Cartilage

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Lecture outline:

I. General organization of cartilage
   A. Relationship of matrix & cells in cartilage
II. Cartilage extracellular matrix
   A. Molecules of cartilage matrix; proteoglycans
III. Growth of cartilage
   A. Interstitial growth
   B. Appositional growth
      1. the perichondrium
IV. 3 types of cartilage
   A. Hyaline
   B. Elastic
   C. Fibrocartilage

Objectives:
When your study of this topic is complete, you should be able to:

1. Compare and contrast the 3 types of cartilage
2. Recognize components of cartilage, i.e., chondrocytes, perichondrium, lacunae
3. Describe the ultrastructure of the chondrocyte and its functional role in the synthesis and maintenance of the extracellular matrix.
4. Explain how nutrients and metabolic waste products are exchanged between cartilage cells (chondrocytes) and the blood
5. Recognize and describe histological distinguishing features between hyaline, elastic and fibrous cartilage
6. Compare and contrast the functions of the 3 types of cartilage and how these relate to their structural characteristics and location in the body.
7. Describe the steps in the histogenesis and growth of cartilage.
8. Explain how cartilage can grow: by both interstitial and appositional processes

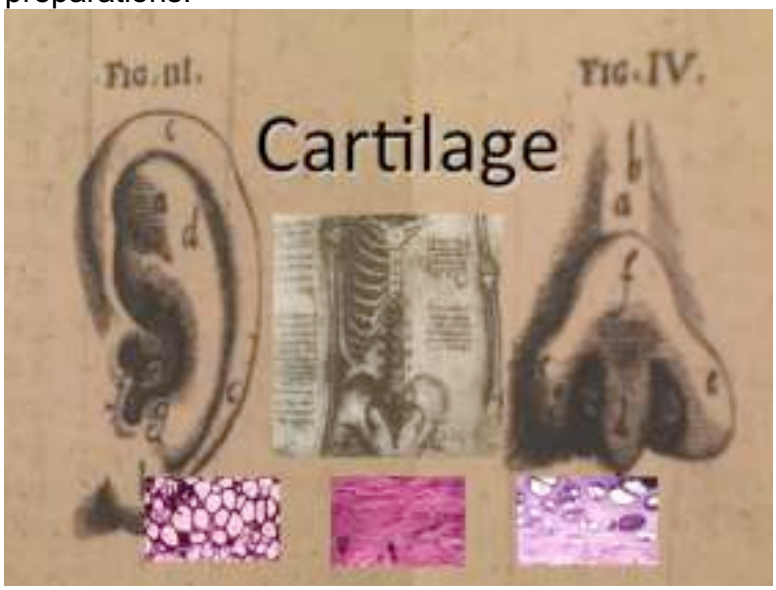
Readings:

Histology: A text and atlas, Pawlina: Chapter 7
Basic Histology, Junqueira et al: Chapter 7

Graphic materials derived from Basic Histology, Junqueira et al 2017; Kierszenbaum, Histology & Cell Biology, 13th Ed., 2013; WebMic Virtual Microscopy & Departmental Collection.

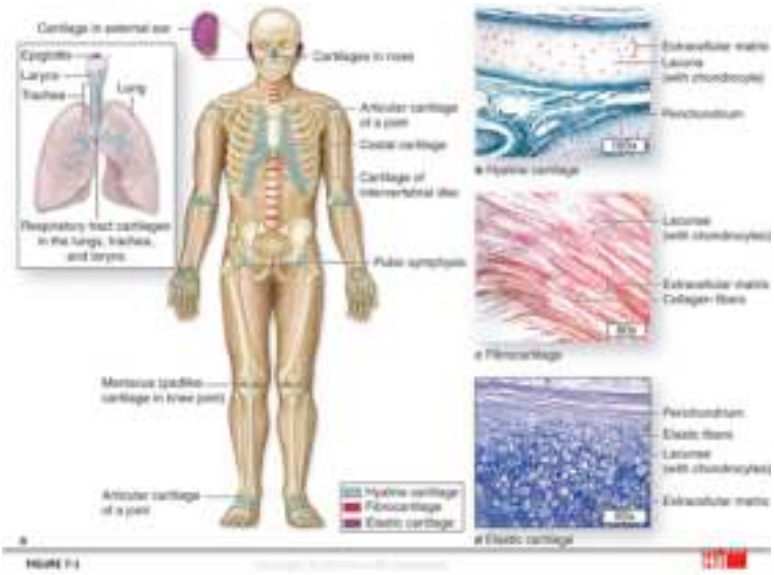
Introduction

The purpose of this lecture is to describe the microanatomical features of the three major types of cartilage. We will discuss 1) the locations of each major type of cartilage in the body; 2) the cellular organization and molecular composition of the extracellular matrix; 3) and the mechanisms of growth of cartilage. Based on our discussion of the histological characteristics of each type of cartilage, you will be able to identify them in histological preparations.



