CARTILAGE, BONE & JOINTS

CARTILAGE

The Basics:

- specialized connective tissue
- rigid, elastic & resilient – RESISTS COMPRESSION
- AVASCULAR – necessary nutrients diffuse through matrix

The Components:

- **perichondrium** – dense irregularly arranged connective tissue (type I collagen); ensheaths cartilage; houses vasculature; home of chondroblast precursors (look like fibroblasts) *Note from Dr. Rothman: this is true of the portion of the perichondrium closest to the cartilage.*
- **chondroblast** – progenitor of chondrocyte; secretes type II collagen and other extracellular matrix components (chondroblasts build); lines the border b/t perichondrium and matrix
- **chondrocyte** – mature cartilage cell surrounded by matrix; reside in spaces called lacunae; active chondrocytes secrete collagen, GAG, and proteoglycans
- **matrix** – composed of fibers (either collagenous or elastic) and ground substance (rich in glycosaminoglycans [GAGs], especially chondroitin sulfates); provides rigidity, elasticity, and resilience
  *Note: GAG are highly negatively charged due to sulfate and carboxyl groups on their sugars; this negative charge causes them to stain basophilic, and it attracts water. The water creates a hydrated gel-like matrix*

Methods of Growth:

- **appositional** – increasing in GIRTH or WIDTH; new cartilage cells, chondroblasts, arise from inner layer of the surrounding perichondrium and secrete extracellular matrix; forms new cartilage at surface of preexisting cartilage
- **interstitial** – increasing LENGTH; forms new cartilage within the cartilage mass; chondrocytes divide and secrete matrix from within their lacunae; This cartilage cell division occurs in all types of cartilage and is associated with endochondral bone formation. *Note from Dr. Rothman: can affect WIDTH in other kinds of cartilage.*

Types of Cartilage:

1) hyaline
2) elastic
3) fibrocartilage
<table>
<thead>
<tr>
<th><strong>HYALINE</strong></th>
<th><strong>ELASTIC</strong></th>
<th><strong>FIBROCARTILAGE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APPEARANCE</strong></td>
<td>Hundreds of eyes staring back at you.</td>
<td>Layers of collagen fibers visible; Chondrocytes aligned between collagen fibers</td>
</tr>
<tr>
<td><strong>FUNCTION</strong></td>
<td>Support of tissues &amp; organs; bone development</td>
<td>Support with flexibility</td>
</tr>
<tr>
<td><strong>LOCATIONS</strong></td>
<td>Nasal septum, larynx, tracheal rings, articular surfaces of joints, sternal margins of ribs</td>
<td>External ear, external auditory canal, epiglottis, part of laryngeal cartilage, Eustachian tubes</td>
</tr>
<tr>
<td><strong>MATRIX</strong></td>
<td><strong>COLLAGEN</strong></td>
<td>Type II (thin fibrils)</td>
</tr>
</tbody>
</table>
| **GROUND SUBSTANCE** | 3 types of GAGS  
- chondroitin sulfate  
- keratin sulfate  
- hyaluronic acid  
(proteoglycan monomer = GAG + core protein) contains a lot of water | (same) | |
| **STAINING** | Matrix – basophilic due to GAGs (neg. charged sulfate grps)  
Territorial matrix – surrounds lacunae; more basophilic due to high concentration of proteoglycans secreted by chondrocytes  
Chondrocytes – active in matrix production; dark blue nuclei; clear areas b/c Golgi apparatus and lipid droplets | Weigert stain – elastic fibers stain black |  
H&E – type I collagen layers are intense pink; matrix is basophilic  
Orcein van Giesen Elastic stain – Fibrocartilage is reddish brown (nucleus pulposus at center); NOTE: hyaline cartilage is yellow |
**Bone**

**What?** specialized connective tissue: cells + mineralized matrix

→ mineralization confers *rigidity* and *strength*

**Functions:**
1) support & protection
2) storage site of calcium & phosphate
3) encloses hematopoietic elements of bone marrow

*Throughout life, bone is in a **dynamic state** of growth & resorption to accommodate changing *mechanical stress* & the demands of *calcium homeostasis.**

