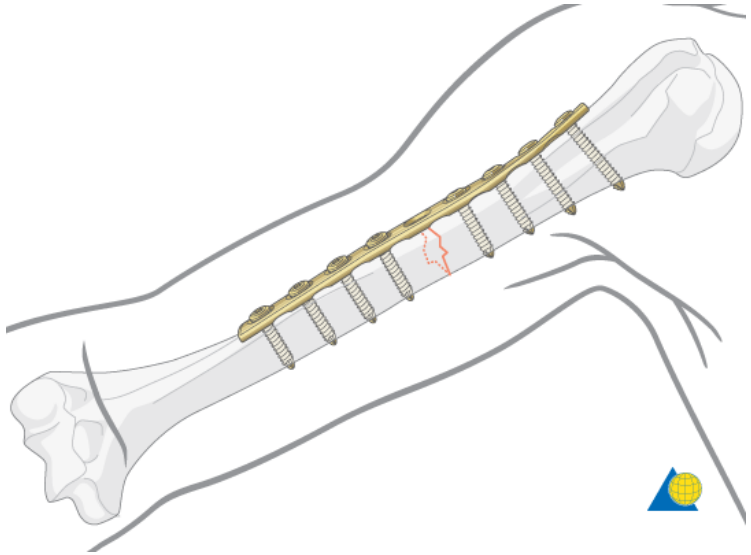
- Displaced vs. non-displaced
- Open vs. closed
- Transverse, spiral, oblique, …

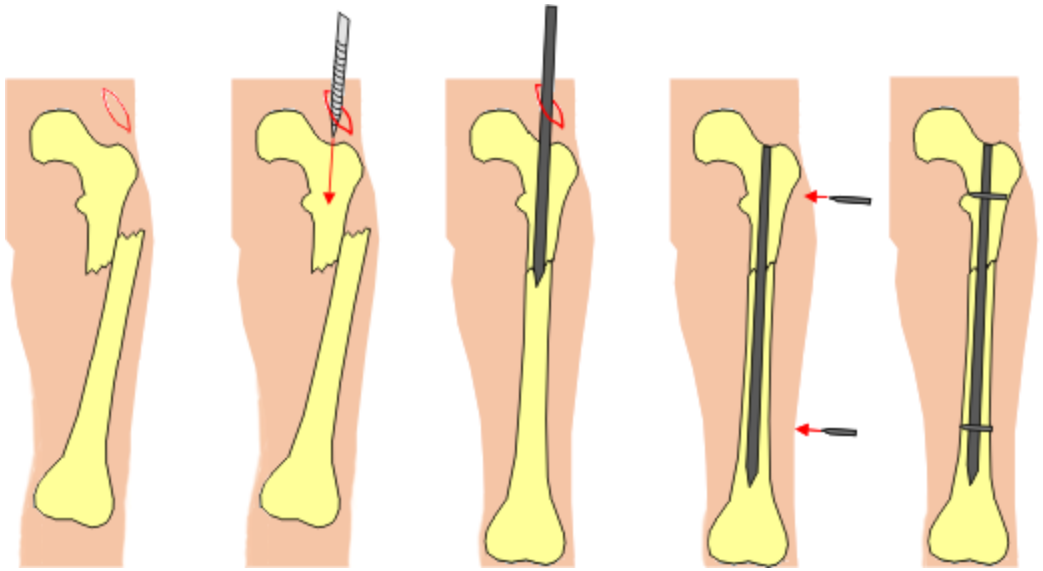


OpenStax http://cnx.org/contents/FPtK1zmh@8.25:no74XEM7@3/Fractures-Bone-Repair

Treatment & Challenges

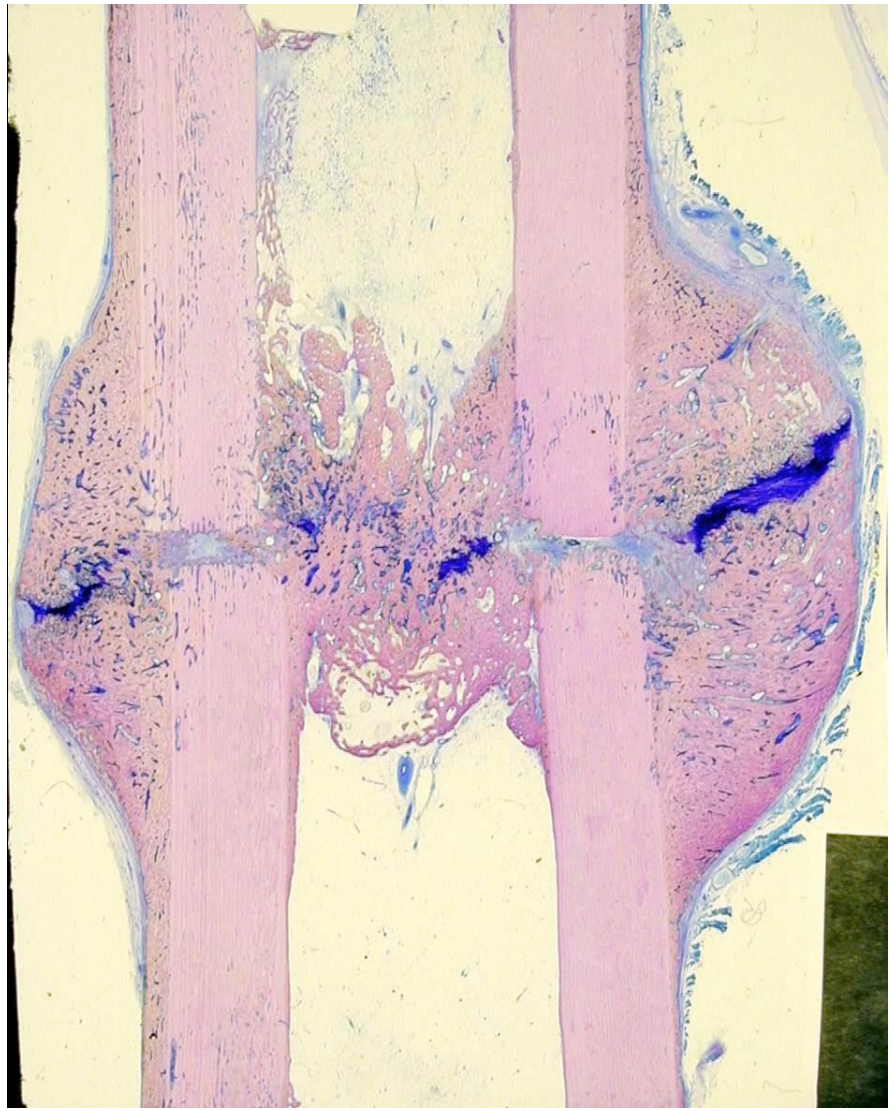
- Fracture fixation
  - Align fragments & stabilize
  - Braces, casts, plates, IM nails, external fixator, …
- Complication rate ~ 10 % (Einhorn et al. 2014)
- Delayed union
- Non-union
  - Hypertrophic pseudarthrosis
  - Atrophic pseudarthrosis
  - Synovial pseudarthrosis
- Causes
  - Excessive motion
  - Large gap
  - Loss of blood supply
  - Severe periosteal and/or soft tissue trauma
  - Systemic (age, malnutrition, …)

