ditki
medical & biological sciences

Bone Development

INTRODUCTION

OVERVIEW

Here, we'll address bone development, called osteogenesis (aka, ossification). Ossification is the formation of new bone. Calcification is the mineralization of soft bone -- the hardening of bone. Bones develop via two different processes.

Endochondral Ossification

• Endochondral ossification is an INDIRECT form of ossification. It originates with mesenchymal tissue, which then transitions to a hyaline cartilaginous model, and then transitions to bone. Most bones develop through this process (think of the long bones (eg, the femur)).

Intramembranous Ossification

• Intramembranous ossification is a DIRECT form of ossification, which originates with mesenchymal tissue that directly differentiates bone. In intramembranous ossification no cartilaginous model is formed. This occurs in only a selection of bones, such as the flat bones of the skull.

DEVELOPMENTAL DIVISIONS

Intramembranous Ossification

Indicate that the majority of the skull and the clavicles form via intramembranous ossification. We can describe, simply, as this as:

• Flat bones of the skull (the cranial vault).

• Facial bones of the skull (including the maxilla and the mandible).

• Clavicle (the majority of it).

Detailed review of the bones of intramembranous ossification

Neurocranium

• Membranous part of the skull: the cranial vault.

- Includes the frontal and parietal bones, the squamous portion of the temporal bone, and the upper part of the occipital bone (the part between the parietal bones ("interparietal").

- It's "membranous" meaning it forms via intramembranous ossification.

• Cartilaginous part of the skull (aka chondrocranium): the skull base.

- The lower portion of the occipital bone.

- It's "chondrocranium" so it forms via endochondral ossification ("chondro" refers to cartilage).

Viscerocranium

• The skeletal bones of the face:

- Includes the maxilla, mandible, and zygomatic portion of the temporal bone.

- Although its beyond our scope here, so as to avoid confusion, be aware that the majority of the bones of the viscerocranium derive from neural crest cells rather than mesoderm.

Clavicles

• The bulk of the clavicle forms via intramembranous ossification but the medial end of the clavicle (which articulates with the sternum) further develops in adolescence and forms via endochondral ossification.

It helps us remember that intramembranous ossification is a more direct form of ossification that doesn't require a transitional model when we consider that the skull bones must ossify prior to delivery of the fetus, so the brain isn't squashed during childbirth.

Endochondral Ossification

We can describe it, simply, as the:

• Axial skeleton (other than the head): the sternum, ribs, and vertebrae.

• Appendicular skeleton (other than the clavicles): the shoulder and hip girdles and the limbs.

To help us remember that the long bones transition from a cartilage model to a bone model, consider that children fall a lot -- so it's better that their bones bounce (flex) and and not break (crack).

INTRAMEMBRANOUS OSSIFICATION

Now, let's learn the key steps in intramembranous ossification. Indicate that intramembranous ossification occurs within a fibrous connective tissue **membrane** (hence it is "intramembranous" - it occurs "within" a "membrane").

OSSIFICATION CENTERS

Mesenchyme

First, draw two pools (condensations) of **mesenchymal cells** within their fibrous connective tissue membranes.

• Although the majority of mesenchymal cells originate from mesoderm, a minority (particularly those of the head) originate from ectoderm (from neural crest cells) that undergo an epithelial-mesenchymal transition (EMT).

• The mesenchymal cells migrate to form these ossification centers.

Indicate that the majority of the skeleton forms via endochondral ossification.

Osteoprogenitor Cells

Show that the mesenchymal cells differentiate into osteoprogenitor cells: bone-forming cells.

Vasculature

Now draw a blood vessel: vascularization is a key for providing the necessary nutrients and supporting cells for bone development.

Osteoblasts

Indicate that the osteoprogenitor cells differentiate into osteoblasts that further organize themselves in the ossification centers.

Osteoid

Show that the osteoblasts secrete osteoid, which is a collagenous precursor to mineralized bone. Think of it as soft goo. It lacks the calcium and phosphate required for mineralization (calcification, hardening). Consider that in osteomalacia, "soft bone disease", the bones lack the necessary vitamin D, calcium, or phosphate to appropriately mineralize, so the bones are too soft.

• The principal inorganic substance (mineral) of osteoid is hydroxyapatite, which is hydroxylated calcium/phosphate.

