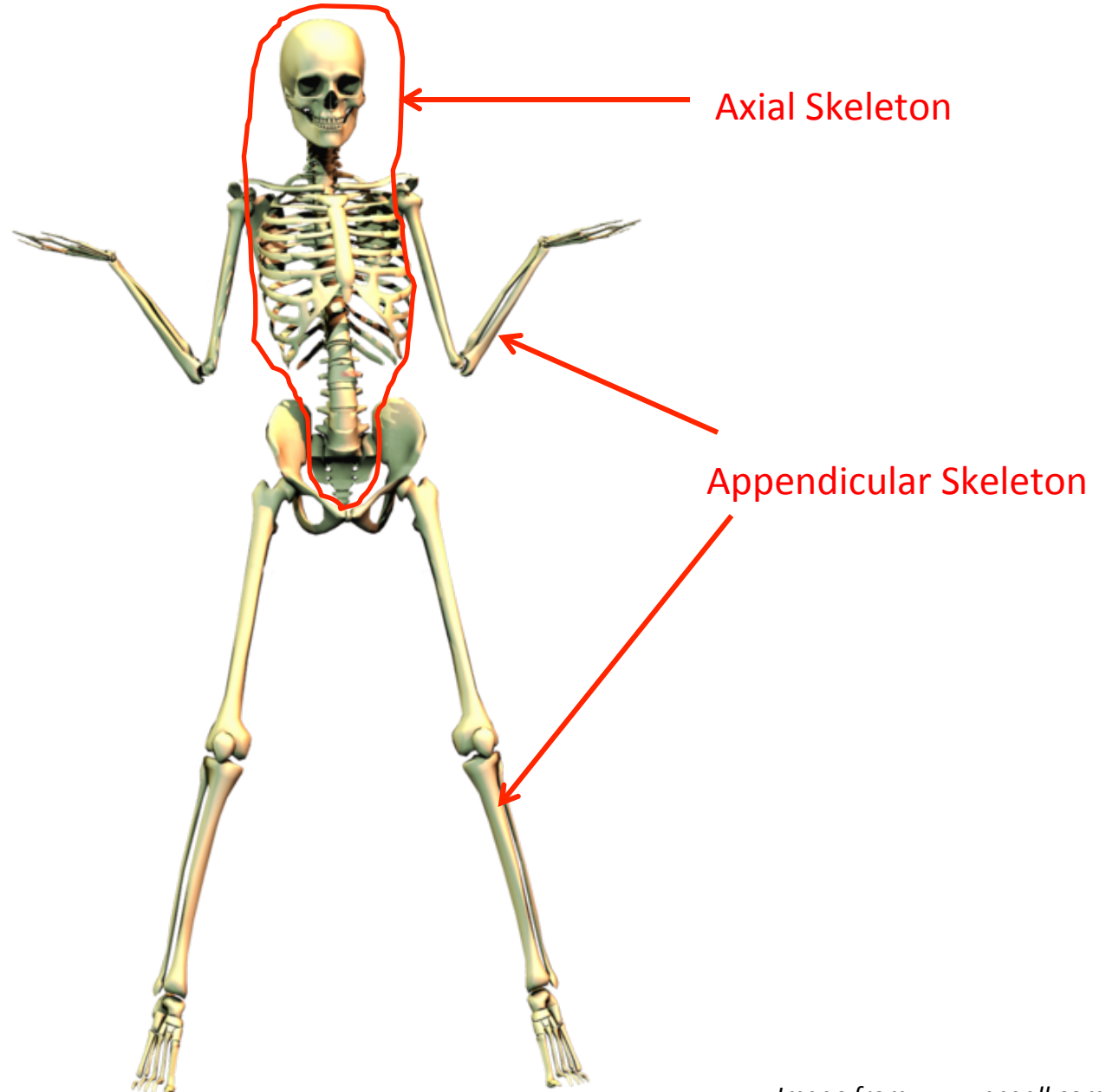# Bone Homeostasis and Pathology

Instructor: Roman Eliseev
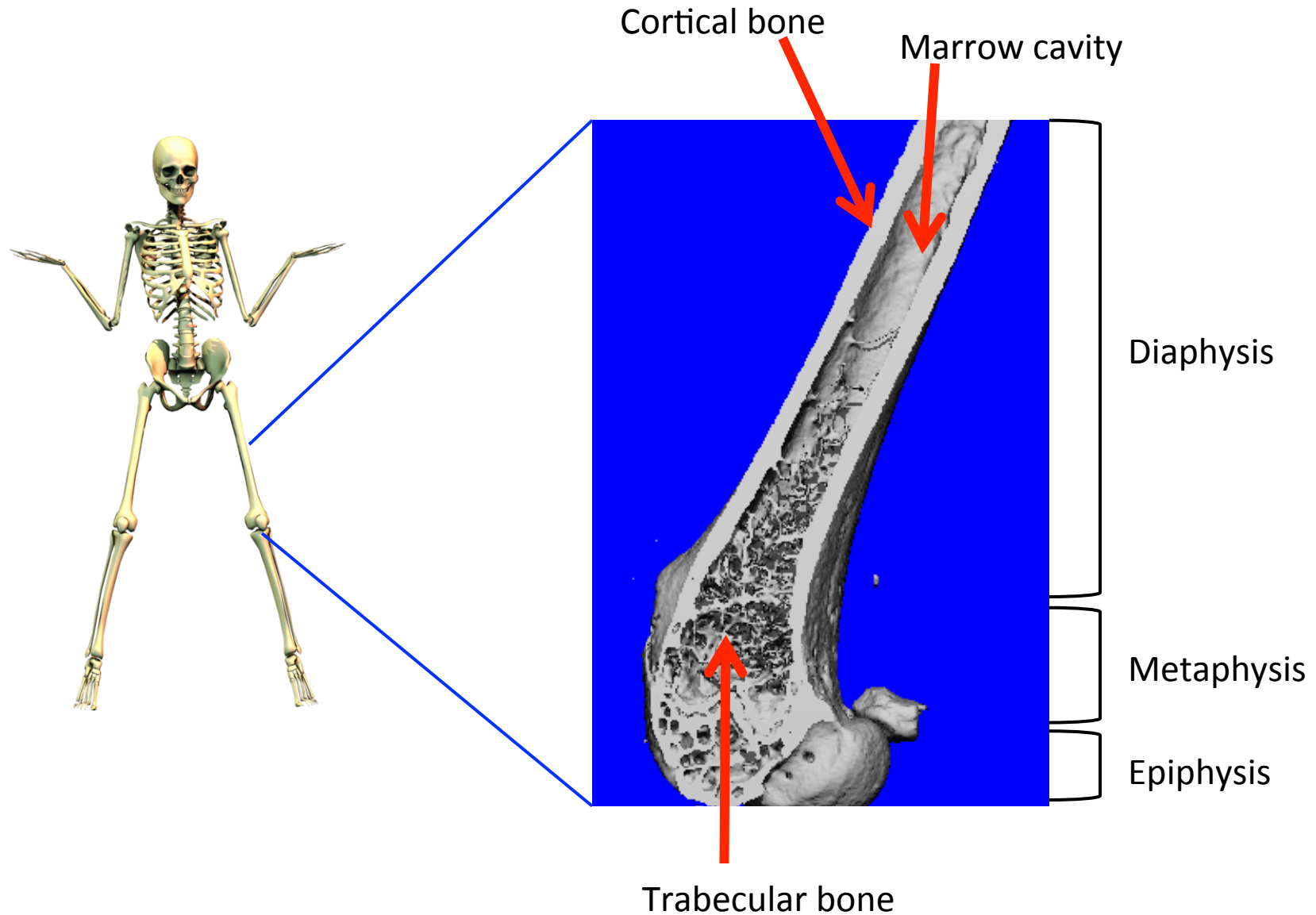
[Outline:](#)

- Bone anatomy and composition
- Bone remodeling
- Factors regulating bone homeostasis
- Disorders of bone homeostasis:
  - -Bone loss
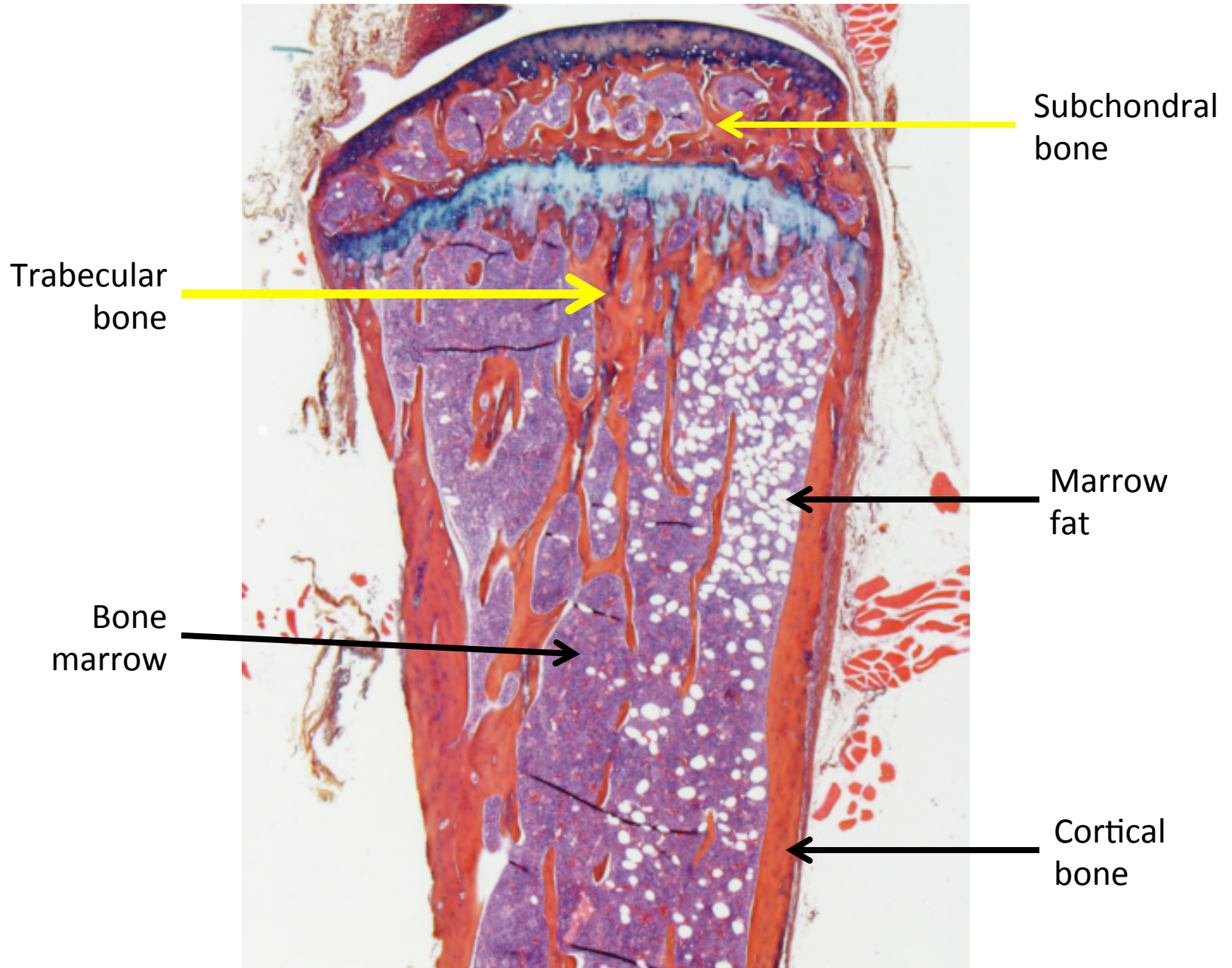 - -Abnormal bone acquisition
- Methods and Mouse Models