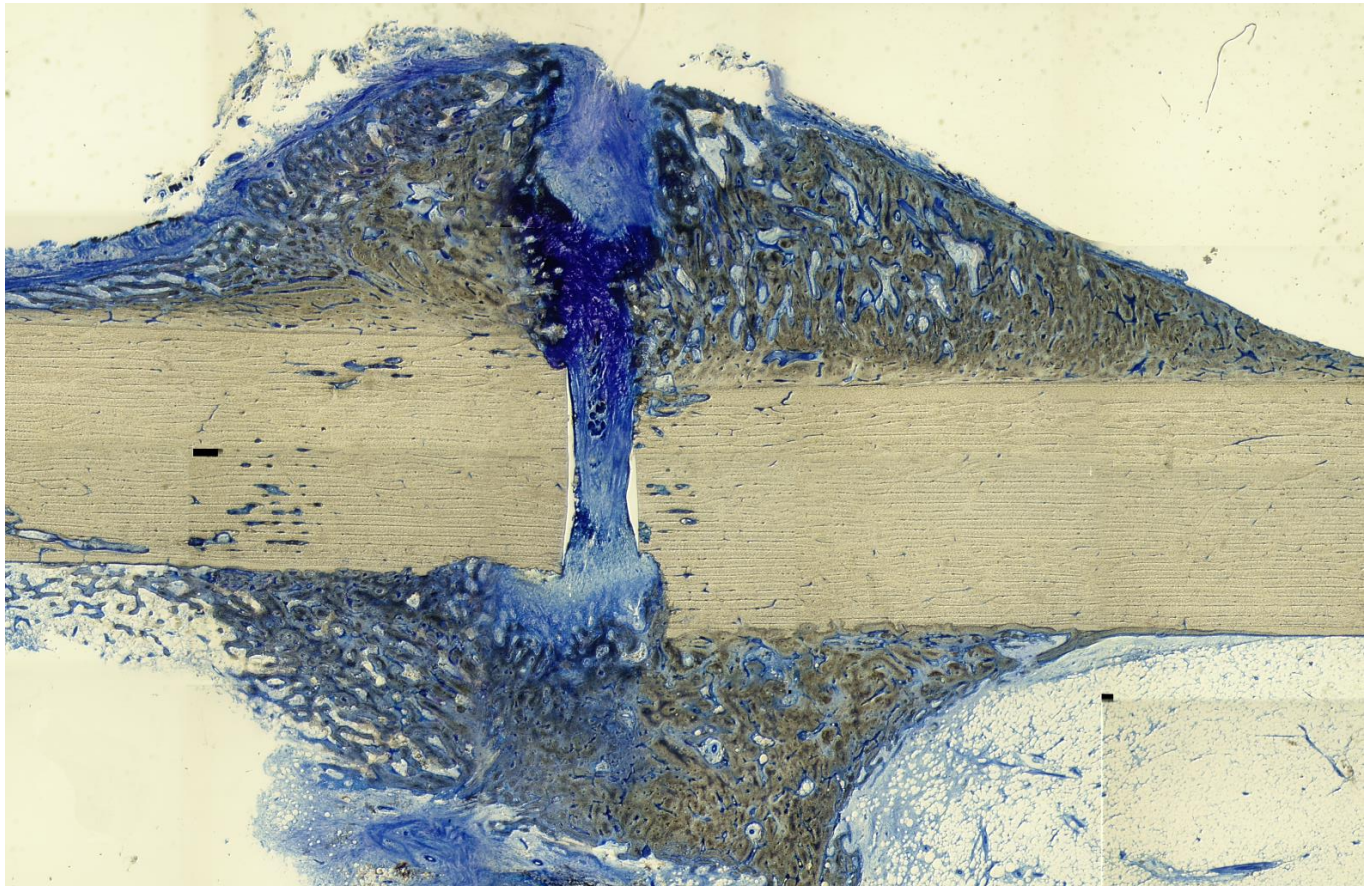




http://www.rob.cs.tu-bs.de/en/research/projects/femur/

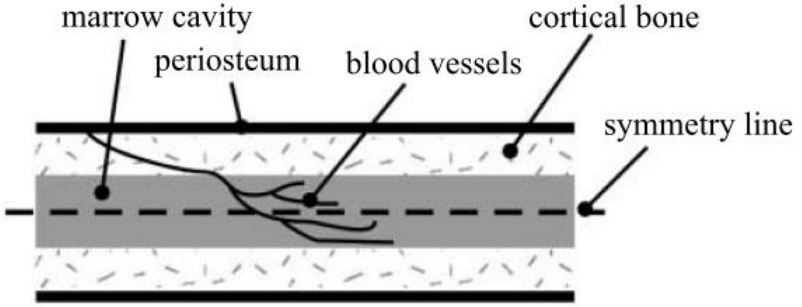
Indirect Fracture Healing

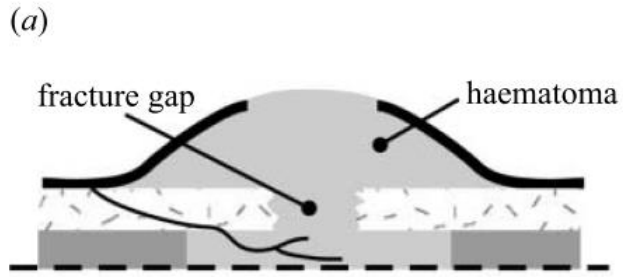




Healing Phases: Inflammation

- Fracture damaged bone, soft tissue & vasculature
- Blood coagulates → blood clot
- Hypoxic, low pH → cells die
- Ruptured blood vessels → osteocytes in cortical ends near fracture die
- Release of proinflammatory cytokines, growth factors, angiogenic factors
  - Sources: platelets, necrotic cells, damaged bone ends, muscles, periosteum, marrow
- Peak within 24 h, completed after ~ 7 days (rats)