- Other constituents include magnesium, potassium, sodium, bicarbonate, and citrate.

• The principal organic substances are collagen type 1 fibers and ground substance.

TRABECULA FORMATION

Bone Matrix

Show that the osteoid hardens to form bone matrix.

Osteocytes

The osteoblasts that are trapped in the bone matrix become osteocytes. They reside in lacunae (cavitations) and possess cytoplasmic processes within canaliculi that help them communicate with the rest of the bone. This can help us remember that although it is a hard substance, bone is an active body tissue.

Osteoblasts at the periphery

Show that the osteoblasts populate the periphery of the swath of bone. They will continue to secrete osteoid and enlarge the bony surface area.

Trabecula

We show that the ossification centers merge into a single structure, which we call a trabecula (plural, trabeculae).

INTRAMEMBRANOUS BONE LAYERS

Periosteum (Outer Layer): Collagen Fiber

Let's show how these ossification centers develop into ossified trabeculae.

Periosteum (Inner Layer): Row of Osteoblasts

Periosteum also possess an inner layer of osteoblasts (the cambium layer). These osteoblasts that are requisite for appositional growth – bone widening/thickening, which continues throughout life.

Compact bone

Note that this is not unique to intramembranous bones. Both intramembranous and endochondral bones possess a covering of periosteum and compact bone that thickens and remodels throughout our lives.

Spongy bone

In the center, show spongy bone, which constitutes trabeculae populated with osteocytes, and is covered in endosteum

Red marrow and Vascularization.

Interspersed show red marrow (hematopoietic stem cells and various other hematopoietic cell types) and blood vessels. Remember that bone is actively being remodeled throughout life.

ENDOCHONDRAL OSSIFICATION

Now, let's address the most common form of ossification: endochondral ossification, in which ossification occurs as a transition through a cartilage template. Indicate that "endo" means "within" and "chondral" means "cartilage".

HYALINE CARTILAGE MODEL

Mesenchymal Cell Model

First draw the outline of a mesenchymal cell model (template) in the shape of a long bone and populated with mesoderm-

Mesenchymal Cells Become Chondrocytes

Now, let's draw out the structure of an intramembranous bone.

Now, show that mesenchymal cells transition into chondroblasts (cartilage-producing cells) and become chondrocytes (cartilaginous cells that reside within the cartilage matrix).

• The transcription factor: SOX9 is a key gene that promotes this differentiation from mesenchyme to chondroblast.

Chondrocytes

Chondrocytes are lipid-containing cartilaginous cells that possess the structural components of cartilage: collagen, proteoglycans, and glycosaminoglycans. They are usually found in clusters (isogenic groups) of recently divided cells.

Perichondrium

Perichondrium surrounds the cartilage (hence "peri" for "around" and "chondrium" for cartilage). The perichondrium is a dense but well-vascularized fibrous tissue both protects the underlying cartilage and supplies key cells for growth and development, namely chondroblasts and osteoblasts.Thus, chondroblasts derive from:

• Mesenchymal cell precursors.

• The perichondrium, itself.

PERIOSTEUM FORMATION

Redraw an outline of the cartilaginous model. The epiphyses are the ends of the bone. The diaphysis is the shaft.

Periosteum (Bone Collar)

At the bone shaft (the diaphysis), show that perichondrium becomes periosteum.Osteoblasts lay down peripheral bone (compact bone) around the shaft (bone collar).

The bone collar is essential for the formation of the primary ossification center because it starves the cartilage of necessary nutrients (limits their diffusion).In endochondral ossification, we see a theme of cartilage growth, death, and ossification. The bone collar provides a key element for cartilage death and thus central ossification.

CHONDROCYTE HYPERTROPHY & CALCIFICATION

Chondrocyte Hypertrophy

Show that chondrocytes hypertrophy at the bone ends (the epiphyses). They replicate and divide to produce bone growth in a linear direction, called interstitial growth, at the epiphyseal ends. Think of these epiphyseal ends as growth cartilage.

Chondrocyte Hypertrophy -> Apoptosis & Ossification

Within the primary ossification center, in the center of the bone, show that the chondrocytes hypertrophy but, here, we will see that they lack the nutrients to support cell division.

Calcified Core

Show that hypertrophic chondrocytes in the ossification center secrete a matrix that becomes calcified (essentially a calcified core), which further starves the ossification center of nutrients and promotes further cartilage-cell death.

