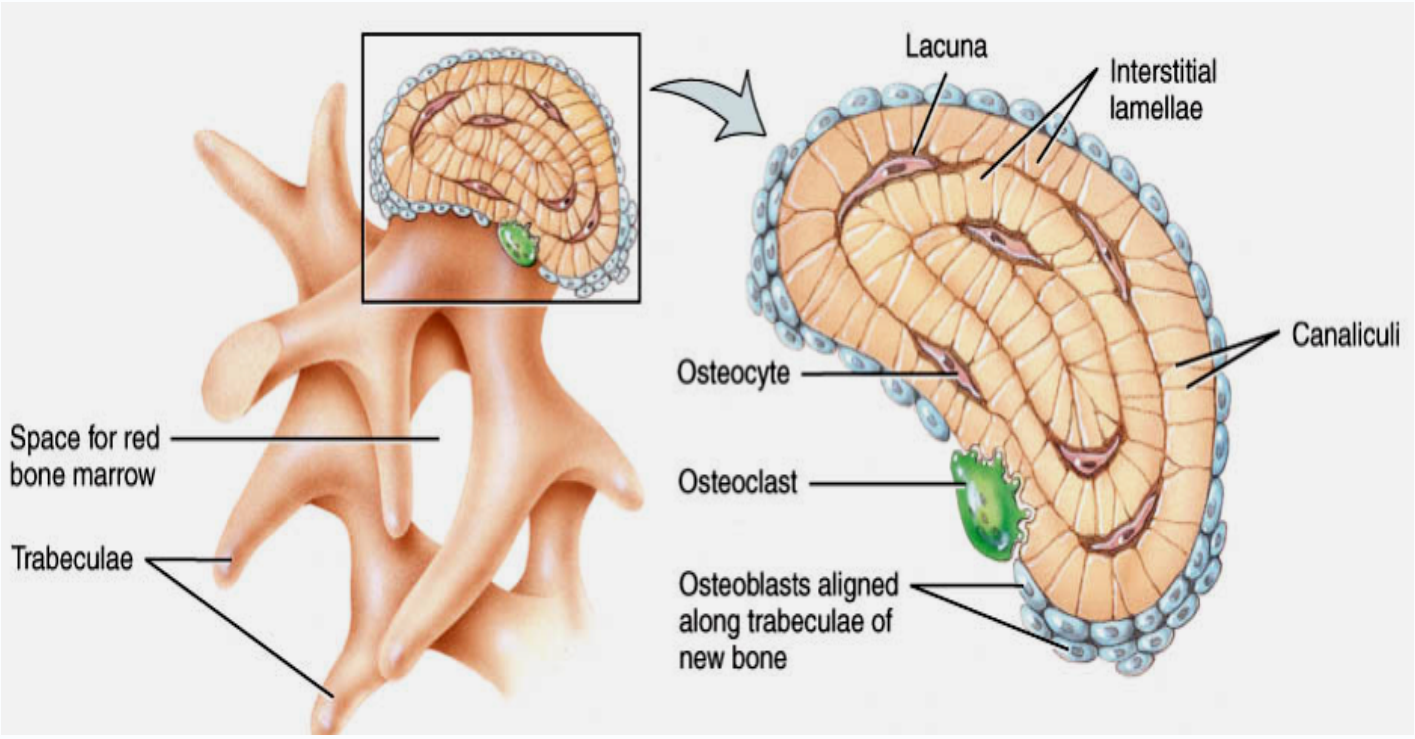
ME 482 Experimental Tissue Mechanics

Professor A. Wagoner Johnson

10/5/16 (started)

Bone: Function, Structure, Bone cell types



Bone – *Incomplete* list of topics we will cover for cortical (compact) and cancellous (trabecular, spongy) bone

- Mechanical and physiological function of bone
- Composition, hierarchical structure
- Mineralization
- Bone cells and their roles in bone formation and maintenance
- Determinants of strength of whole bone

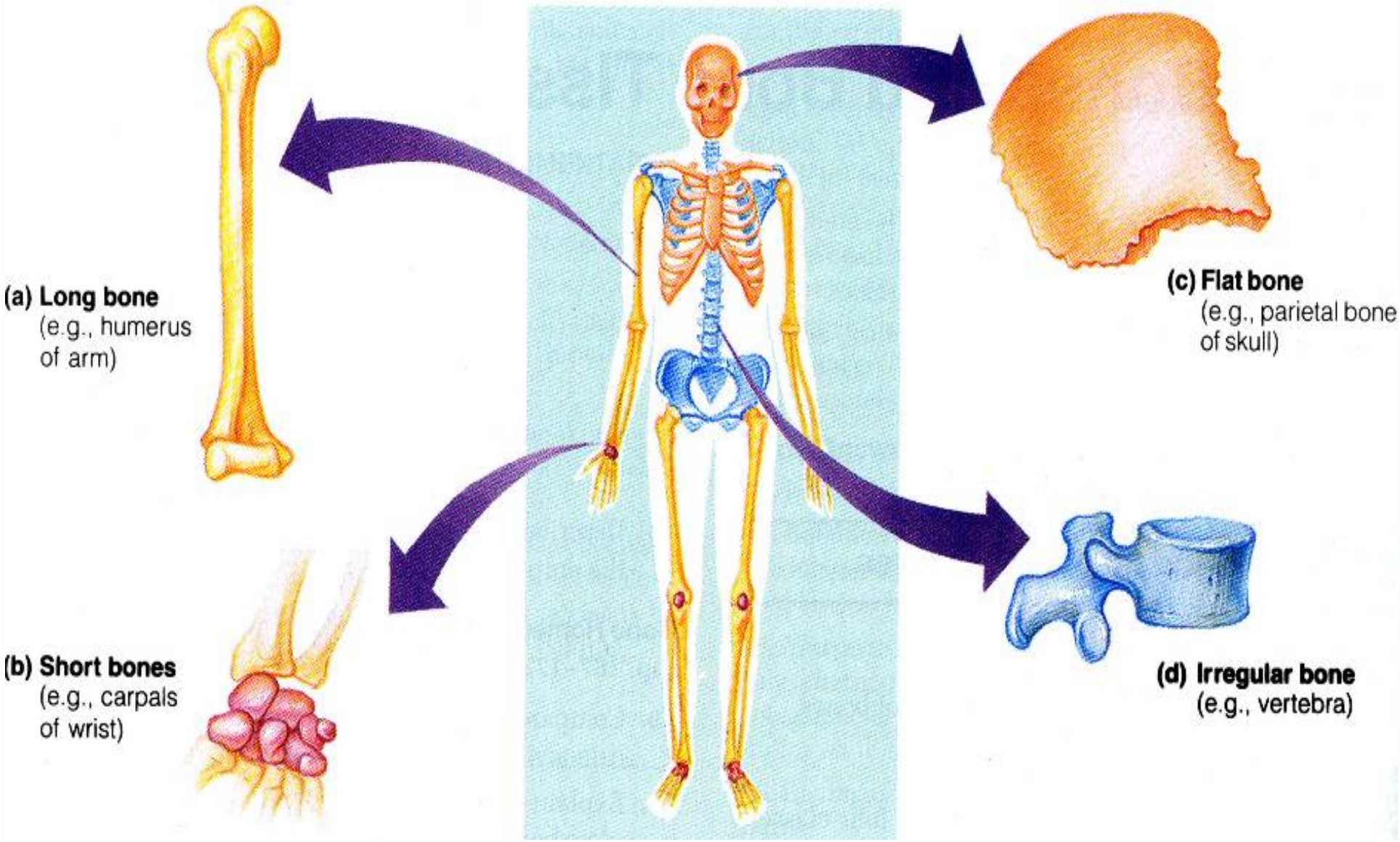
- **Trabecular bone**
  - Mechanical properties – magnitudes, anisotropy
  - Determinants of material properties and apparent properties
  - Deformation mechanisms (i.e. cellular solids)
  - Damage mechanisms, measuring damage

- **Cortical bone**
  - Mechanical properties and determinants
  - Fracture and fatigue
    - Review basic fracture mechanics
    - Fracture in bone
    - Fracture mechanisms in bone

Mechanical and Physiological Function of Bone

- <u>Mechanical Function</u>
  - To provide support for body against external forces
    - (both cortical and trabecular)
  - To act as a lever system to transfer forces –
    - bones and muscle function together as a lever system to transfer forces thru muscle tendon units that attach to bone.
  - To protect vital organs –
    - flat bones (two cortices with trabecular bone – schematics on next slides) protect organs such as heart, lung, brain, internal reproductive organs
- <u>Physiological Function</u>
  - To form blood cells (hematopoiesis)
    - *Hematopoeisis* occurs in bone marrow, which is located in regions containing trabecular bone
  - To store calcium (mineral homeostasis)
    - 99% of body's calcium is stored in skeleton as are K, Zn, P, and Mn.
    - Ca is critical for many cell (and there foretissue) functions

Types of bone

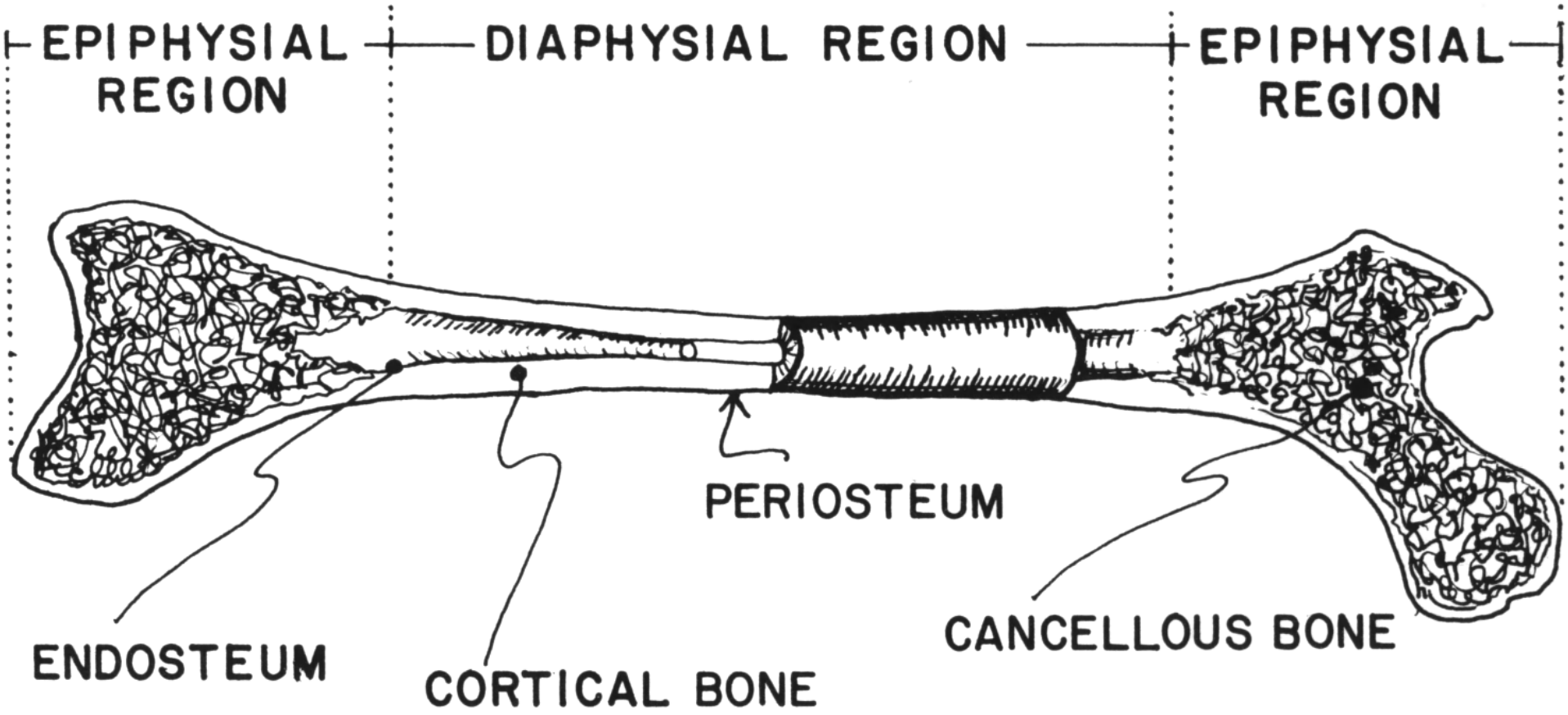


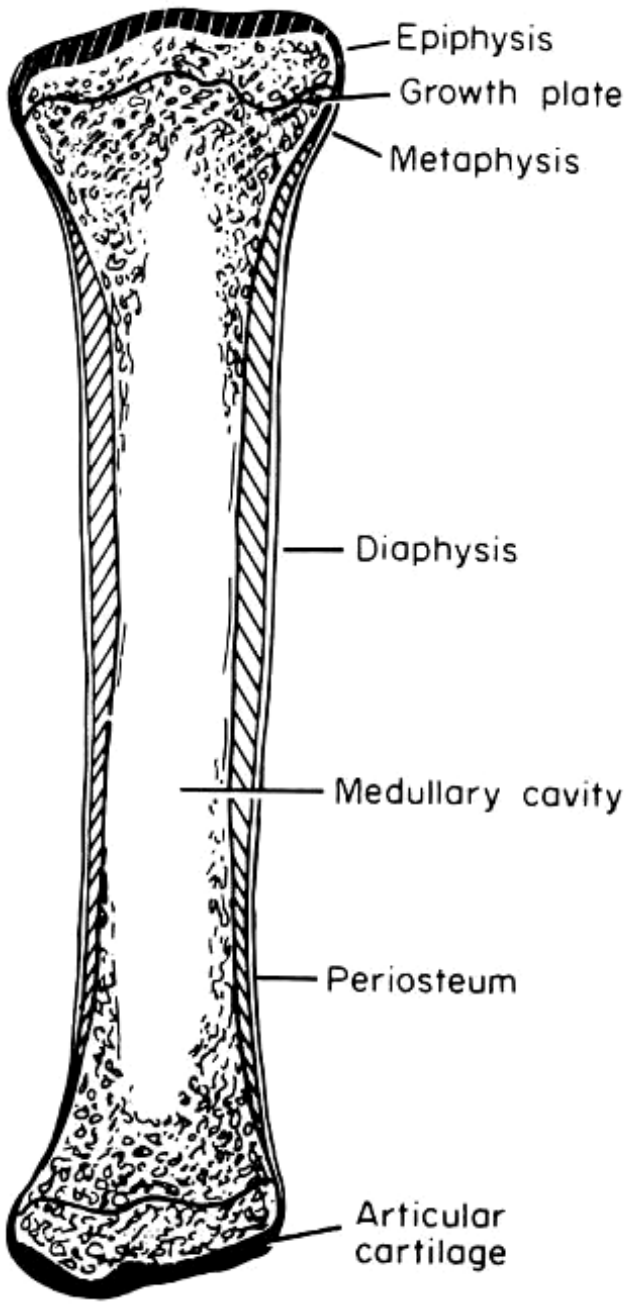
References: Nigg & Herzog, Marieb

Classification of bone (shape, size, location, function)