Adult Skeleton

Axial Skeleton

Appendicular Skeleton

206 bones

Adult Bone Architecture

Cortical bone

Marrow cavity

Diaphysis

Metaphysis

Epiphysis

Trabecular bone

Bone Histology

Subchondral bone

Trabecular bone

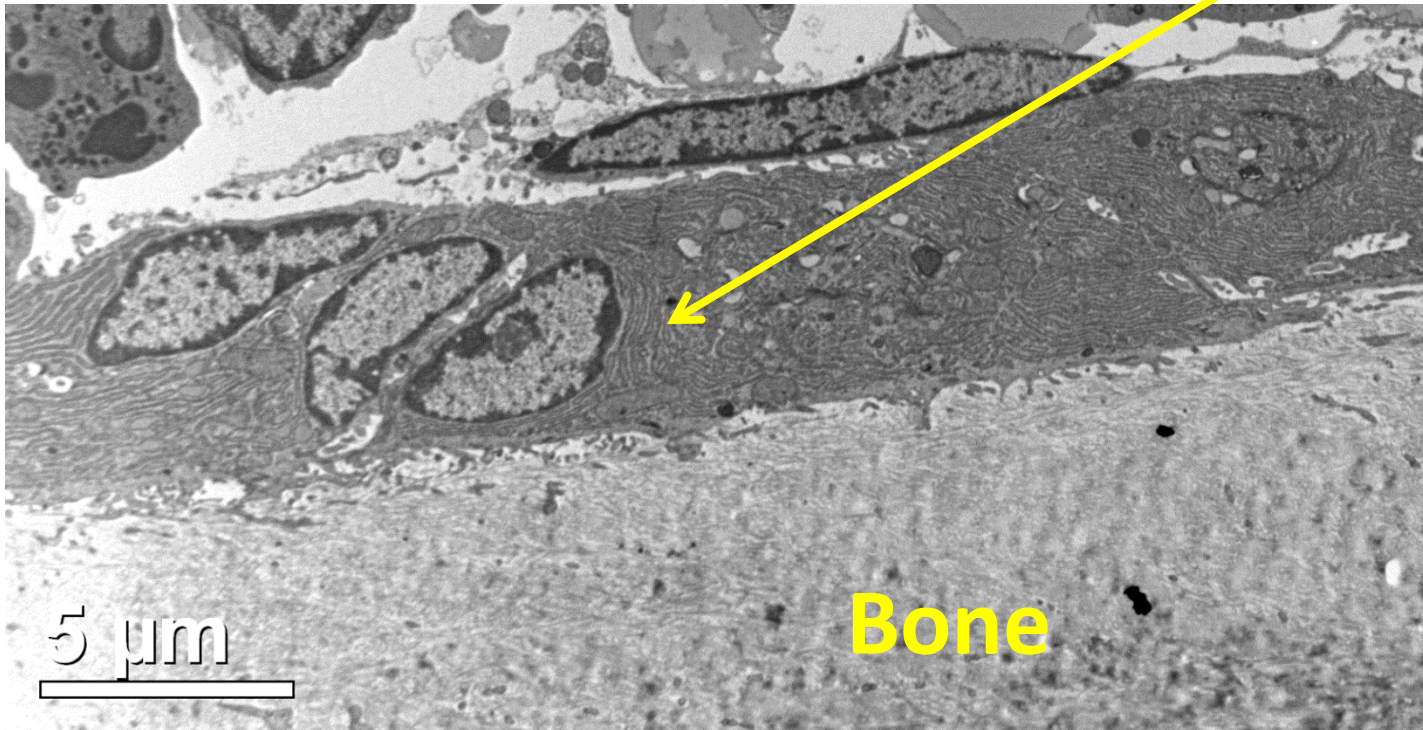Marrow fat

Bone marrow

Cortical bone

Bone Composition

Bone is a mineralized organic matrix composed of:

- Type I collagen and non-collagenous proteins (osteoid)
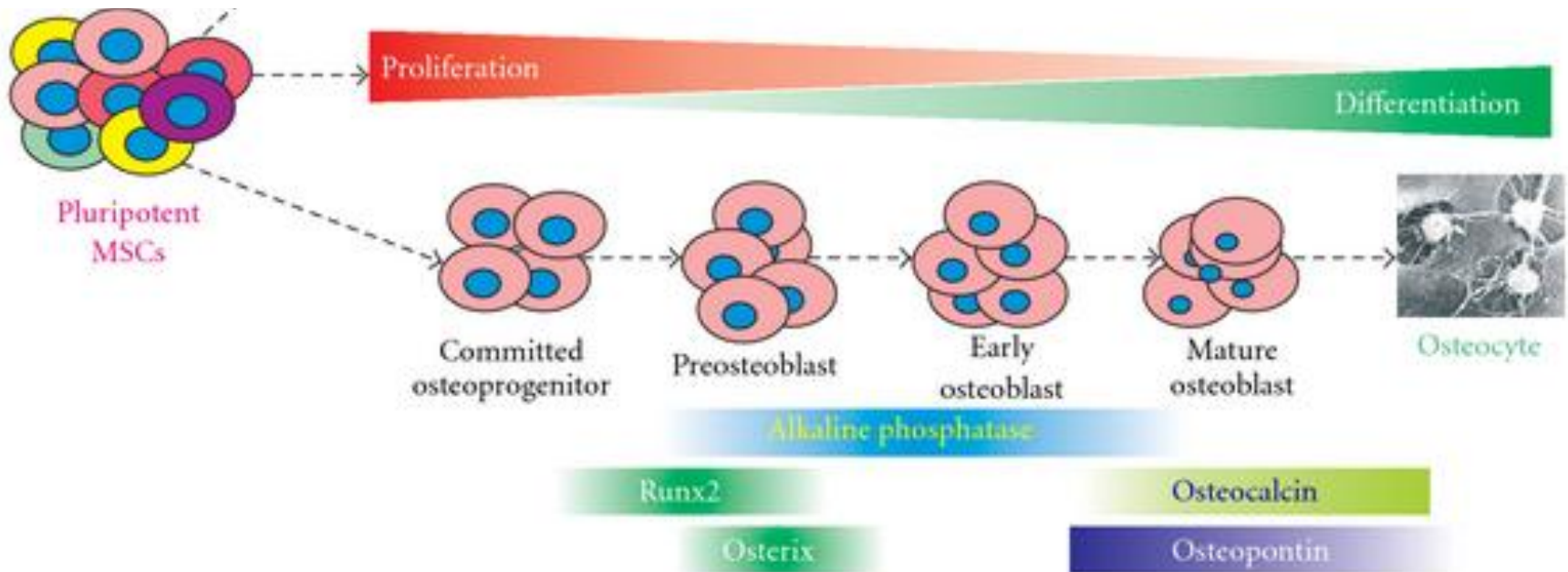- Hydroxyapatite crystals ($Ca_5(PO_4)_3(OH)$)

Osteoblasts

Bone

5 µm

Bone-forming cells are osteoblasts (OB) that produce collagen I and deposit HA

Bone is Formed by Osteoblasts

OBs originate from Bone Marrow Stromal (a.k.a. Mesenchymal Stem) Cells (BMSC) and terminally differentiate into osteocytes (OT).

Wagner et al., PPAR Res., 2010

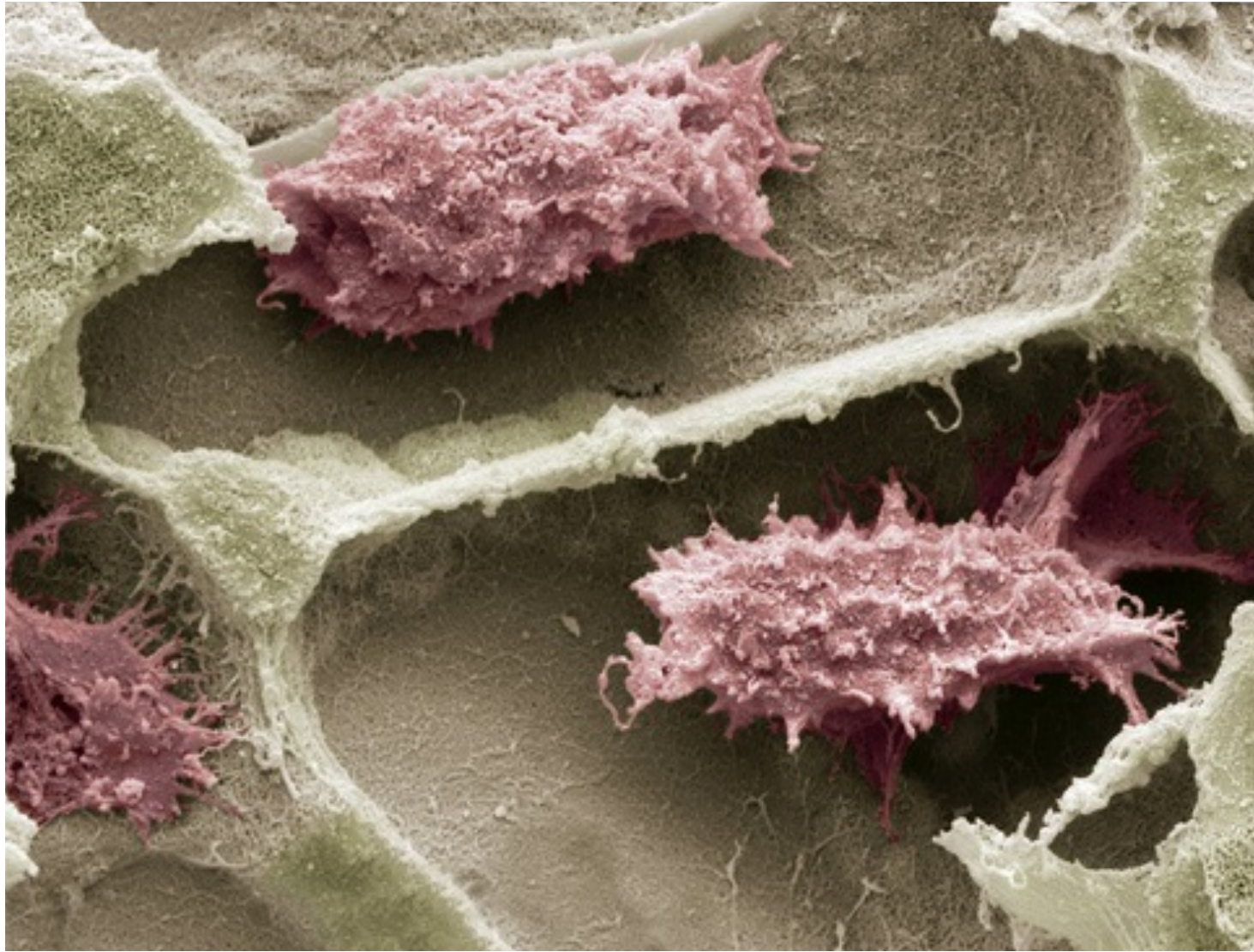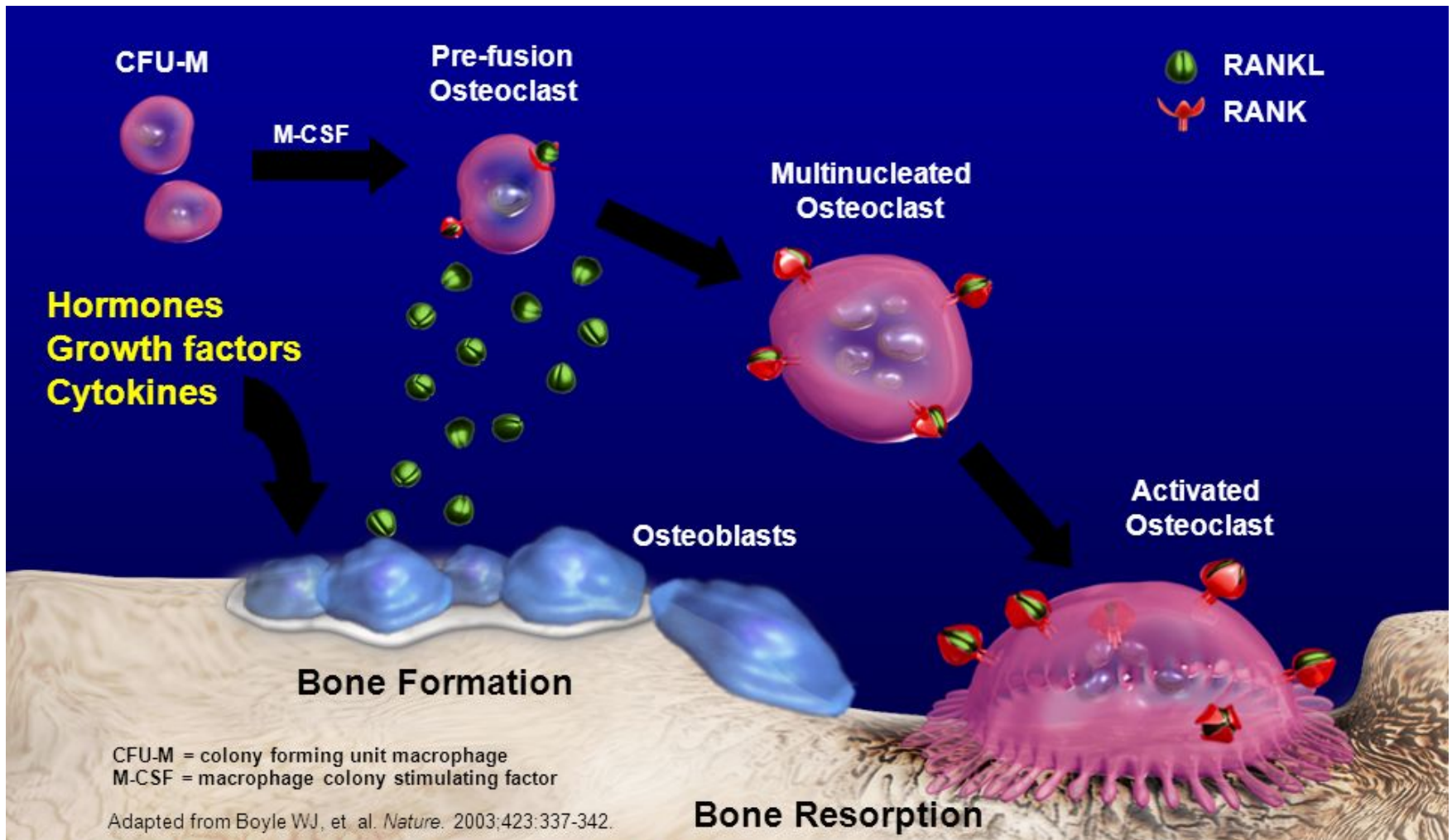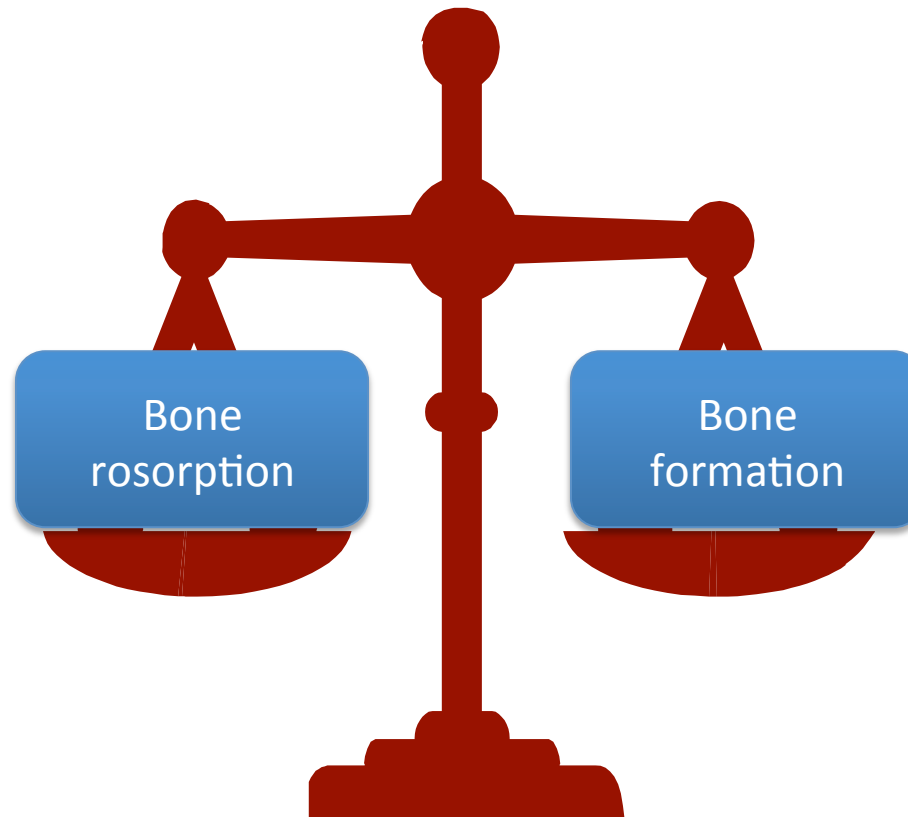# Bone is Resorbed by Osteoclasts

