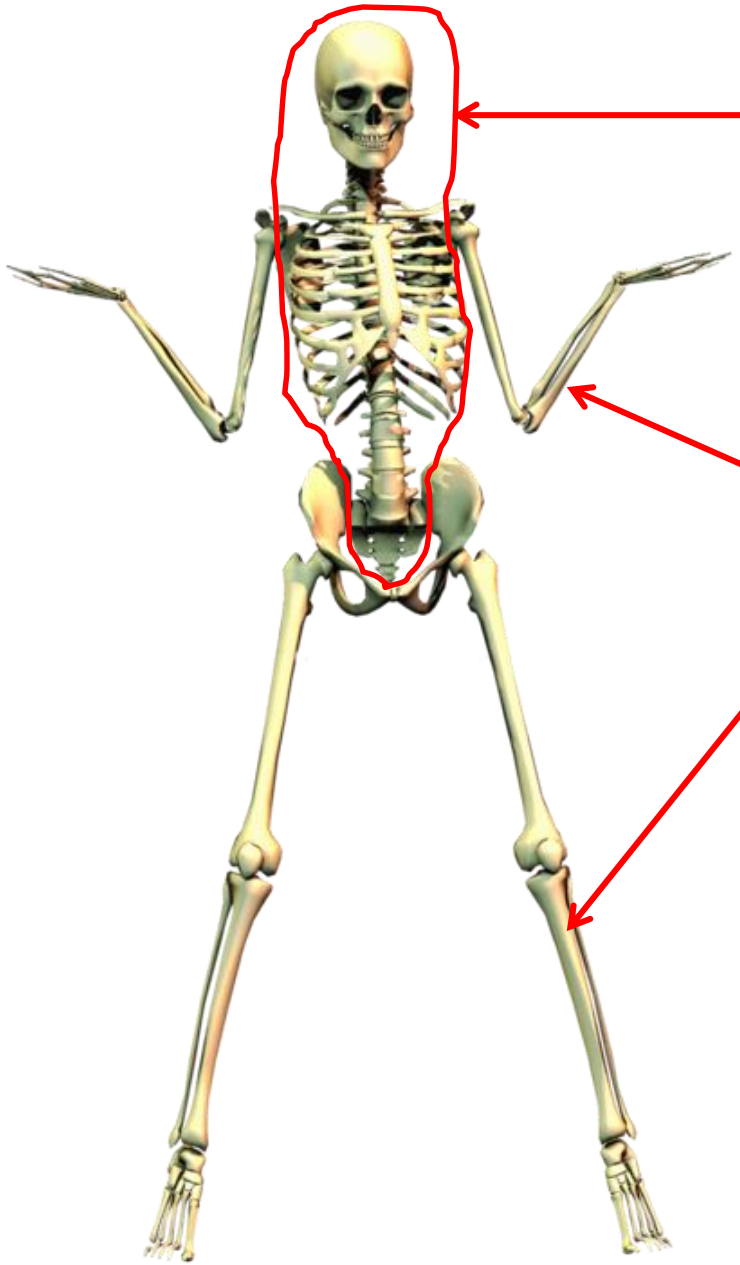
Bone Homeostasis and Pathology

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

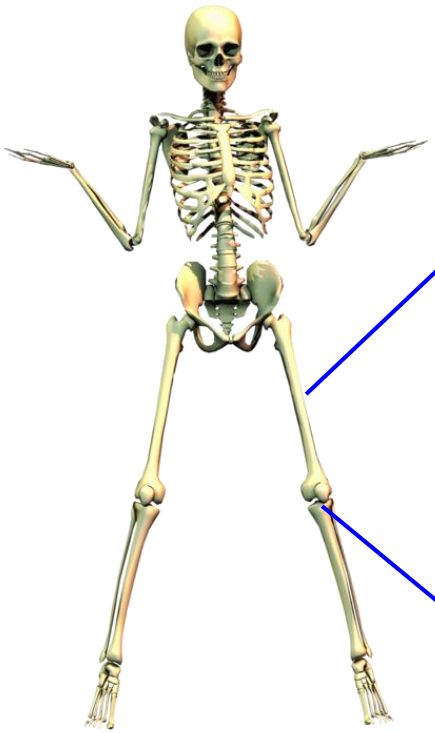
Adult Skeleton

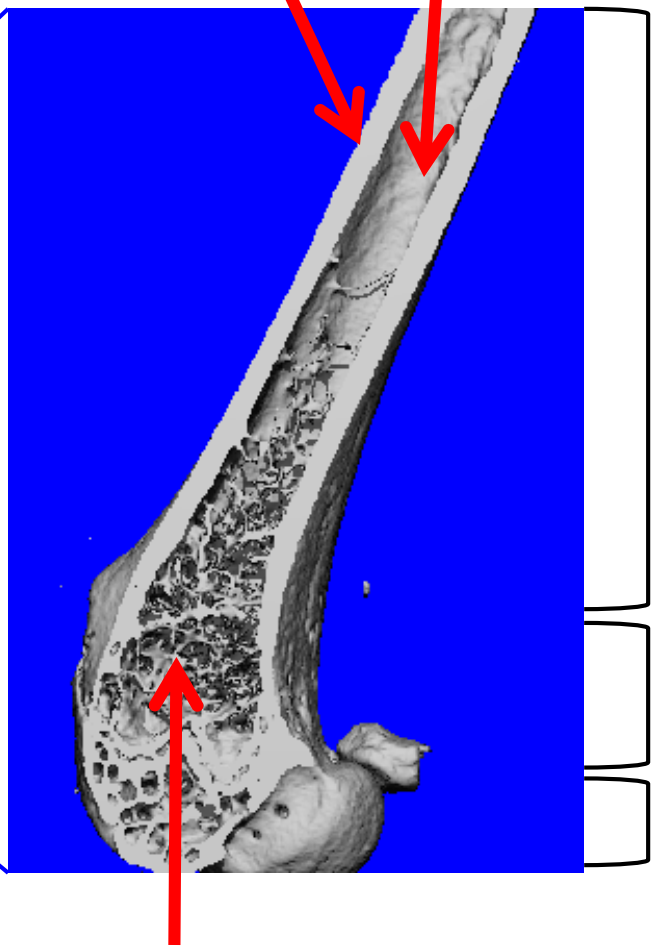


206 bones

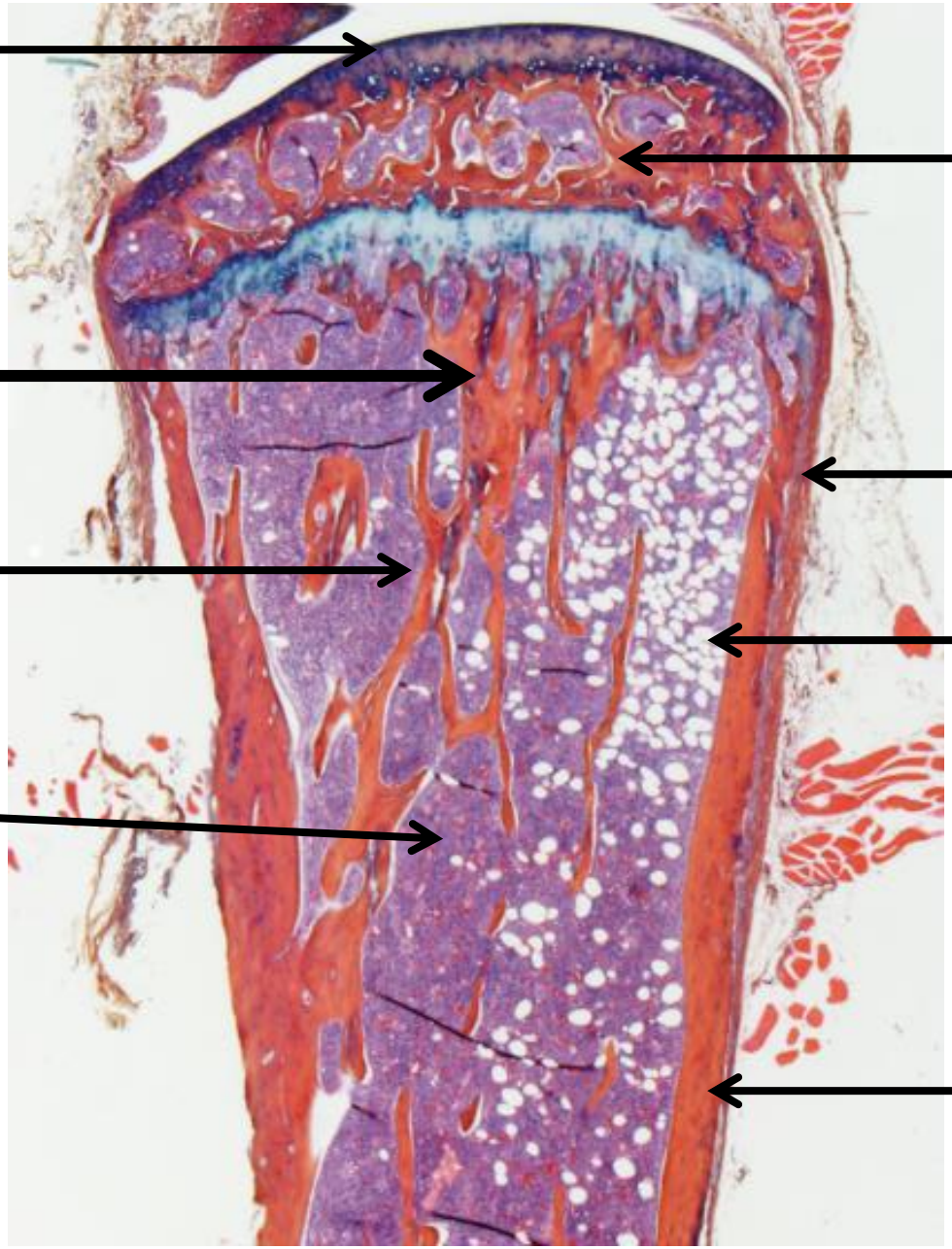
Image from www.pngall.com

Adult Bone Architecture





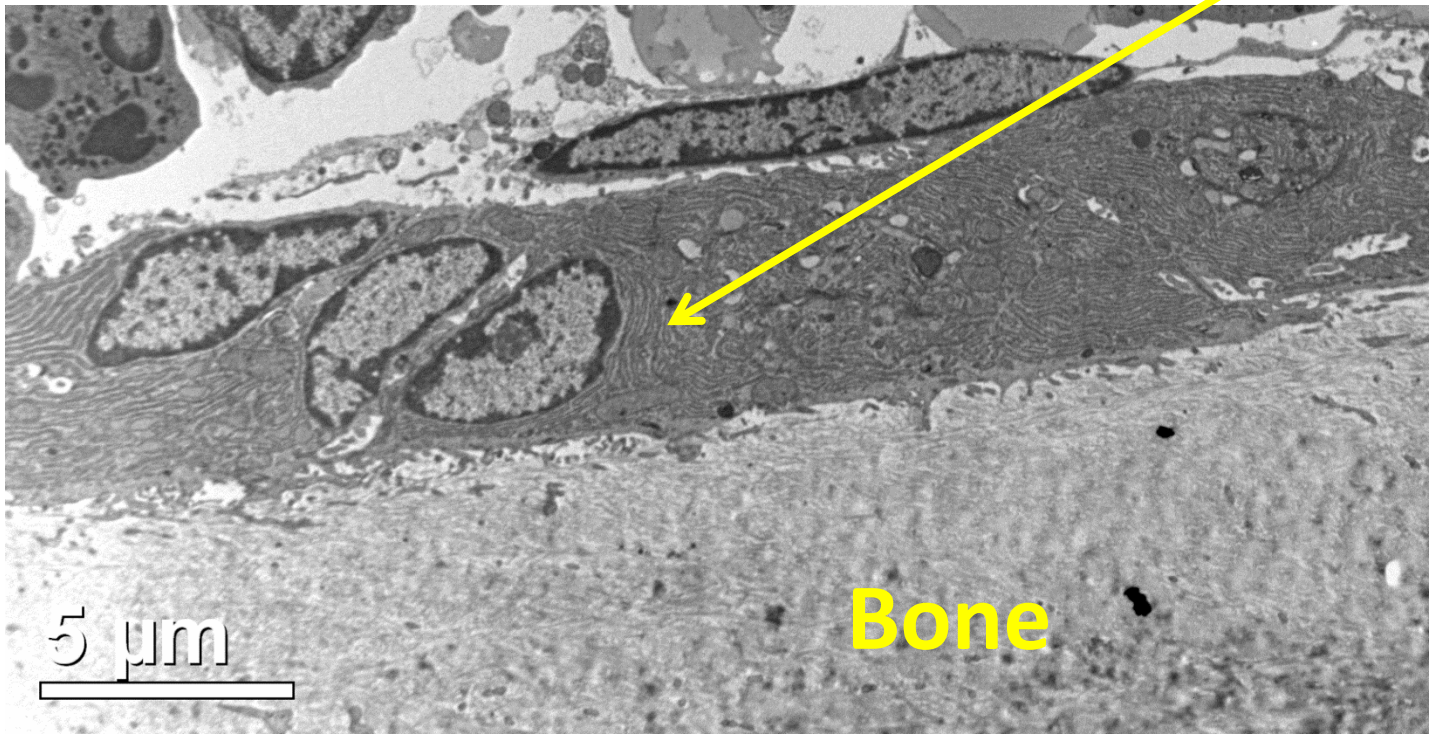