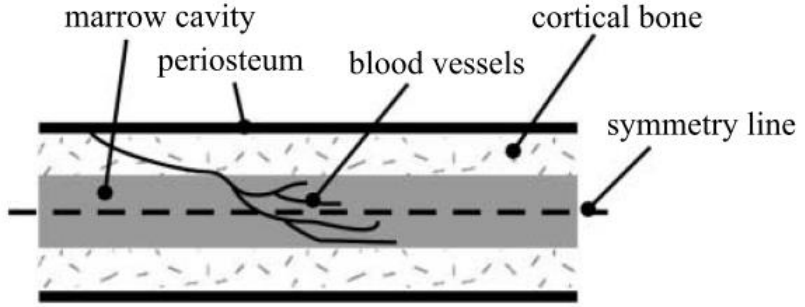


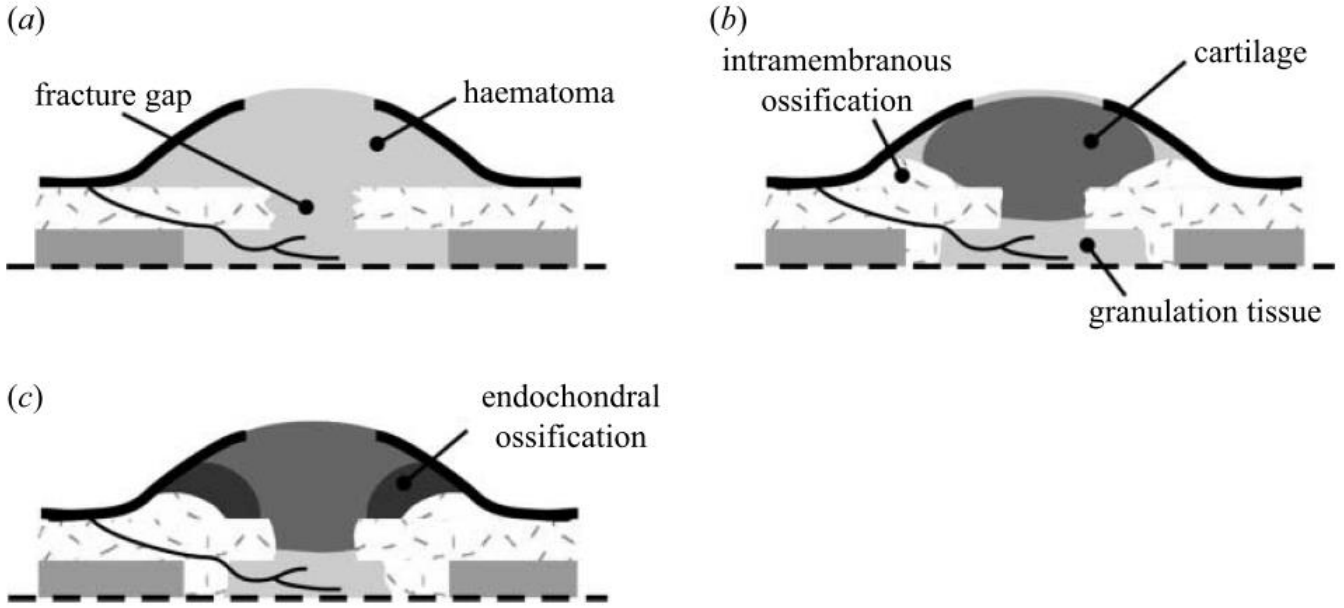


Bailón-Plaza & van der Meulen 2001, Geris et al. 2009

Healing Phases: Repair

- Primary callus response
  - At some distance to the gap, beneath periosteum
  - Intramembranous ossification
  - Lasts ~ 2 weeks
- Revascularization of the hematoma commences
- MSCs & fibroblasts (blood vessels & soft tissues) invade
- Fibroblasts replace hematoma gradually by granulation tissue → soft callus
- Near/inside the gap: MSCs differentiate into chondrocytes → endochondral ossification
- Result: hard callus, stabilized fracture
- Bony bridging, given the right conditions