**Cells in Bone:**

<table>
<thead>
<tr>
<th>Osteoblasts</th>
<th>Osteocytes</th>
<th>Osteoclasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocyte progenitors</td>
<td>Mature, non-dividing bone cells</td>
<td>Remodeling cells</td>
</tr>
<tr>
<td>Secrete collagen I &amp; ground substance for <em>osteoid</em> (unmineralized bone matrix)</td>
<td></td>
<td>Release lysosomal enzymes to digest bone; bone remodeling/ resorption</td>
</tr>
<tr>
<td>Initiate matrix calcification</td>
<td>Enclosed in calcified bone matrix</td>
<td></td>
</tr>
<tr>
<td>Single layer on surface of <em>forming</em> bone</td>
<td>Lacunae</td>
<td>Howship’s lacunae</td>
</tr>
<tr>
<td>Basophilic*</td>
<td>Less basophilia than osteoblasts</td>
<td>Acidophilic*</td>
</tr>
<tr>
<td>Cuboidal, Polygonal</td>
<td></td>
<td>Large cells, ruffled border on EM (membrane foldings around bone seal off spaces for localized enzymatic digestion)</td>
</tr>
<tr>
<td>Eccentric, Euchromatic nuclei</td>
<td></td>
<td>Multinucleated</td>
</tr>
<tr>
<td>Prominent nucleoli &amp; Golgi</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Osteoid newly-secreted from osteoblasts is light-staining (not calcified) | Canaliculi connect osteocytes and can be well-visualized in ground bone sections. | Parathyroid hormone → increase osteoclast activity Calcitonin → decrease osteoclast activity |

*Rather than memorize details, think about patterns: rER and other necessary synthetic components stain *basophilic* to H&E, so cells producing high levels of proteins, etc., will have basophilic cytoplasm. Cells with high levels of lysosomal enzymes, like osteoclasts, tend to be *acidophilic.*
Matrix:

- **type I collagen**
- ground substance w/ proteoglycans & non-collagenous glycoproteins (ALL MINERALIZED)
- mineral = calcium phosphate in form of hydroxyapatite crystals

Types of Bone:

**Mature vs. Immature Bone**

**Mature (Adult bone; Lamellar)**
- Compact
  - *Haversian systems*
  - *Dense layer covering bone exterior*

**Immature**
- WOVEN BONE
  - *Deposited in fetal skeleton/ following a fracture*
  - *Nonlamellar*
  - *Irregular collagen in proteoglycan matrix (unmineralized)*
  - *More cells & ground substance than mature bone*
  - *Stains more intensely w/ H&E than mature bone*

*Can also classify bones as long vs. flat.*

**Long Bones**
- *Growth = endochondral ossification*
- *Examples: Tibia, Metacarpals*
- *Components:*
  - **diaphysis** = shaft
    - marrow cavity surrounded by compact bone
  - **epiphysis** = expanded bone end
    - spongy bone surrounded by thin compact bone shell
  - **metaphysis** = flared portion b/t diaphysis & epiphysis
  - **epiphyseal plate** = cartilage separating epiphyseal & diaphyseal cavities; maintains growth process

**Flat Bones**
- *Growth = intramembranous ossification*
- *Examples: Skull, Sternum*
- *Thin & plate-like*
Methods of Bone Growth:
- **appositional vs. interstitial**
  - appositional = growth of bone tissue on pre-existing surface of bone, beneath periosteum
  - interstitial = bone growth via formation of new cartilage within existing cartilage mass
  - ALL GROWTH OF BONE TISSUE IS APPPOSITIONAL
- Long Bone Growth in.....:
  - WIDTH $\rightarrow$ appositional growth
  - LENGTH $\rightarrow$ interstitial growth