SLIDE 2: This first slide shows images of an external ear and nose from the 16th century that were used in a medical school in Padua, Italy. The center drawing (by Leonardo DaVinci) is from approximately the same period. A medical student in the 17th century would probably know that cartilage is found in the external ear (pinna), the nose, and in the intervertebral disks between the vertebral bodies. But students at that time would not have known that the three images shown here are representative anatomical locations for the three major types of cartilage. This is because the microscope was just being invented and the science of histology, used to distinguish different features of cartilage, did not exist.

Histological images of the three types of cartilage are included in the figure. The nose ear contains elastic cartilage, the intervertebral discs contain fibrocartilage, and the nose contains hyaline cartilage. Morphological similarities and differences are likely apparent, even if you have no training in histology. By the end of this lecture, you should be able to easily differentiate between all three types.

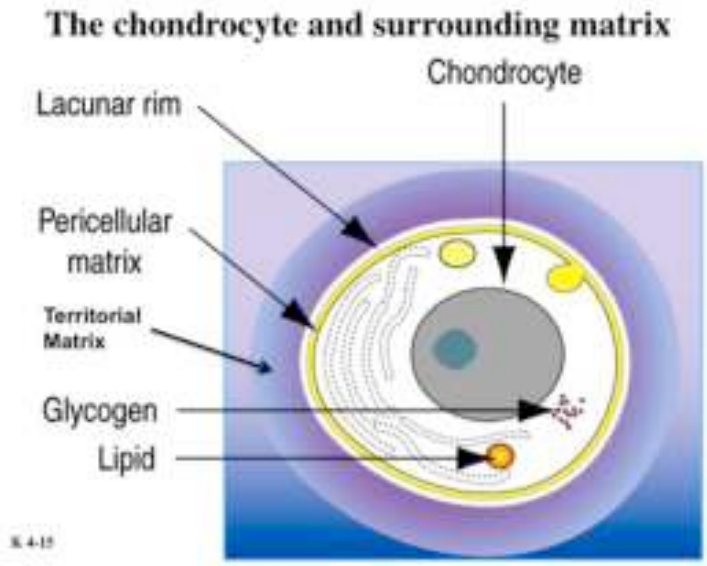


SLIDE 3: These images illustrate the distribution of cartilage in the adult. Histological images of the 3 types of cartilage are shown at the right. The three types of cartilage are hyaline cartilage, elastic cartilage, and fibrocartilage. The upper right panel depicts hyaline cartilage.

Hyaline cartilage is the most abundant cartilage type found in the body. It is coded in light blue in the illustration. Hyaline cartilage is found in all the articular joints and throughout the respiratory tract, including the nose and bronchi of the lungs (see the upper left panel).

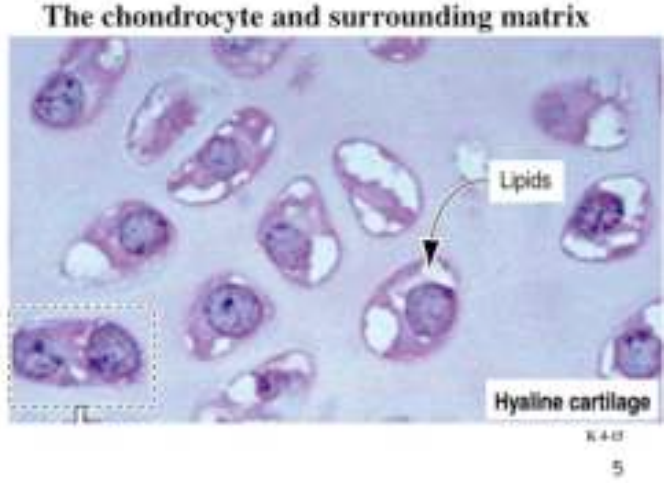
Fibrocartilage is the second most abundant cartilage in the body. It is coded in red in the illustration. Fibrocartilage is found between the vertebral bodies as part of the intervertebral disks. It is also found in the menisci of the knee joints and at the temporomandibular joint in the head.

Elastic cartilage is the least abundant type of cartilage in the body. It is coded in purple. Elastic cartilage is found only in structures of the head, including the external ear, a portion of the auditory (Eustachian) tube, and the epiglottis of the orolarynx.