SHAPE	EXAMPLES	FUNCTION
LONG – tubular with long shaft, limbs	Femur, tibia, radius	Levers to transmit longitudinal force
SHORT – bones in hands and feet	Carpal, tarsal	Same as above
FLAT	Sternum, ribs, skull, illiac crest	Protection, points of attachment for ligaments
IRREGULAR	Vertebrae	Various functions
SESAMOID	Patella	Improved lever

Some anatomy of long bone



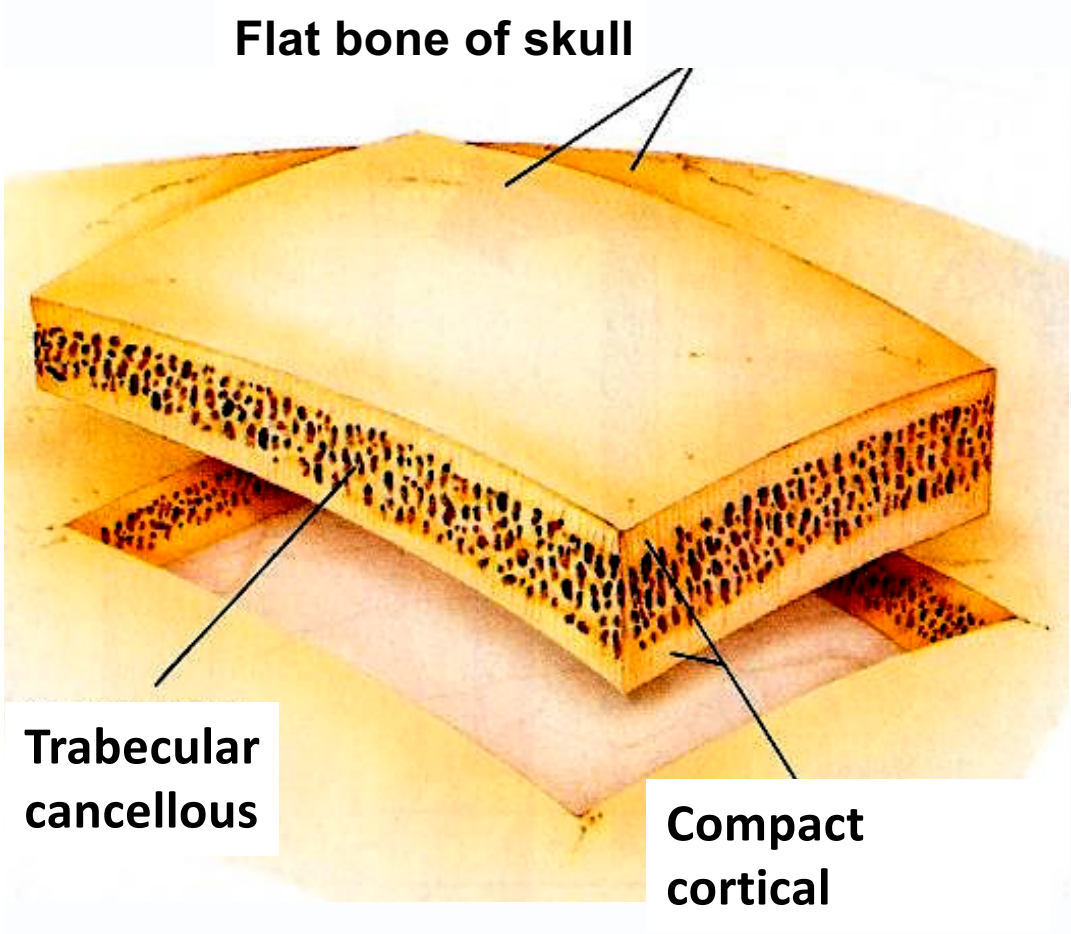


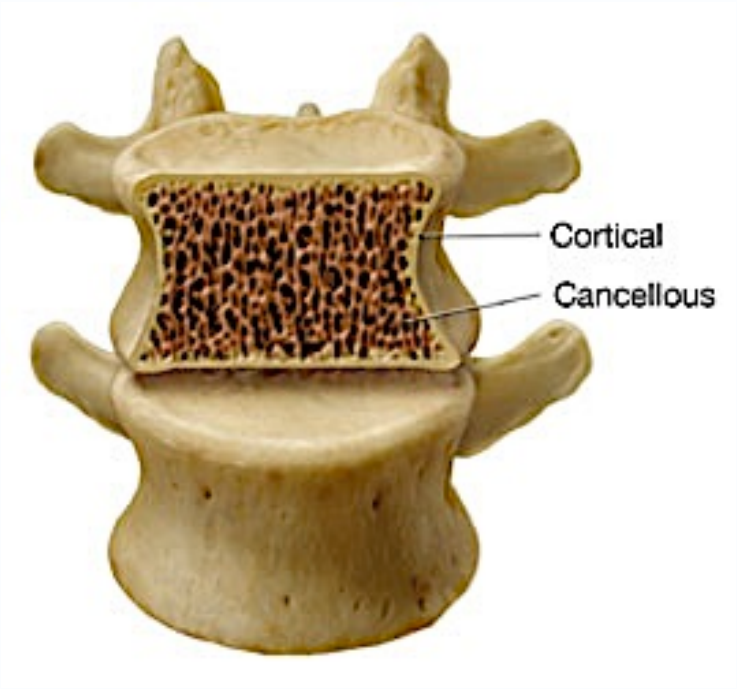
From Fung, orig Rhinelander (1972)

Anatomy of Long Bone (Fung):

- ***epiphysis**:* region between growth plate and expanded end of bone; mostly trabecular bone with thin shell of cortical bone
- ***growth plate**:* unites epiphysis and metaphysis, location of calcification of cartilage
- ***metaphysis***: bulbous end, thinner shell, between growth plate and diaphysis, fuses with diaphysis at end of growth stage
- ***diaphysis***: shaft of long bone, thick
- ***medullary cavity**:* bone marrow cavity
- ***periostium**:* dense fibrous tissue surrounding bone, abundant vasculature, stem cells, and growth factors that are relevant to remodeling and healing
- ***articular cartilage***: at the extremities, gliding surface of joint, possibly lowest coefficient of dry friction for any material

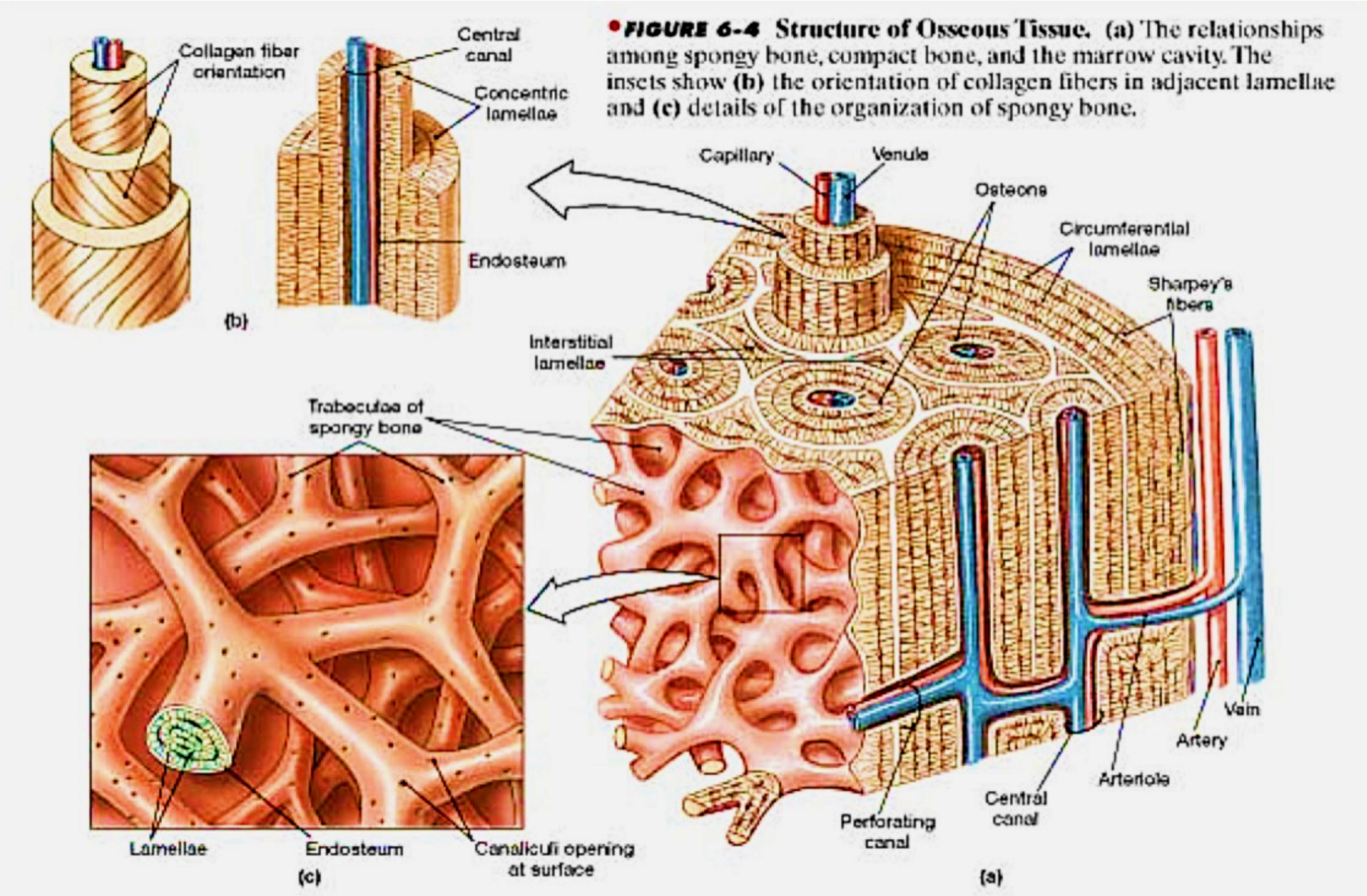
Two types of bone structure: cortical and cancellous





www.ama-cmeonline.com/.../m3_02path_02.jpg

Summary of bone structure

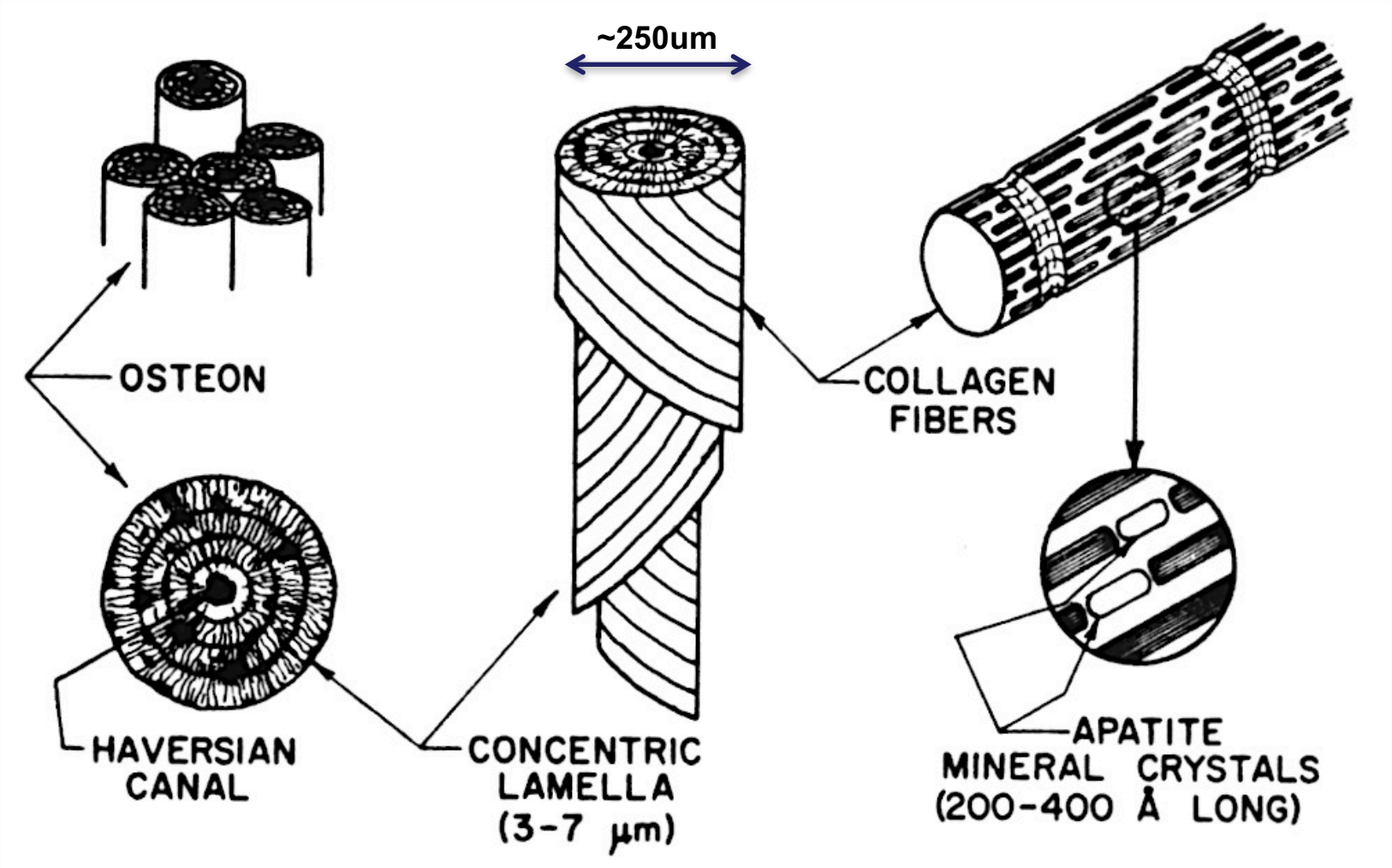


•FIGURE 6-4 Structure of Osseous Tissue. (a) The relationships among spongy bone, compact bone, and the marrow cavity. The insets show (b) the orientation of collagen fibers in adjacent lamellae and (c) details of the organization of spongy bone.