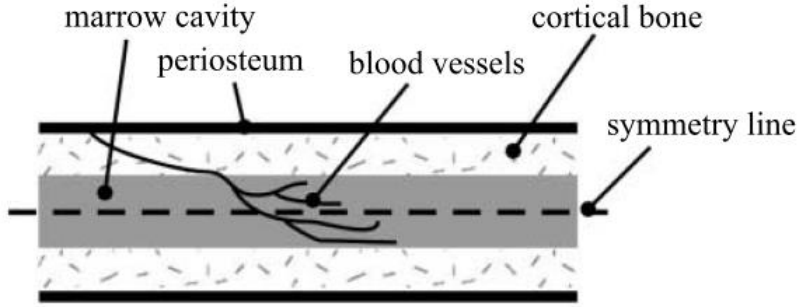


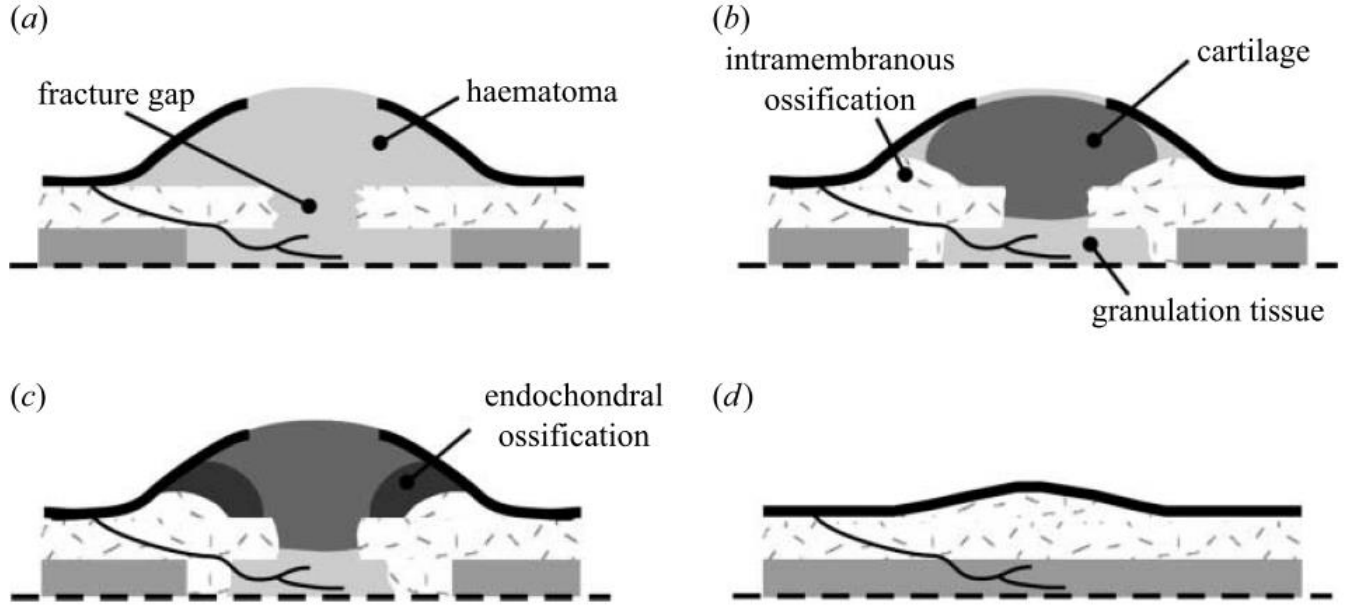


Bailón-Plaza & van der Meulen 2001, Geris et al. 2009

Healing Phases: Remodeling

- Maturation
  - Resorption of woven bone
  - Lamellar bone deposition
- Osteoclastic resorption of superfluous bone tissue
- 5 – 8 weeks (rats; humans: years)
- Result
  - Restored bone architecture, anatomy
  - Restored stability
  - Blood supply normalized to pre-fracture levels