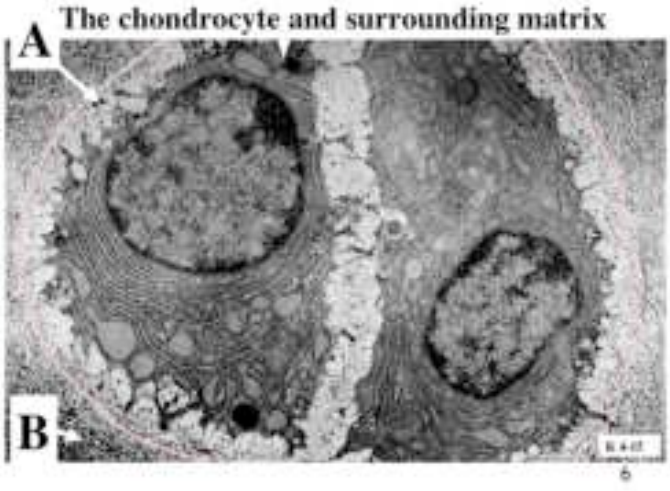


SLIDE 4: Cartilage is composed of specialized extracellular matrix and cells. The cells found in cartilage are called chrondrocytes. During cartilage development, the progenitor of the chrondrocyte is called a chrondroblast. Chrondroblasts synthesize type II collagen and other matrix components to eventually isolate themselves completely from surrounding cells. Once the chrondroblast becomes completely isolated by matrix, it is called a chrondrocyte; the space it resides within is called a lacuna. This image depicts the features of a typical chrondrocyte within hyaline cartilage tissue. Note the large round nucleus with a prominent nucleolus (characteristic of hyaline cartilage). Abundant profiles of RER and secretory vesicles can be visualized; they mediate the production of extracellular matrix. Glycogen granules and lipid vesicles are also present.

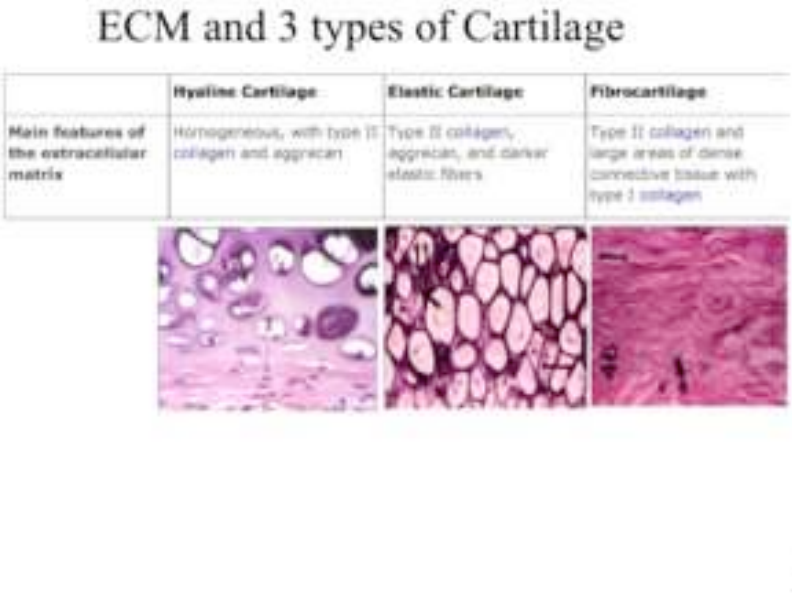
The space the chrondrocyte resides within is called the lacuna. On the surface of the cell's plasma membrane is the pericellular matrix that is intimately associated with the cell and best visualized at the ultrastructual level of resolution. External to the pericellular matrix is the lucunar rim. The lacunae themselves, containing chrondrocytes, are closely surrounded by a region of matrix called the territorial matrix. Between adjacent lacunae is a region of extracellular matrix called the interterritorial matrix. The territorial matrix is made up of loose collagen type II fibrils and higher concentration of proteoglycans. It can be differentiated from the interterritorial matrices containing collagen (type II) and proteoglycans histologically by their different staining properties.



SLIDE 5: This is a low magnification photomicrograph of hyaline cartilage. Note the prominent round nuclei and nucleolus that are characteristic of hyaline chondrocytes and the lacunae separated by a relatively homogenous interterritorial matrix (light blue background). The territorial matrix is evident as a more darkly stained ring circumscribing the lacunae (pink layer). Clear regions within the chondrocytes are due to the extraction of lipid vesicles during histological processing. In some lacunae, notice that more than one chondrocyte is resident. These are cells that have divided by mitosis to generate what is termed an isogenous group (Gr. *Isos*, equal and *genos*, family/kind). Chondroblasts will divide and continue to secrete extracellular matrix until they have individually isolated themselves. This growth mechanism is called interstitial growth. Note that several chondrocytes (often 4 to 6) may reside in a single lacuna as an isogenous group.