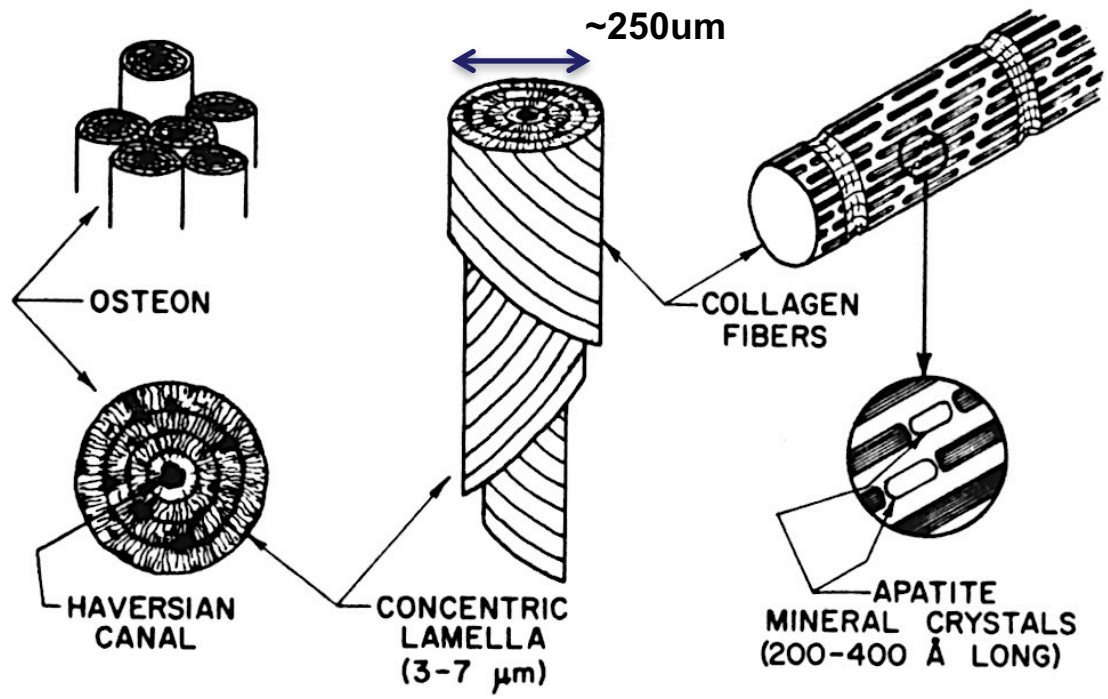
Haversian canal

Volkman's canal

The *osteon* is the structural unit of cortical bone



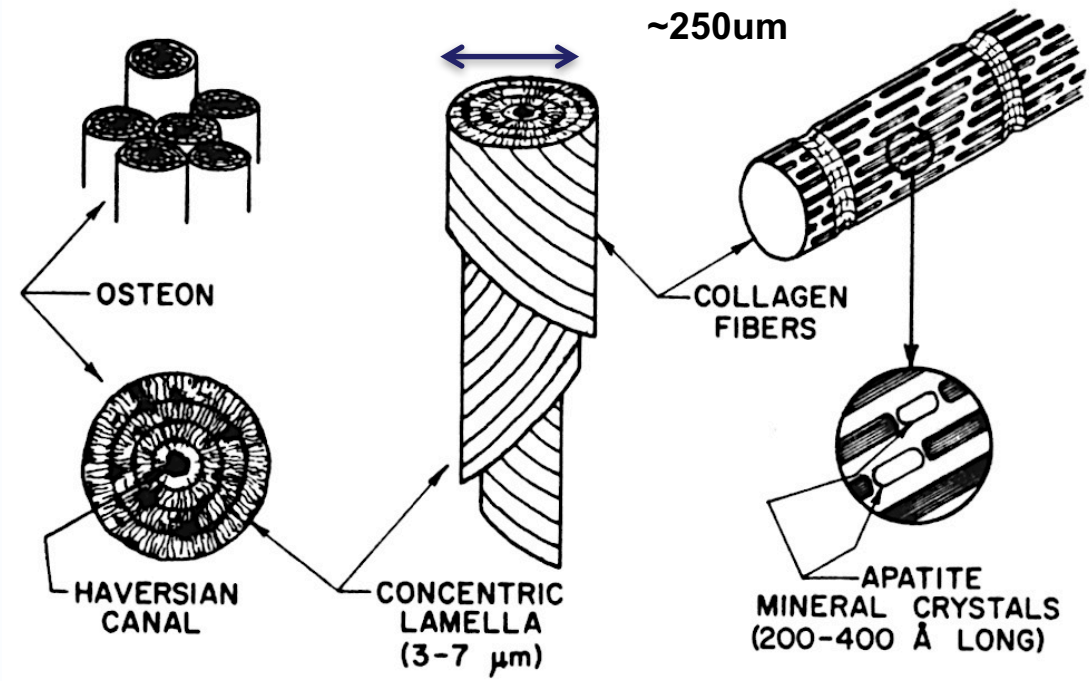
The osteon is the structural unit of cortical bone



Microstructure (~10s – 100s microns)

- Osteons contain blood vessels and nerves. Connected laterally by Volkman's canals.
- Osteons run parallel to the long axis of cortical bone in long bones. Also exist in cortical bone in flat bones
- Osteons (cortical bone) are ~250 micron in diameter.
  - The wall is about 70-100 μm thick.
  - 20-30 rings, or lamellae, surround a central canal called the Haversian canal (HC).

Osteon is the structural unit of cortical bone



CONTINUED

Microstructure (~10s – 100s microns)

- Lamellae ~3-10 microns thick, same arrangement of collagen fibers within a single layer
- Collagen fibers are oriented differently from layer to layer in order to optimize mechanical properties.
- Osteons are often separated by cement lines are 1-2 microns thick.
  - layers of mineralized, collagen deficient matrix, i.e. high inorganic content
  - Mark region of remodelling
- Woven bone is less organized and is formed quickly during growth or repair and then remodeled to be more organized.

Mineral-Collagen (Type I) Composite

- 40 nm gaps
- Overlap or offset between adjacent molecules is ~30nm
- CaP mineral in gaps between collagen molecules
  - 50 x 25x 3 nm (plate)
- Type I Coll
  - 300 nm tropocollagen
  - ~1-3nm dia

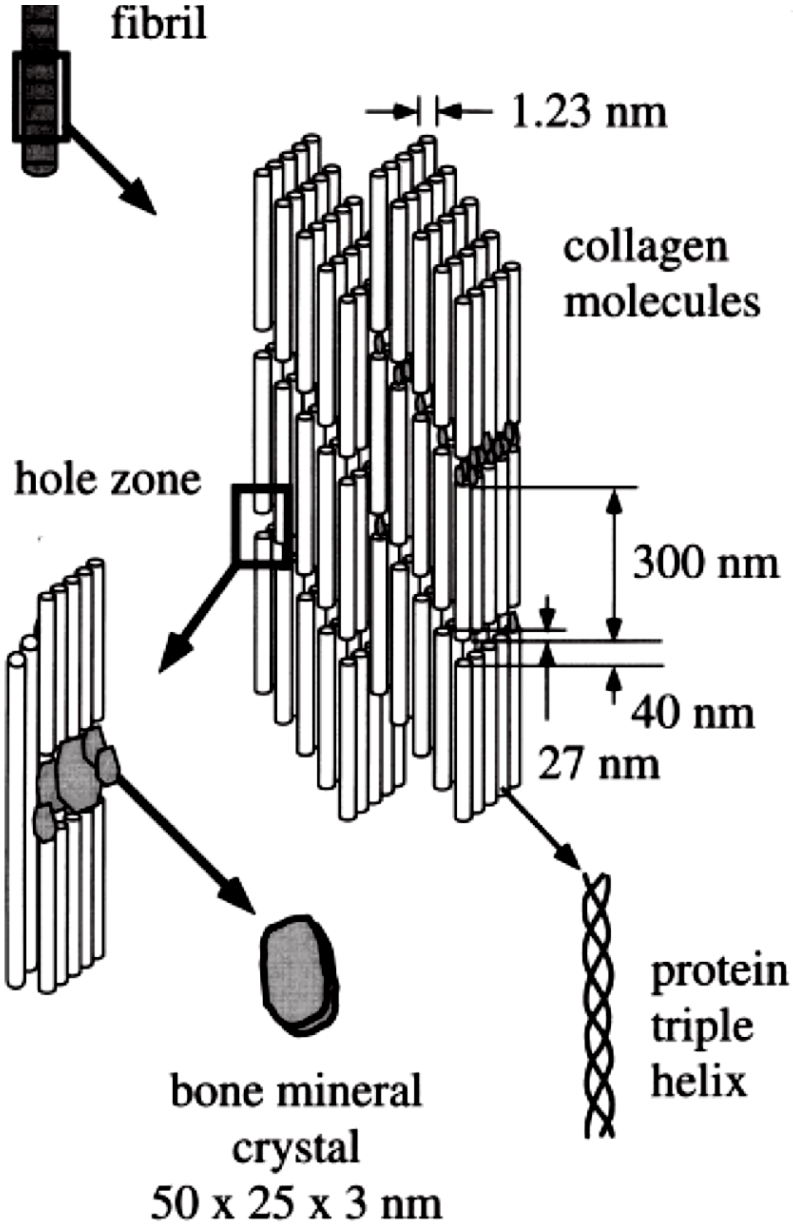
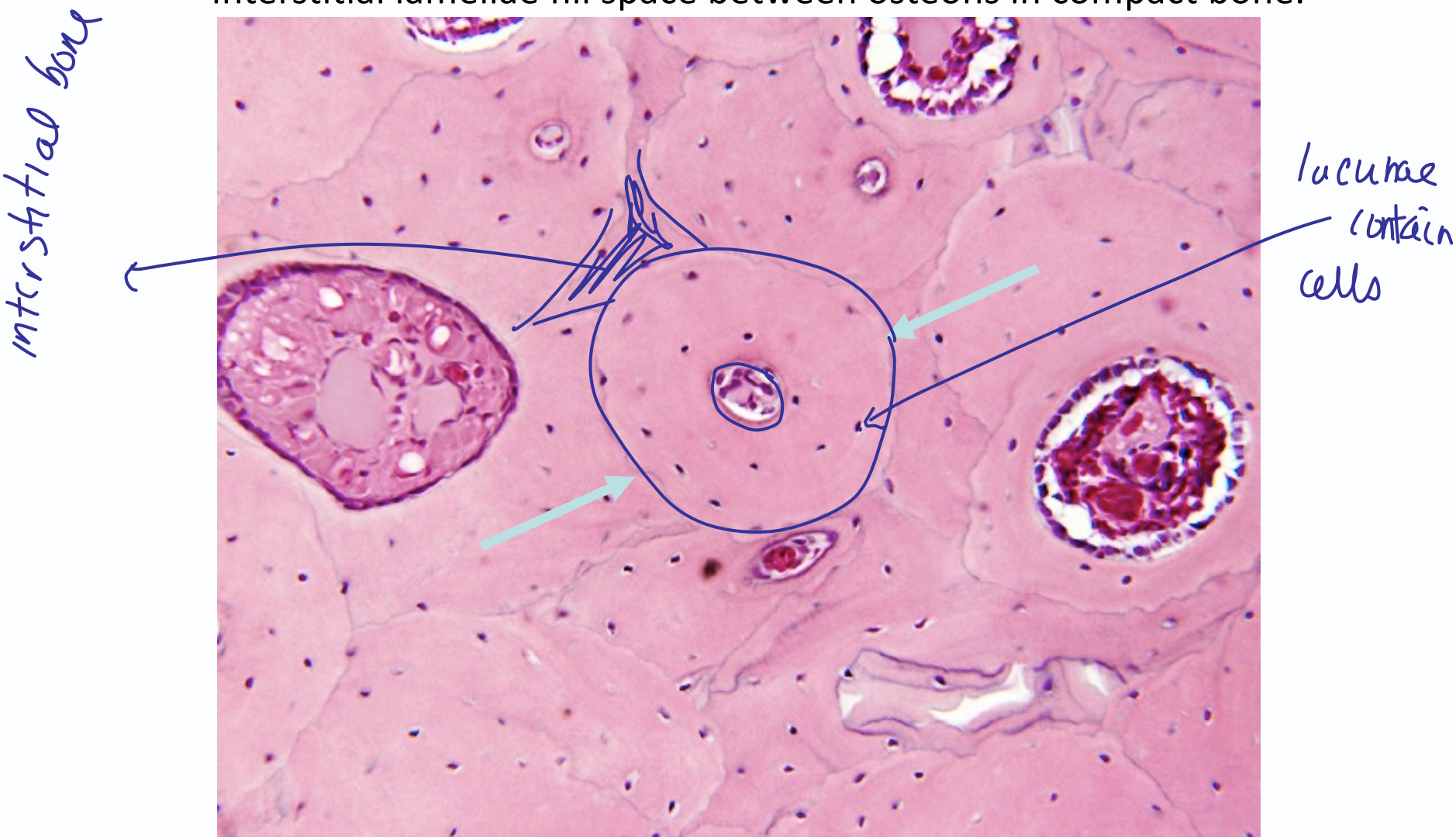


Fig. 2. A schematic diagram illustrating the assembly of collagen fibrils and fibers and bone mineral crystals. The well known 67 nm periodic pattern results from the presence of adjacent hole (40 nm) and overlap (27 nm) regions of the assembled molecules.

Rho et al 1998

Histology of an osteon and interstitial lamellae within Compact Bone

Interstitial lamellae fill space between osteons in compact bone.