Bone Histology



Bone Composition

Bone is a mineralized organic matrix composed of:

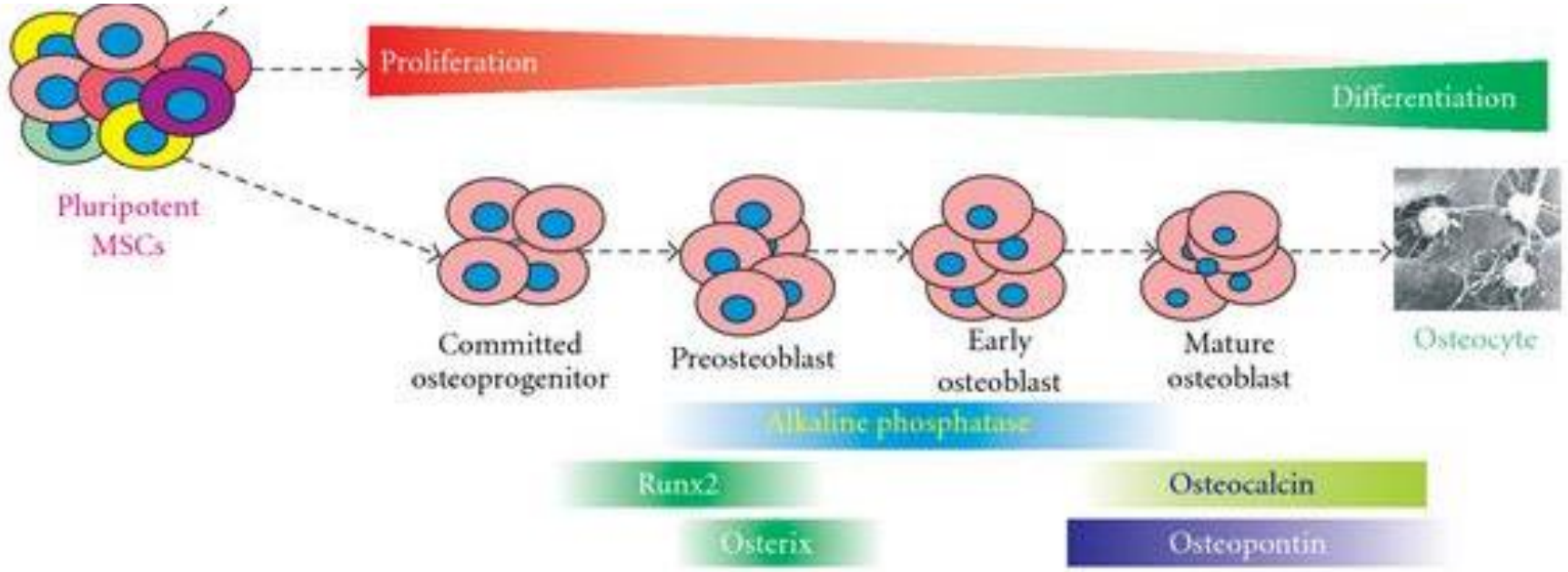
- Type I collagen and non-collagenous proteins (osteoid)
- Hydroxyapatite crystals ($Ca_5(PO_4)_3(OH)$)



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

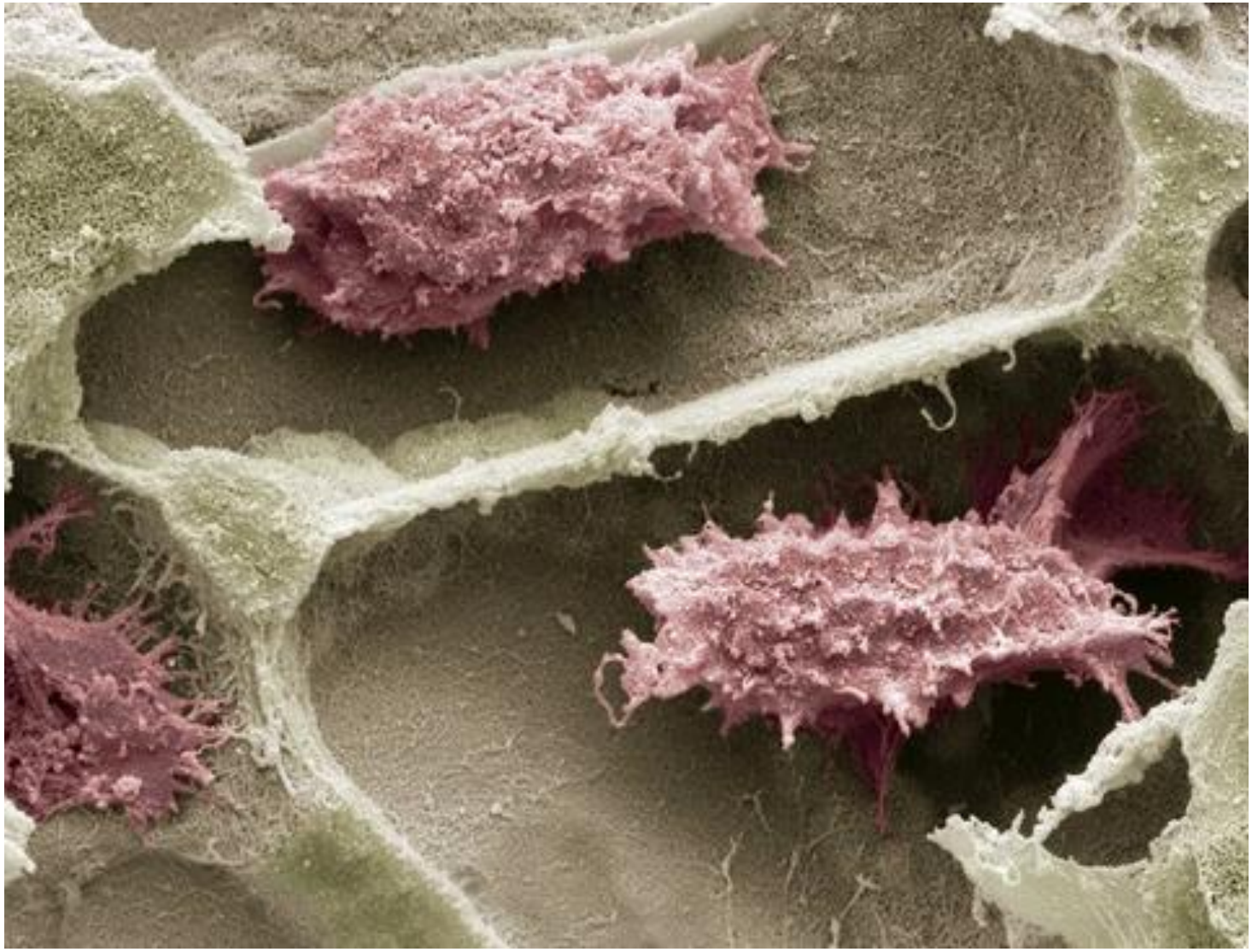
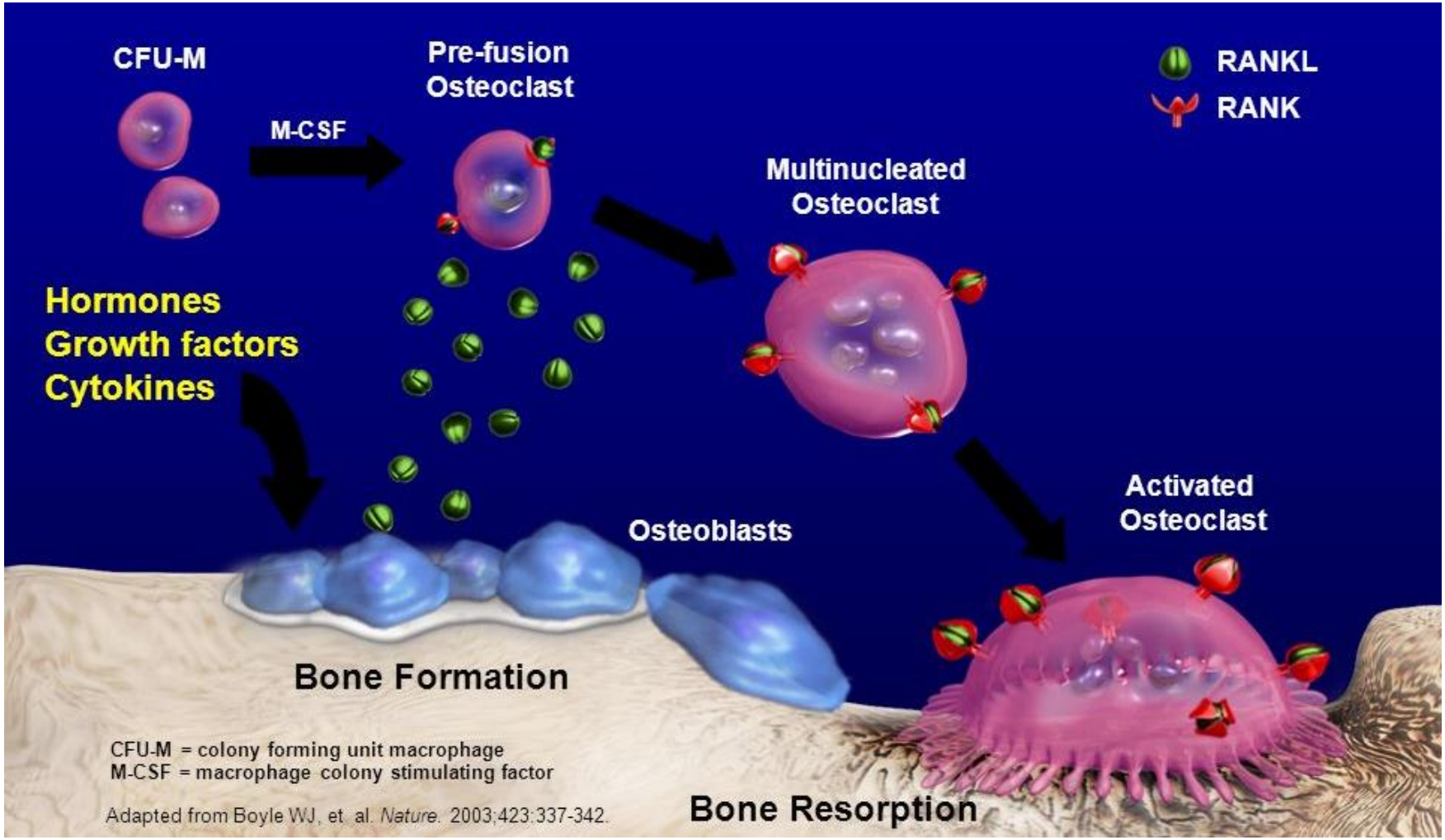