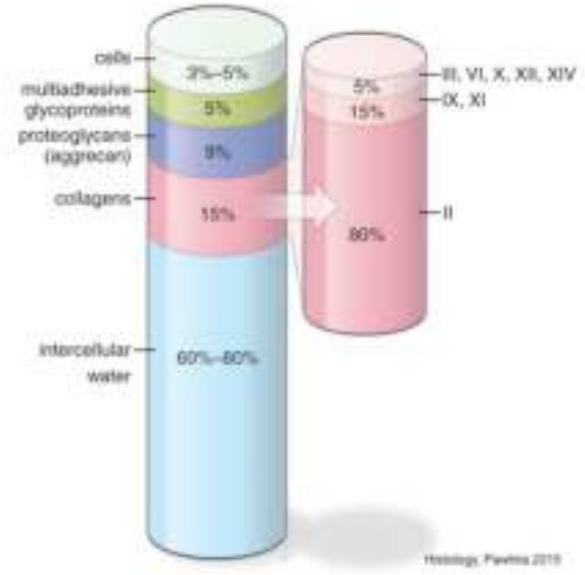


SLIDE 6: This is an electron micrograph of an isogenous group containing two cells. At this resolution, a prominent round nucleus is clearly seen in each cell. Note also the extensive profiles of RER and matrix containing vesicles that are characteristic of cells involved in the very active protein synthesis needed to build the extracellular matrix of the cartilage. A darkly stained (electron dense) layer can be seen along the plasma membrane. This is labeling the pericellular matrix. The lacunar rim's inner edge is delineated by the pink line and marked by the A arrow. The B arrow indicates part of circumferential territorial matrix that is composed of randomly arranged type II collagen.

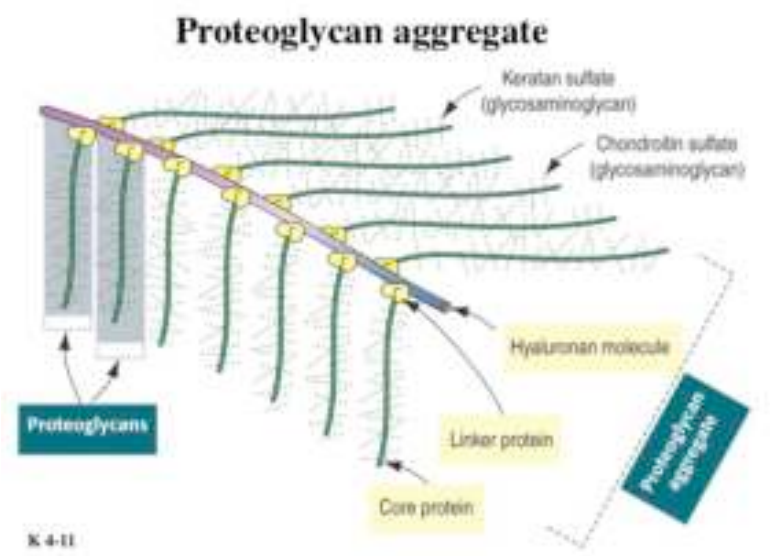


SLIDE 7: The extracellular matrix (ECM) components that comprise the territorial and interterritorial matrix differ for each of the 3 types of cartilage and this is the basis for differences in their physical properties. It is the extracellular matrix that defines the type of cartilage.

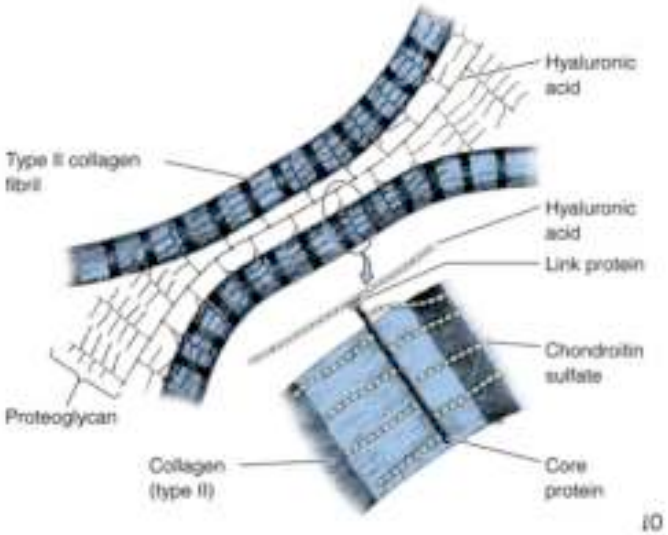
All three types of cartilage have in common collagens, hyaluronan, proteoglycans and other glycoproteins. Collagen is type II is the most abundant collagen in all types of cartilage. All three types of cartilage contain the proteoglycan aggrecan. Hyaline cartilage is the most common throughout the body. The other types are more limited in distribution and have specialized molecular composition containing elastin (elastic cartilage) and type I collagen (fibrocartilage) that give the tissue their specialized physical characteristics. The chondrocytes themselves occupy a very small mass in the cartilage tissue.