Trabeculae **are the structural units of *trabecular bone***

- Trabecula is singular
- Trabeculae can be in the form rods or plates
- ~50-300 microns.
- Avascular
- woven or lamellar bone.
- lamellae typically run parallel to the trabecula that contains them

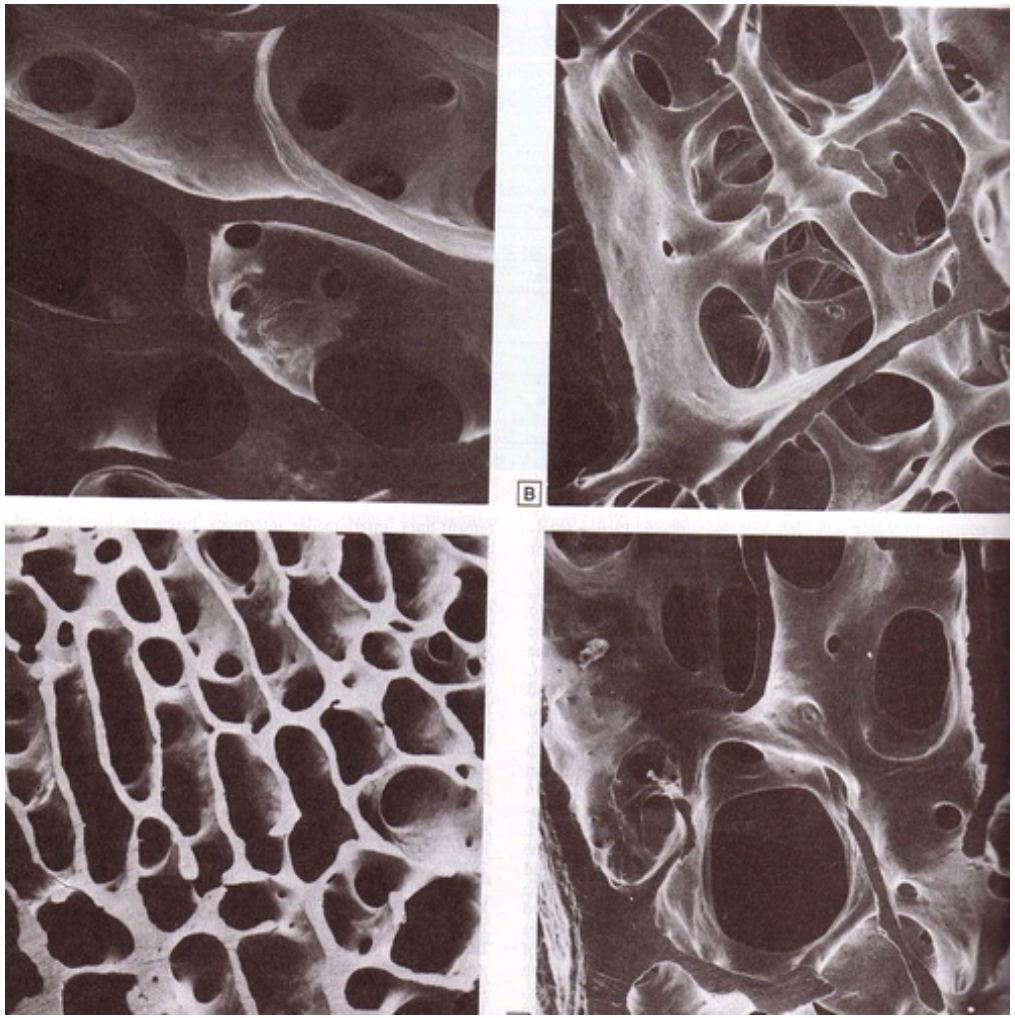
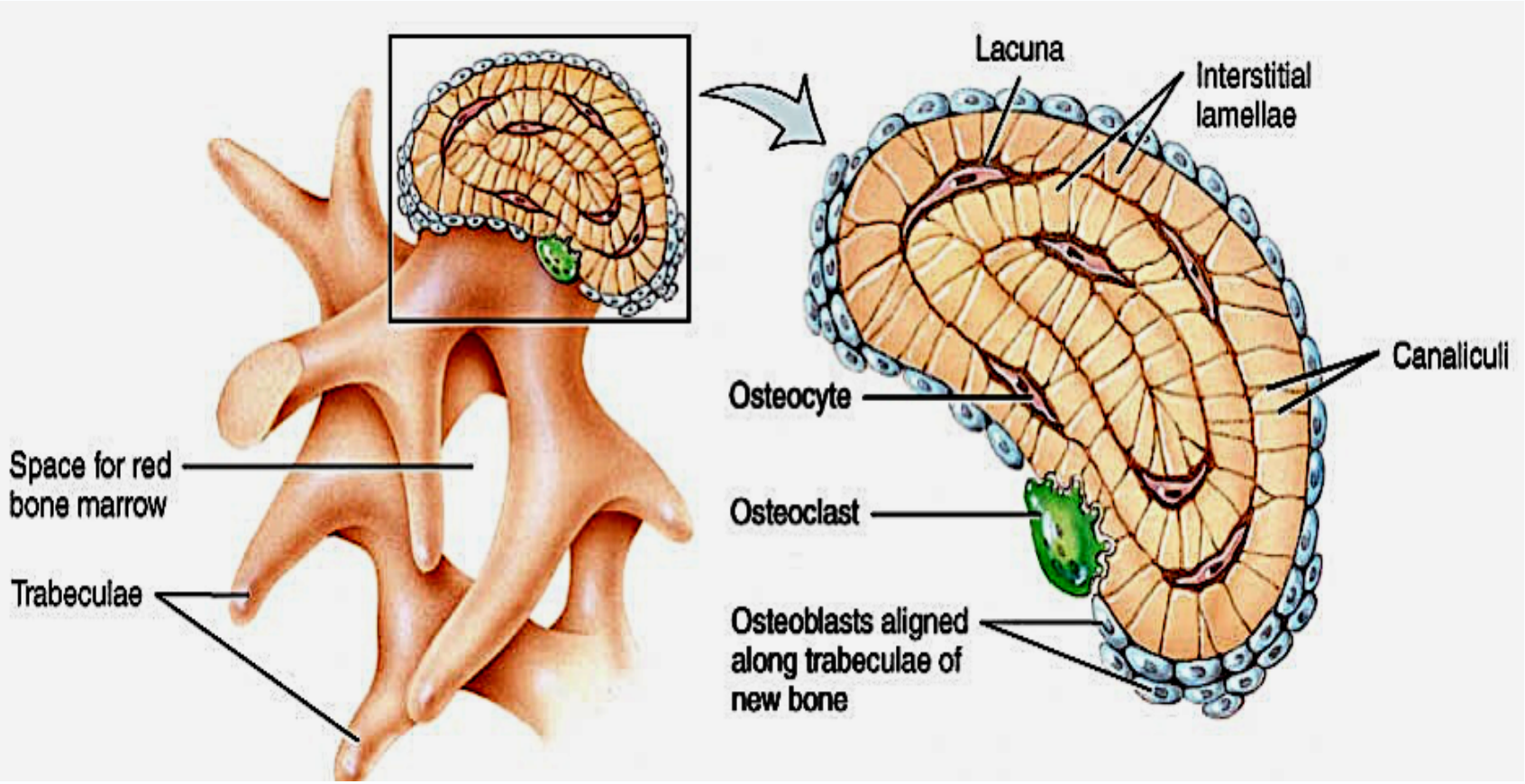
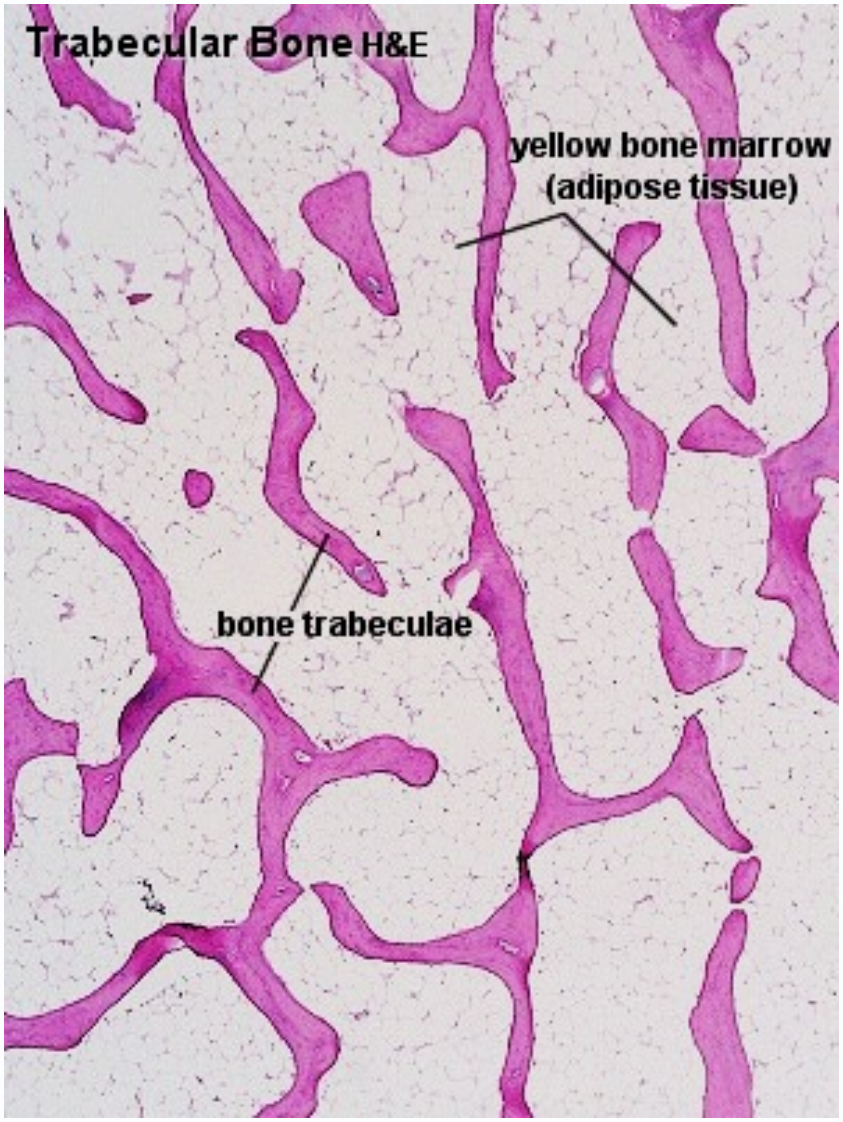


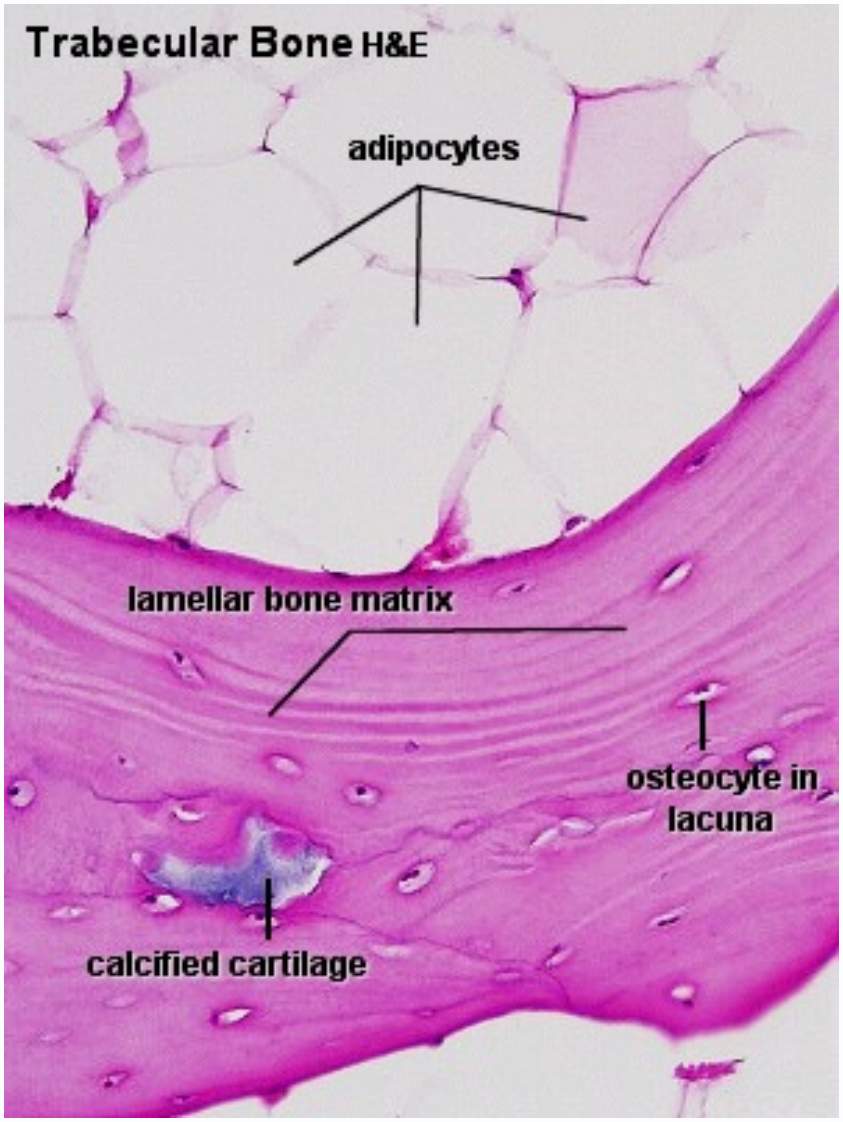
Image obtained from Gray's Anatomy, Churchill Livingstone Publishers, 38th Edition, 1995

Schematic of trabecular bone and cross-section



Histology of Spongy Bone
(also called trabecular or cancellous bone)





http://www.lab.anhb.uwa.edu.au/mb140/CorePages/Bone/Bone.htm

TABLE 1.4 Comparison of Cortical and Trabecular Bone Volume and Bone Surface

Factors	Cortical	Trabecular
Fractional volume (mm^3/mm^3)	0.95	0.20
Surface/bone volume (mm^2/mm^3)	2.5	20
Total bone volume (mm^3)	1.40×10^6	0.35×10^6
Total internal surface (mm^2)	3.50×10^6	7.0×10^6
Forming (%)	3.0	6.0
Resorbing (%)	0.6	1.2
Quiescent (%)	96.6	92.8
Function	Primarily biomechanical, supportive	Mainly mineral homeostasis, supportive