- **endochondral vs. intramembranous**
  - These describe mechanism of growth only – not type of existing bone. The remodeling process replaces the initial bone laid down by these processes.
  - endochondral – CARTILAGE MODEL SERVES AS PRECURSOR
    - fetal development
    - mesenchymal cells condense, aggregate, and differentiate into chondroblasts
    - chondroblasts lay down cartilage model; cartilage model grows in length by interstitial growth and width by appositional growth
    - bony collar develops around shaft of growing bone
    - calcification of cartilage matrix occurs in this region causing death of chondrocytes
    - lacunae become confluent, creating larger cavity in center of model
    - periosteal cells migrate in; differentiate into osteoblasts, and begin to lay down osteoid on calcified spicules that remain in cavity
    - *Calcified cartilage that remains is basophilic. New bone is eosinophilic.*
  - growth in young adulthood.
    - Occurs @ epiphyseal plate
    - from epiphysis to diaphysis, the zones of growth are:
      - zone of reserve cartilage – randomly arranged chondrocytes; no proliferation; area to be “tapped” for bone-destined chondrocytes
        - HALLMARKS: Cells most sparse; appears like “normal” cartilage; closest to distal edge of epiphyseal plate
      - zone of proliferation – chondrocytes undergo division and are organized in distinct columns (stacks of poker chips); actively producing matrix.
        - HALLMARKS: Look for cells of “normal” size that have increased in number & appear to stack.
      - zone of hypertrophy – chondrocytes and lacunae are enlarged
        - HALLMARKS: Clear cytoplasm from glycogen accumulation; matrix compressed between columns of large cells.
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- **zone of calcification** – matrix begins to mineralize, causing chondrocyte death (cuts chondrocytes off from nutrients)
  - HALLMARKS: Huge dying cells; empty lacunae; lacunae invaded by blood vessels.
- **zone of ossification** – osteoblasts deposit osteoid on exposed cartilage
  - HALLMARKS: Look for layer of osteoblasts.
- **zone of resorption** – nearest diaphysis; osteoclasts absorb oldest bone on spicules
  - HALLMARKS: Cells look irregular, warped.
    Osteoclasts present; look for bone marrow nearby.

- **intramembranous** – NO CARTILAGE MODEL
  - Mesenchymal cells begin to condense, area becomes vascularized.
  - Mesenchymal cells become larger, rounder. Cytoplasm changes from eosinophilic to basophilic as cells differentiate into osteoblasts.
  - Osteoblasts secrete collagen and proteoglycans of matrix (osteoid). When surrounded by matrix, these osteoblasts become osteocytes and maintain bone.
  - Matrix is calcified and forms shape of spicules, which enlarge and interconnect, forming trabeculae
  - Osteoblasts on surface of spicules reproduce to maintain population capable of growth.
  - Fibrous periosteum surrounds growing bone.
  - As bone continues to grow, it undergoes remodeling via resorption by osteoclasts.

**Preparation of Samples for Microscopy: Ground bone vs. Decalcified bone:**

- **Ground bone**
  - Dried and finely ground preparations of bone that are not decalcified
    - *Black and tan*
    - Allows visualization of **Haversian systems** (a.k.a. osteons)
      - = Structural unit of compact bone
      - Consists of **Haversian canals**: blood vessels, nerves, lymphatics
      - Surrounded by concentric **lamellae** of bone. The outermost rings are oldest
      - **Lacunae** = w/in lamellae; contain osteocytes. Lacunae and osteocytes are interconnected via thread-like **canaliculi** that contain the cytoplasmic processes of osteocytes. Allow gap junction communication between osteocytes, circulation of extracellular fluid, wastes and metabolites.
      - **Volkmann’s canals** run **perpendicular to Haversian canals**, passing through the lamellae. They carry neurovascular bundles from endosteum and periosteum into Haversian canals.
      - **Interstitial lamellae** are remnants of Haversian systems that have been resorbed. Lie between osteons.
• **Cementing lines** delimit Haversian systems. *Basophilic due to proteoglycans.*

• **Decalcified bone**
  o Demineralized with acid and then stained with H&E
  o Able to see cells, organic matrix & periosteum
    - **Periosteum** – sheath of dense connective tissue surrounding outer surface of bone containing osteoprogenitor cells
    - **Endosteum** – lines bone cavities (marrow cavity of compact bone & the marrow spaces between trabeculae of spongy bone). Contains endosteal cells which can differentiate into osteoblasts