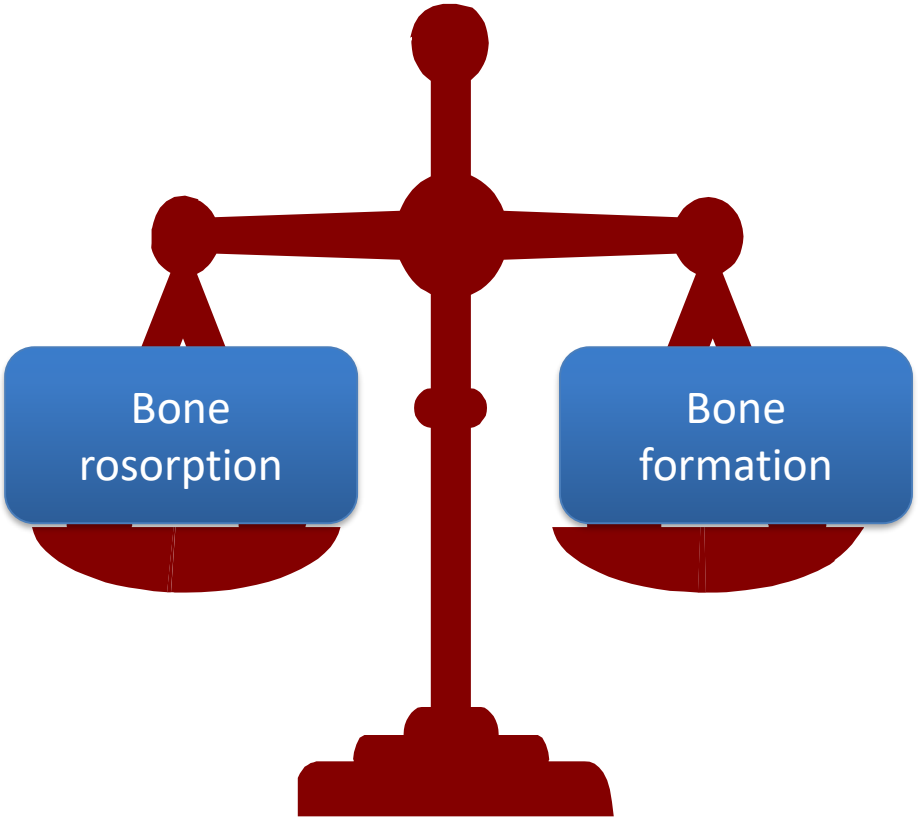
Image from SciencePhotoLibrary

Osteoclasts (OC) are bone resorbing multinucleated cells that originate from hematopoietic cells (monocyte/macrophage)



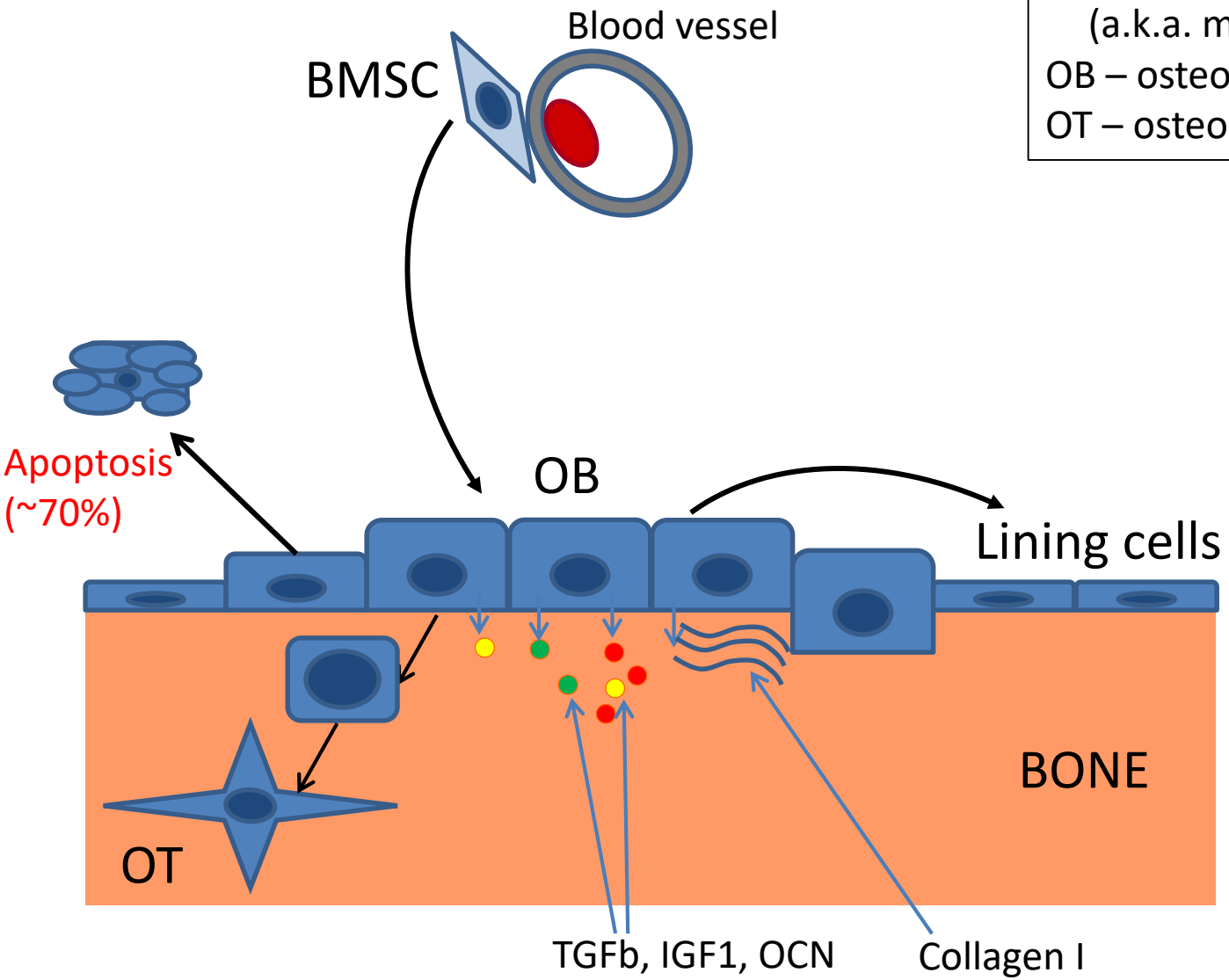
Homeostasis = equilibrium (Greek: ὁμοίως + στάσις)