Adapted from Bone Mechanics Handbook, Cowin

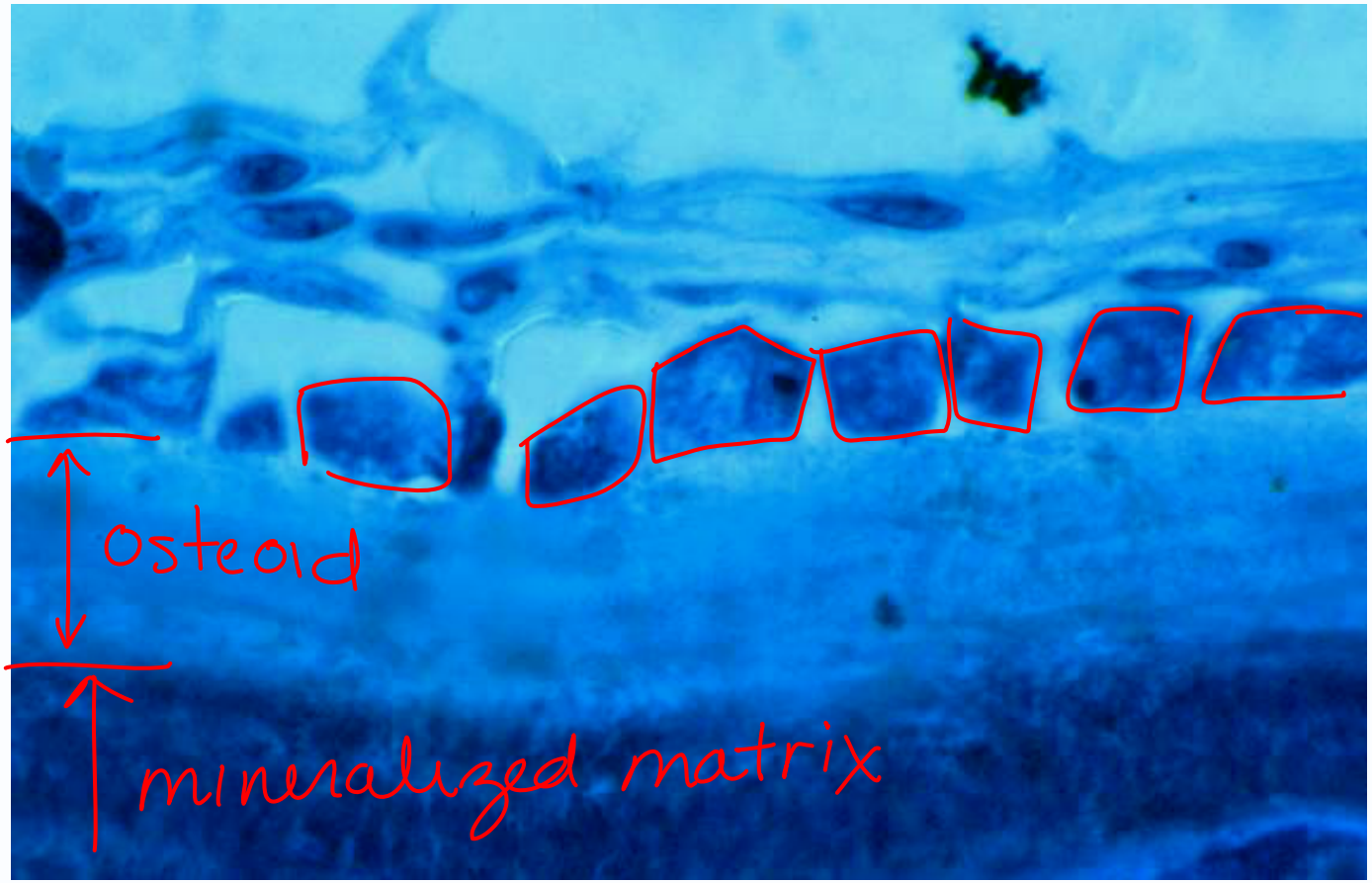
Summary of Cortical and Cancellous Bone Structural Length scales

Structure	Size
Whole bone	mm - m
• Osteons (in cortical) • Trabeculae (in cancellous) *trabecula is singular*	• ~100 - 300 microns • ~50-300 microns • Length scale dependent on trasport of nutrients and waste by diffusion.
• lamellae • Lacunae • Cement lines	• 3 – 20 microns • 10-20 microns x 5-10 microns (oblate spheroid) • 1-2 microns
• Collagen – Mineral Composite	10's - 100's nm

Bone Cells

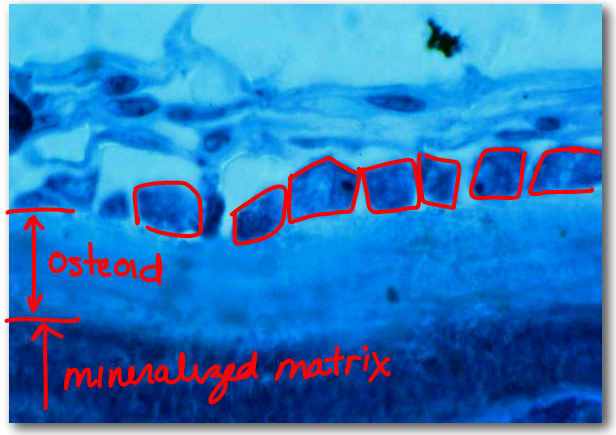
- Osteoprogenitor cells
  - in marrow or periostium
  - "stem cells" for bone
  - differenitate into osteoblasts (OBs)
- Osteoblast (OB) – bone forming
- Bone lining cell (BLC) – inactive osteoblasts
- Osteocyte (OC) – mature osteoblasts
- Osteoclast (OCL) – bone resorbing

Osteoblasts (OB)



Eurell

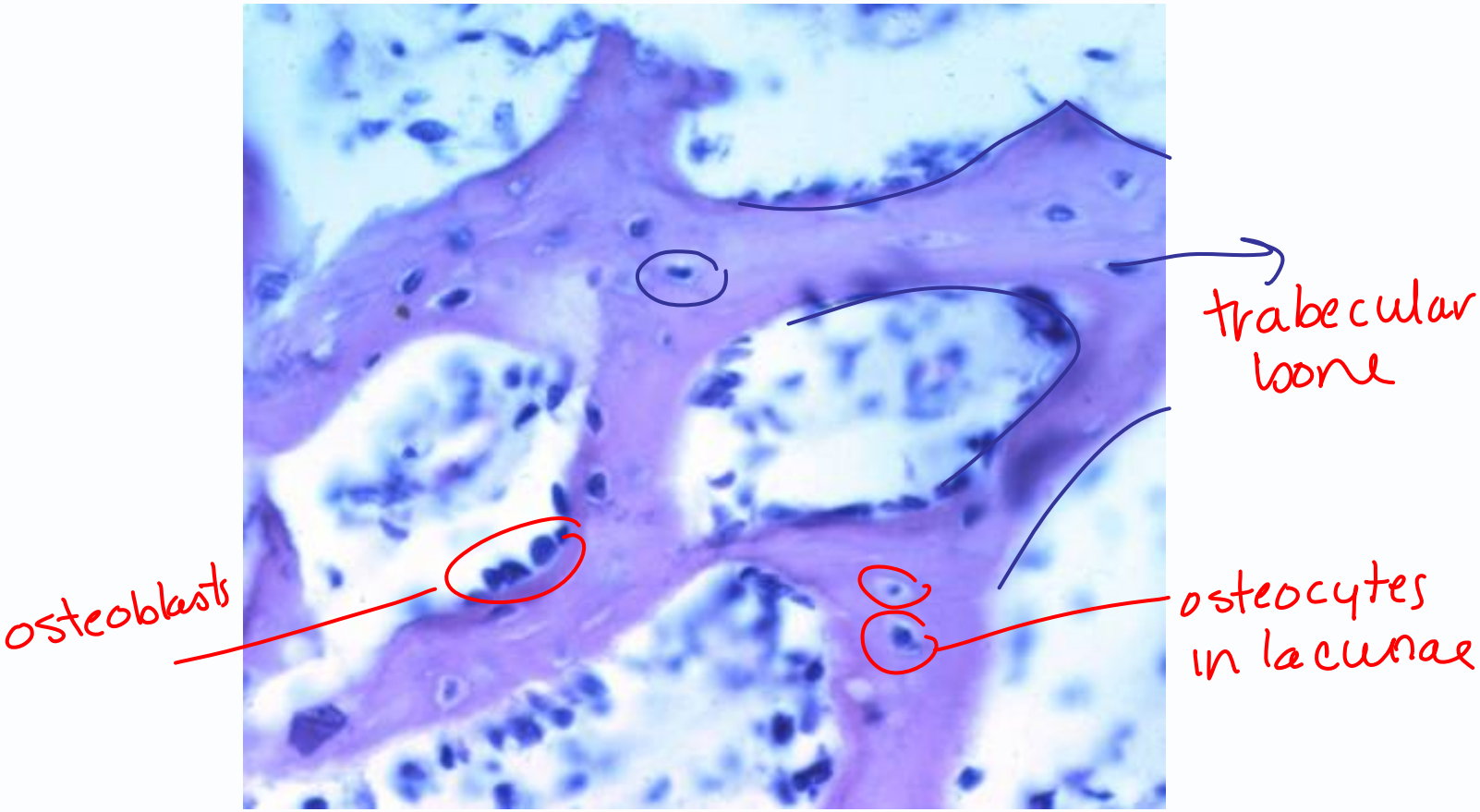
Osteoblasts (OB)



Eurell

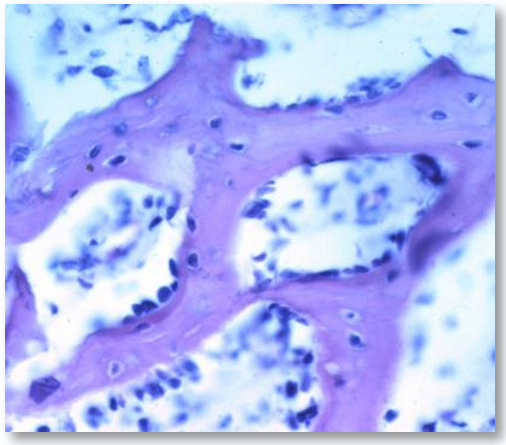
- Mononuclear, cuboidal, on surface of bone
- Derived from osteoprogenitor cells in marrow or the periostium
- Regulate mineralization, but the mechanism is not understood.
- Synthesize and secrete osteoid (unmineralized bone matrix)
  - Osteoid consists of mostly type I collagen + ground substance + water
  - Osteoid is “reinforced” by mineral during calcification.
  - Bone apposition rate (growth rate on a surface) ~ 1 micron/day

Osteocytes (OC)



Eurell

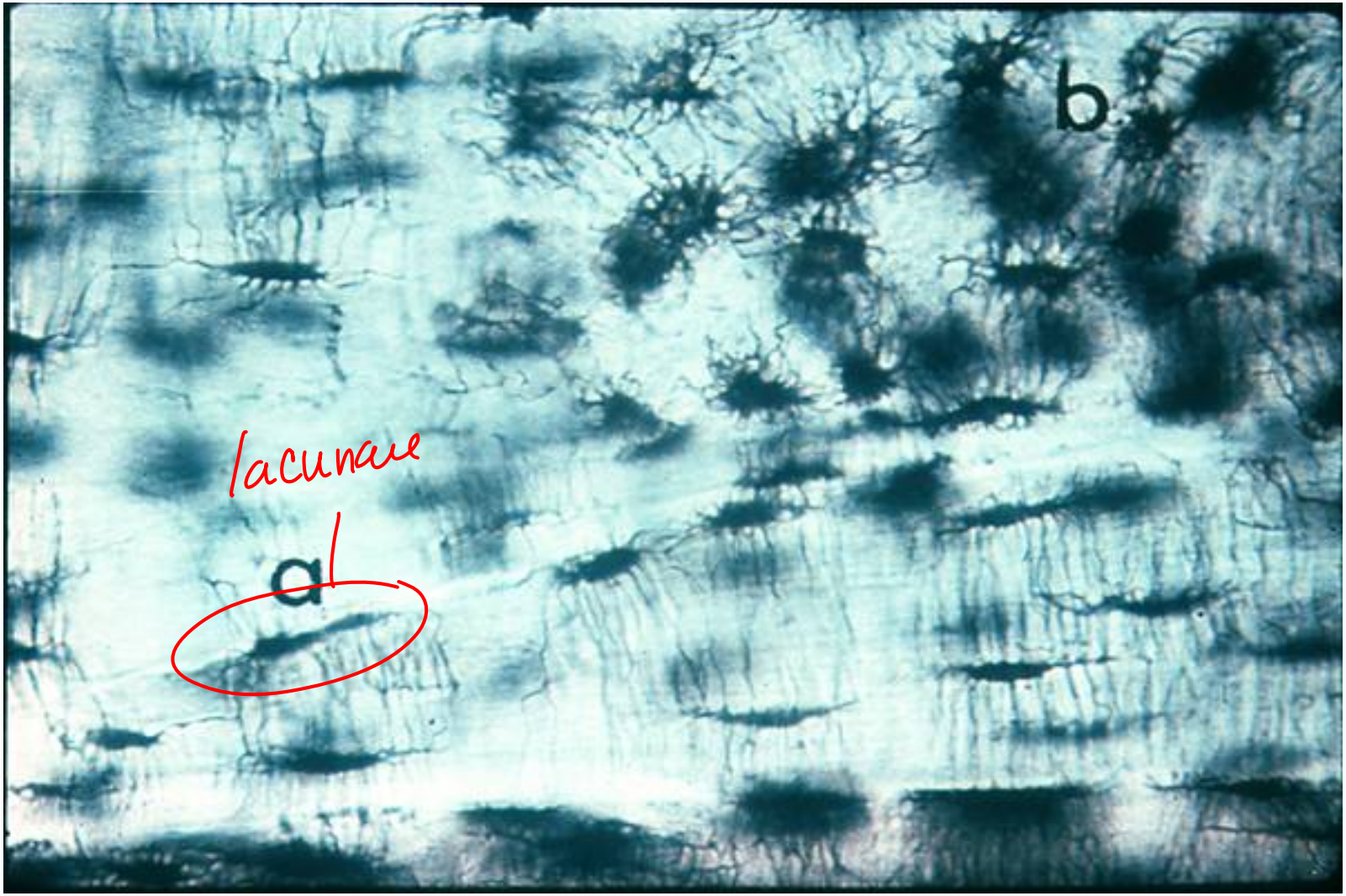
Osteocytes (OC)