Image from SciencePhotoLibrary

Osteoclasts (OC) are bone resorbing multinucleated cells that originate from hematopoietic cells (monocyte/macrophage)
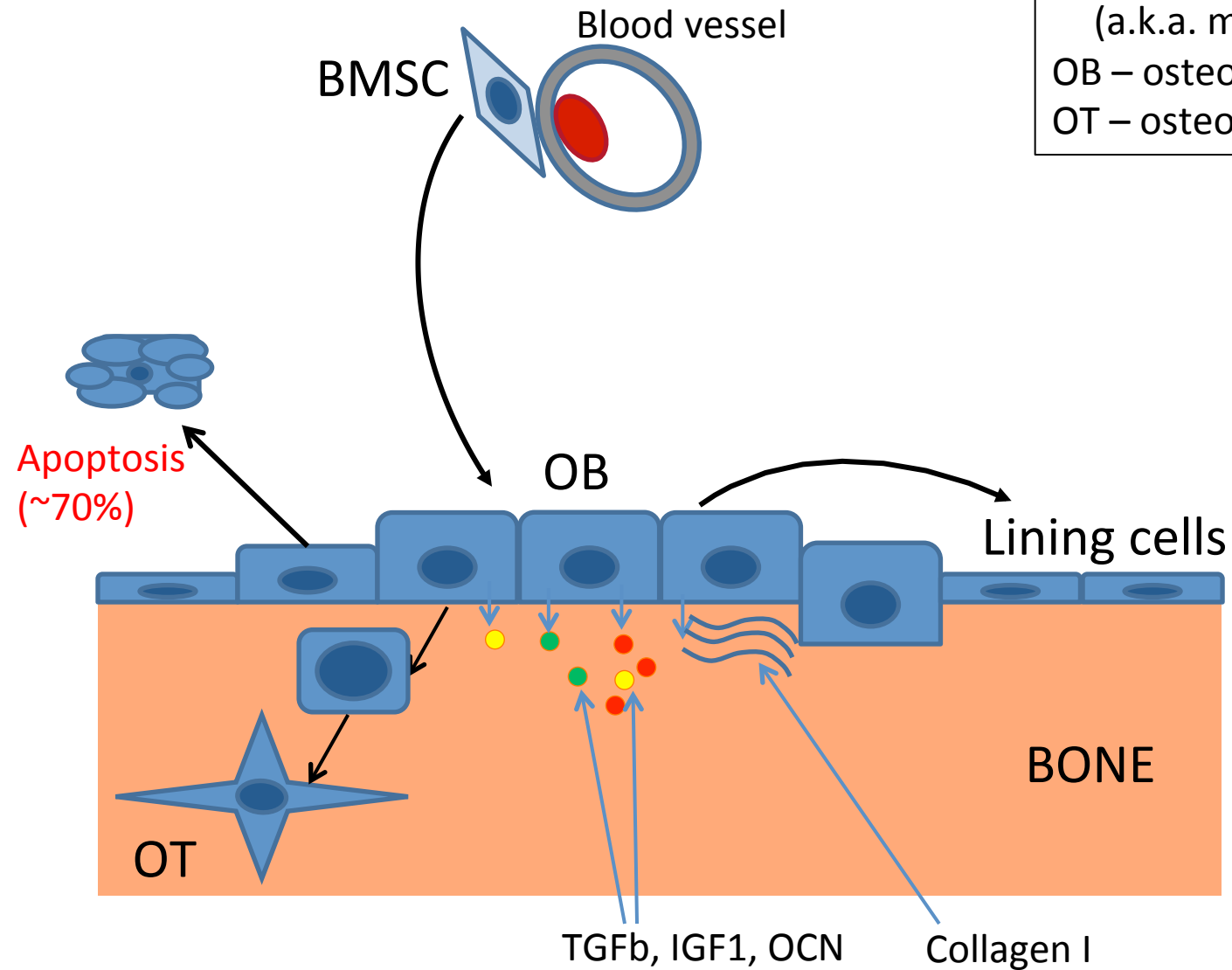
Homeostasis = equilibrium (Greek: ὁμοίως + στάσις)

Bone Formation vs Resorption = Dynamic Equilibrium (~10% of adult human skeleton is replaced annually)

Bone rosorption

Bone formation

Intact Bone

Blood vessel

BMSC

BMSC – bone marrow stromal
   (a.k.a. mesenchymal stem) cell
OB – osteoblast
OT – osteocyte

Apoptosis
(~70%)

OB

Lining cells

BONE

OT

TGFb, IGF1, OCN

Collagen I

Remodeling: Initial Phase

Blood vessel

BMSC

HSC

?