SLIDE 8: Chondrocytes occupy only about 3-5% of the total mass of cartilage. Collagen is the most abundant of the proteins in cartilage (15% total weight). Of these, type II is the most predominant, representing 80% of the total collagen with other collagens (contributing to the other 20%). 60-80% of cartilage is water that is bound in the matrix by hydrophilic proteoglycan aggregates.



SLIDE 9: You recall that a proteoglycan is a very large extracellular matrix molecule that consists of a proteoglycan core protein made up of several functional domains. One most abundant types of functional domains is the glycosaminoglycan attachment domain that binds to glycosaminoglycan repeating disaccharide chains (GAG chains). These are found along the entire central length of the core protein. Proteoglycan types vary between tissues and have GAG chain differences. For example, the dominant glycosaminoglycans in the connective tissue of skin (both loose and dense) is dermatan sulfate. In cartilage, the dominant glycosaminoglycan molecules is chondroitin 6-sulfate and keratan sulfate. Two representative proteoglycans are shown in greyed boxes in this drawing. The core protein is drawn in green and the GAG chains are depicted as dotted lines decorating each proteoglycan molecule to create a bottle brush-like appearance. At the amino terminal end of the core protein is a hyaluronan attachment domain that attaches each proteoglycan to a much longer repeating disaccharide called hyaluronan (hyaluronic acid). Hyaluronan is a non-sulfated glycosaminoglycan, made of ~2500 repeating disaccharide units of N-acetylglycosamine and D-glucuronic acid. The molecular interaction between the core protein and hyaluronan is stabilized by another protein called a link (or linker) protein. Together, the macromolecular complex consisting of the long hyaluronan molecule and attached proteoglycan molecules constitute a proteoglycan aggregate. Because of the abundance of negatively charged groups (hydroxyl, carboxyl, and sulfate) on the GAG chains that decorate each proteoglycan, this macromolecular complex is extremely hydrophilic. It is within this hydrophilic proteoglycan matrix that the water is cartilage is bound and retained.



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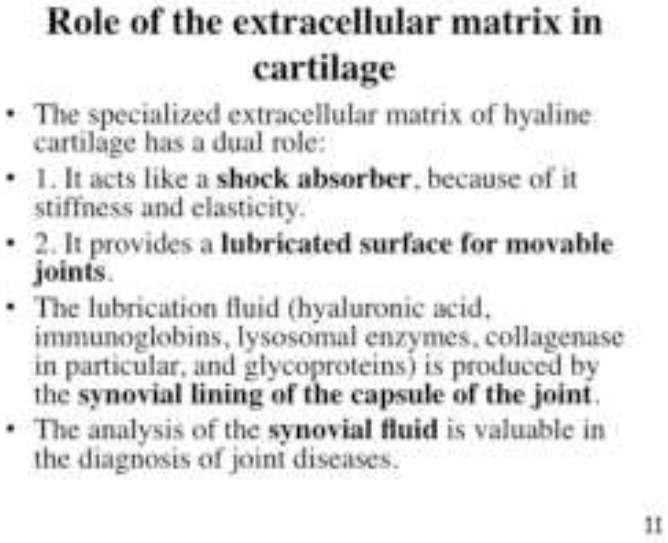
SLIDE 10: This is a lower magnification representation of the molecular organization in cartilage matrix. Note the multiple proteoglycan molecules attached to the long hyaluronan molecule that forms the proteoglycan aggregate. The chondroitin sulfate side chains of the proteoglycan GAGs electrostatically bind to the collagen fibrils, forming a structured cross-linked matrix. The oval outlines the area shown larger in the lower part of the figure.