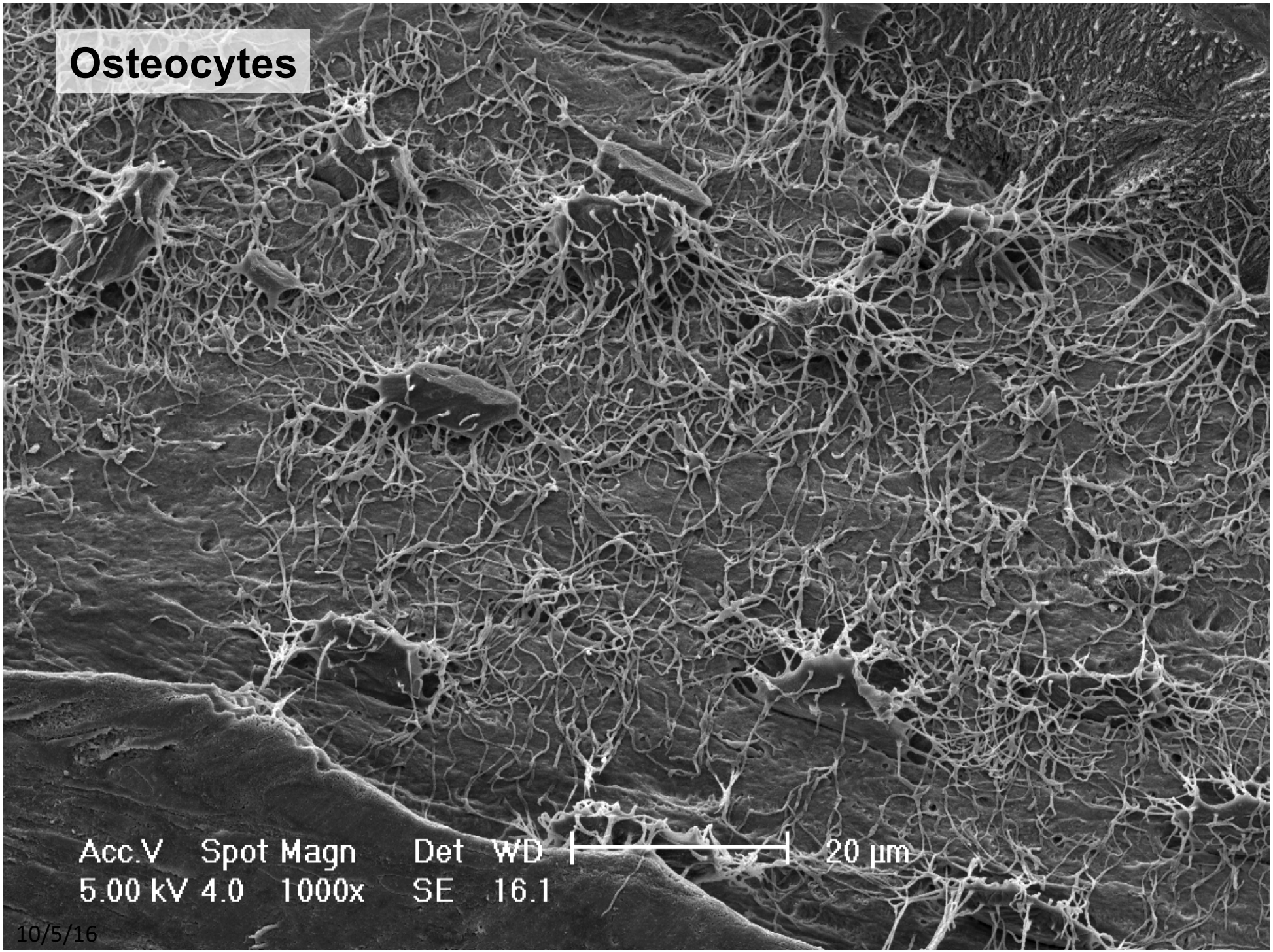
Eurell

- Former OBs that have since been buried.
- Housed in lacunae with cell processes extending through ECM via canaliculi (SEE NEXT SEVERAL SLIDES).
- Processes between cells connect via gap junctions, through which they communicate and exchange 'substances.'
- There are 15,000 lacunae (or osteocytes) per mm^3 of bone.
- Canaliculi and lacunae occupy only ~1% of bone volume.
- Surface areas
  - canaliculi is estimated to be 1200 m^2,
  - haversian and Volkman's canals occupy ~3 m^2
  - cancellous bone surfaces occupy 9 m^2.