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



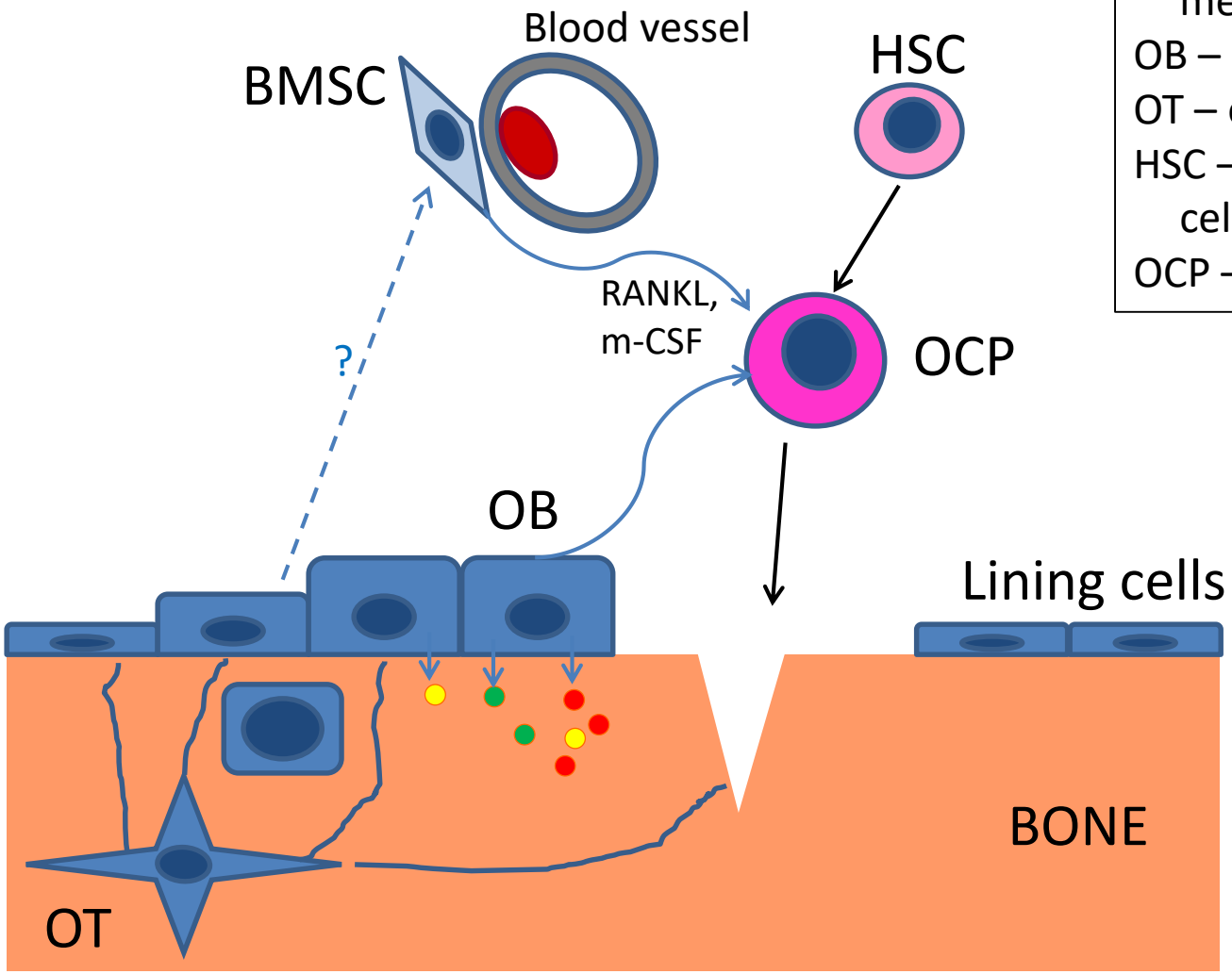
Intact Bone

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



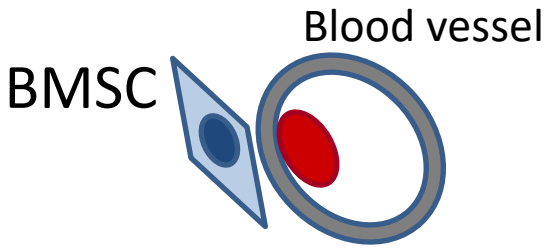
Remodeling: Initial Phase

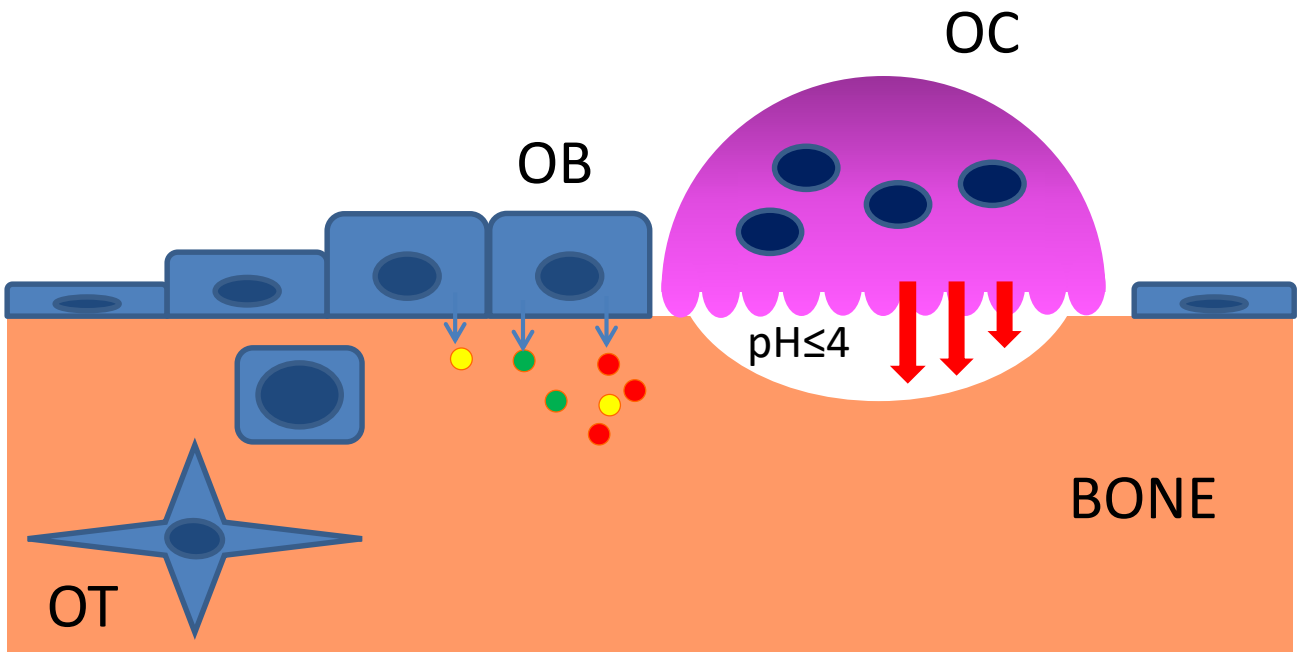
BMSC – bone marrow stromal (a.k.a. mesenchymal stem) cell
OB – osteoblast
OT – osteocyte
HSC – hematopoietic stem cell
OCP – osteoclast precursor



