Stages of Intramembranous Ossification: This is the type of ossification that occurs in the flat bones of the skull and also the mandible and clavicle. This type of ossification also occurs during healing following a bone injury.

1. In the area where bone is to form, the mesenchyme becomes richly vascularized. Mesenchymal stem cells (MSCs) start replicating until there is a dense aggregate of cells called a osteogenic center. At this stage the cells stop replicating and differentiate into osteogenic cells and then osteoblasts. The differentiation into osteoblasts is thought to be due to the influence of factors such as bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF) and transforming growth factor beta (TGF β). Remember that the osteoblasts are the bone building cells, so following their formation they begin to secrete matrix containing collagen fibers. At this point the matrix is not mineralized, so we refer to it (the matrix) as osteoid.

2. Osteoblasts release factors like osteocalcin and alkaline phosphatase into the osteoid, which after some lag time promote calcification of the matrix. Once the osteoblasts are completely surrounded by matrix, they stop secreting matrix and become osteocytes. These osteocytes are contained within lacunae, and send out cytoplasmic processes through canaliculi to allow communication between cells “trapped” within the calcified matrix.

3. As the matrix forms, we begin to see trabeculae that will form spongy bone. Blood vessels grow into the spaces between the trabeculae, and the connective tissue associated with those vessels becomes the red bone marrow.

4. Mesenchyme condenses at the periphery of the bone and the periosteum is formed. The superficial layers of spongy bone will eventually be replaced with compact bone (produced by osteoblasts of periosteum), but the interior of the bone will still contain spongy bone tissue.
Steps of Endochondral Ossification: This involves formation of bone from a hyaline cartilage model. All bones from the base of the skull down, except for the clavicles, are formed in this way.

1. Development of the cartilage model. At the site where the bone is going to form, mesenchymal cells differentiate into chondroblasts. The chondroblasts secrete cartilage extracellular matrix, resulting in a cartilage model of the bone. The perichondrium forms over the hyaline cartilage bone model.

2. Growth of the cartilage model. Once the chondroblasts are surrounded by matrix, they are called chondrocytes. The model grows in length as the chondrocytes divide and more matrix is secreted. This is called interstitial growth. New chondroblasts in the perichondrium secrete matrix on the periphery of the model, resulting in appositional growth. As the model continues to grow, chondrocytes deep in the center of the model hypertrophy. They stop secreting collagen and begin secreting alkaline phosphatase, an enzyme essential for mineral deposition. As a result, the surrounding matrix begins to calcify. Chondrocytes within the areas of calcification die, as it is now much harder to transfer nutrients through the calcified matrix. As the cells die they leave lacunae which merge into small cavities. Because of the loss of the chondrocytes in the center of the model, the matrix in that area begins to weaken.

3. Development of the primary ossification center. A nutrient artery penetrates the perichondrium (@ 8 weeks of development) resulting in osteoblast rather than chondroblast formation, and thus the perichondrium becomes the periosteum. A “bone collar” forms along the surface of the diaphysis to help provide structural support for the model. Capillaries penetrate the disintegrating calcified center of the model. As the capillaries continue to invade the model, erosion of the calcified matrix continues. The osteogenic cells associated with the connective tissue sheath of the invading blood vessels differentiate into osteoblasts. The osteoblasts start to secrete bone matrix around the remnants of the calcified cartilage, forming the primary ossification center. The primary ossification center is usually present @ 12 weeks of age. The primary ossification continues to spread towards the ends of the models.

4. Development of the medullary cavity. As the primary ossification center spreads, osteoclasts break down some of the newly formed spongy bone. This causes formation of the medullary cavity in the diaphysis. The walls of the diaphysis will eventually be replaced by compact bone.

5. Around the time of birth, secondary ossification centers form in the epiphyses following invasion by branches of the epiphyseal artery. Bone formation is similar to primary ossification, with the exception that spongy bone is not removed.

F. Formation of articular cartilage and epiphyseal plate. The hyaline cartilage covering the ends of the epiphyses becomes the articular cartilage, and the cartilage between the diaphysis and epiphysis becomes the epiphyseal (growth) plate.

G. Closure of the growth plate. In the late teens/early twenties the cartilage in the epiphyseal plate is replaced with bone tissue. Once the bone tissue is present, the bone can no longer increase in length.
Fracture repair steps:

1. **Formation of fracture hematoma** (6-8 hours post injury) - blood vessels crossing the fracture line are broken, blood leaks out and a clot forms around the fracture site. Because there is no longer circulation in the area of the fracture, nearby bone cells die. Inflammation occurs... redness and heat due to increased blood flow to affected area. This is beneficial since the increased blood flow will bring along cells to clean up the damaged tissue. Swelling due to increased leakiness of vessel walls – fluid starts moving into surrounding tissue. The clean-up may last several weeks.

2. **Fibrocartilage callus formation** (takes @ 3 weeks) – fibroblasts from a nearby area of the periosteum migrate to the fracture site and produce collagen fibers. Other cells in the periosteum differentiate into chondroblasts, which begin to produce fibrocartilage. The callus is the collection of collagen fibers and cartilage that bridge the gap in the injured tissue.

3. **Bony callus formation** (lasts 3-4 months) – in areas close to healthy tissue, osteogenic cells differentiate into osteoblasts which begin to lay down spongy bone. Over time, the fibrocartilage callus is replaced by spongy bone tissue.

4. **Bone remodeling** – Osteoclasts resorb the dead pieces of broken bone, and compact bone replaces spongy bone along the periphery of the fracture. A thickened area on the surface of the bone remains as evidence of a healed fracture.