PERIOSTEAL BUD

Again, redraw the cartilage model.

Chondrocyte Hypertrophy -> Apoptosis & Ossification

Show that a hypertrophic chondrocyte undergo apoptosis.Chondrocyte apoptosis releases factors like vascular endothelial cell growth factor (VEGF), which induces the sprouting of blood vessels (vascular invasion).

(compact bone) around the shaft (bone collar).

Calcium Cavitation -> Medullary cavity.

Show that the calcified core now cavitates. The cavity in the center of bone is called the medullary cavity. It will fill with spongy bone, bone marrow, and related hematopoietic cells.

Periosteal Bud & Primary Spongiosa

Now, in the center, draw a periosteal bud, which is a descriptive term (rather than a singular anatomical structure) for a collection of key materials for production of primary spongiosa (new spongy bone).

• Vasculature

• Osteogenic cells

- Osteoblasts (bone-producing)

- Osteoclasts (bone-resorbing)

• Hematopoietic stem cells.

The periosteal bud vasculature possesses key factors that allow it to pass through the hard compact bone and feed the ossification center, so that spongy bone forms.

Primary Spongiosa

The osteoblasts form new bone (primary spongiosa).

SECONDARY OSSIFICATION CENTERS

We can think of secondary ossification in the same way we do primary ossification except there is no bone collar. Thus, there is no outer layer starving the epiphysis of nutrients. Rather the rapid growth and cartilage-cell death leads to ossification at these centers.The only remaining growth cartilage is found at the epiphyseal plates, in between the diaphysis and the epiphyses. These epiphyseal plates (growth plates) persist until the completion of interstitial growth (we reach our final height), after which time they become the epiphyseal lines.

Finally show vascular invasion at the epiphyses and draw the secondary ossification centers.

WOVEN VS LAMELLAR BONE

Note that you'll commonly see the terms woven bone and lamellar bone, which refers to the degree of bone maturation.

Woven Bone

Indicate that woven bone is an immature (newly-developed) form of bone. Think of it as loosely-arranged (disorganized).

Lamellar Bone

Indicate that lamellar bone is the mature form of bone; it has an organized structure.

• Compact bone has a classic and well-recognized concentric ring organization to its lamellae (bone tissue).

• Spongy bone is linearly arranged; its structure is generally less-well recognized.

INTERSTITIAL GROWTH VS APPOSITIONAL GROWTH

Interstitial growth is linear growth (bone lengthening) whereas appositional growth (at the bone collar) is bone widening

Clinical Correlations

ACHONDROPLASIA

Genetics/Pathophysiology

Achondroplasia is an autosomal dominant disorder of the fibroblast growth factor receptor 3 (FGFR3) that results in excessive inhibition of chondrocyte proliferation, which is essential for endochondral ossification (linear bone growth).

Clinical Effects

It primarily affects endochondral growth at the epiphyseal plates (growth plates) and accounts for ~ 90% of cases of disproportionately short stature (dwarfism).

OSTEOMALACIA ("SOFT BONES")

General

Vitamin D deficiency results in inadequate bone mineralization and bone softening. There is a high proportion of non-mineralized osteoid present.In children, this leads to rickets, in which there are significant skeletal deformities, because the growth plates are affected.In adults, it leads to osteomalacia.

Symptoms

Patients suffer from bone pain, weakness, and fractures.

Labwork

• Calcium, Phosphate, and 25-hydroxyvitamin D (calcidiol) are all typically LOW

(thickening).

• Alkaline phosphatase is very HIGH

Causes of Vitamin D Deficiency (& Osteomalacia)
There are a variety of causes of inadequate vitamin D, below are some notable causes:

• Reduced intake and intestinal malabsorption (eg, celiac disease)

• Reduced sunlight exposure

• Reduced skin synthesis of vitamin D

• Reduced vitamin D metabolism

- Medications that impact vitamin D metabolism include seizure medications, steroids, certain antivirals (eg, AIDS medications (HAART), bisphosphonates, barbiturates, etc…

• Phosphatase deficiency

• Kidney diseases, including genetic defects in vitamin D hydroxylation in the kidney and chronic kidney disease (in general).

• Various tumors (mesenchymal, metastatic, leukemic, etc…)

• Lead and aluminum toxicity