

Differential staining of cartilage & Bone

Types of stains used { Alcian Blue
Alizarin Red

Why the staining is used:

- 1) study ossification (osteogenesis)
- 2) study abnormal skeleton, which occur due to:
 - ↳ intrinsic - genetics
 - ↳ extrinsic - drugs...

→ study of abnormal development known as tetragony

* embryo is up to week 10-11 after that it is called fetus
↳ due to organogenesis

Principle of the stain:

• Alizarin Red - ionic stain "negatively charged"

↳ will stain positively charged ions

→ such as Ca^{++}

↳ so, all BONY structures will stain Red

→ occurs in intramembranous ossification AKA flat bones

↳ lacrimal bones

↳ sternum

↳ hip bone

↳ scapula

↳ skull (cranial bone)

↳ clavical

↳ vomer

↳ Mandible

Steps of Intramembranous ossification

- 1) Anesthetize the pregnant rat (by diethyl anesthesia)
- 2) dislocate the head
- 3) Dissect & isolate the embryos
- 4) Identify the fetus & the umbilical cord & placenta

→ isotonic solution

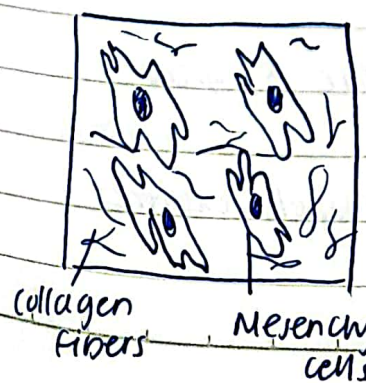
- 5) wash embryos with saline (0.9% sodium chloride)
- 6) Fix the fetuses in 95% ethyl alcohol (or formalyn) for 7 days
↳ to prevent the fetuses from lysis (preserve the fetus)
- 7) Remove the skin & remove all internal organs ^{internal thoracic}
- 8) Place the fetus in acetone
↳ to remove fats
- 9) place fetus in the Alizarin Red & Alcian Blue stain at 37°C (in an incubator) for 7 days
- 10) wash with distilled water
- 11) clear the muscles in the fetus by placing in 1% potassium hydroxide
- 12) Place fetus in 25% glycerin
- 13) Add 50% glycerin, gradually dilute the 1% potassium hydroxide & until we get 100% glycerin

Types of Osteogenesis

- 1) Intramembranous ossification - takes place within fibrous connective tissue
↳ occur in many flat bones
- 2) Endochondral ossification - takes place within cartilage model
↳ occur in the remaining part of the skeleton