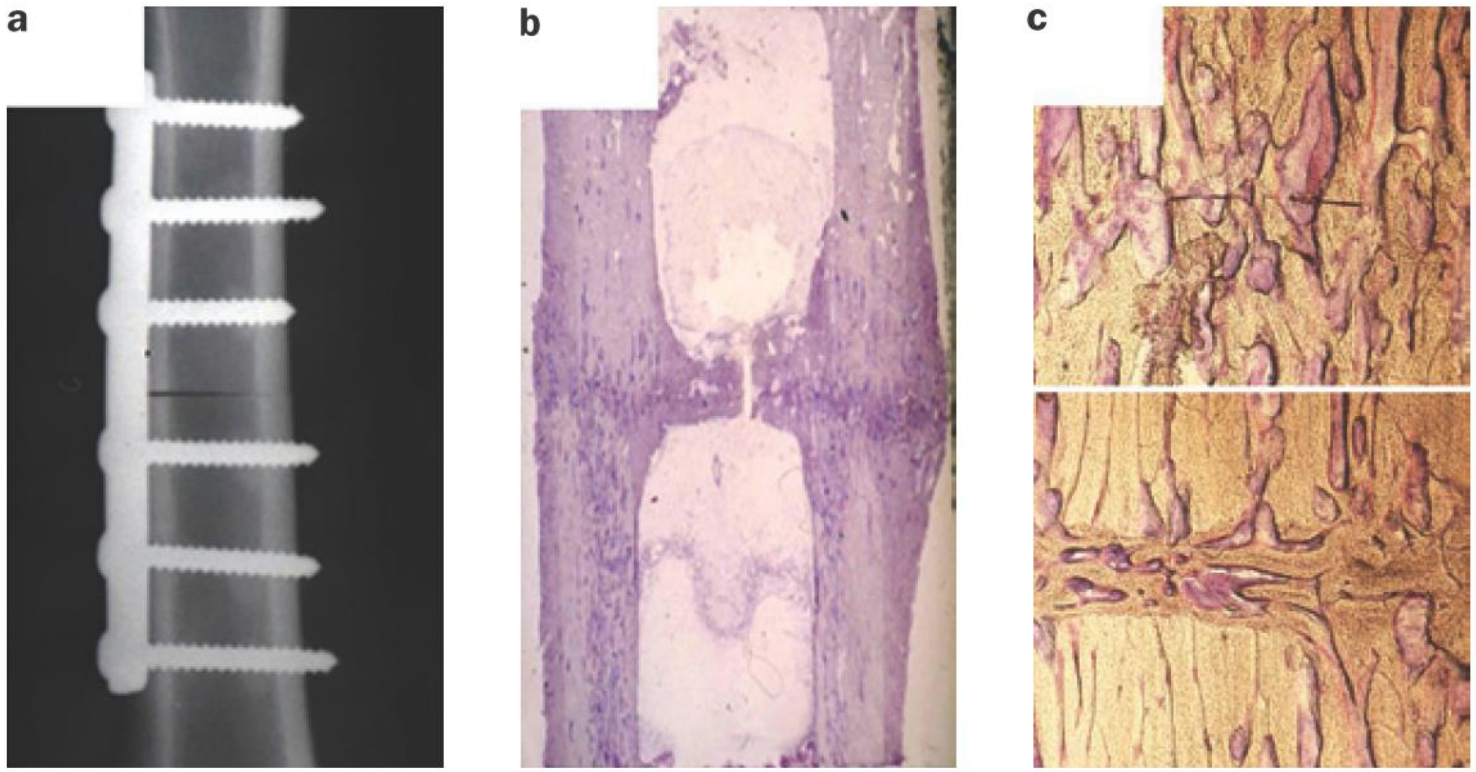




Bailón-Plaza & van der Meulen 2001, Geris et al. 2009

Direct Fracture Healing

- Requires very stable fixation
- Tiny gaps, no inflammation, no callus formation
- Contact healing
  - Gap < 0.01 mm
  - BMUs directly remodel lamellar bone cross-fracture
  - Bony union and restoration of Haversian system
- Gap healing
  - Gap < 0.8 mm
  - Gap filled with woven bone
  - Gradually replaced by oriented revascularized osteons