RANKL,
m-CSF

OCP

OB

Lining cells

OT

BONE

BMSC – bone marrow
stromal (a.k.a.
mesenchymal stem) cell
OB – osteoblast
OT – osteocyte
HSC – hematopoietic stem
cell
OCP – osteoclast precursor
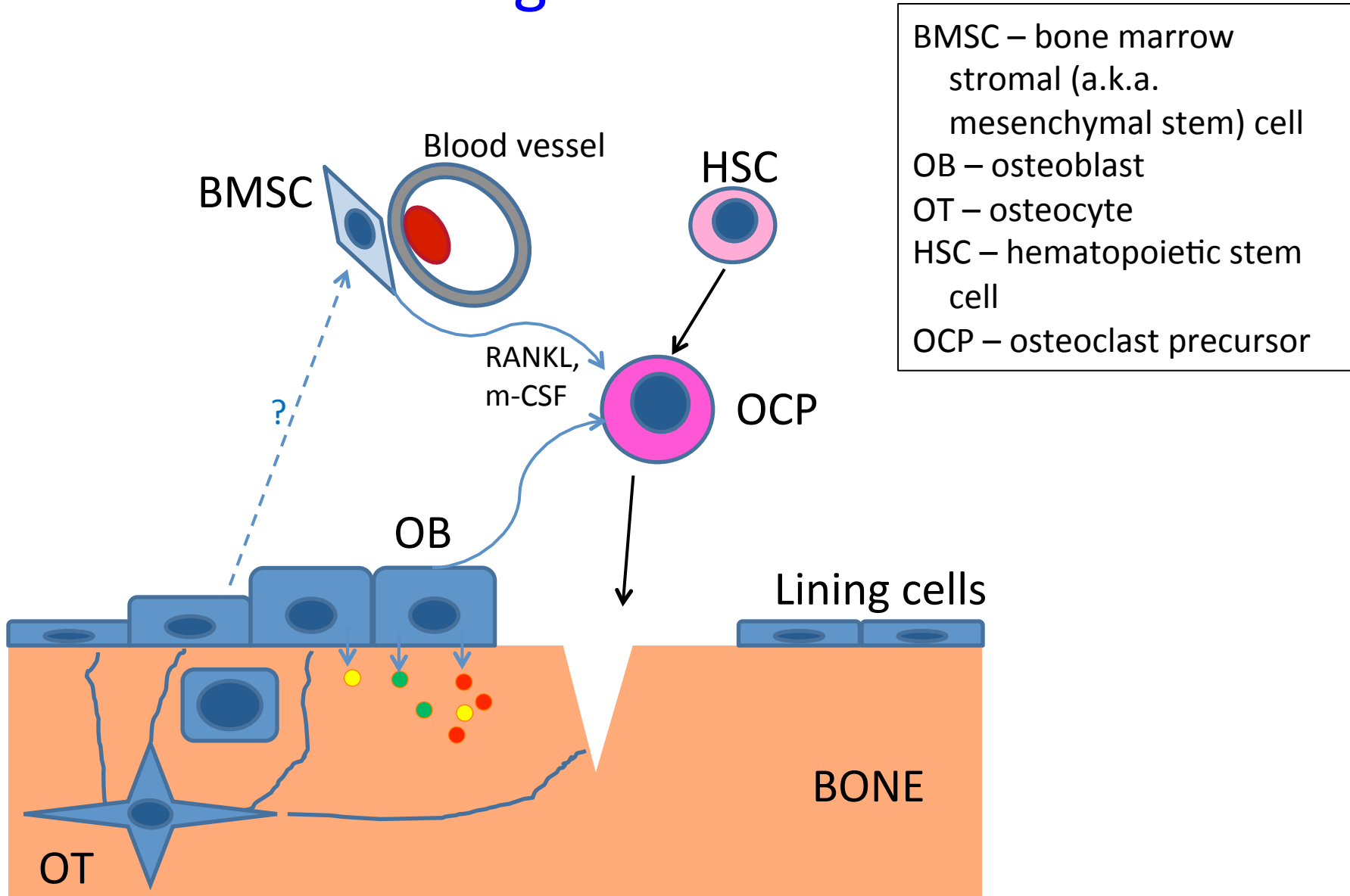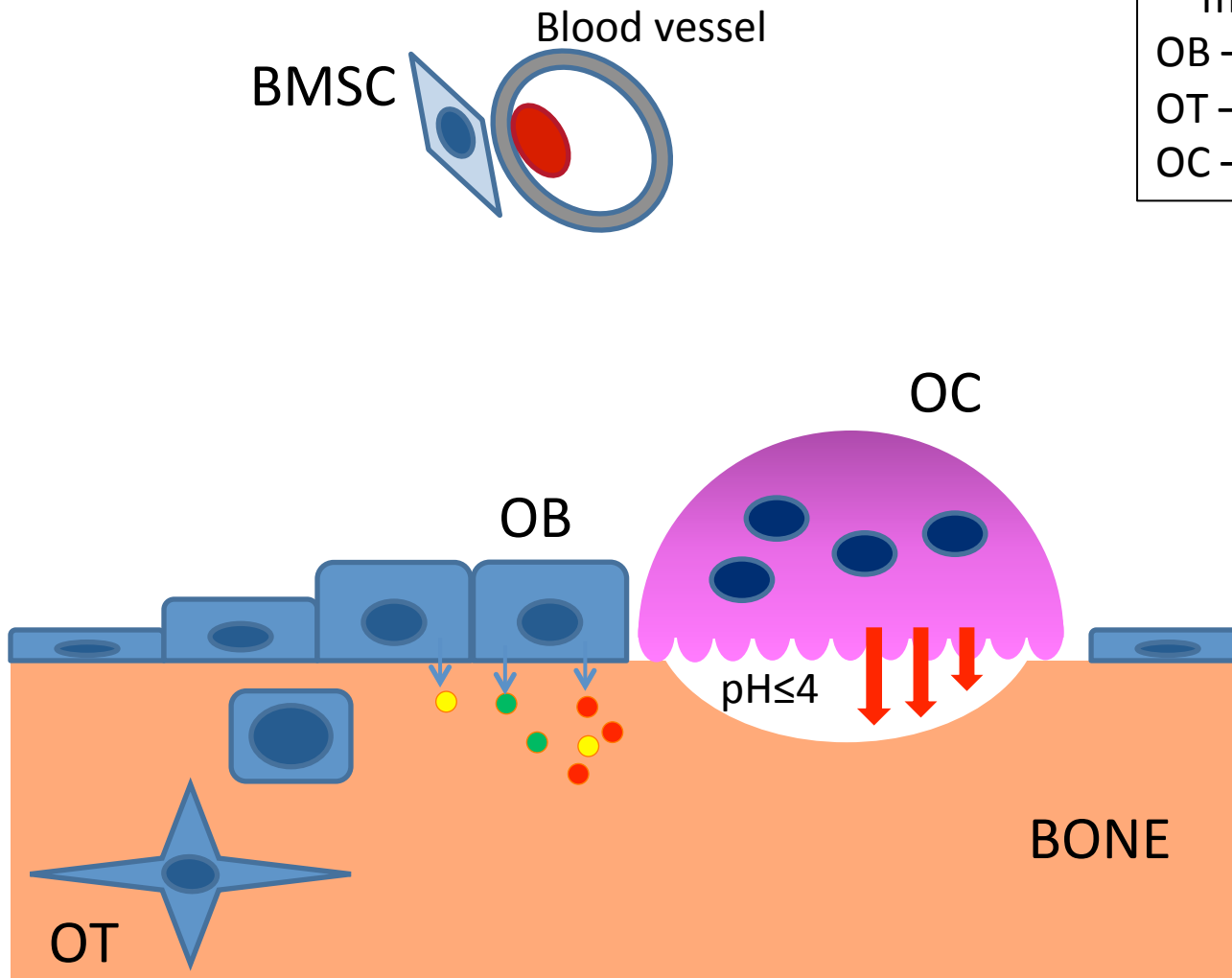
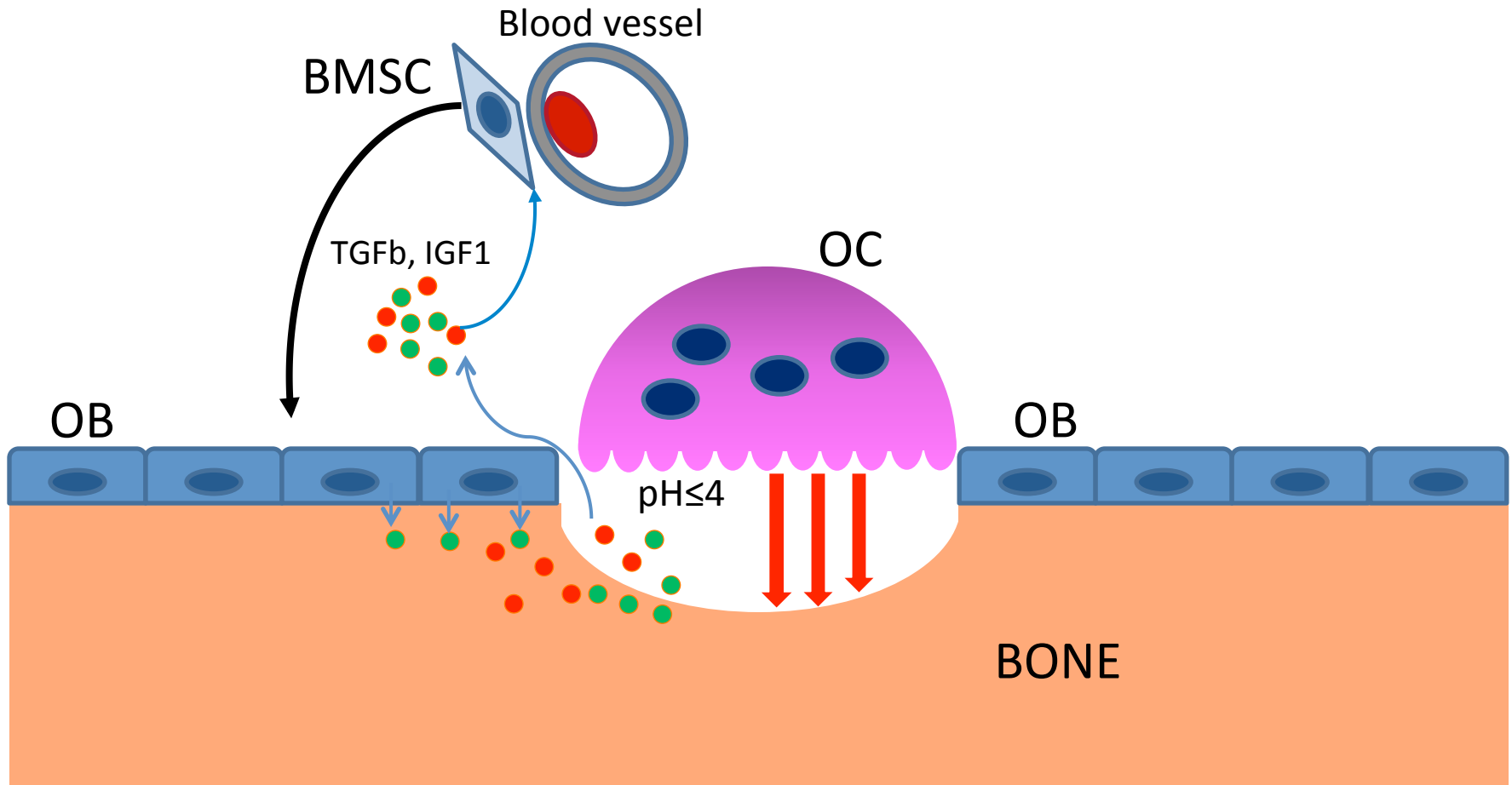# Remodeling: Resorption Pit

BMSC – bone marrow stromal (a.k.a. mesenchymal stem) cell
OB – osteoblast
OT – osteocyte
OC – osteoclast