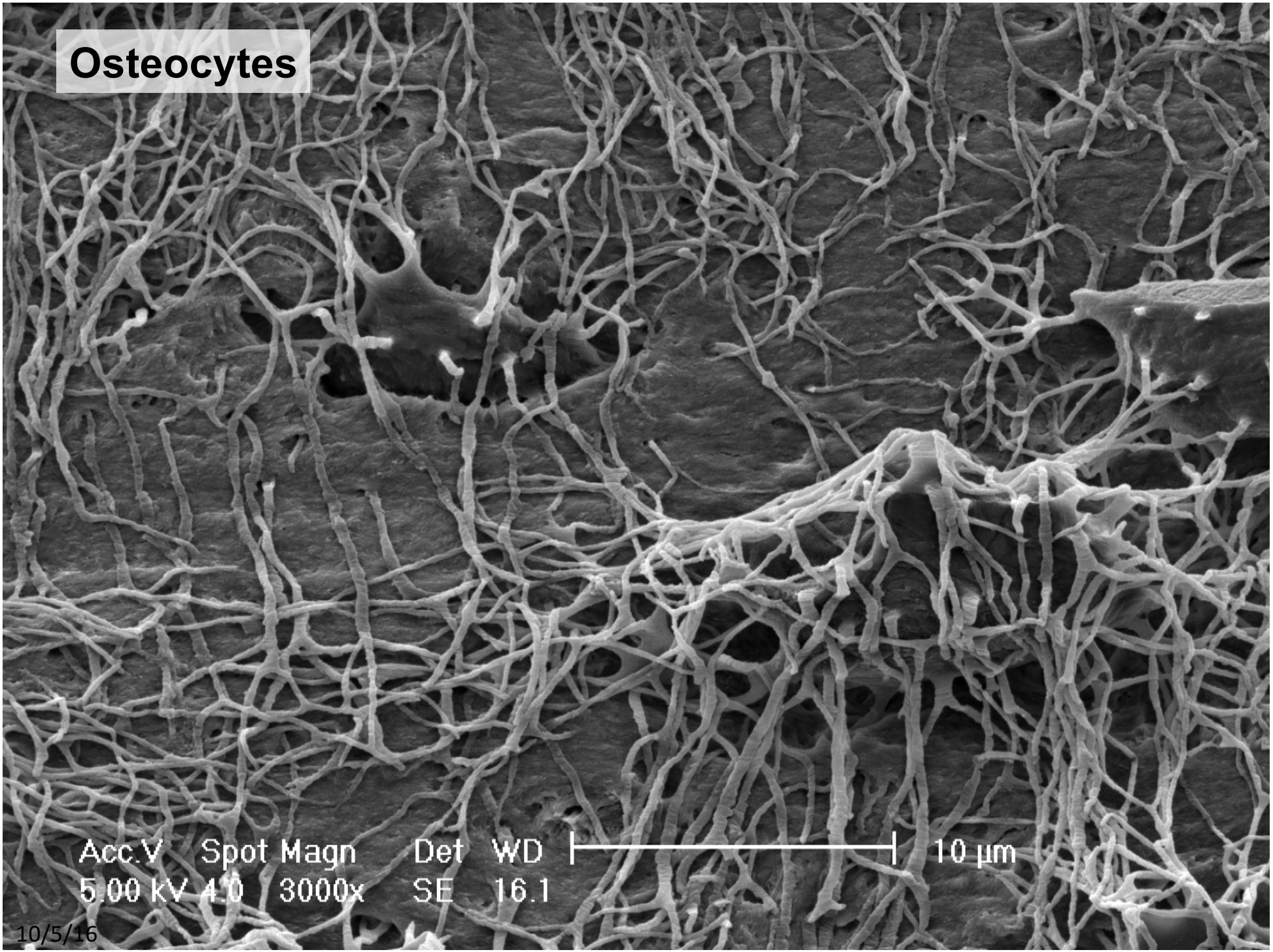
Osteocytic Lacunae and Canaliculi

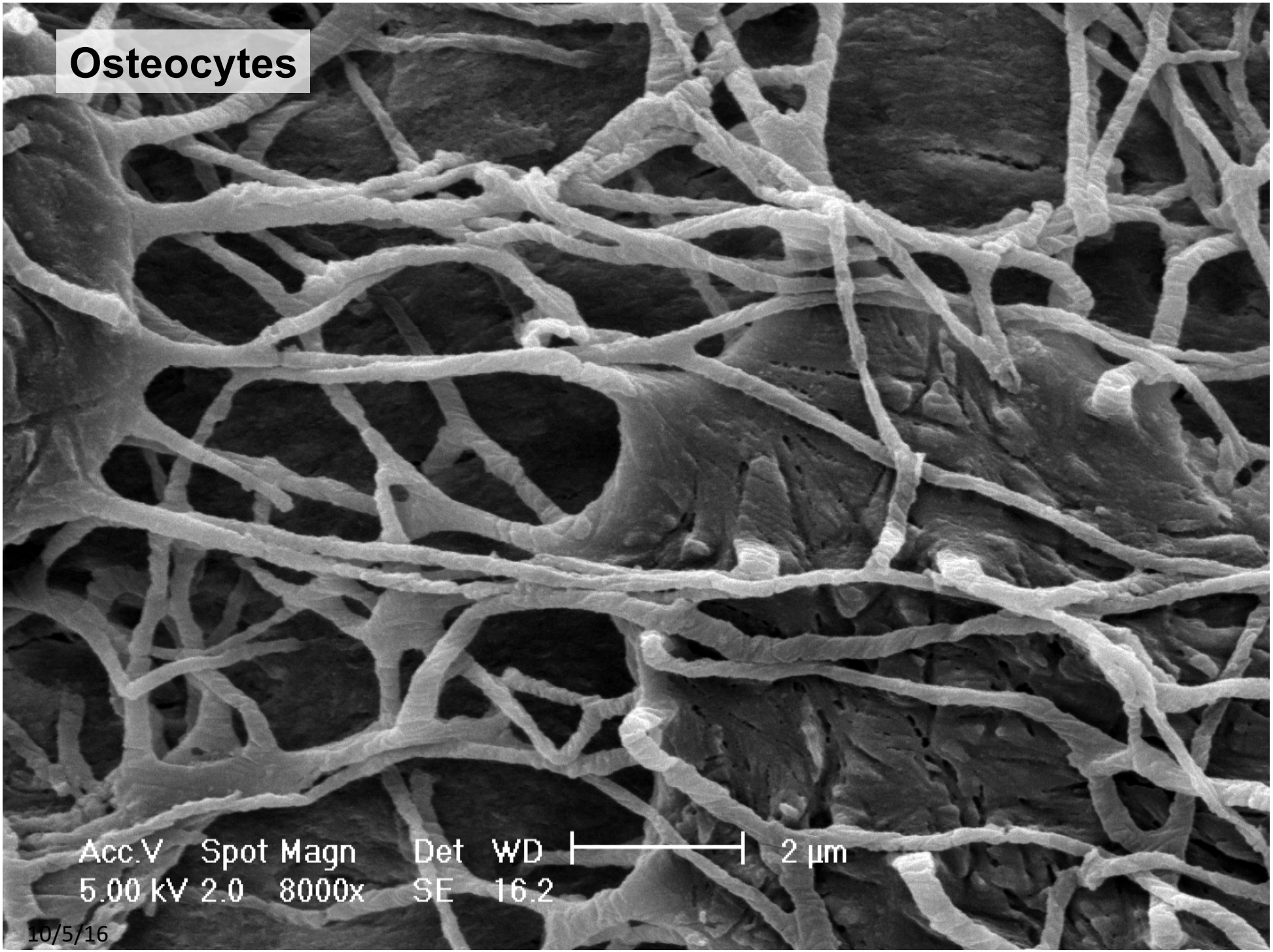


A Visual Approach to Histology Eurell

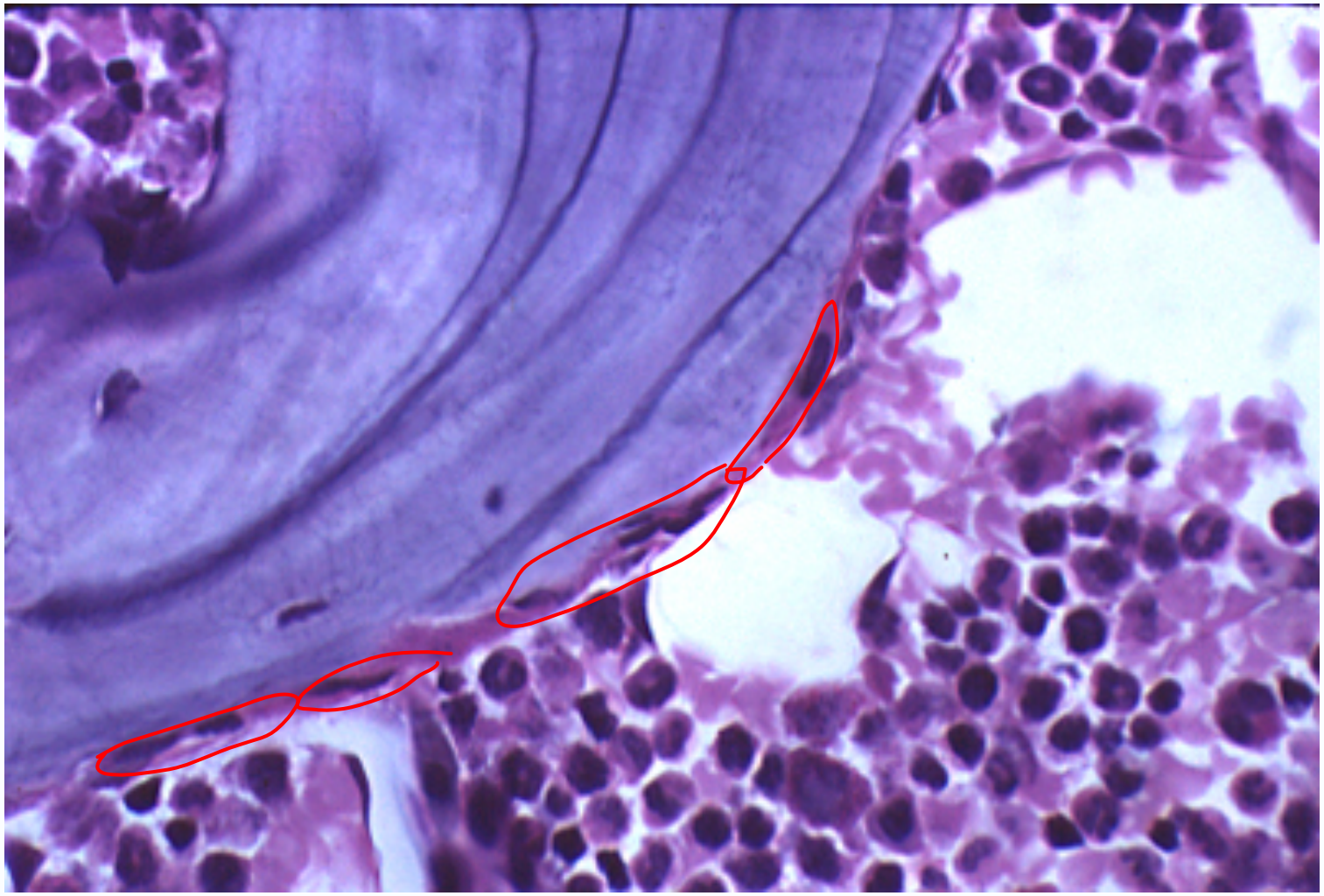

Osteocytes
Acc.V Spot Magn Det WD
5.00 kV 4.0 1000x SE 16.1
20 µm

Osteocytes



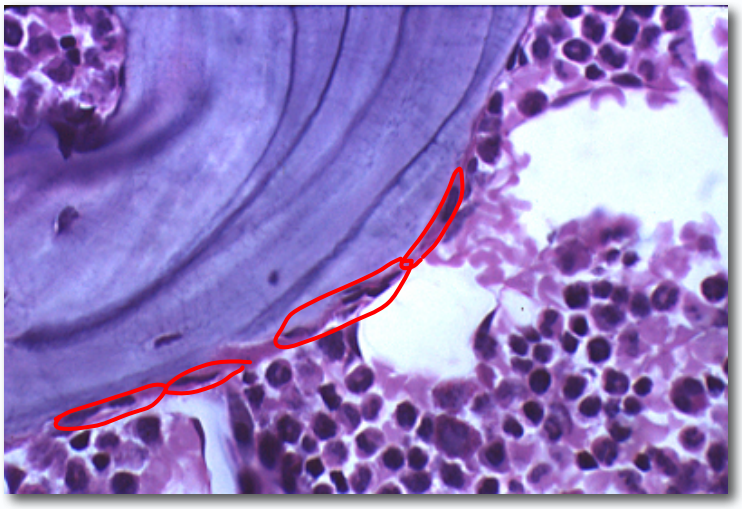

Osteocytes
Acc.V Spot Magn Det WD
5.00 kV 2.0 8000x SE 16.2
2 µm

Bone lining cell (BLCs)



Eurell

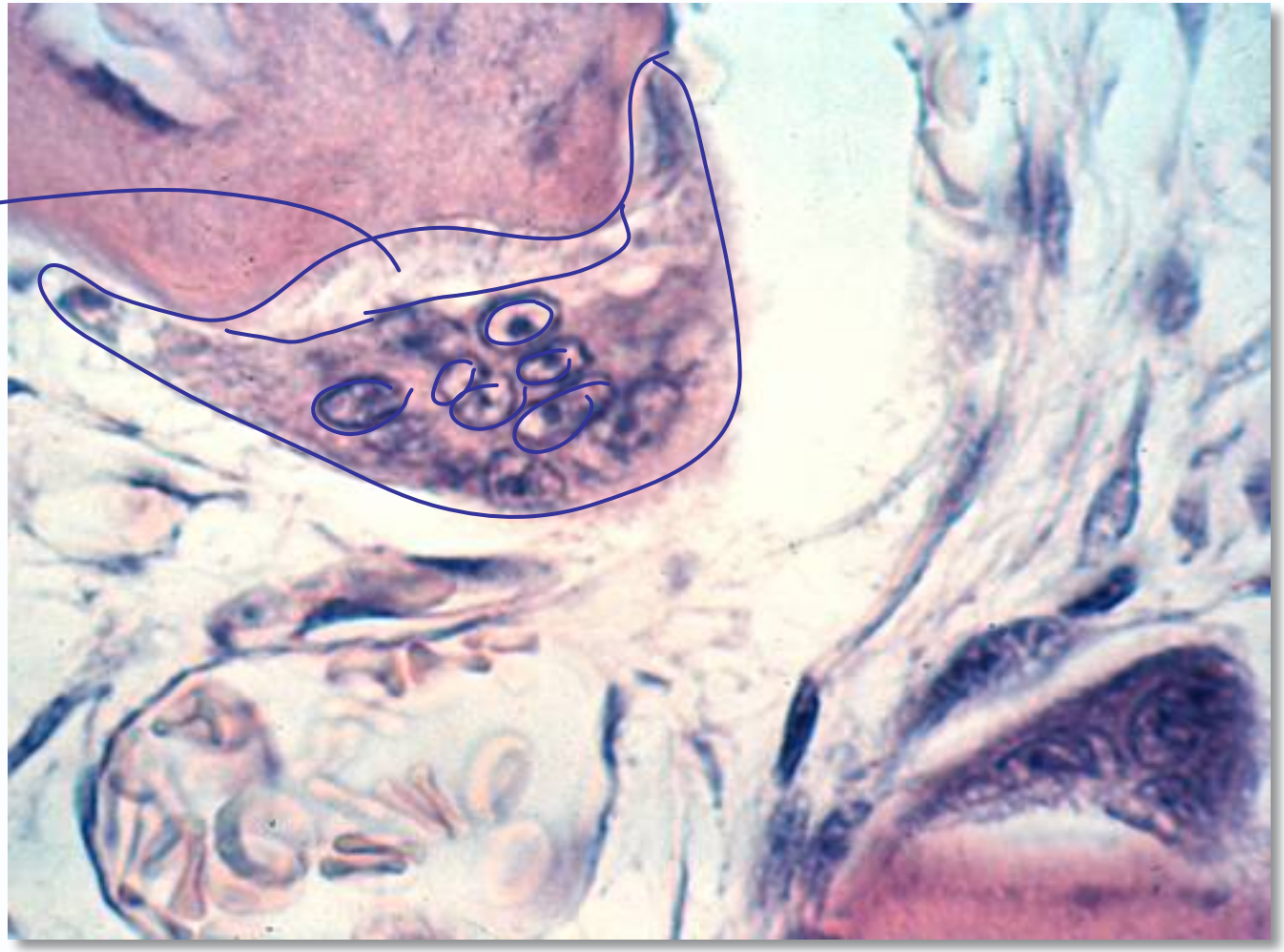
Bone lining cell (BLCs)



Eurell

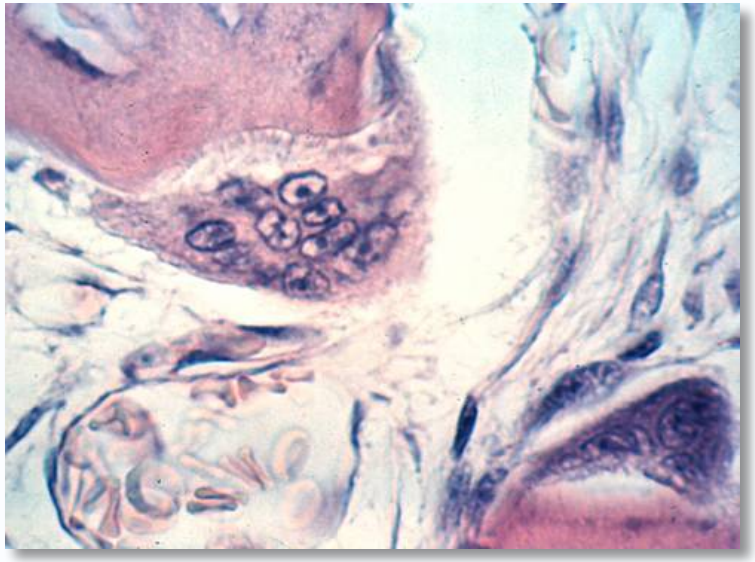
- Flattened spindle-shaped cell on bone surface
- Quiescent OBs
- Can divide and differentiate into OB if needed
- Cells that were not buried in matrix when bone formation stopped
  - remained on the bone surface
  - ***1/10 OB become OCs, rest BLCs***
- Flatten against the bone when not producing osteoid, but not continuous over the surface.
- Able to maintain communication via processes
- Also thought to be mechanosensors.

Osteoclasts (OCL)



Eurell

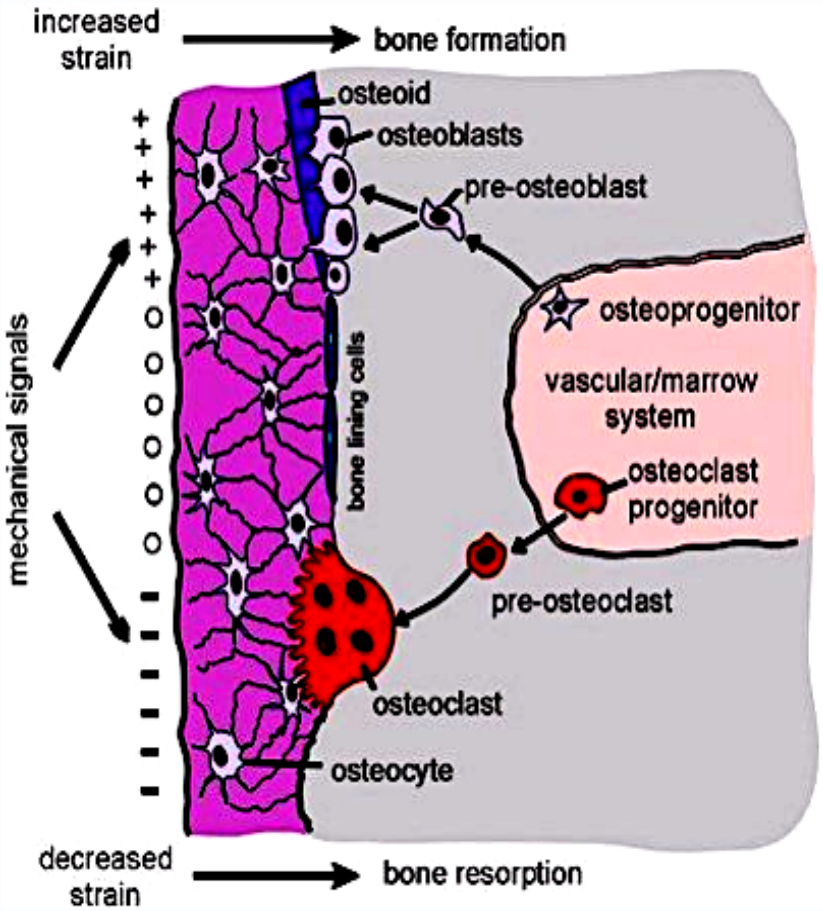
Osteoclasts (OCL)



Eurell

- Related to macrophages
  - cells that remove debris in the body.
- large, multi-nucleated cells
- originate in the bone marrow
- ruffled border and seal-off a region of bone.
- demineralize the matrix
  - acid
  - then dissolve the collagen using enzymes
  - removing bone at a rate of 10's um/day
  - recall apposition rate 1um/day

Simple model for bone formation – stimulated by mechanical strain



- **Signal – increase in strain**
  - Osteoprogenitors (aka preosteoblasts)
  - Differentiate to OBs
  - Produce matrix, then mineralize
  - Differentiate into OCs
- **Signal – decrease in strain**
  - Osteoclast progenitor (aka preosteoclasts)
  - Differentiate to multinucleated cell – OCL
  - Resorb bone
- **Steady-State Signal**
  - Quiescence – BLCs

Bone composition – Organic matrix

- **Collagen type I: 25-30% *dry* wt bone**
  - Provides sites for nucleation - Believed to be a template for mineral deposition
  - Provides tensile strength and flexibility
- **Ground Substance: ~5% *dry* wt bone**
  - Protein polysaccharides or glycosaminoclycans (GAGs)
  - GAGs between layers of mineralized collagen fibers
  - Proteoglycans (PGs) that can play a role in collagen assembly
- **Non collagenous proteins (examples – there are MANY!)**
  - osteocalcin: manufactured only by osteoblasts (bone building cells) - presence indicates that bone is being formed and can be used to measure bone formation rate
  - osteopontin: late stage marker for osteoblast differentiation (to osteocytes)
- ***WET* WEIGHT**
  - 70% MINERAL
  - 20% ORGANIC (18% COLLAGEN, 2% NONCOLLAGENOUS PROTEINS)
  - 10% WATER

Bone composition – Mineral (general)

- 60-70 *dry* wt% in bone
- Gives bone strength and stiffness
- hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ or apatite-like material
  - single crystal rods or plates plus some poorly crystalline material
  - Nanometer-sized
  - mineral also contain impurities
    - carbonate CO_3^{2-}, fluoride F^-, citrate $C_6H_5O_7^{4-}$
    - affect solubility of bone mineral.

Differential thermal analysis – Based on mass loss at different Ts

- Water at 110C (measure dry mass after this step)
- Organic matrix at ~600C
- Carbonate at ~900C
- Residual is called ASH after organic burnout
  - most common measure of mineral content
  - Ash fraction = mass of ash/dry mass
- 60% of the volume of water is bound to collagen, the rest is free
- Mineralization of osteoid displaces some of the water, therefore water content continuously changing

Bone composition – Mineral (human vs bovine)

TABLE 5.1 The Chemical Composition of Bone

	Bovine Cortical Bone (wt% of whole bone)	Range in Literature for Healthy Adult Whole Bone (wt% of ash)
Calcium	26.7 ± 0.15	32.6–39.5
Magnesium	0.436 ± 0.009	0.32–0.78
Sodium	0.731 ± 0.015	0.26–0.82
Potassium	0.055 ± 0.0009	—
Strontium	0.035	—
Phosphorus	12.47	13.1–18.0
Carbonate	3.48	3.2–13
Citrate	0.863	0.04–2.67
Chloride	0.077	—
Fluoride	0.072	0.02–0.207[a]

[a] Additional data from Zerwekh.[12]

Source: Modified from Driessens, F. C. M. and Verbeeck, R. H. M., *Biominerals*, CRC Press, Boca Raton, FL, 1990, tables 10.5 and 10.6. With permission.