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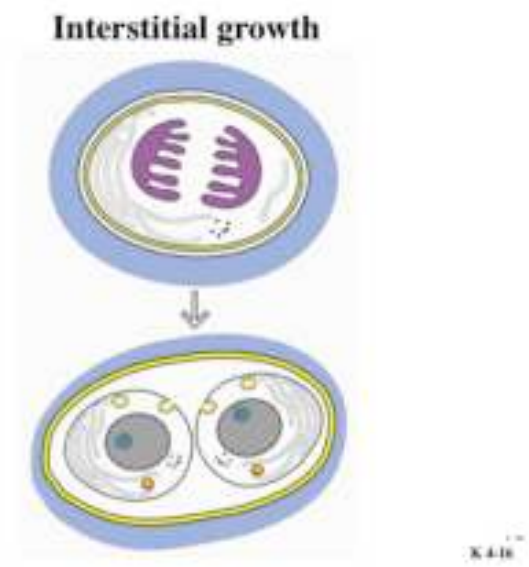
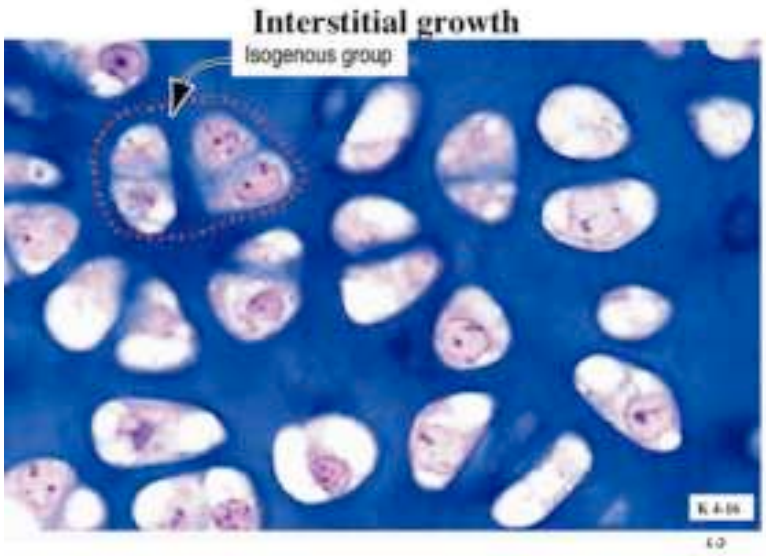
SLIDE 11: The molecular organization of its extracellular matrix gives cartilage its specialized physical/mechanical characteristics. Hyaline cartilage acts like a shock absorber because of its stiffness and elasticity. It also provides a lubricated surface for movement at articulations. The lubrication fluid is predominately hyaluronic acid that contains immunoglobins, lysosomal enzymes, collagenase, and glycoproteins. It is produced by the synovial lining of the capsule of the joint. The analysis of the synovial fluid is valuable in the diagnosis of articular diseases (blood, uric acid crystals, bacteria, viruses, fungi, inflammatory cells etc.)

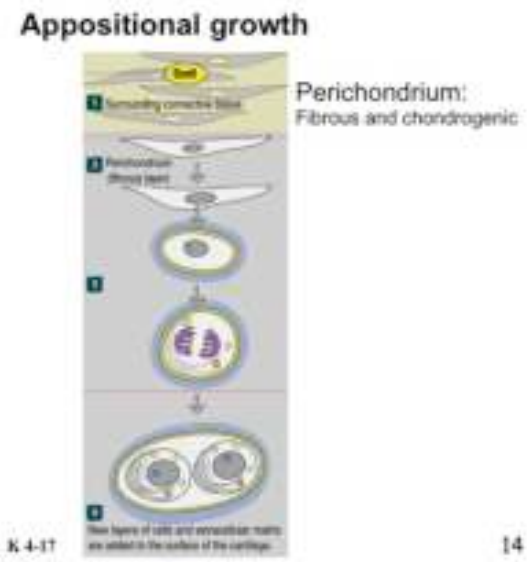


SLIDE 12: Cartilage growth occurs through two different mechanisms: interstitial growth and appositional growth.

Interstitial growth (as shown in this diagram) occurs when daughter cells of dividing chondroblasts remain within the same lacuna to form an isogenous group. The isogenous group is surrounded by a single territorial matrix.