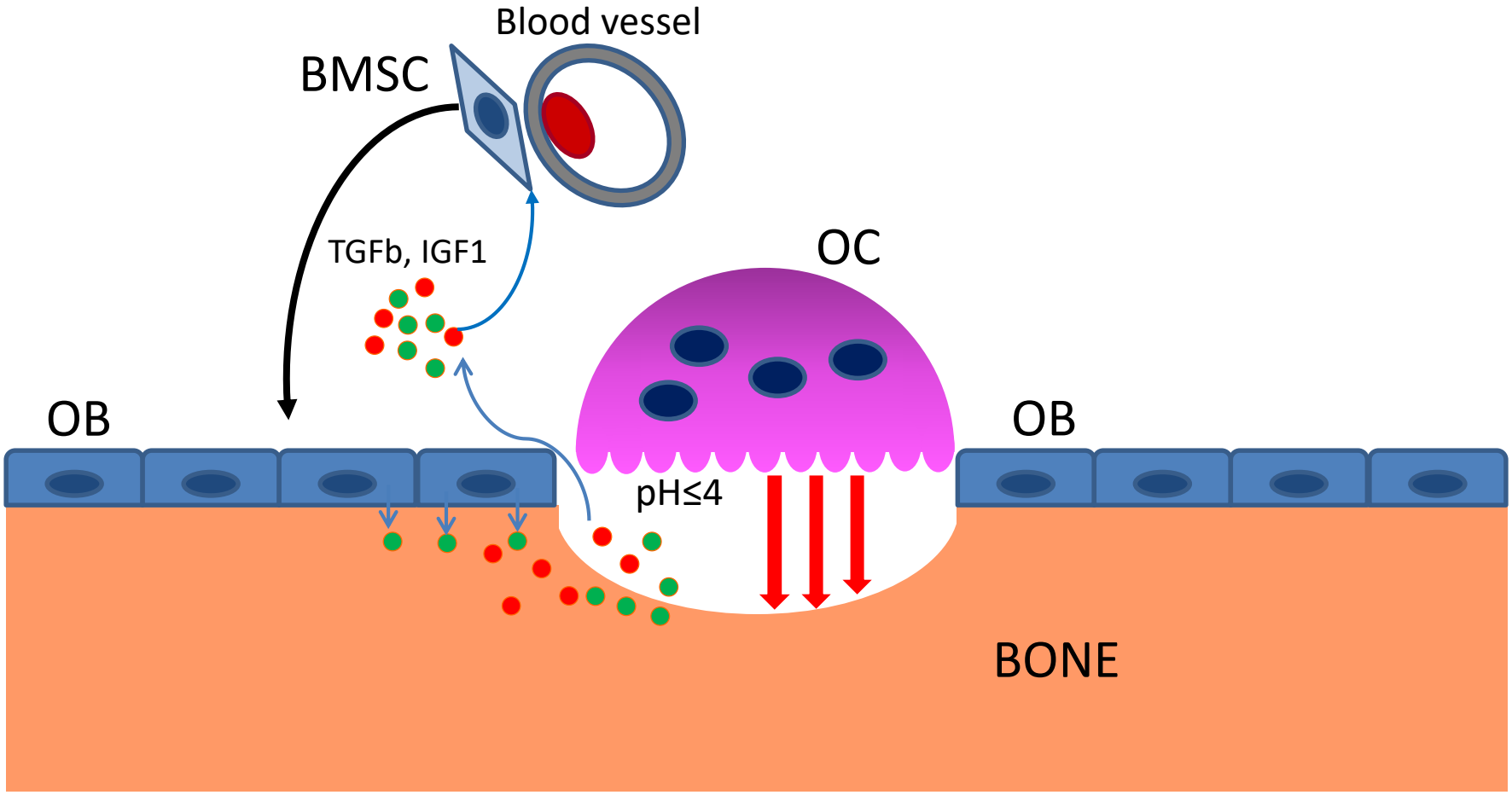
Remodeling: Resorption Pit

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

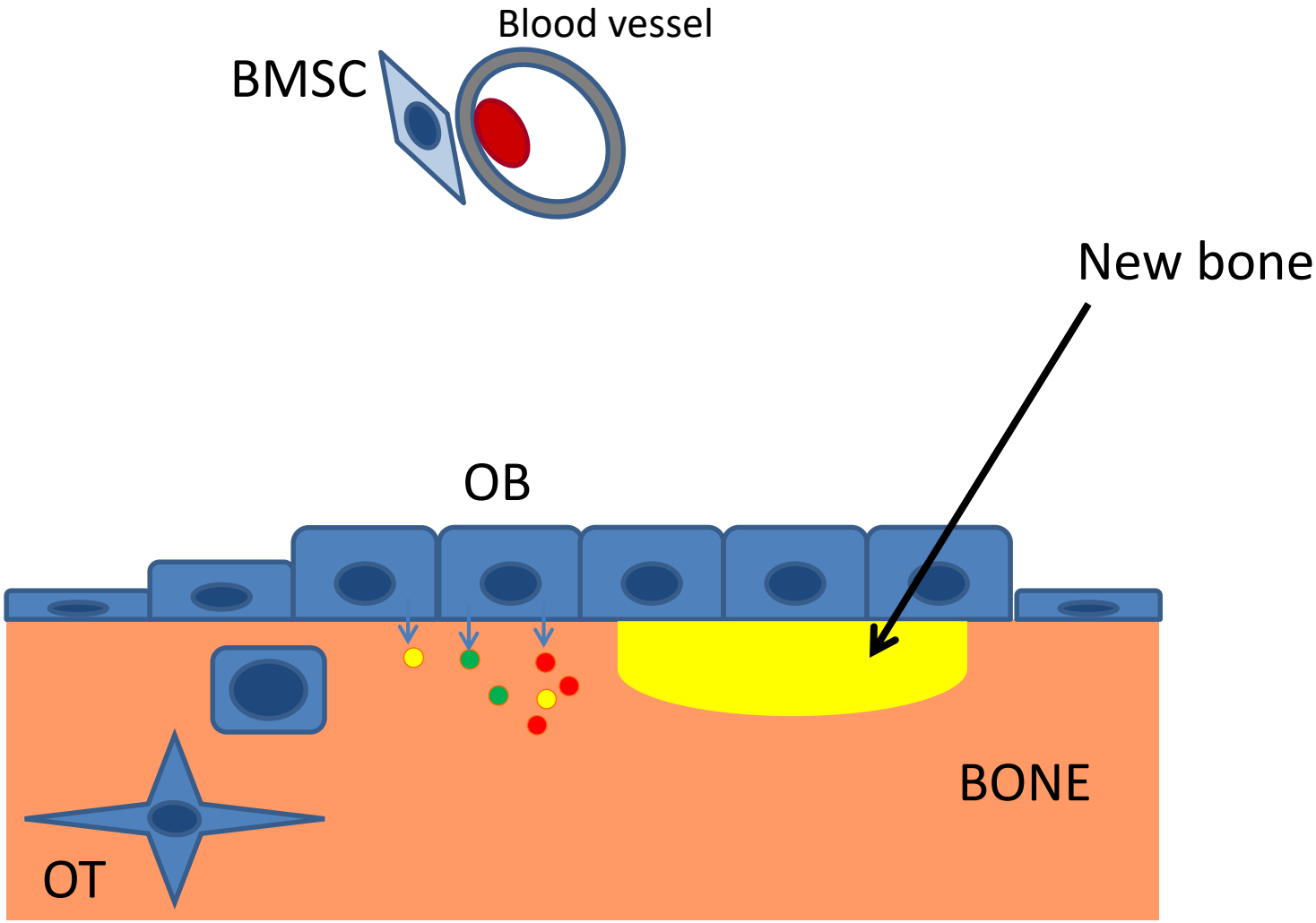




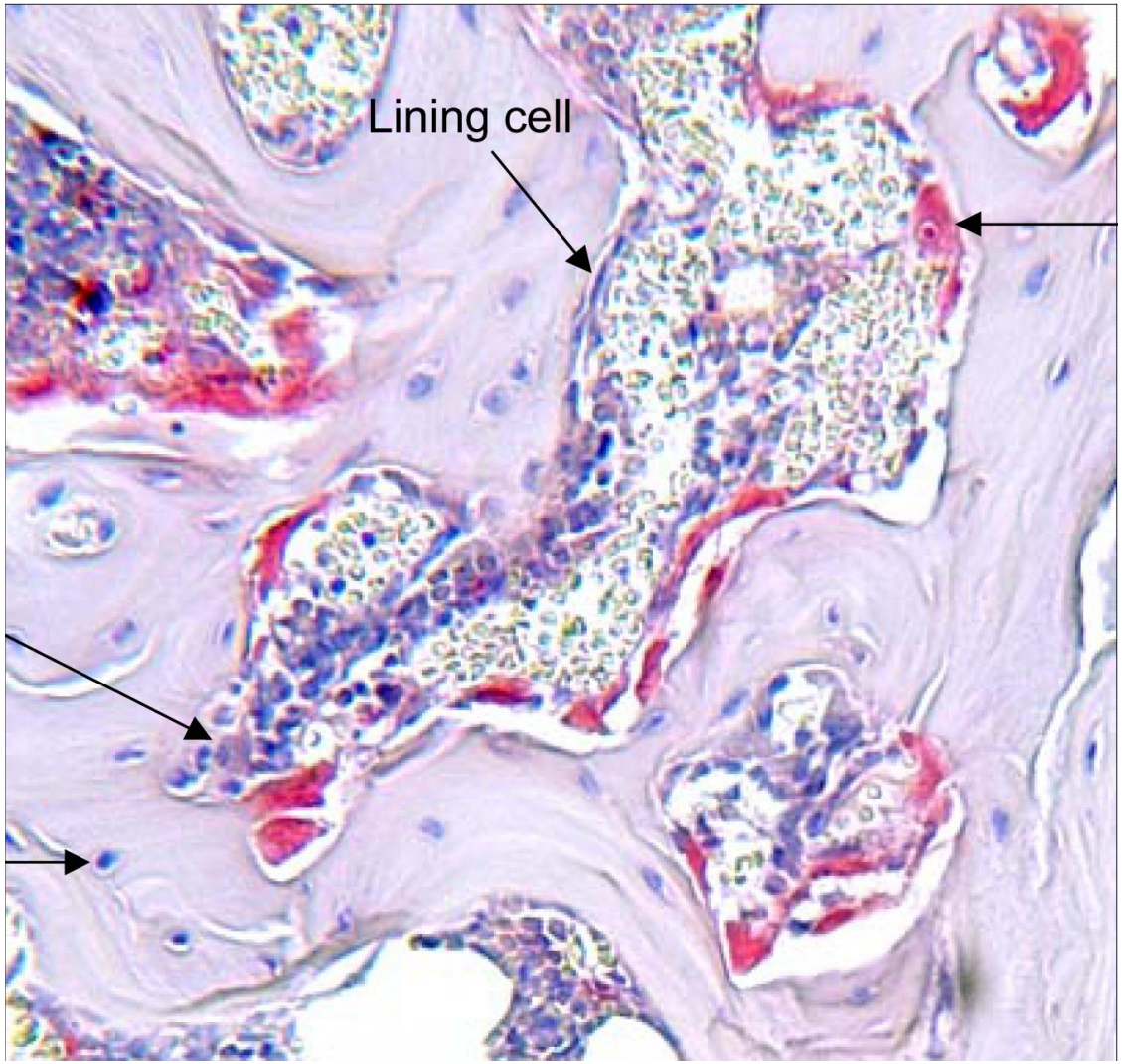
Remodeling: Recruitment/Activation of BMSCs into Osteogenic Lineage