Cowin Bone Mech Handbook
10/5/16

Factors Affecting Bone Mineral

- Mechanical properties vary with mineral content and density, but not all bone mineral is the same
- Also mineral is important for regulation of mineral homeostasis – regulating ionic calcium and magnesium.
- There are variations in
  - Composition and crystal size
  - Spatial variations
    - Periosteal to endosteal surfaces
    - Within single osteon –
      - mineral/matrix increases from center,
      - crystal "quality" decreases from center (most recently deposited mineral has lower quality)
    - Outer surface of trabecula to inside
  - Age-related changes (i.e. time since formation)
      - Rapid periods of growth it is hypomineralized
      - Slow periods – hypermineralized
  - Person age
  - Gender differences
  - Ethnicity

Variations with age, species etc.
(There are tables at the end of these slides. We won't go through in detail in class).

- **Differences with Age**
  - Ash weight increases (what is left after organic burnout)
  - Mineral to matrix ratio increases (which increases with ash weight)
  - Mineral crystal size in early stages of development increases anisotropically, rate slows with maturity
  - Carbonate content increases
  - Most recently deposited mineral (eg in osteon vs interstitial regions) is smallest, least perfect crystal, lowest carbonate content
- **Differences with gender (just a couple of examples, there are more differences)**
  - Bone density generally greater extent in males
  - Bone mineral content and mineral properties during pregnancy and lactation can change (bone is more fragile)

Factors Affecting Bone Mineral – Differences with Diet

Take away

- Ca and D deficiency
- Differences with Diet – chemical composition reflects dietary history
  - Malnutrition
    - Decrease bone mineral density
    - Decrease Mineral content
    - Change mineral composition and crystal size
  - Some dietary supplements can substitute for Calcium: Sr, Ga, Mg
  - Vitamin D deficiency (or changes in Ca P that depend on vit D)
    - Reduces mineral content
    - Increase mineral crystal size
    - Generally more brittle bones
  - Vitamin B6 deficiency – affects collagen synthesis
    - B6 deficient chick has no change in mineral content, crystal size, mineral composition
    - but decreased elastic properties b/c of collagen structural changes
  - See Table 5.3 and Chapter 5 of Cowin's Bone Mechanics Handbook for more details. Chapter 5 will be posted on Piazza

Some diseases that change bone characteristics

TABLE 2 TYPICAL MINERAL CHANGES IN HUMAN BONE AND DENTAL DISEASES RELATIVE TO AGE AND SEX-MATCHED HEALTHY INDIVIDUALS

Disease	Description	Prevalence	Mineral Content	Crystal Size	Other Features and Relevant Review
Osteoporosis	Increased porosity with tendency to fracture	High	Variable	Increased	Collagen maturity increased (Boskey et al. 2006)
Amelogenesis imperfecta	Impaired enamel mineralization	High	Decreased	Variable	Hypomineralization; oftenX-linked (higher prevalence in males) (Robinson et al. 2003)
Osteomalacia	Poorly mineralized bone with tendency to fracture	High	Decreased	Increased	Associated with vitamin D deficiency (Faibish et al. 2005)
Osteogenesis imperfecta	Brittle bone disease due to abnormal collagen synthesis	Low	Decreased	Decreased	Abnormal collagen gene expression (Zhu et al. 2007)
Osteopetrosis	Rock-like bone with increased tendency to fracture	Low	Increased	Decreased	Impaired bone remodeling (Boskey et al. 2006)
Osteonecrosis	Dead bone	Low	Increased	Variable	Lack of viable cells (Weinstein et al. 2000)
Renal osteodystrophy	Kidney malfunction leading to osteoporotic bone	Low	Decreased	Increased	(Sanchez 2006)

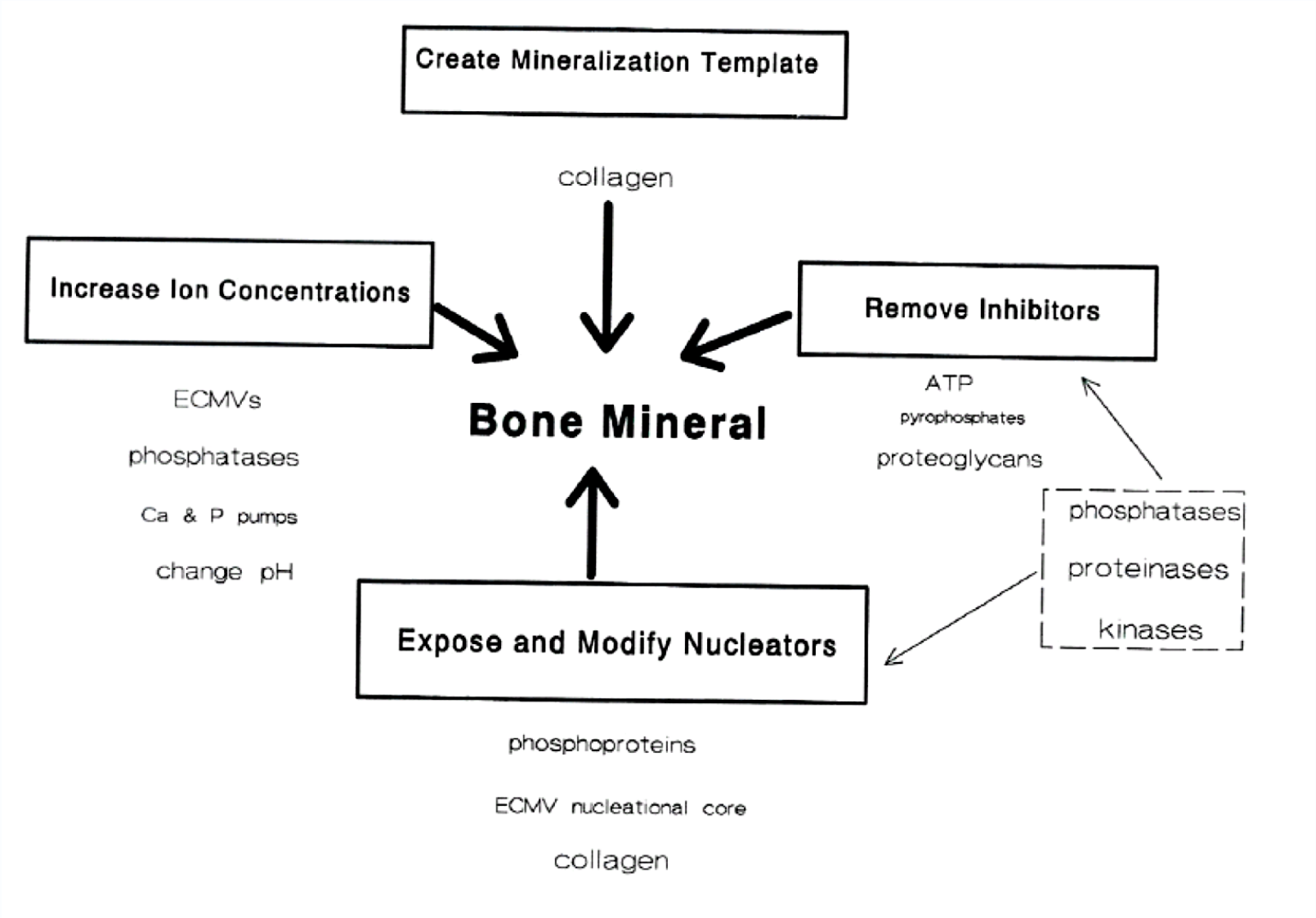
From A. Boskey, Elements, Vol 3, 2007

SOME other Bone Diseases Related to Mineral Variation

- Untreated diabetes
  - Crystal size and CaP ratio decrease
- Paget's disease – abnormal bone remodeling process
  - Crystal size decreased
- Osteopetrosis – bones are overly dense because of osteoclast malfunction
  - Crystal size, mineral content increased
- Osteogenesis imperfecta – brittle bone disease
  - Defective collagen fibers

Bone composition – Mineral formation schematic

(CH5 Cowin Bone Mechanics Handbook is good resource).



Bone composition – Mineral formation

(CH5 Cowin Bone Mechanics Handbook is good resource).

- Bone mineral formation occurs by nucleation and growth (like engineering materials), exact mechanisms are not understood.
- The deposition sites, or nucleation sites, are believed to be associated with collagen fibrils, and likely in the spaces between tropocollagen molecules in the fibrils. Other matrix proteins are also thought to help to nucleate mineral crystals.
  - Nucleation accelerated by increasing conc of component ions, increasing ion collision rate (temp eg), more nucleation sites…
- Mineral formation and growth are regulated by local ion concentration, removal of inhibitors (type I collagen is all over the body, but mineral crystals are not – there must be inhibitory mechanisms, PGs are known inhibitors), and appropriate nucleators.
- ECMVs are extra cellular matrix vessicles – pouches of ions ~70nm
- pH ~7.2-7.8 optimum

The next slides are table showing variations in mineral due to diet and other factors.

Bone Diseases Related to Mineral Variation

10/5/16

Part of Table 5.3 – Cowin Bone Mech Handbook

TABLE 5.4 Mineral Variation[a] in Bones of Diseased Animals

Disease /Site		Age	Ash (%)	Ca:P	Min:Mat	CO_3/PO_4	Size (Å)
			Hypophosphatemic (hyp) mouse				
Distal	hyp	35 d	41.4	1.64 ± 0.041	—	—	157
metaphysis	Control		60 ± 2	55 ± 0.1			151
Proximal	hyp	35 d	46 ± 1	1.67 ± 0.07			173
metaphysis	Control		61 ± 5	1.55 ± 0.1			151
Diaphysis	hyp	35 d	64 ± 6	1.71 ± 0.04			189
	Control		69.3 ± 0.8	1.5 ± 0.2			125
	hyp	12 wk	56.1 ± 0.1		1.01 ± 0.27	0.0036	168
	Control		68.4 ± 0.6		3.57 ± 0.52	0.0019	168
			Brachymorphic Mouse				
Diaphysi	bm/bm	10 wk	62			0.00353	178
	Control		60			0.00451	164
			Osteopetrotic Rats				
Metaphysis	ia/ia	11 d	42 ± 1.2				144
	Control		36 ± 1.2				113
	ia/ia	52 d	62 ± 1.2				149
	Control		46 ± 1.2				131
Calvaria	ia/ia	11 d	42 ± 2				138
	Control		43 ± 5				131
	ia/ia	52 d	62 ± 4				143
	Control		57 ± 1				143
Diaphysis	tl/tl	6 wk	65				
	Control		61				
			Diabetic Rat—Metaphysis				
	Experimental	Adult	67 ± 2	1.39 ± 0.07			173
	Control		64 ± 2	1.49 ± 0.09			185
			Ovariectomized primates—Vertebra, Trabecular Bone				
	Experimental	Adult		1.7	5.9 ± 1[c]	0.0137	189
	Control			1.8	6.8 ± 0.5	0.0095	202

[a] Parameters: ash weight (ash %) as percent of non-defatted dry weight, Ca:P molar ratio by analysis of the ash, mineral:matrix ratio (Min:Mat) and carbonate-to-phosphate ratio determined by FTIR, carbonate listed as (%) determined by chemical analysis and expressed as percent of dry weight, size refers to the c-axis (002) length as determined by X-ray diffraction line-broadening analysis.

[b] Crytallinity of tl/tl estimated from EPR to be 30 to 50% wild type.

Factors Affecting Bone Mineral – Differences with Diet

TABLE 5.3 Diet Alters Bone Mineral Composition[a]

Dietary Manipulation	Species/Site	Ash (%)	Ca:P(molar)	Size (Å)
Excess calcium (normal vitamin D)				
Experimental	Chick diaphysis	61 ± 1	1.55 ± 0.2	173
Control		61.9 ± 0.3	1.67 ± 0.04	161
Vitamin D deficiency (normal phosphate)				
Experimental	Chick diaphysis	45.9 ± 5	2.4 ± 0.5	193
Control (see above)				
Low phosphate diet (normal vitamin D)				
Experimental	Chick diaphysis	60.1 ± 0.6	1.78 ± 0.3	193
Control (see				

[a] Parameters: ash weight (ash %) as percent of non-defatted dry weight, Ca:P molar ratio by analysis of the ash, size refers to the c-axis (002) length as determined by X-ray diffraction line-broadening analysis.

Part of Table 5.3 – Cowin Bone Mech Handbook

Factors Affecting Bone Mineral – Differences with Diet

TABLE 5.3 Diet Alters Bone Mineral Composition[a]

Dietary Manipulation	Species/Site	Ash (%)	Ca:P(molar)	Size (Å)
Low phosphate diet (normal vitamin D)				
Experimental	Chick diaphysis	60.1 ± 0.6	1.78 ± 0.3	193
Control (see above)				
Calcium deficiency and vitamin D deficiency				
Experimental	Chick diaphysis	44.5 ± 1.3	1.90 ± 0.06	181
Control (see above)				
Vitamin D deficiency (+ low phosphate)				
Experimental	Rat metaphysis	40.3 ± 2		151
Control		54 ± 0.4		153
Experimental	Rat diaphysis	54 ± 2		153
Control		65.2 ± 0.4		146
Vitamin D deficiency (second generation)				
Experimental	Rat diaphysis	62 ± 1		144
Control		63 ± 1		135
Vitamin B6 deficiency				
Experimental	Chick diaphysis	63 ± 6		169
Control		58 ± 3		173

[a] Parameters: ash weight (ash %) as percent of non-defatted dry weight, Ca:P molar ratio by analysis of the ash, size refers to the c-axis (002) length as determined by X-ray diffraction line-broadening analysis.

Part of Table 5.3 – Cowin Bone Mech Handbook

Factors Affecting Bone Mineral – Differences with Diet

TABLE 5.3 Diet Alters Bone Mineral Composition[a]

Dietary Manipulation	Species/Site	Ash (%)	Ca:P(molar)	Size (Å)
Trace element supplementation				
Strontium				
Experimental	Rat Metaphysis	55.5 ± 1.5		137
Control		60.0 ± 3.8		166
Gallium				
Experimental	Rat Metaphysis	54.5 ± 0.5		138
Control		56.8 ± 0.8		144
Mg deficiency				
Experimental	Rat Metaphysis	53 ± 4	1.50 ± 0.01	164 ± 11
Control		59 ± 4	1.59 ± 0.05	152 ± 7
Experimental	Rat Diaphysis	66 ± 4	1.63 ± 0.01	152 ± 17
Control		67 ± 2	1.60 ± 0.06	147 ± 7

[a] Parameters: ash weight (ash %) as percent of non-defatted dry weight, Ca:P molar ratio by analysis of the ash, size refers to the c-axis (002) length as determined by X-ray diffraction line-broadening analysis.

Part of Table 5.3 – Cowin Bone Mech Handbook