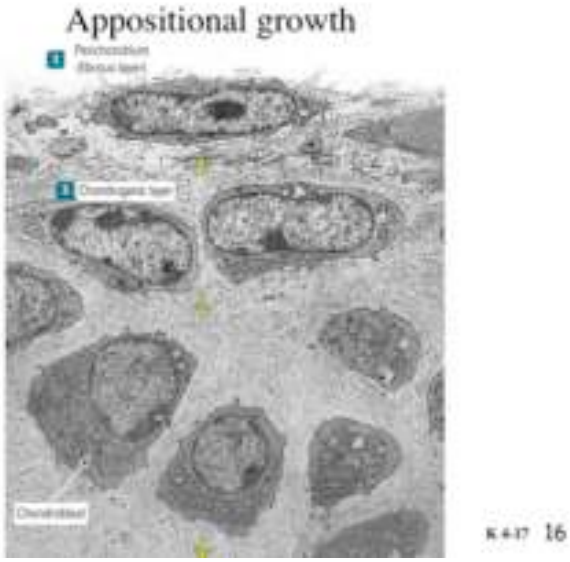
SLIDE 13: This photomicrograph shows an example of interstitial growth of cartilage. After a cartilage cell divides, the two new cells (chondrocytes) share a common lacuna, to form an isogenous group (more than two cells may be found within a single isogenous group). As chondrocytes secrete extracellular materials, the cells move apart, and the cartilage grows. Interstitial growth occurs ***within*** the mass of cartilage. This type of cartilage growth provides the basis for the lengthening of long bones during the process leading to endochondral ossification. Interstitial growth is especially evident in the zone of resting cartilage and zone of proliferation in bone growth plates during this type of ossification.



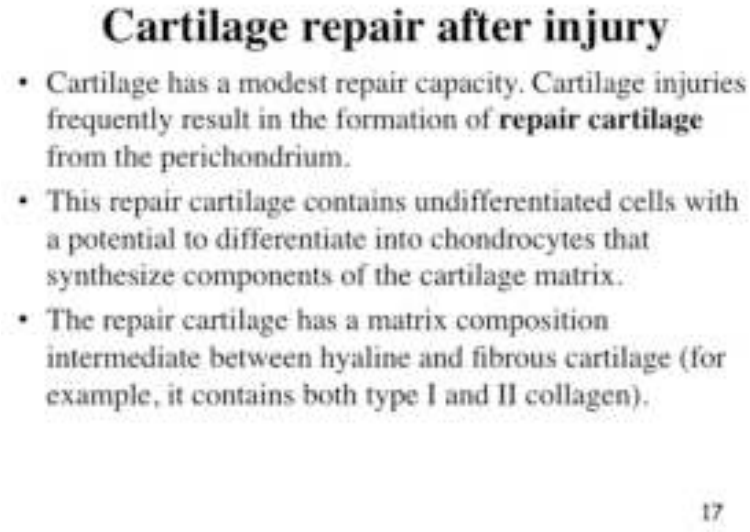
SLIDE 14: The second type of cartilage growth is called, appositional growth. This occurs from the perichondral edge of the cartilage, from the cells of the perichondrium.

The perichondrium consists of two layers: an outer fibrous and an inner chondrogenic/cellular layer. The inner layer has cells that can develop into chondroblasts. The chondroblasts secrete extracellular matrix that will completely encase the cell forming a lacuna. Cells within lacunae can then divide and contribute to the cartilage by interstitial growth. Two types of cartilage, hyaline and elastic (but not fibrocartilage), have a perichondrium. Fibrocartilage does not have a perichondrium. Another exception is that hyaline cartilage lacks a perichondrium in the region of an articular joint surface.

SLIDE 15: The left panel is a light micrograph of hyaline cartilage. Chondrocytes are located within the matrix lacunae. The upper border of the figure shows the perichondrium. Note the gradual differentiation of cells from the perichondrium into chondrocytes. The perichondrium consists of a fibrous portion and a chondrogenic portion with cartilage subjacent as indicated in the blue diagram. The fibrous and chondrogenic portions of the perichondrium can be better seen in a higher magnification electron micrograph shown in the next slide.



SLIDE 16: Electron micrograph of the perichondrium overlying cartilage. Cells in the outermost fibrous layer are spindle shaped with oval nuclei and appear as fibroblast-like chondrogenic precursor cells. As the fibrous layer cells divide and enter the chondrogenic zone, the cell body and the nucleus become rounder, and the fibroblast-like cells become chondroblasts that secrete the extracellular matrix that will form the territorial matrix that will delimit the lacunae of the chondrocyte.