Remodeling: New Bone Formation



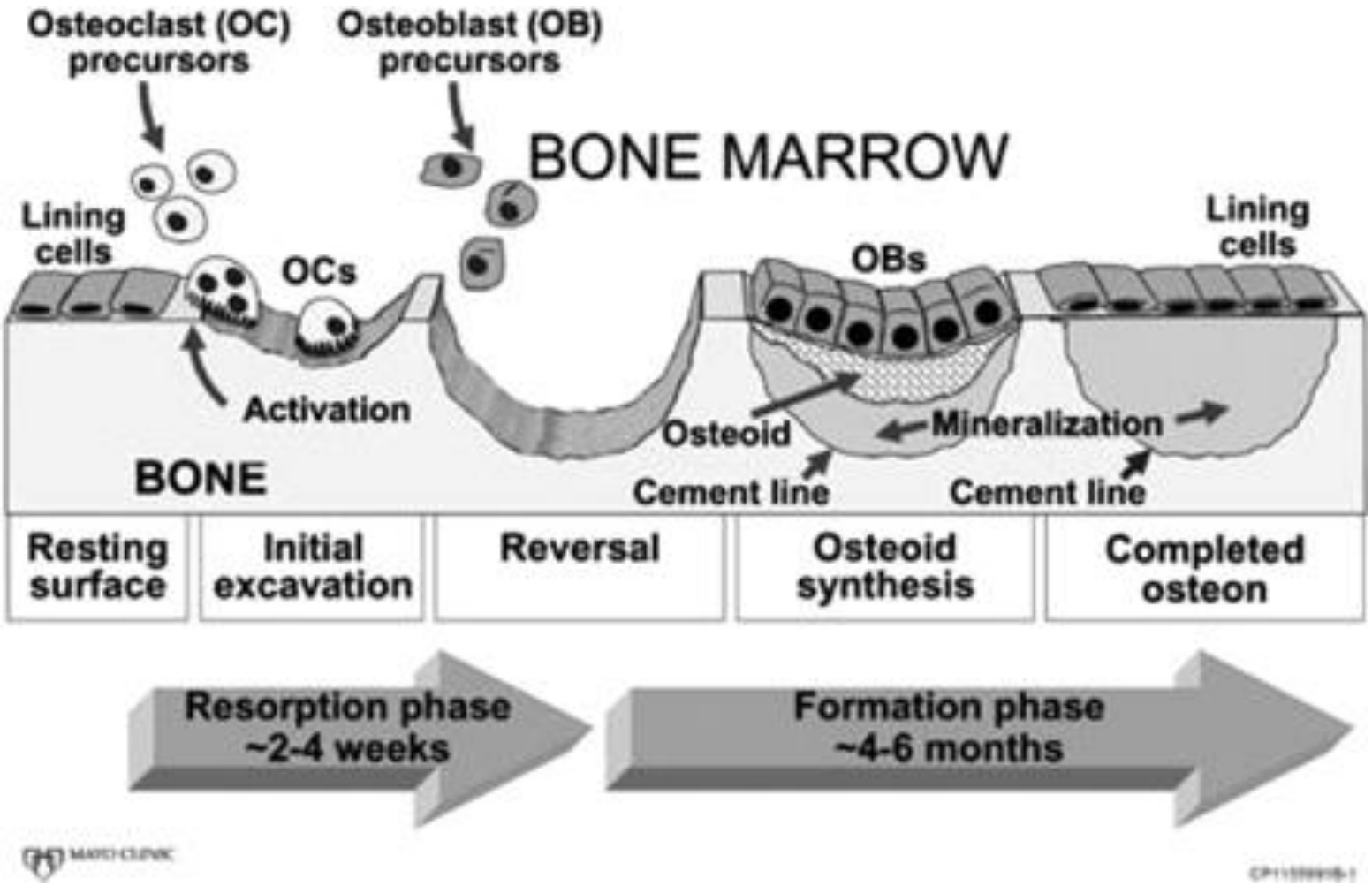
Histology of Bone Remodeling



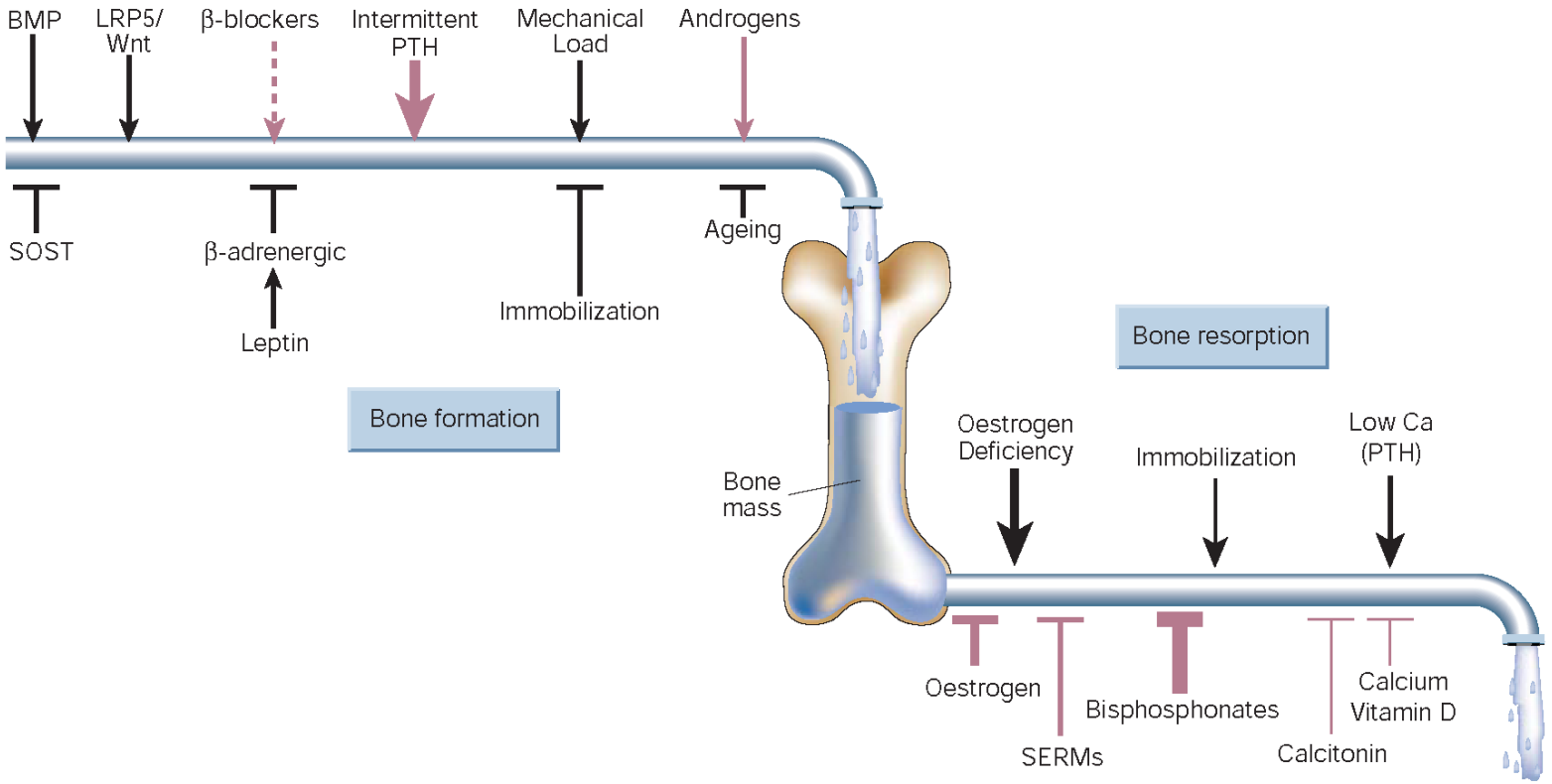
TRAP Staining

Image – courtesy of Dr. J. Jonason

Phases of Remodeling

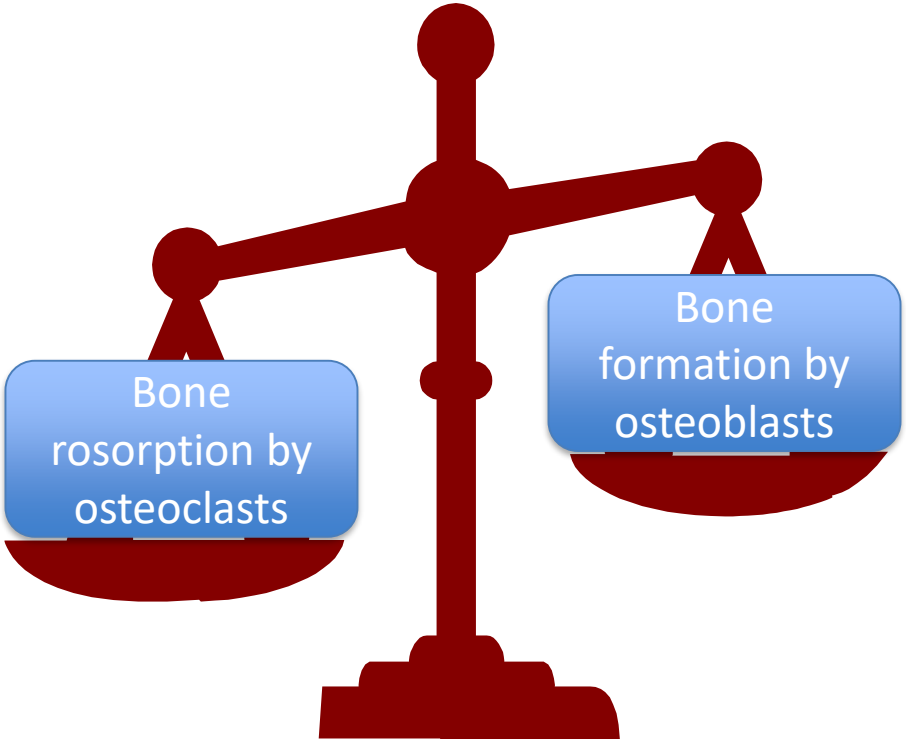


Factors Regulating Bone Homeostasis

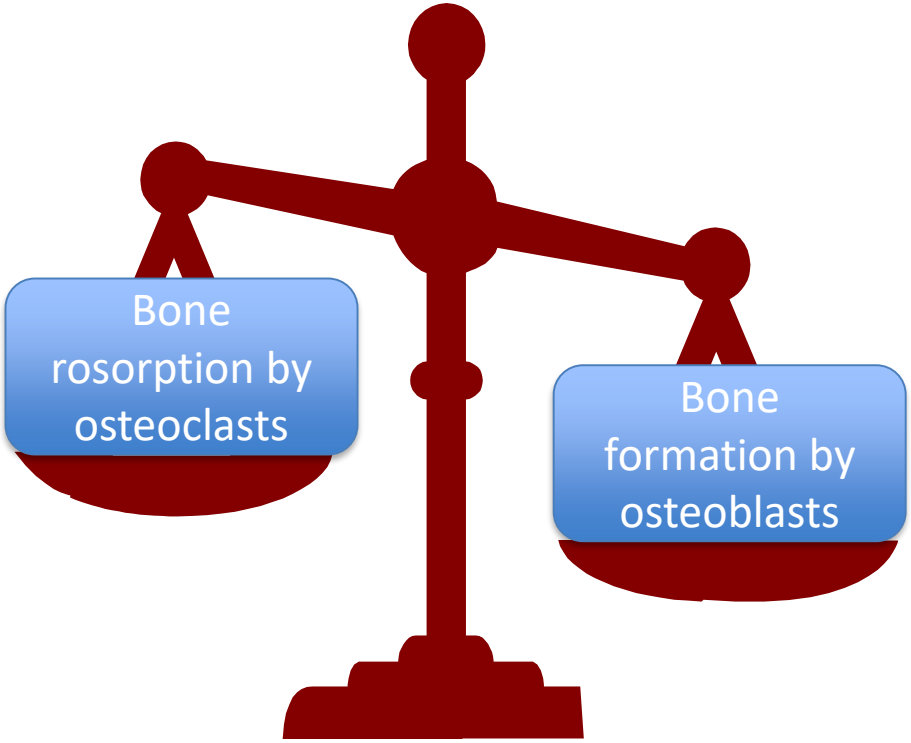


Harada S and Rodan GA., (2003) Nature 423: 349-355.

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 α1 and α2 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**

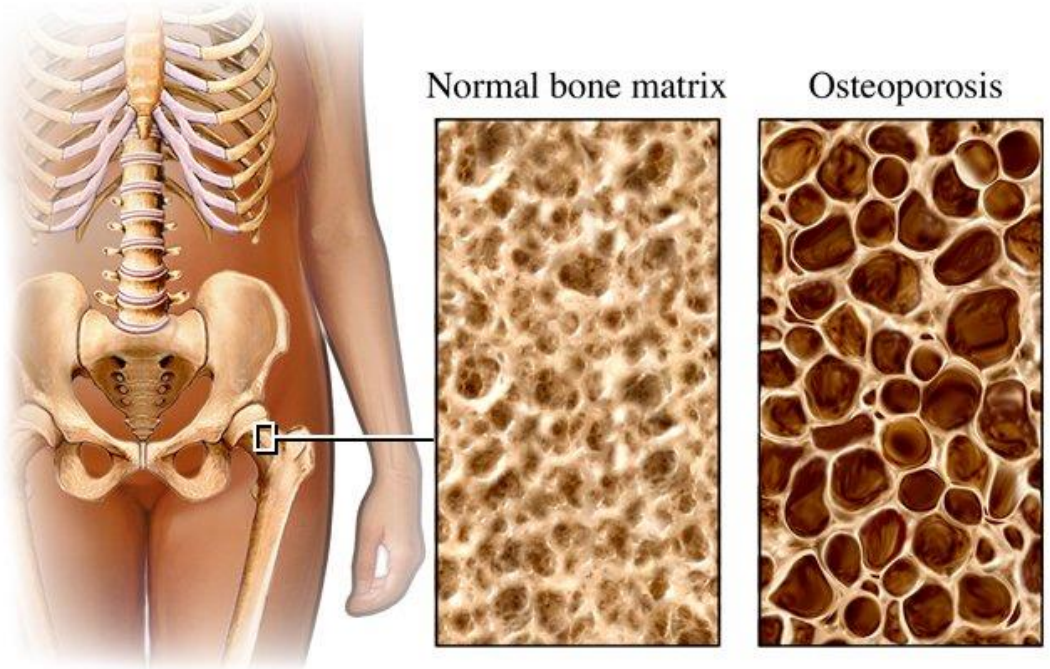
Courtesy of Dr. J. Jonason

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 primary (due to hyperplasia or tumor of the glands) or secondary (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