Claes et al. 2012

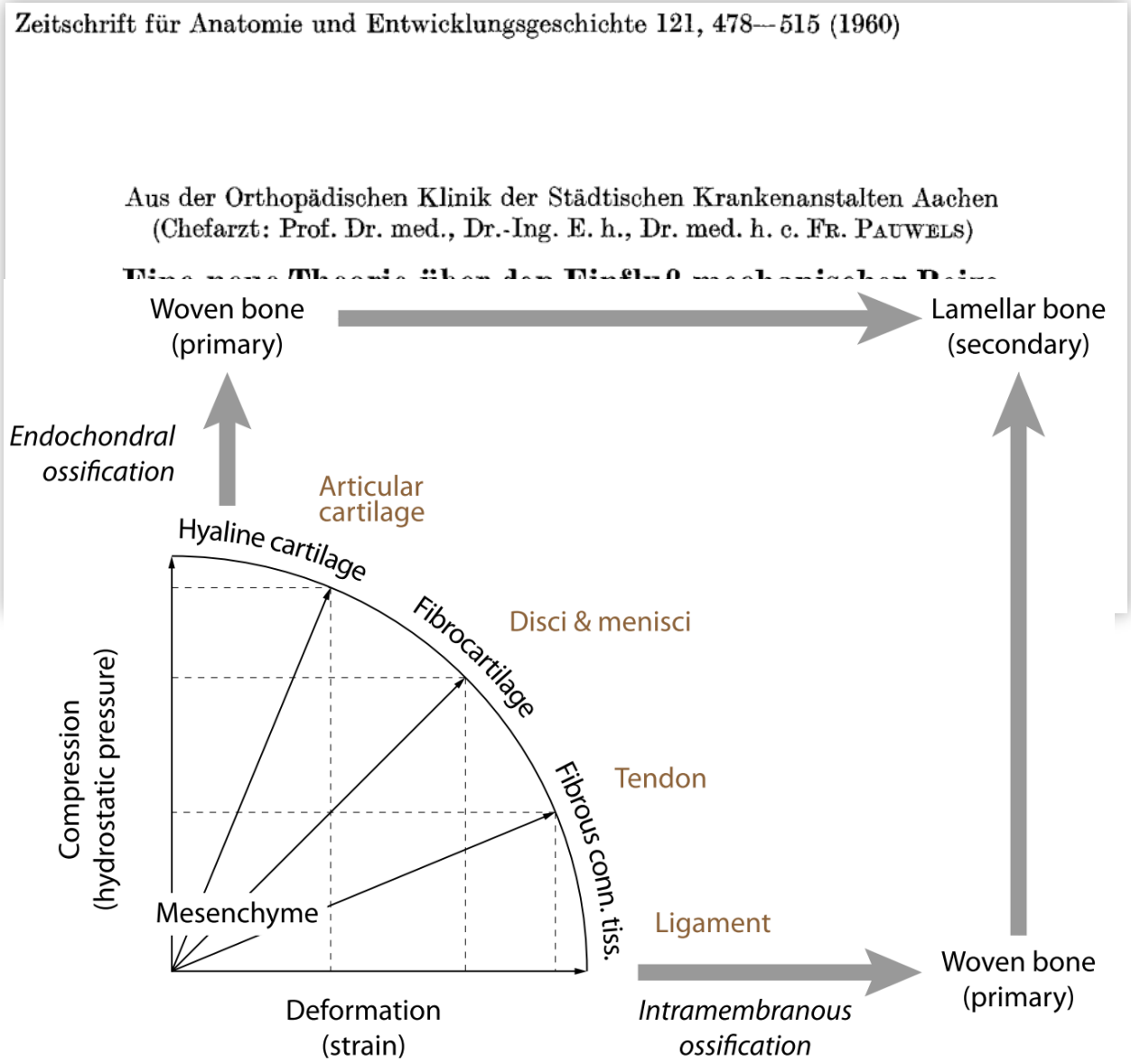
Roux & Krompecher

- Roux (1881): specific stimulus → specific tissue type
  - Proposed that "cells within tissues engage in a competition for the functional stimulus" (Weinans & Prendergast 1996)
    - „Differenzirende u. gestaltende Wirkungen der function. Reize."
    - → „Selbstgestaltung" (self-organization)
  - Compressive → bone
  - Tensile → fibrous connective tissue
  - Compressive/tensile + high shear stress → cartilage
- Krompecher (1937)
  - Agrees with Roux, but
  - … Hydrostatic pressure → cartilage

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

Pauwels

- „Eine neue Theorie über den Einfluss mechanischer Reize auf die Differenzierung der Stützgewebe“ (1960)
- Challenges Roux’s hypothesis
  - Tensile stimuli also stimulate bone formation
  - Long bones: bending loads
  - Refutes Roux’s specific stimulus for cartilage formation
- New hypothesis
  - Bone deposit on an existing framework protecting it from non-physiological deformations
  - Cell-level combinations of pure distortional strain & pure volumetric strain determine differentiation



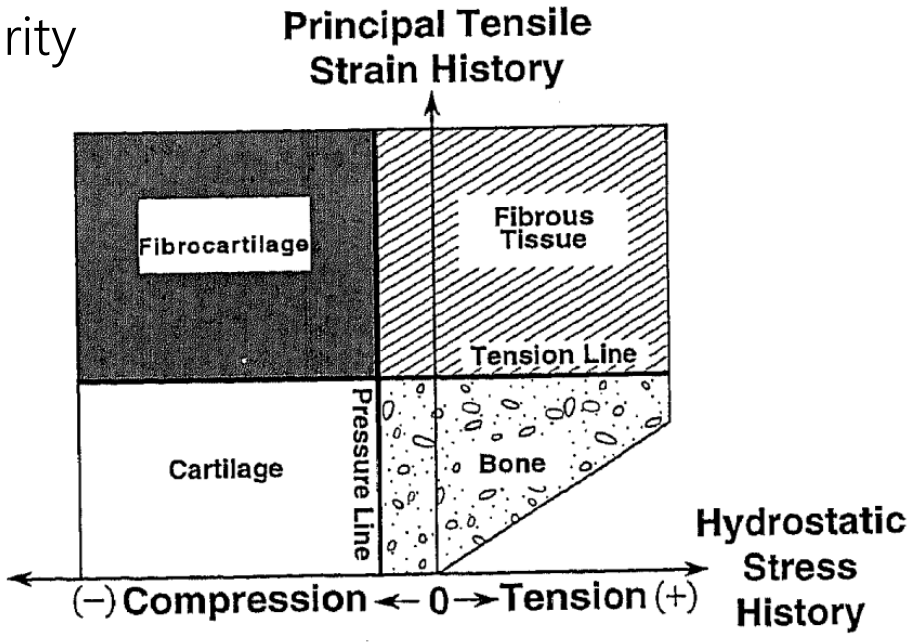
Niemeyer 2013 (after Pauwels 1973)

Carter et al.