### Bone vs. Cartilage

<table>
<thead>
<tr>
<th></th>
<th>Bone</th>
<th>Cartilage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H&amp;E Staining Properties</strong></td>
<td>Eosinophilic</td>
<td>Basophilic</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Rigid structure &amp; support</td>
<td>Shape, precursor to bone</td>
</tr>
<tr>
<td><strong>Nutrients</strong></td>
<td>HC, VC, &amp; canaliculi</td>
<td>Diffusion across matrix</td>
</tr>
<tr>
<td><strong>Growth</strong></td>
<td>Appositional</td>
<td>Appositional &amp; interstitial</td>
</tr>
<tr>
<td><strong>Cells</strong></td>
<td>Osteocytes, -blasts, -clasts</td>
<td>Chondrocytes</td>
</tr>
<tr>
<td><strong>Matrix</strong></td>
<td>Mineralized; type I collagen</td>
<td>GAGs, type II collagen (hyaline &amp; elastic)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and type I collagen (fibrocartilage)</td>
</tr>
</tbody>
</table>

### Joints

Types of Joints:
- immovable or slightly movable
  - **syndesmoses** – *fibrous* joints; bone connected to connective tissue
  - **synchondroses** – *cartilaginous* joints; bone connected to cartilage
  - **synostoses** – *osseous* joints; bone connected to bone
- freely movable joints
  - **synovial (diarthroid)** - articulating bones separated by a fluid-filled cavity; articular surfaces covered by hyaline cartilage

Terms:
- **synovial cavity** – fluid-filled space between two bones
- **synovial fluid** – comprised of water and GAGs; maintains articular cartilage (provides nutrients than enter cartilage through diffusion)
- **synovial membrane** – specialized secretory connective tissue; consists of collagenous fibers and fibroblasts; fibroblasts secrete synovial fluid; highly vascular; may be attached to perichondrium at lateral regions of articular cartilage
- **synovial villi** – folds of the synovial membrane; project into the synovial cavity to allow increased secretion/absorption of synovial fluid
CARTILAGE

QUESTION #1
Panel C is a low magnification micrograph of the tissue shown in Panel B.

Provide the letter or letters (A and/or B) (or none) of the panel to which the following apply.

a. normally calcified  
b. vascularized  
c. collagen type II  
d. cells capable of division  
e. matrix contains proteoglycans  
f. lacunae present

Questions 2 and 3:

2. The three tissues shown have all of the following properties in common EXCEPT:
   
a. They contain capillaries.  
b. They contain proteoglycans.  
c. They can increase in size by interstitial growth.  
d. They can increase in size by appositional growth.

3. Which tissue is the most highly specialized to resist compression?
   
a. A  
b. B  
c. C

4. What cartilage nourishing tissue is missing at the interface shown?
   
a. Blood vessels  
b. Perichondrium  
c. Synovial membrane  
d. Chondroblasts
Answers

Question #1: A. Slide #5 (medium power) Hyaline cartilage (trachea), B. Slide #10 Long bone cross-section (high power), C. Slide #10 Long bone cross-section (low power

   a. B – hyaline cartilage is not calcified – it is made up of type II collagen and ground substance; the bone shown in slide B and C is calcified.
   b. B– hyaline cartilage is not vascularized – vessels run only in the perichondrium and nutrients and waste diffuse through the ground substance; bone is made up of Haversian systems through which vessels travel via the Haversian canals
   c. A – hyaline cartilage is made up of type II collagen, bone is made up of type I collagen
   d. A – cartilage is capable of cell division via proliferation of chondroblasts and chondrocytes. Mature osteocytes, as seen in this image of calcified bone, cannot proliferate.
   e. A, B – the matrix for both hyaline cartilage and bone is made up of proteoglycans
   f. A, B – chondrocytes in cartilage reside in lacunae, surrounded by matrix; osteocytes are also found in lacunae within the lamellae of the Haversian systems

Questions 2 and 3: A. Slide #104 Fibrocartilage; B. Slide #5 Hyaline Cartilage; C. Slide #6 Elastic Cartilage

   Question 2: a – these three tissues are all cartilage, which does not have capillaries; the blood supply to all types of cartilage runs in the perichondrium. All types of collagen contain proteoglycans in their ground substance, and are capable of both interstitial and appositional growth.

   Question 3: a – fibrocartilage is highly specialized to resist compression – as it functions in the intervertebral disc, supporting the weight of our bodies

Questions 4: Slide #95 finger (medium power)

   b. At the articular surface of the joint there is no perichondrium. (The perichondrium typically ensheaths the cartilage and contains the blood vessels that chondrocytes depend on for their nourishment). In the joint the cartilage at the articulating surface is instead nourished by the synovial fluid secreted by the synovial membrane.
**BONE**

**QUESTION #1**

Panel B is a low magnification micrograph of the tissue shown in Panel A.