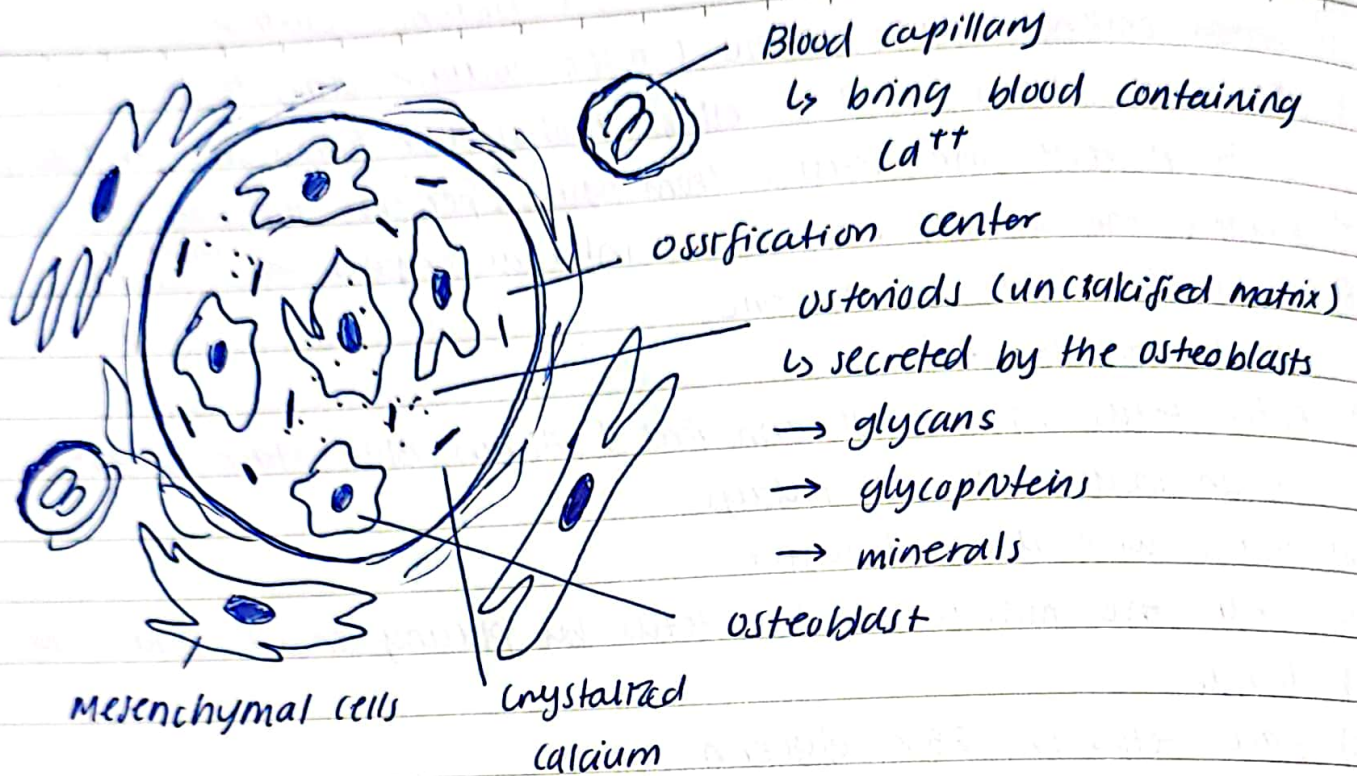
Steps of Intramembranous ossification

• The (FCTM) Fibrous connective tissue membrane is formed of mesenchymal cells, collagen fibers & blood vessels

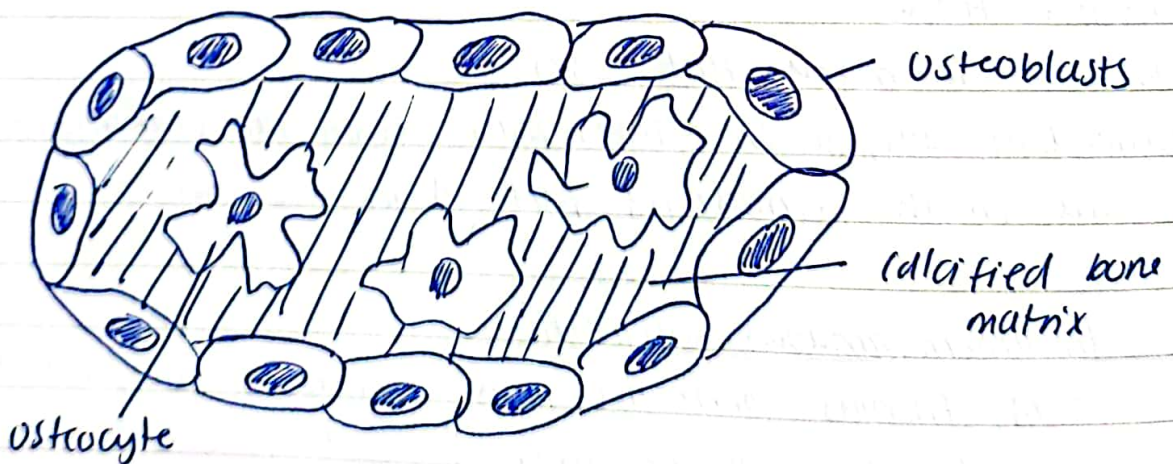


collagen fibers Mesenchymal cells

- 1) the mesenchymal cells will aggregate
↳ start to proliferate by mitosis
→ in a region known as ossification center
↳ result in a bone spicule that contain differentiated mesenchymal cells known as osteoblasts bone-forming cells