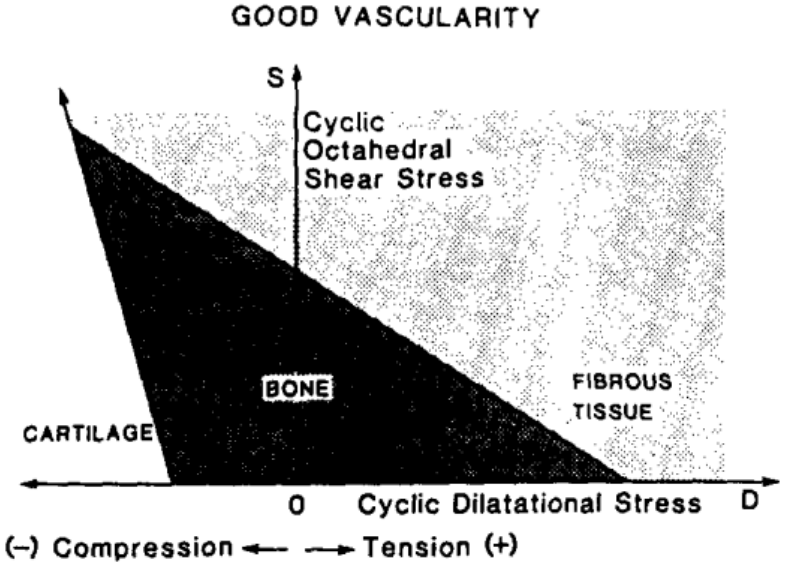
- Proposes "osteogenic index" as a function of peak cyclic shear and peak cyclic hydrostatic stress