Blood vessel

BMSC

OC

OB

pH≤4

OT

BONE

Remodeling: Recruitment/Activation of BMSCs into Osteogenic Lineage

Remodeling: New Bone Formation

BMSC

Blood vessel

New bone

OB

BONE

OT
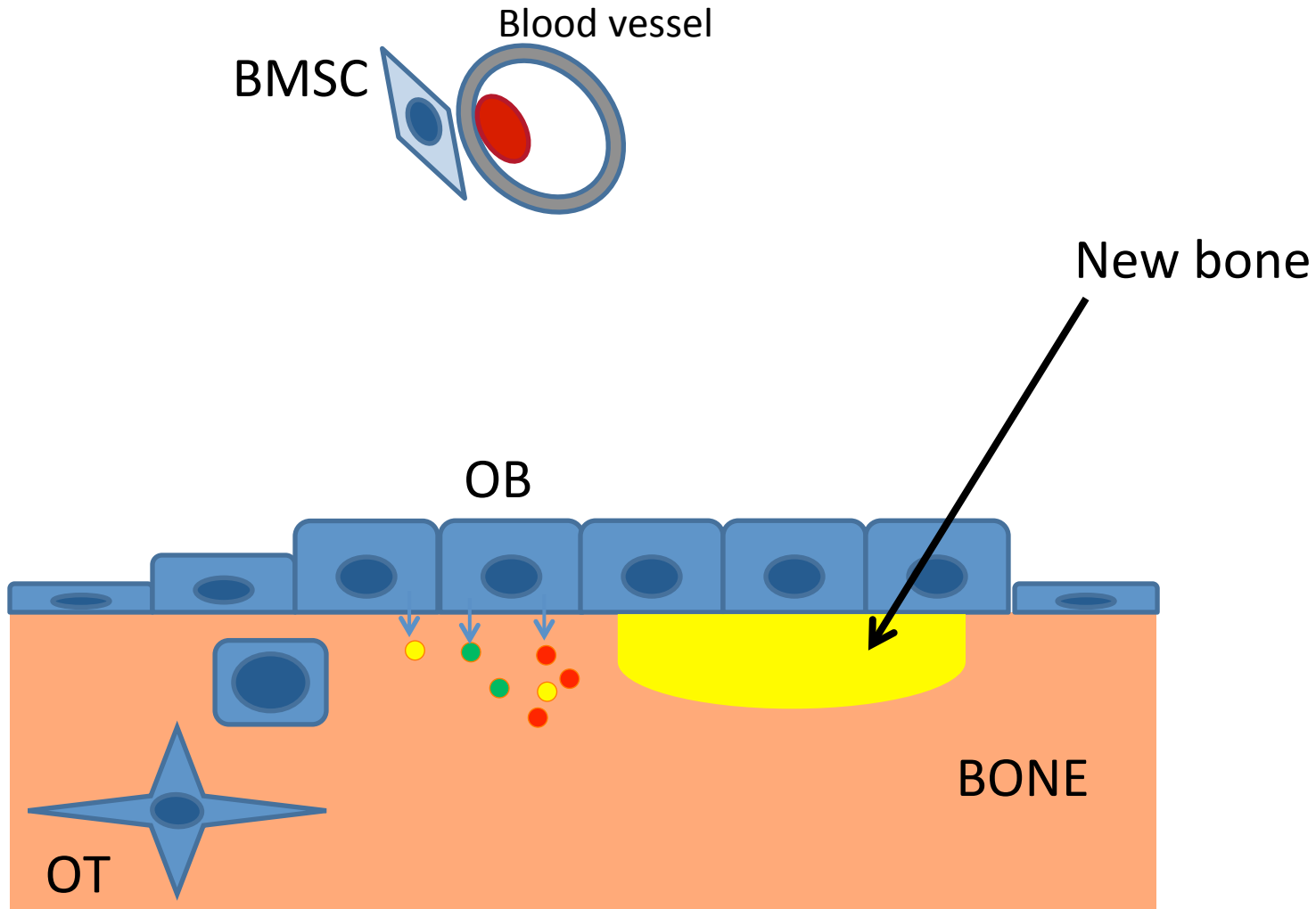
Histology of Bone Remodeling
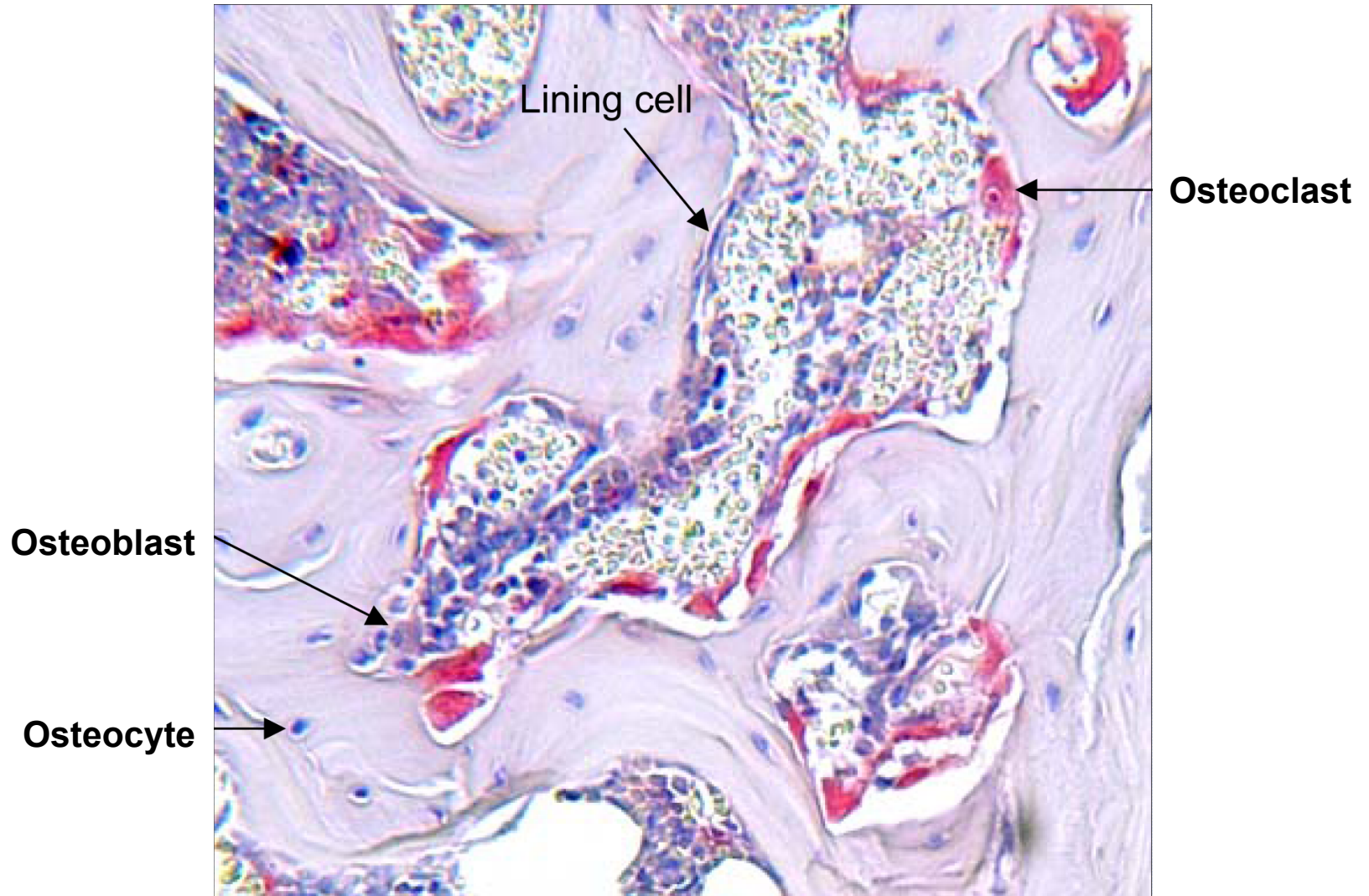
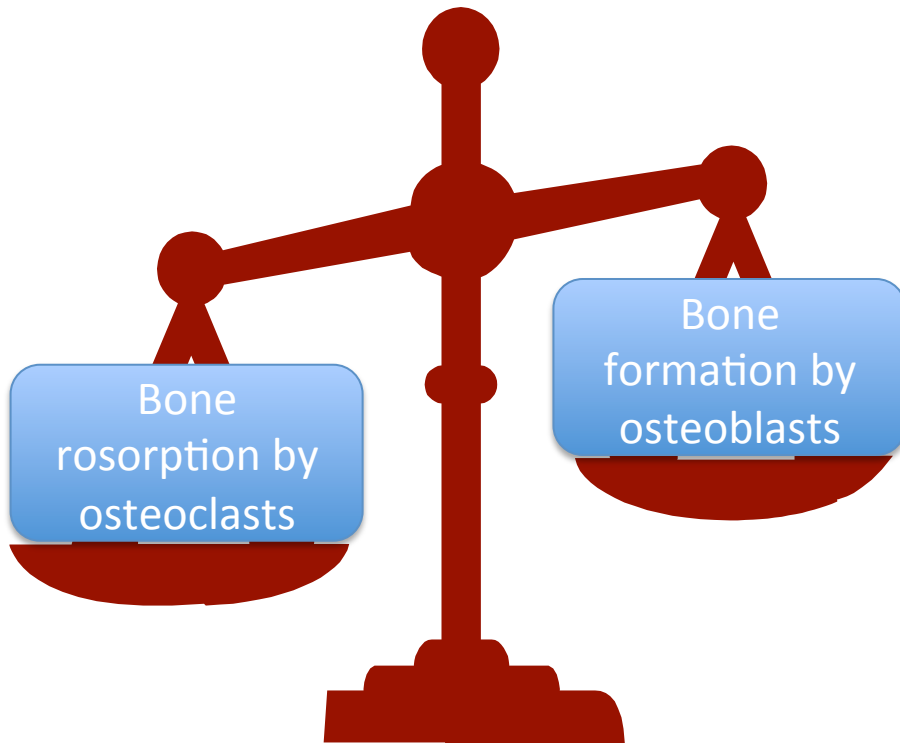**TRAP Staining**

Phases of Remodeling
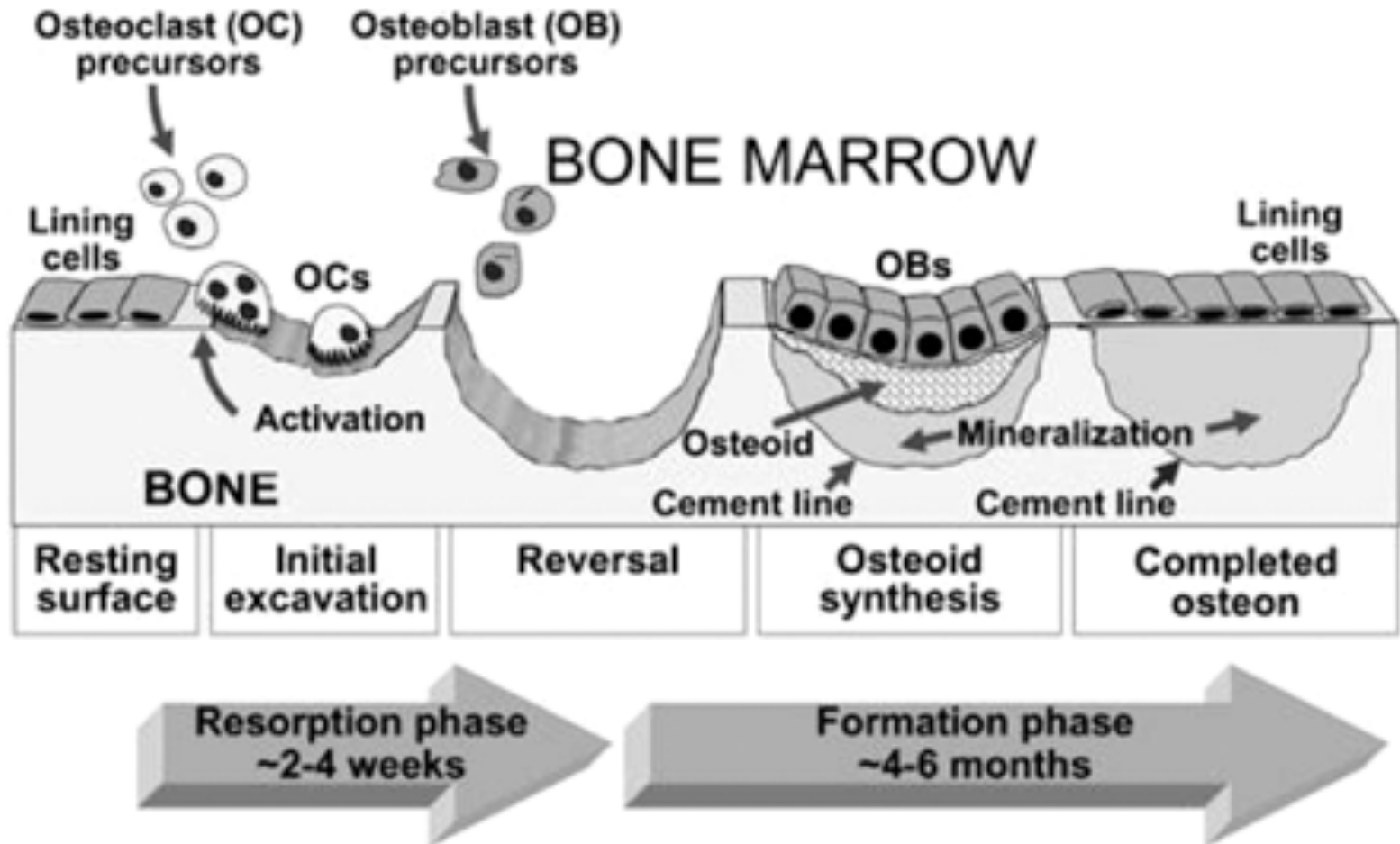
Factors Regulating Bone Homeostasis
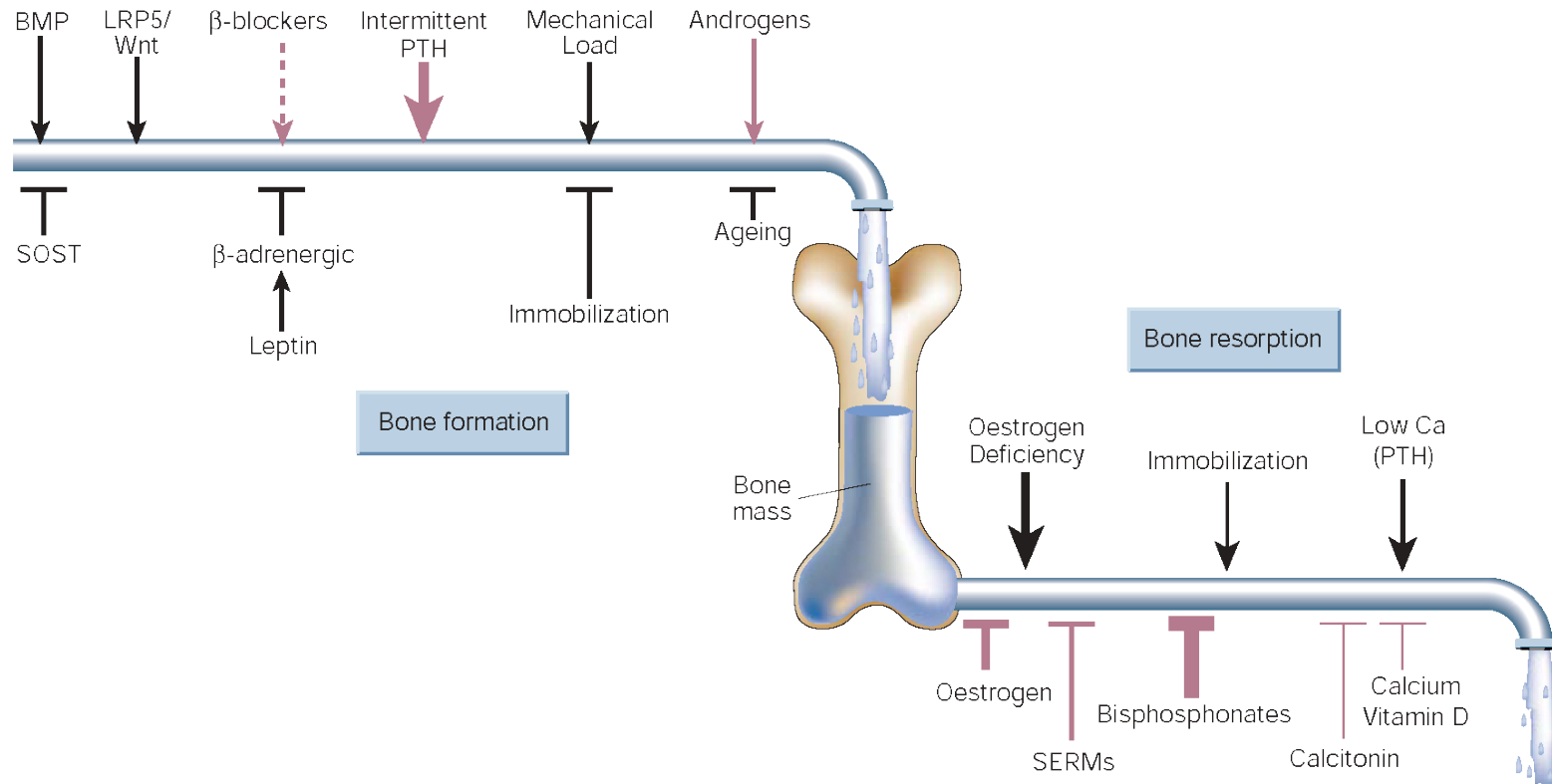
Harada S and Rodan GA., (2003) Nature 423: 349-355.