- Osteoblasts use the blood Ca^{++} & crystallize the Ca^{++} ions using the enzyme Alkaline phosphatase
 - ↳ the crystallized Ca^{++} make the matrix hard
 - the osteoblasts are trapped, forming a cavity around it
 - ↳ differentiate to ~~osteocyte~~ osteocyte
 - cavity known as lacunae



- ↳ mature bone cells - live inside the cavity
- * Many bone spicules will form, they will fuse & grow around blood vessels → bone trabeculae
 - formation of bone bars around blood vessels cavities
 - AKA spongy (cancellous) bones

In humans, ossification begins about the third month of fetal life & is completed by late adolescence

- Bone spicules will fuse together & form bone trabeculae around blood vessels

- ↳ Bones are highly vascularized

- supplying nutrients, minerals & oxygen

Bone Remodeling

- ↳ osteocytes rearrange at the periphery of the bone into a compact bone structures known as osteons

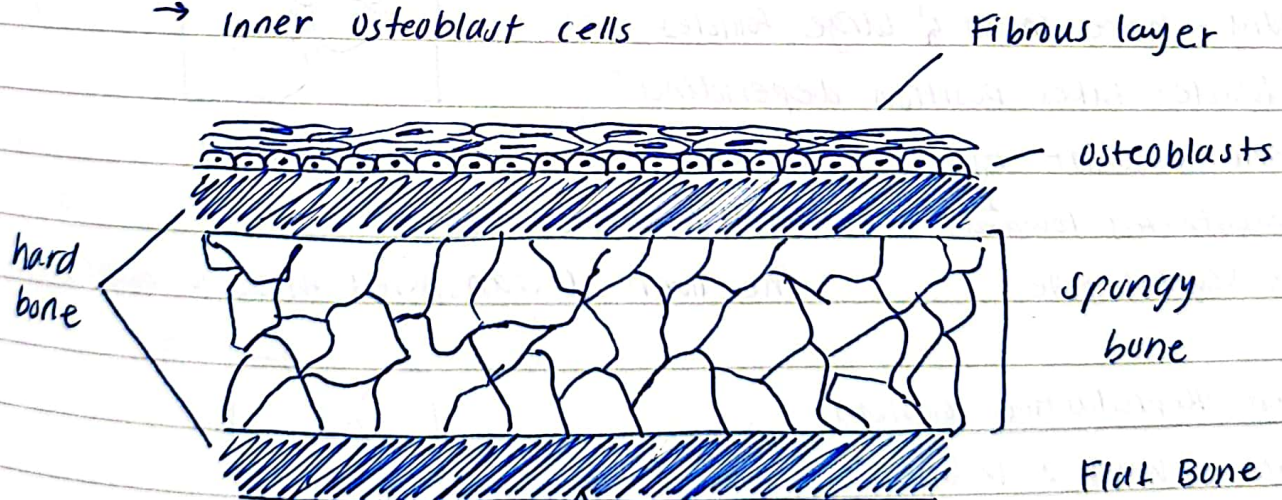
- forming compact (hard) bone

- The mesenchymal cells at the periphery condense & develops into periosteum

- ↳ The periosteum is formed of:

- Outer fibrous layer of collagen fibers

- Inner osteoblast cells



- ↳ osteoclasts cells help in bone remodeling
- ↳ form Medullary cavity

form Compact bone

Osteon
(Haversian system)