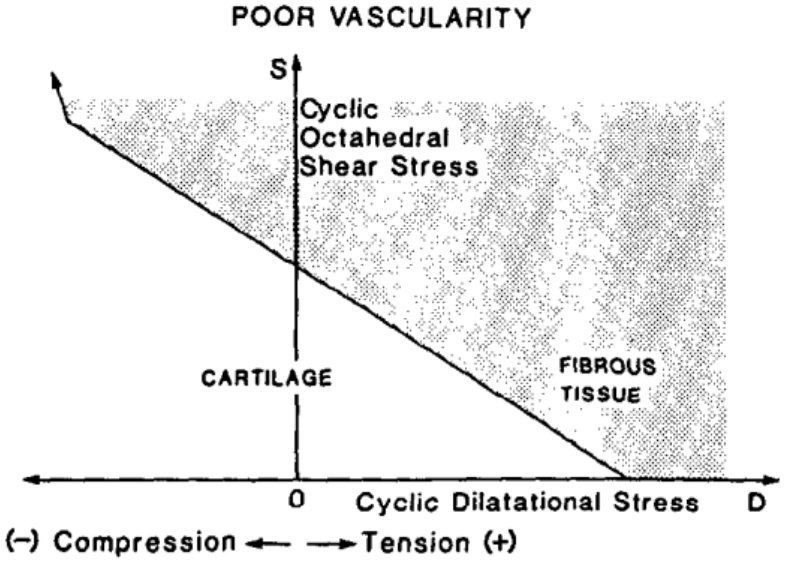
$$I = \sum_i n_i(S_i + kD_i)$$

- Influence of vascularity



Carter et al. 1998

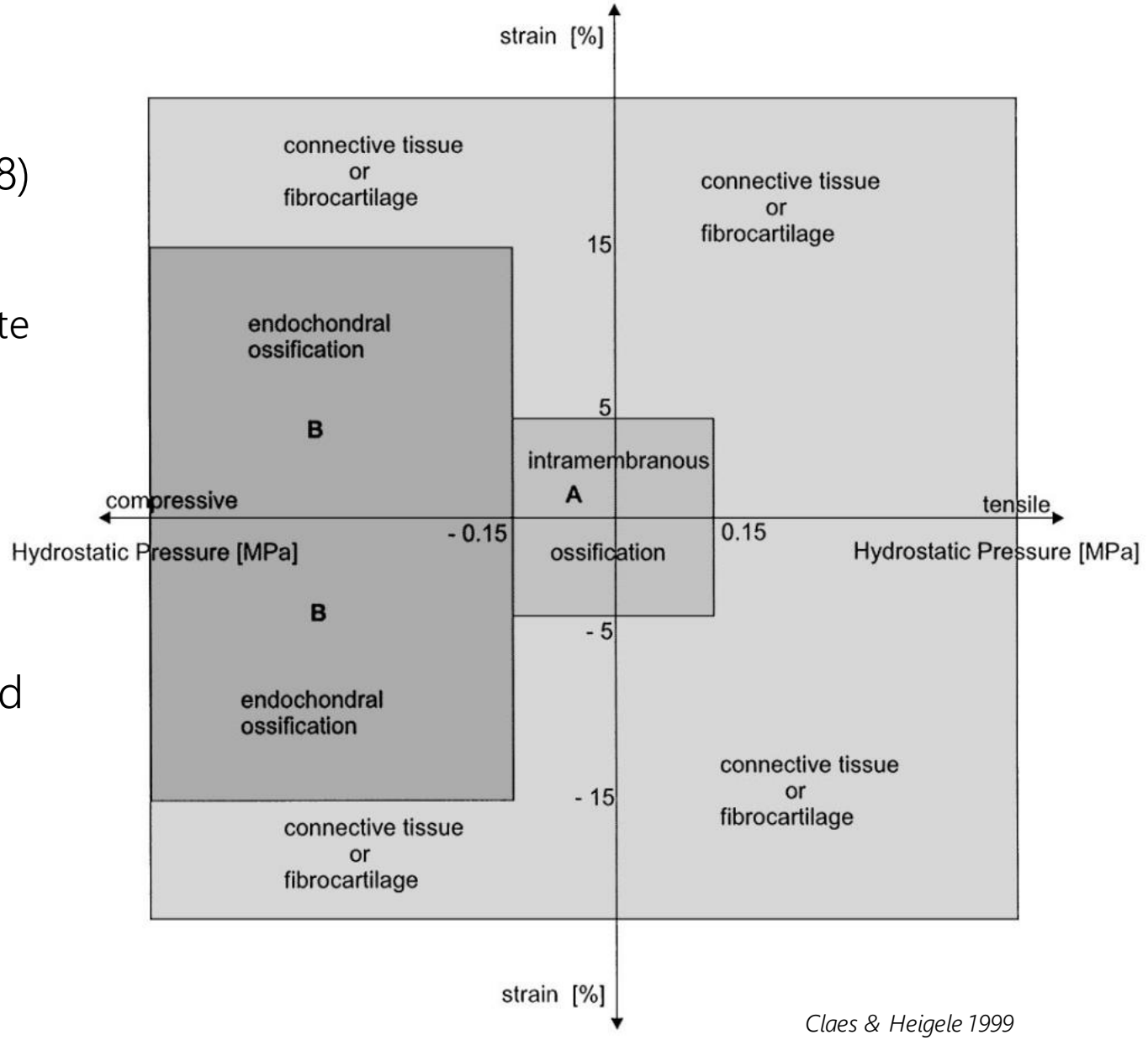




Carter 1987

Claes & Heigele

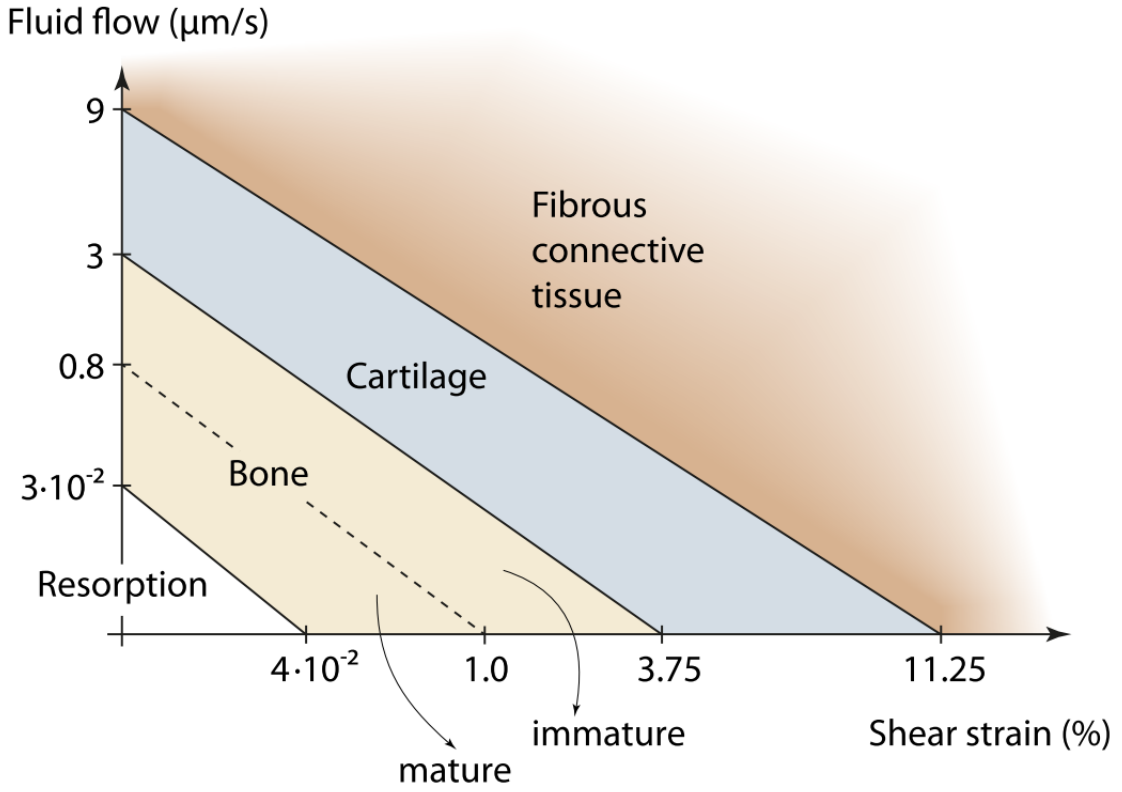
- "Reinterpretation of Pauwels" (Heigele 1998)
- Assumptions
  - Local hydrostatic stress and local strain state as determining stimuli
  - Bone formation on existing bony surfaces
  - … if both hydrostatic stress and shape changing strains stay below certain thresholds
- Thresholds determined based on combined *in vivo* & FE investigation
- Vaguely defined "strains"
  - Probably normal strain of max. absolute value along *x/y* axes



Claes & Heigele 1999

Prendergast et al.

- Biological tissue as biphasic material (poroelastic)
  - Solid phase (matrix)
  - Fluid phase (interstitial fluid)
- Tissue differentiation guided by
  - Octahedral shear strain γ
  - Fluid flow (flow velocity) v
- Combined stimulus $S = \gamma/a + v/b$



Niemeyer 2013 (after Lacroix et al. 2002)

Simulating Remodeling vs. Fracture Healing

- Remodeling: Dynamics of a single "species" (typically bone density)
  → Single ODE/PDE
  → Single mechanical stimulus
  → Osteocytes as mechanosensors

- Fracture Healing: Multiple interacting "species" (tissue and/or cell types)
  → System of coupled PDEs (or other equivalent formalization)
  → Multiple mechanical and biological stimuli
  → Tissue differentiation & maturation
  → Growth
  → Additional mechanosensors required (fracture gap?)