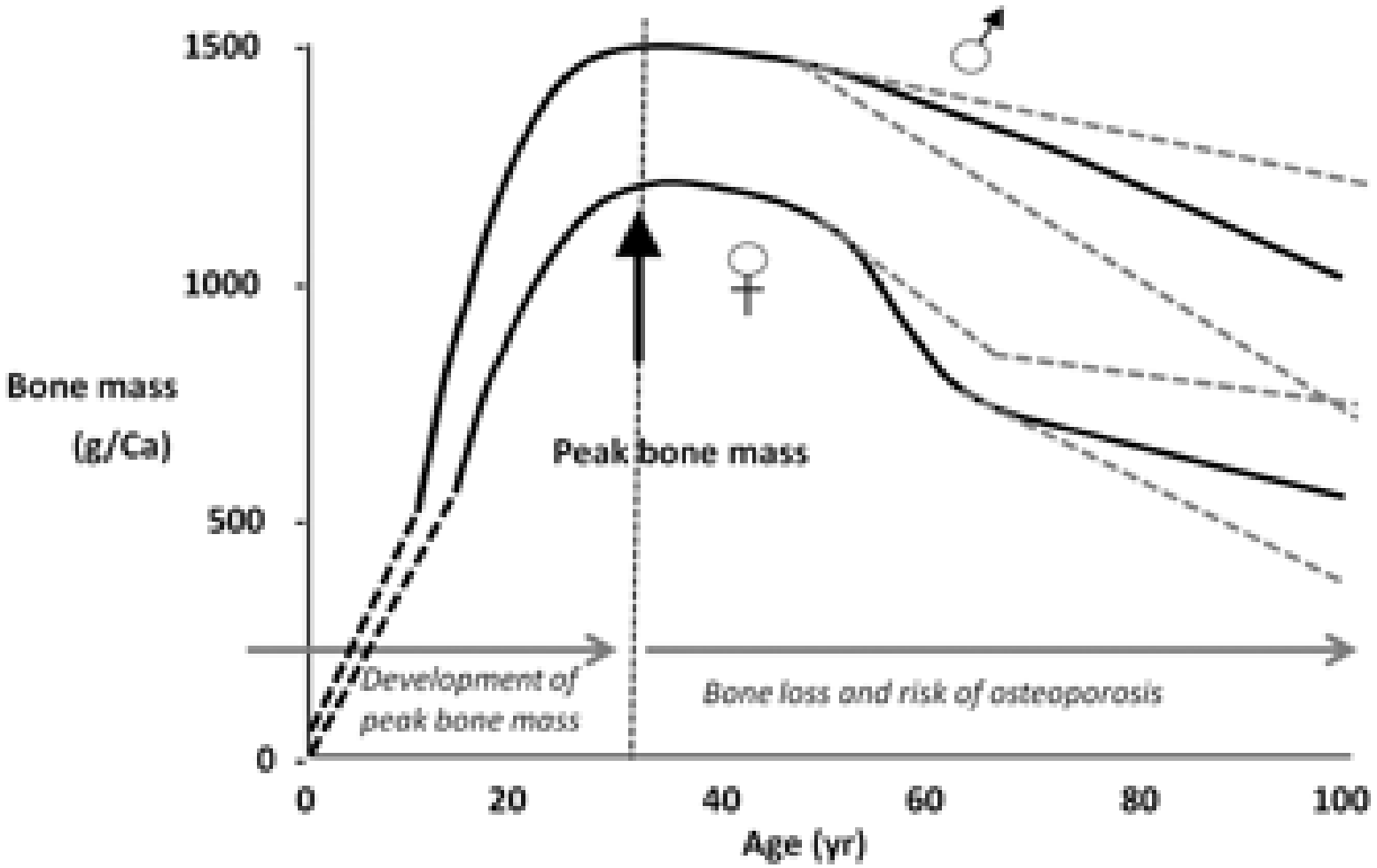
-Fracture
-Delayed repair

Images from Bionews Texas

Epidemiology of Osteoporosis

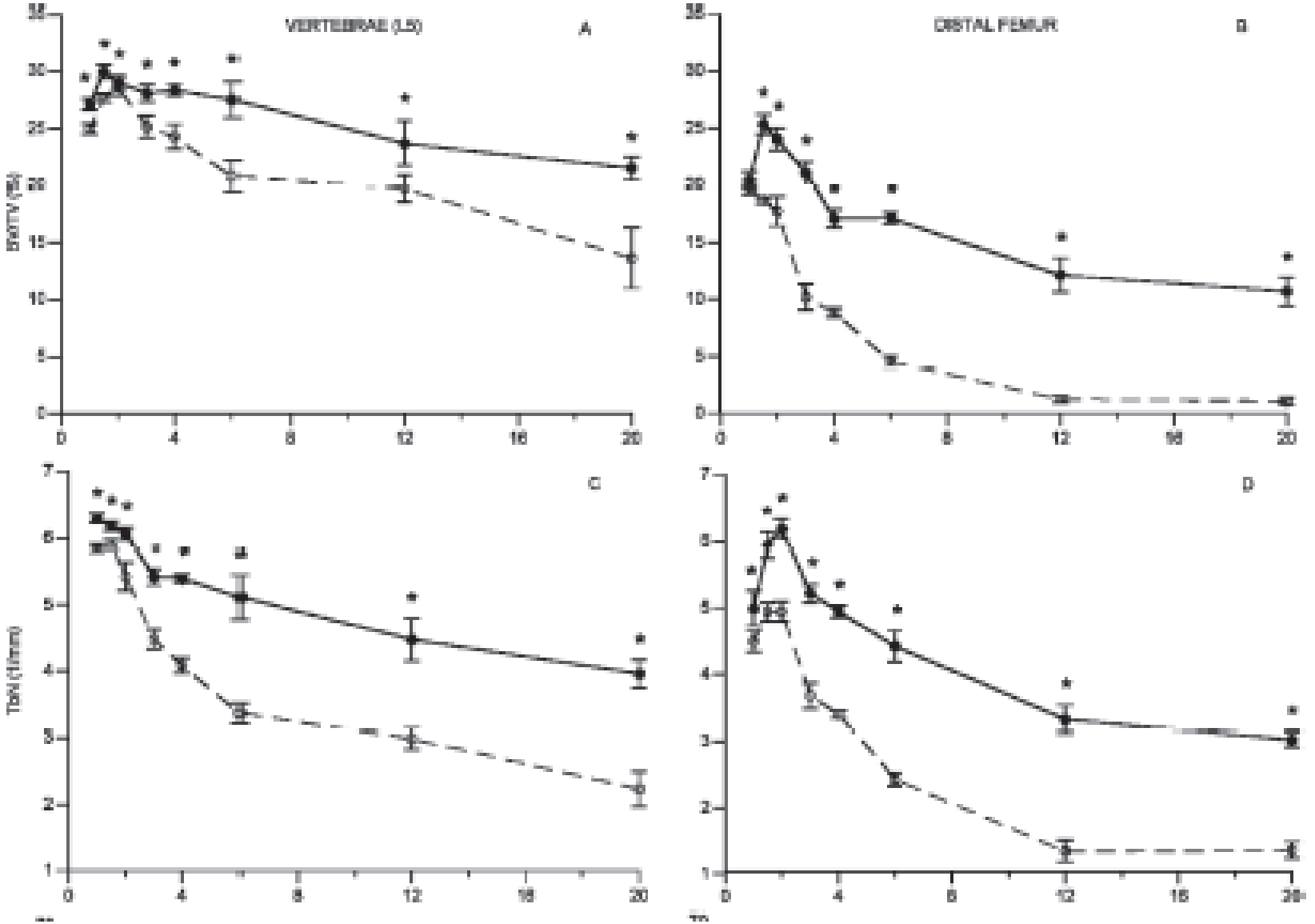
- **Osteoporosis is found in 70% of the elderly population**
- **Post-menopausal women are most affected (25 million American women with Osteoporosis)**
- **1.9 million fractures annually due to low bone mass.**
- **1 of 3 women will develop a vertebral fracture by age 65, and 1 of 3 women will develop a hip fracture by age 85.**
- **$22 billion dollars per year is spent on treating Osteoporosis.**

Epidemiology of Osteoporosis



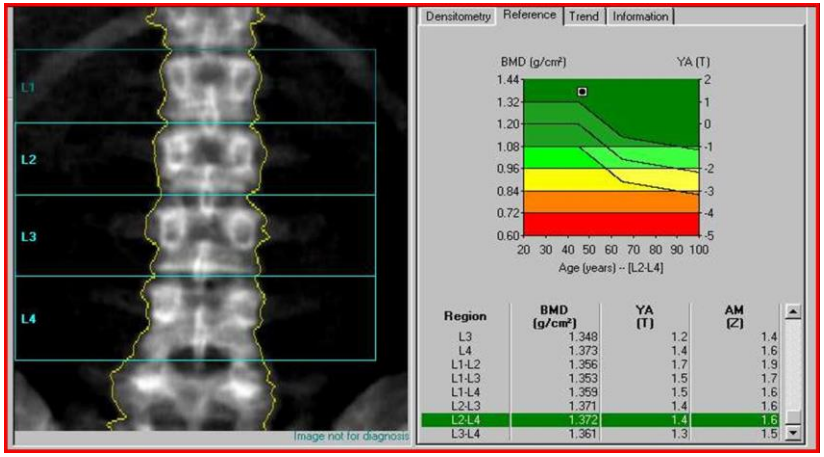
Journal of Bone and Mineral Research
Volume 29, Issue 9, pages 1917-1925, 20 AUG 2014 DOI: 10.1002/jbmr.2286
http://onlinelibrary.wiley.com/doi/10.1002/jbmr.2286/full#jbmr2286-fig-0001

C57Bl/6J mice:



JOURNAL OF BONE AND MINERAL RESEARCH
Volume 22, Number 8, 2007

Diagnosis of Osteoporosis

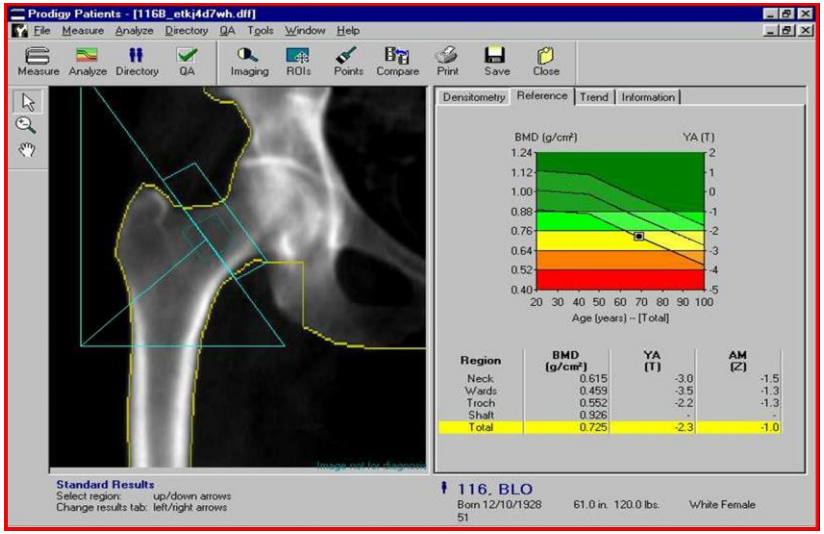


DEXA

Dual Energy X-ray Absorptiometry

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:

Bisphosphonates

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

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

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

Antibody:

Denosumab (Prolia)

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

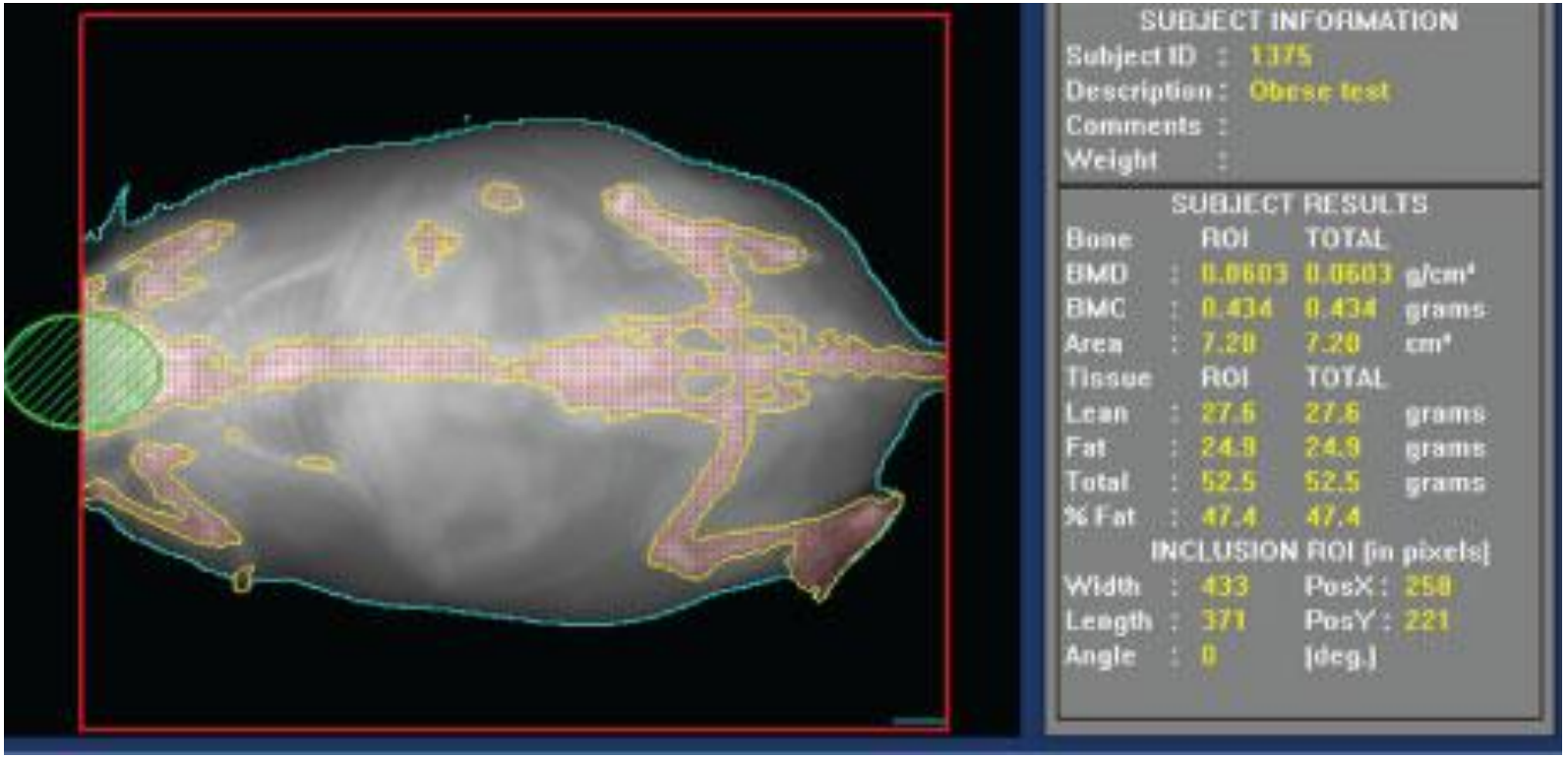
PTH: rPTH(1-34); Forteo. FDA-approved for the use in fracture non-union repair, treatment of osteoporosis.