Circle all that apply to the projected slides.

- g. normally calcified
- h. vascularized
- i. collagen type II
- j. cells capable of division
- k. matrix contains proteoglycans
- l. lacunae present

Questions 2-3: For questions 2 and 3 select the correct combination of terms.

2. Figure A illustrates ______________ and ______________.
   - a. membrane bone formation, interstitial growth
   - b. formation of Haversian systems, appositional growth
   - c. endochondral bone formation, interstitial growth
   - d. membrane bone formation, appositional growth

3. Figure B illustrates ______________ and ______________.
   - a. membrane bone formation, interstitial growth
   - b. formation of Haversian systems, appositional growth
   - c. endochondral bone formation, interstitial growth
   - d. membrane bone formation, appositional growth

Questions 4 and 5:

4. The progenitor that gave rise to the type of cell at the pointer in Figure A was a:
   - a. chondroblast.
   - b. osteoclast.
   - c. osteoblast.
   - d. chondrocyte.

5. The eosinophilic component of the matrix in Figure B provides for:
   - a. tensile strength.
   - b. diffusion.
   - c. interstitial growth.
   - d. protein synthesis.
Question 6:
6. Which of the following is NOT true about the cells at the pointer?
   a. they are accumulating glycogen
   b. they originate from hyaline cartilage
   c. the matrix surrounding them is completely mineralized
   d. they are actively secreting matrix

Answers

Question #1: A. decalcified bone, tibia (high mag), B. decalcified bone (low mag)
   a. Yes - the bone shown in slide B is decalcified in this preparation, but would normally be calcified
   b. Yes - bone is made up of Haversian systems through which vessels travel via the Haversian canals
   c. No - bone is made up of type I collagen
   d. No - Mature chondrocytes, as seen in this image of calcified bone, cannot proliferate.
   e. Yes - the matrix for both hyaline cartilage and bone is made up of proteoglycans
   f. Yes - osteocytes are found in lacunae within the lamellae of the Haversian systems

Questions 2-3: Figure A. #93 fetal endochondral bone formation; Figure B #94 parietal membrane bone with osteoblasts; Figure C same as Figure B but with pointer on osteoclasts.

   Question 2: c – The tissue shown in Figure A was endochondral bone formation, which is the formation of new bone by growth of a cartilage model via interstitial growth. A is wrong because membrane bone does not form from a cartilage model. B is wrong because although appositional growth is the way the bone is growing (vs. the cartilage also shown), we are not seeing the formation of Haversian systems. D is incorrect because, once again, this is endochondral bone, not membrane bone.

   Question 3: d – Figure B shows parietal membrane bone with osteoblasts. Membrane bone grows without cartilage model, growing via appositional growth. A is wrong because interstitial growth applies to cartilage only. B is wrong because we are not
seeing Haversian channels, and C is wrong because this is not endochondral bone (since there’s no cartilage model) and thus there is no interstitial growth.

Questions 4 and 5 Slides: Figure A #9 ground bone, pointer on osteocyte; Figure B #10 decalcified bone

Question 4: c – Figure A shows ground bone, pointer on osteocyte, which started out as an osteoblast, but as it secreted bone matrix, it became embedded in bone and confined to a lacunae where it remained as an osteocyte. Chondroblasts and chondrocytes are found in cartilage only, and osteoclasts break down bone and do not form osteocytes.

Question 5: a – Figure B shows decalcified bone, which appears eosinophilic due to collagen (matrix is basophilic). Collagen is important for tensile strength of bone. Bone is not capable of diffusion (except via Haversian systems) nor of interstitial growth. Collagen cannot produce proteins as it is itself a long polymer of proteins.

Question 6: c – Pointer on zone of hypertrophy within the epiphyseal plate. These stacked cells are very large and actively secrete unmineralized matrix. Once cells enter the zone of calcification, the newly-mineralized matrix isolates chondrocytes from their nutrient supply, leading to cell death and vacant lacunae.