Disorders of Bone Homeostasis

BONE LOSS
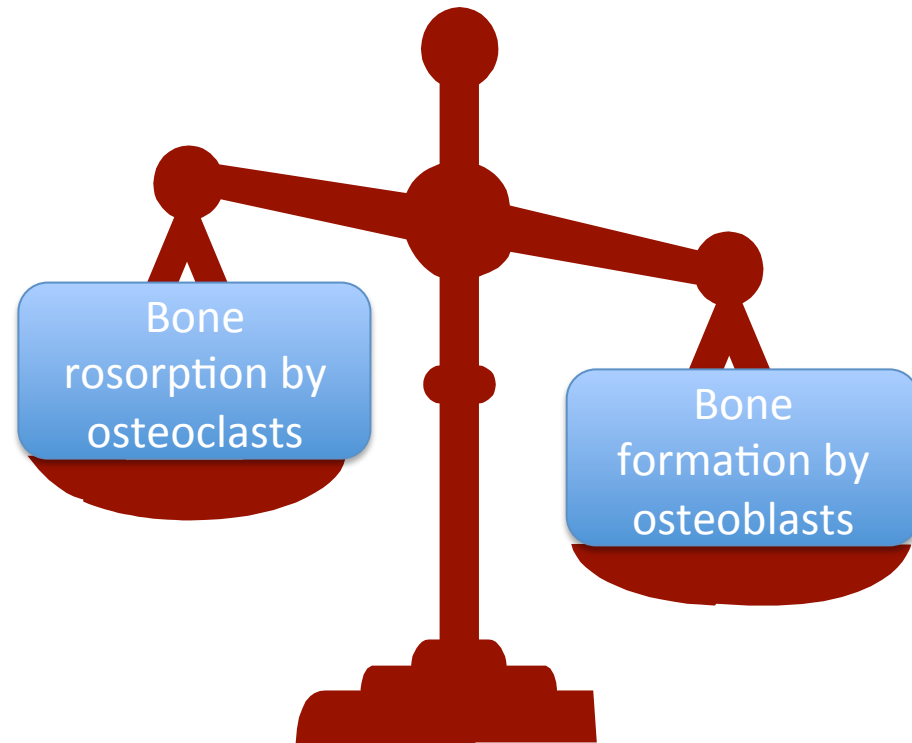
BONE GAIN

Disorders of Bone Homeostasis

- **Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)**

  - Most commonly inherited bone disorder
  - Autosomal dominant mutations (over 800 identified) in the genes encoding the $\alpha1$ and $\alpha2$ chains of type I collagen
  - Clinical features include; skeletal fragility with increased fracture risk, blue sclera, hearing loss, dental imperfections, joint laxity

- **Osteopetrosis (*aka*, Marble Bone Disease)**

- **Osteoporosis**

- **Paget Disease**

- **Hyperparathyroidism**

Courtesy of Dr. J. Jonason

Disorders of Bone Homeostasis

- **Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)**

- **Osteopetrosis (*aka*, Marble Bone Disease)**
    - Rare genetic disease characterized by reduced bone resorption
    - Autosomal dominant or recessive mutations affecting osteoclast numbers or function
    - Clinical features include; frequent fractures, scoliosis, cranial nerve deficits, anemia
    - First genetic disease treated with a bone marrow transplant

- **Osteoporosis**

- **Paget Disease**

- **Hyperparathyroidism**

Courtesy of Dr. J. Jonason

Disorders of Bone Homeostasis

- **Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)**

- **Osteopetrosis (*aka*, Marble Bone Disease)**

- **Osteoporosis**
  - Common disease associated with decreased bone mass and increased fracture risk
  - Caused by both genetic and environmental factors
  - High societal burden with ~2 million fractures in the US annually due to osteoporosis, at a cost of around 22 billion dollars to treat

- **Paget Disease**

- **Hyperparathyroidism**

Courtesy of Dr. J. Jonason

Disorders of Bone Homeostasis

- **Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)**

- **Osteopetrosis (*aka*, Marble Bone Disease)**

- **Osteoporosis**

- **Paget's Disease**
    - Uncontrolled bone remodeling leading, ultimately, to areas of high bone mass that are architecturally unsound
    - Onset in late adulthood with causes unknown (likely to be a combination of genetic and environmental factors)
    - Most prominent clinical feature is bone pain, but can also lead to fracture, bone deformities, and nerve compression

- **Hyperparathyroidism**

Courtesy of Dr. J. Jonason

Disorders of Bone Homeostasis

- **Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)**

- **Osteopetrosis (*aka*, Marble Bone Disease)**

- **Osteoporosis**

- **Paget's Disease**

- **Hyperparathyroidism**
    - Excess parathyroid hormone (PTH) in the bloodstream due to over-activity of the parathyroid glands
    - Can be <u>primary</u> (due to hyperplasia or tumor of the glands) or <u>secondary</u> (due to hypocalcemia and compensatory increases in PTH secretion)
    - Results in increased bone resorption leading to increased fracture risk

Courtesy of Dr. J. Jonason

Osteoporosis

Normal bone matrix — Osteoporosis

-Fracture
-Delayed repair
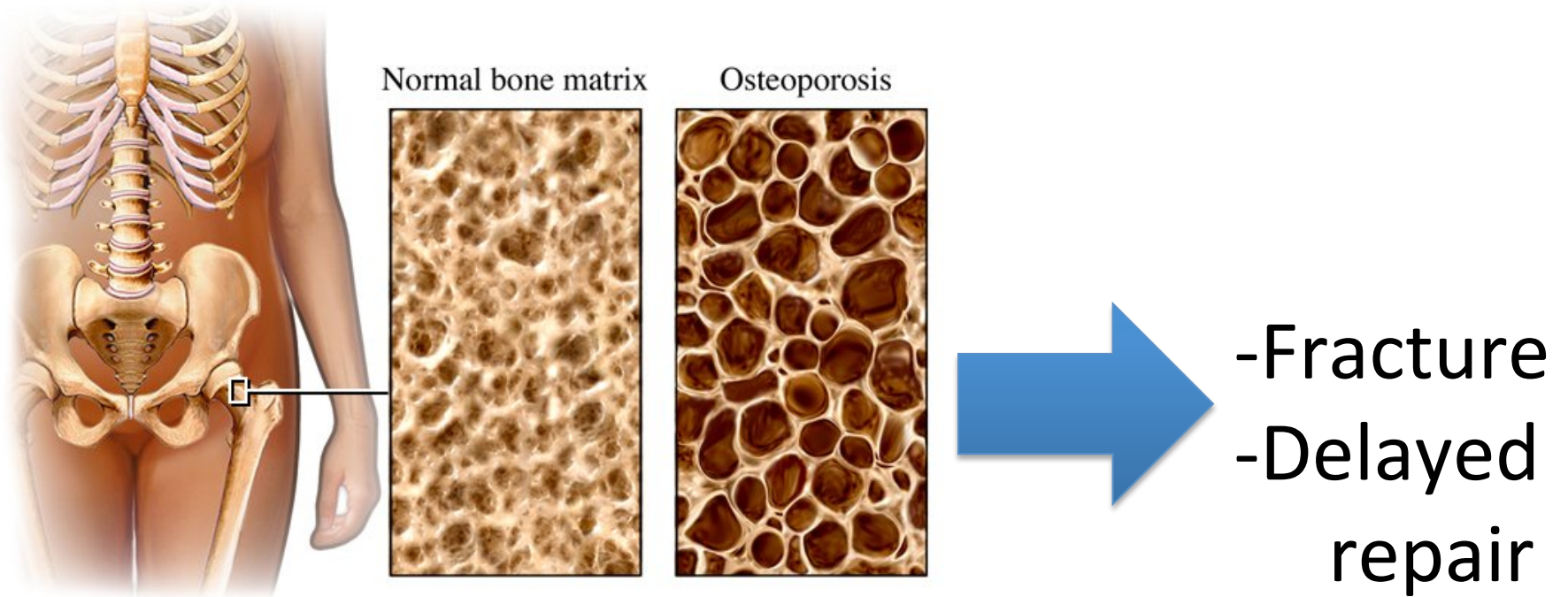
Images from Bionews Texas

Epidemiology of Osteoporosis

- **Osteoporosis is found in 70% of the elderly population**

- **Post-menopausal women are most affected (25 million American women with Osteoporosis)**

- **1.9 million fractures annually due to low bone mass.**

- **1 of 3 women will develop a vertebral fracture by age 65, and 1 of 3 women will develop a hip fracture by age 85.**