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-SOST Romosozumab (Evenity). FDA approved for treatment of osteoporosis.

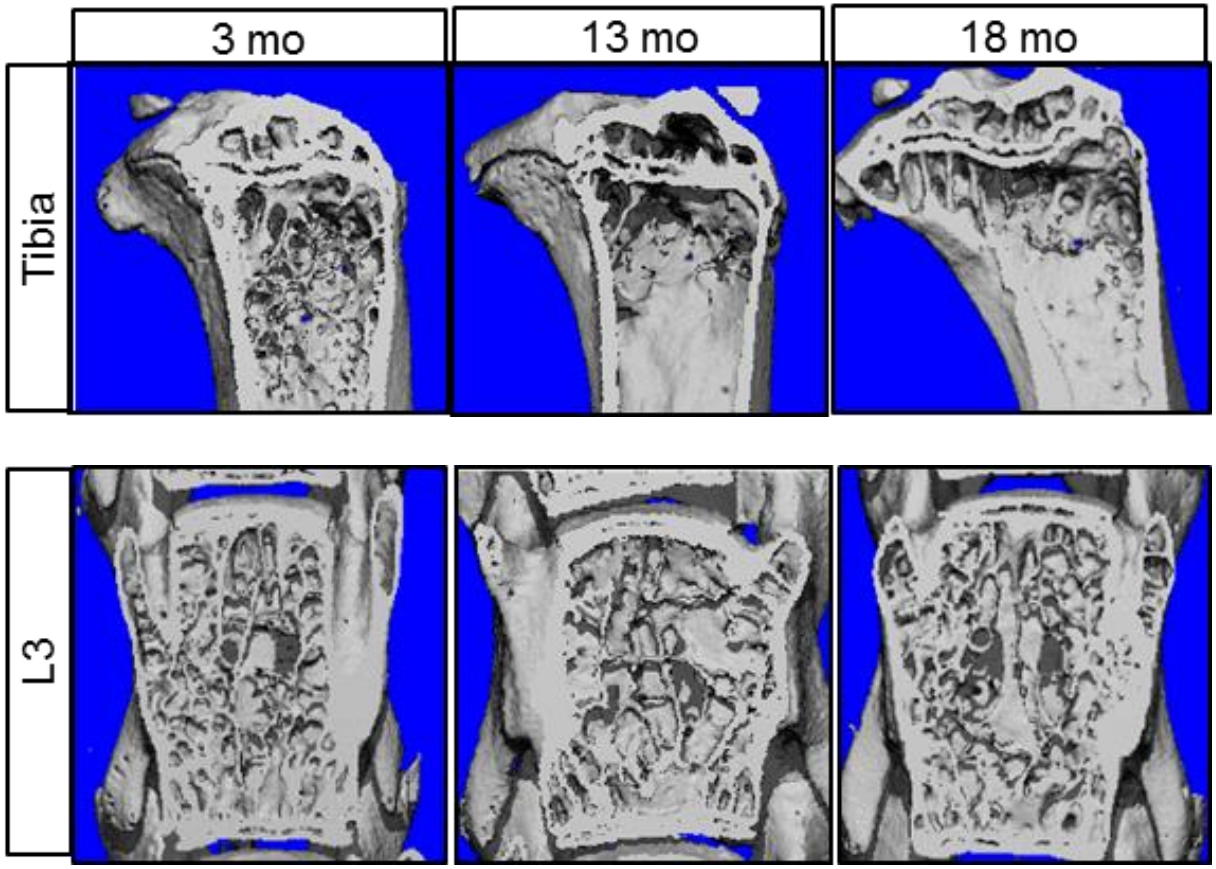
Lab Methods to Assess Bone Homeostasis

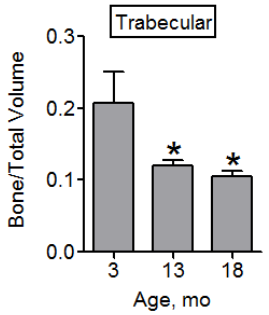
DEXA

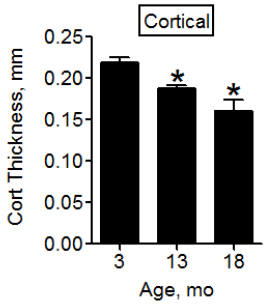


Lab Methods to Assess Bone Homeostasis

micro-CT



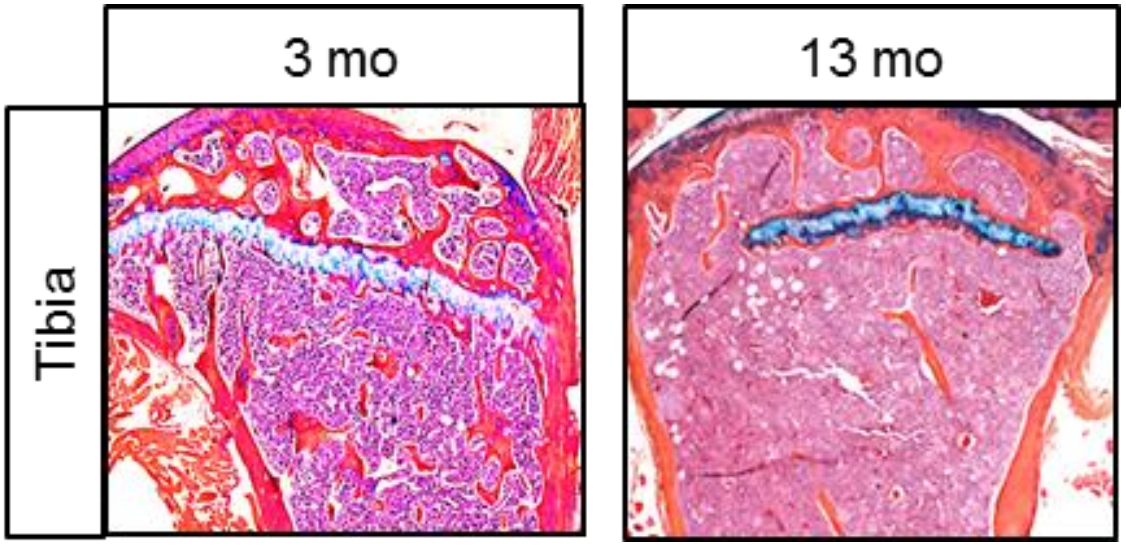


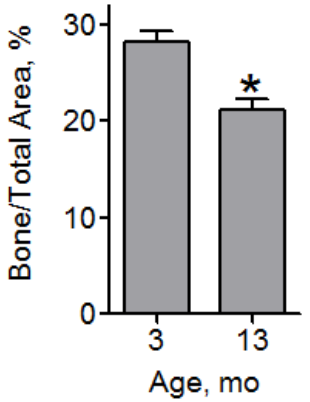


Shum et al., PLoS One, 2016

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

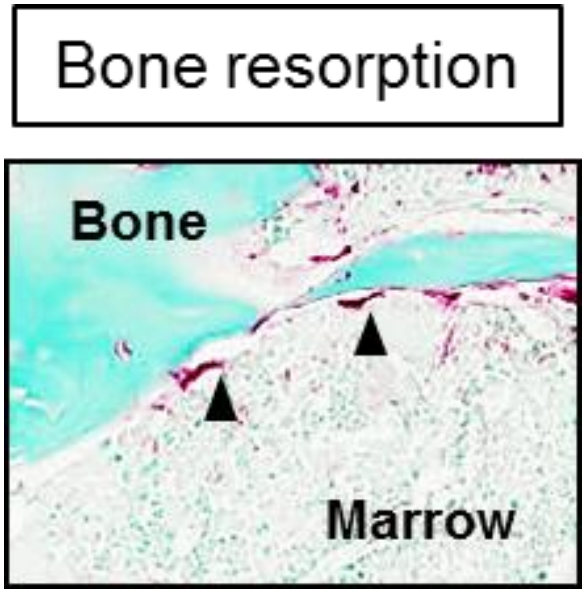
=OB.N.

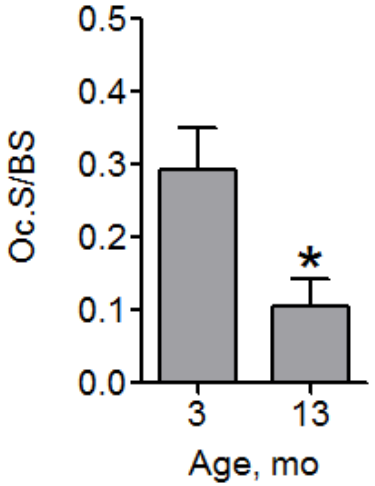
=OT.N.

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)

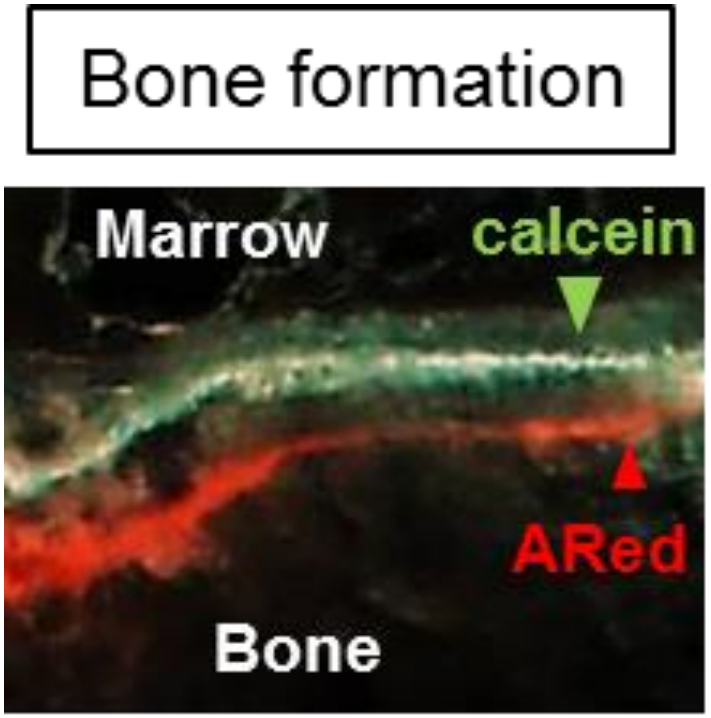
Mice are injected with Alizarin Red 10d before sacrifice & with Calcein 5d before sacrifice. Bone are frozen and sectioned. Labeled bone is visualized using fluorescence microscopy.

Derived Indices:

-Mineralizing Surface (MS/BS)

-Mineral Apposition Rate (MAR)

-Bone Formation Rate (BFR)



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:

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

Useful Mouse Models

Osteoclast-specific Cre Models:

- CD11b
- Lyzm
- Trap

Take-Home Message

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