- **$22 billion dollars per year is spent on treating Osteoporosis.**

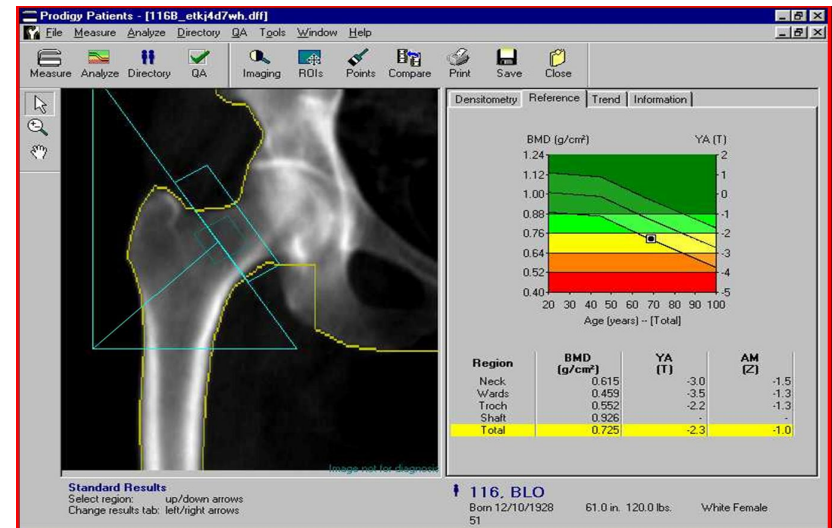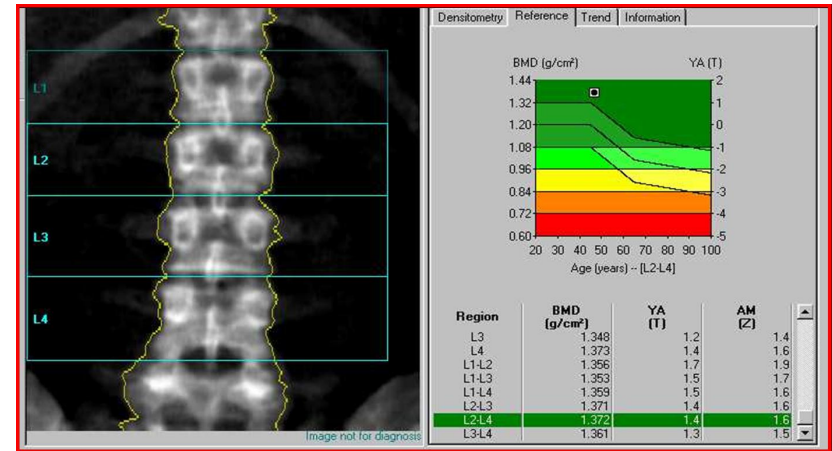# Epidemiology of Osteoporosis
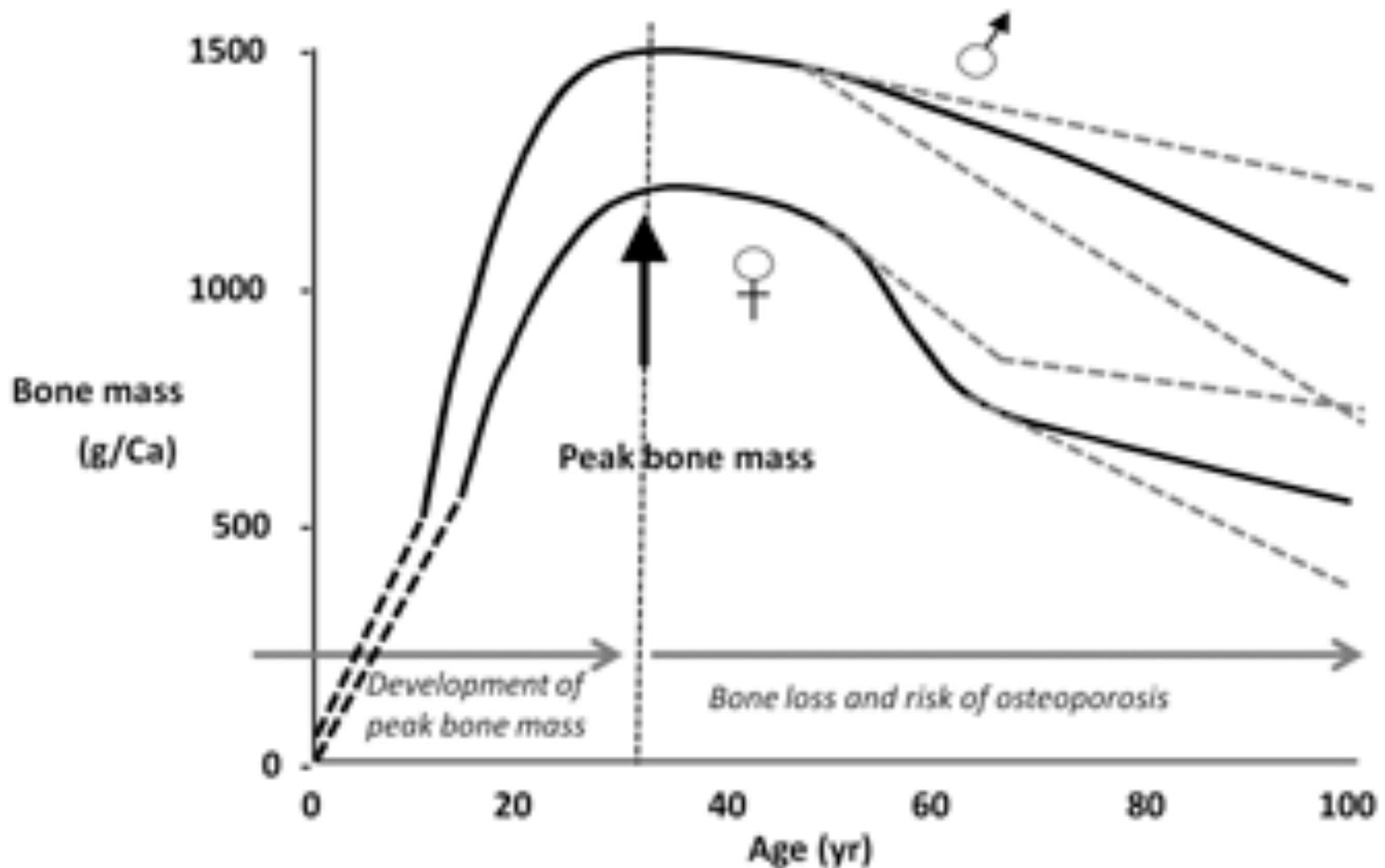
C57Bl/6J mice:
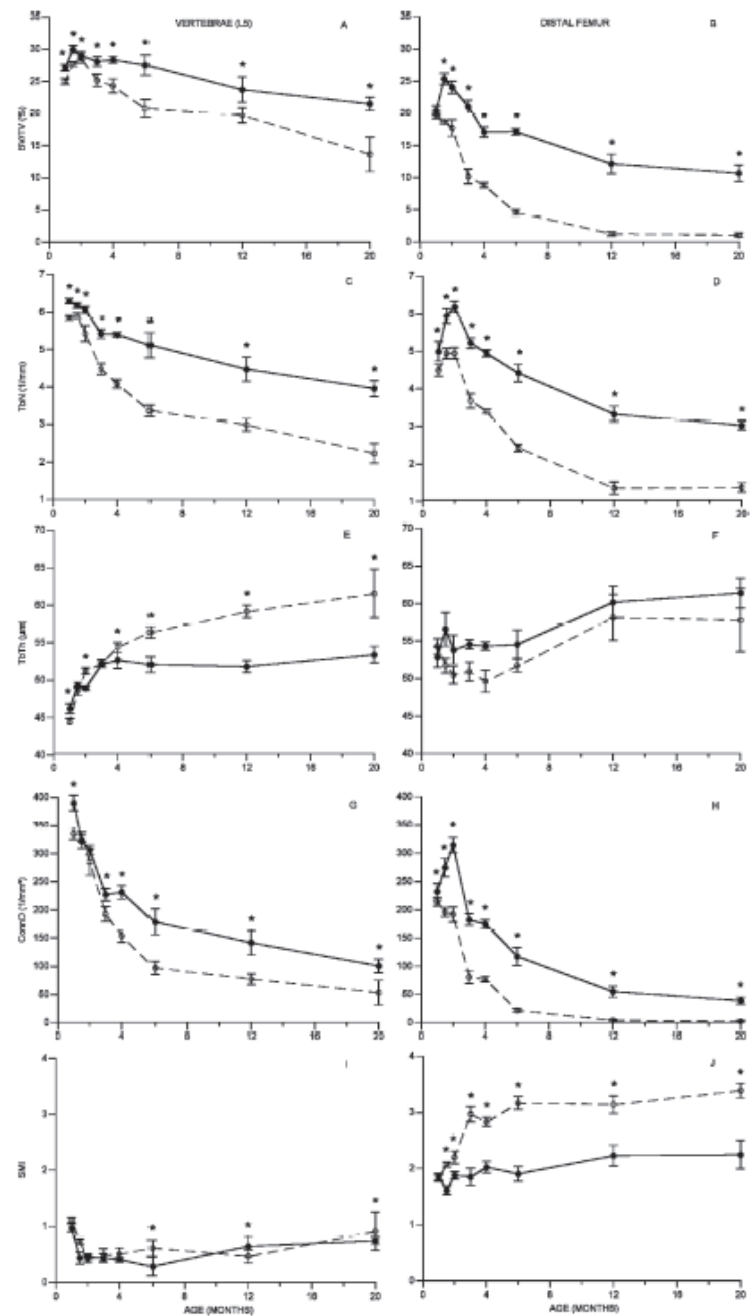
Diagnosis of Osteoporosis

DEXA
Dual **E**nergy **X**-ray **A**bsorptiometry

T-score = comparison to healthy young bone
Z-score = comparison to a reference group of
the same age, race, and gender as
the patient

Courtesy of Dr. J. Jonason

Treatment of Osteoporosis

Anti-resorptive drugs: Bisphonates

TABLE 3. FDA-approved indications for nitrogen-containing bisphosphonates

Drug	Postmenopausal osteoporosis		Glucocorticoid-induced osteoporosis		Men
	Prevention	Treatment	Prevention	Treatment	
Alendronate (Fosamax)	√	√		√	√
Risedronate (Actonel)	√	√	√	√	√
Ibandronate (Boniva)	√	√			
Zoledronate (Reclast)	√	√	√	√	√

Watts NB and Diab DL., (2010) Journal of Clinical Endocrinology and Metabolism. (95)4: 1555-1565.

Treatment of Osteoporosis

SERMS

SERMS: Selective Estrogen Receptor Modulators

Action: Drugs that act as estrogen agonists, activating the estrogen receptor in order to reduce osteoclast activity and bone resorption.

Drug: Raloxifene (Evista)

Dose: 60mg/day for many years

Notes: Not as effective as bisphosphonates. Only appears to protect spine and not hips from fracture.

Treatment of Osteoporosis

Bone anabolic agents

PTH: rPTH(1-34); Forteo. Under clinical investigation for the use in fracture non-union repair, treatment of osteoporosis.

BMPs: rBMP2 and rBMP7. Under clinical investigation and use for the repair of fracture non-union (InFuse). Recent complications in spinal fusion.

Neutralizing antibodies for Wnt inhibitors: anti-Dkk1 and anti-SOST. Under research and pre-clinical investigation for treatment of OA and fracture repair.
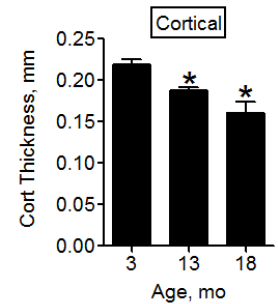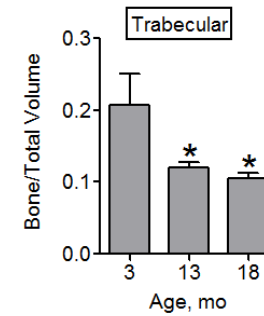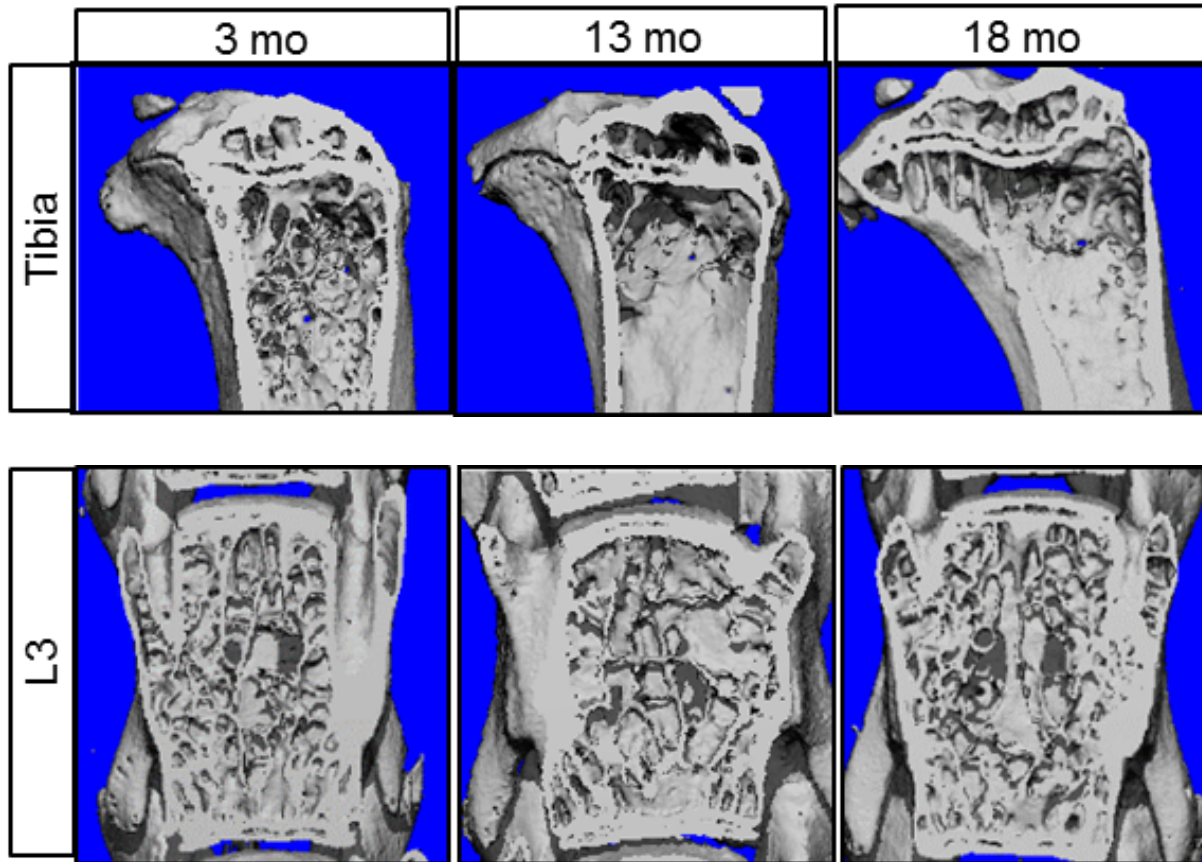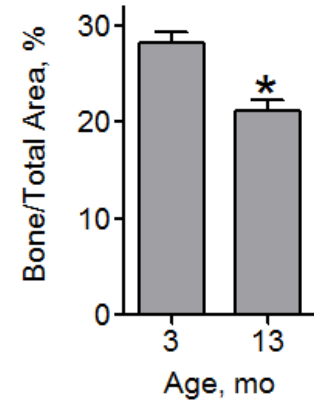
Lab Methods to Assess Bone Homeostasis

DEXA
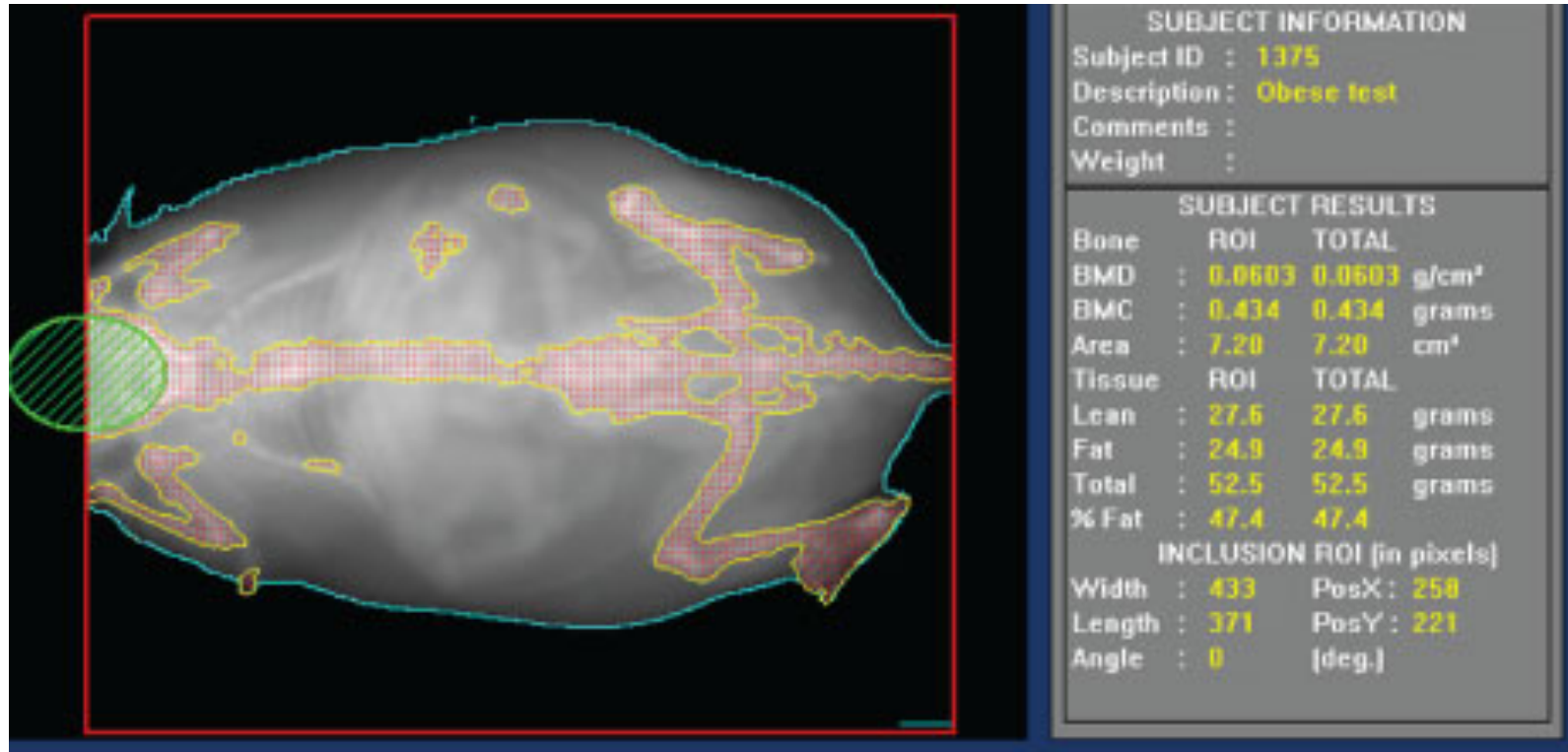
Lab Methods to Assess Bone Homeostasis

micro-CT

Shum et al., PLoS One, 2016

Lab Methods to Assess Bone Homeostasis

Histology/Histomorphometry
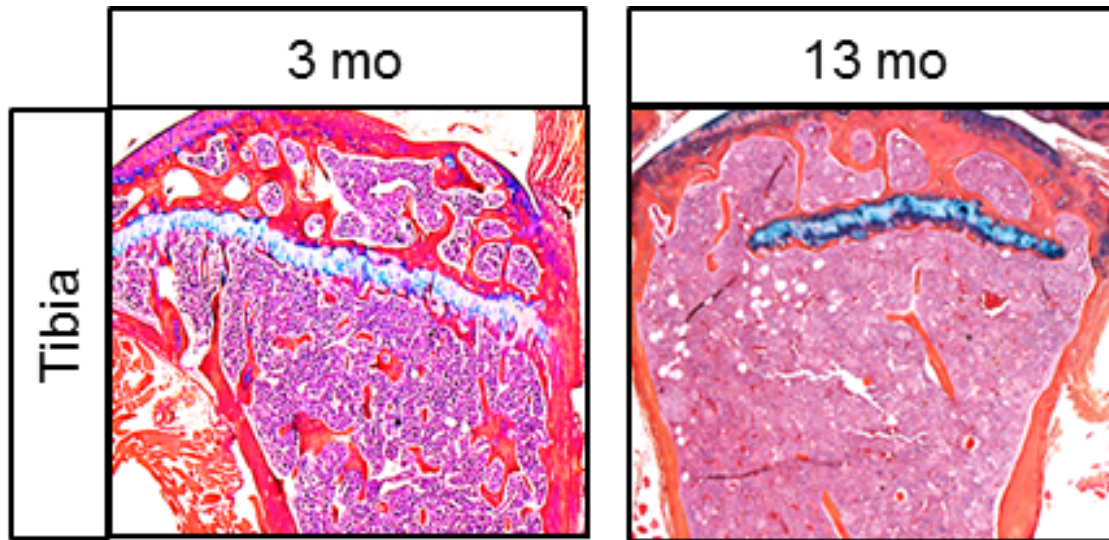
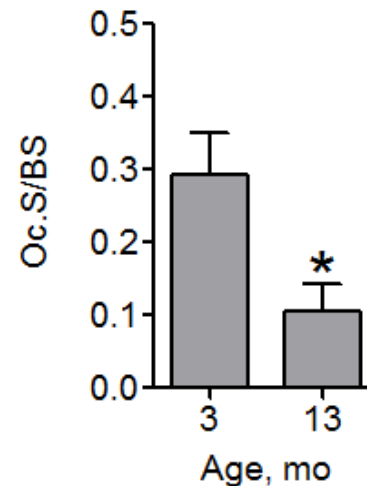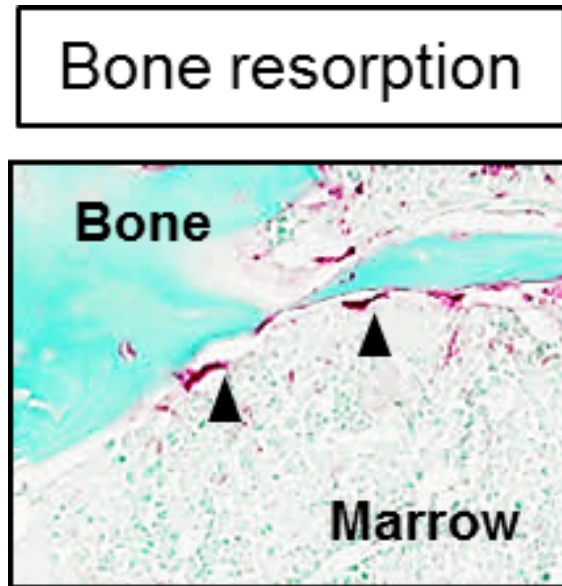*Shum et al., PLoS One, 2016*

Primary Measurements:
- Bone Area (B.Ar.) vs Total Area (T.Ar.)
- Surface, i.e. Bone Surface (BS)
- Cell Number per Surface/Area
    - =OB.N.
    - =OT.N.

OsteoMeasure or Visiopharm

Lab Methods to Assess Bone Homeostasis

Histology/Histomorphometry

Shum et al., PLoS One, 2016

Primary Measurements:

-Surface: Osteoclast Surface per Bone Surface (OC.S./BS)

-Cell Number: Osteoclast Number per Bone Surface (N.OC/BS)

Lab Methods to Assess Bone Homeostasis

Dynamic Bone Labeling (Bone Formation)

Mice are injected with Alizarin Red 10d before sacrifice & with Calcein 5d before sacrifice. Bone are frozen and sectioned. Labeled bone is visualized using fluorescence microscopy.

Derived Indices:
-Mineralizing Surface (MS/BS)
-Mineral Apposition Rate (MAR)
-Bone Formation Rate (BFR)
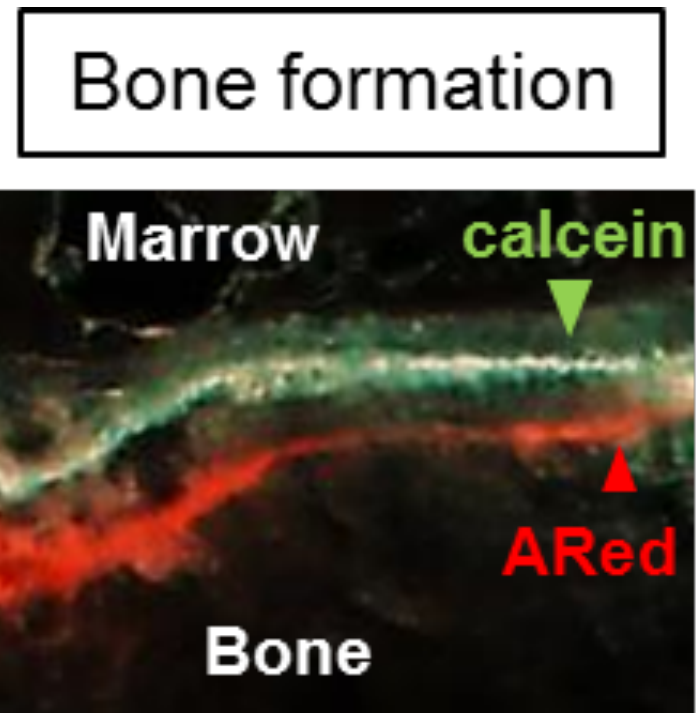
Bone formation

Shum et al., PLoS One, 2016

Lab Methods to Assess Bone Homeostasis

Bone Turnover Serum Markers

Formation: Resorption:

P1NP   CTX

Per recommendation of the International Osteoporosis Foundation (IOF) and the International Federation for Clinical Chemistry (IFCC)

Useful Mouse Models

Osteolineage-specific Cre Models:

- <u>BMSC</u>: Prx1, Nestin, LepR, aSMA, Osx
- <u>OB</u>: Osx (early), Col1 3.2kb (early), Col1 2.3kb (late), OCN (late)
- <u>OT</u>: DMP1

Osteoclast-specific Cre Models:

- CD11b
- Lyzm
- Trap

Bone homeostasis is a dynamic equilibrium due to opposing actions and coupling of bone-forming OBs and bone-resorbing OCs.