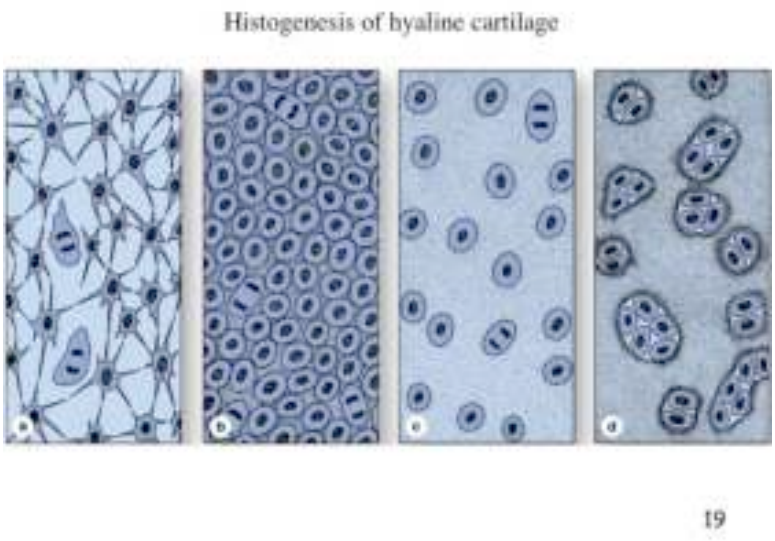


SLIDE 17: Although most cartilage benefits from two modes of growth (interstitial and appositional), cartilage only has a modest repair capacity primarily due to its lack of vascularization.

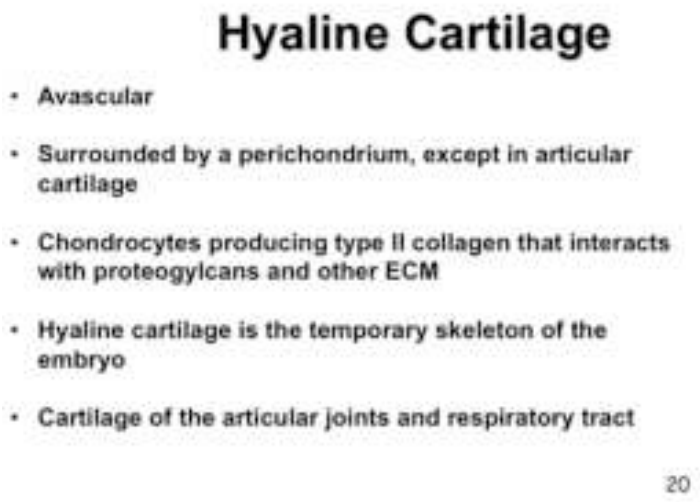
Cartilage injuries frequently result in the formation of **repair cartilage** from the perichondrium.

This repair cartilage contains undifferentiated cells with a potential to differentiate into chondrocytes that synthesize components of the cartilage matrix. The repair cartilage has a matrix composition intermediate between hyaline and fibrous cartilage (for example, it contains both type I and II collagen).

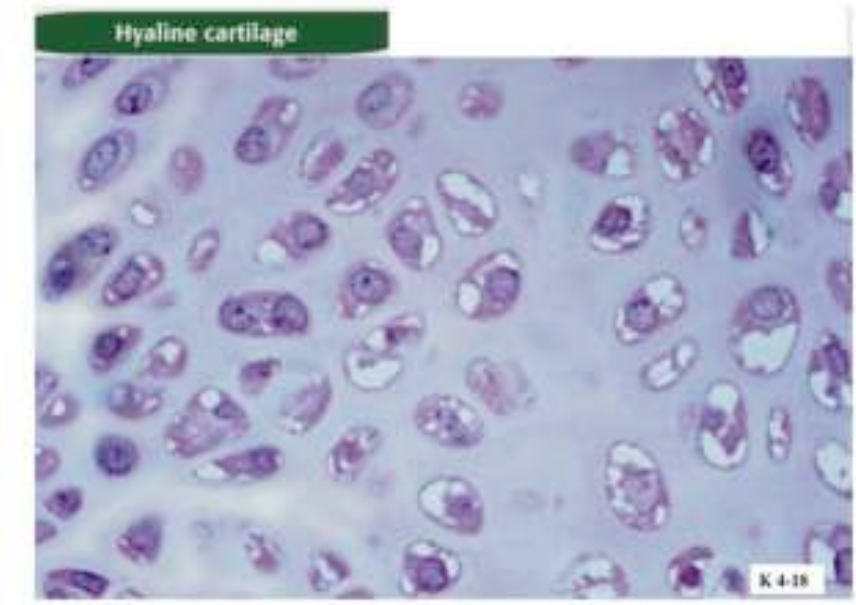
SLIDE 18: **<u>HYALINE CARTILAGE</u> -** Let's now focus on the distinctive histological features of each of the three major cartilages beginning with Hyaline cartilage. Hyaline cartilage is the first cartilage formed and is the cartilage found in the developing embryo. The initial process/mechanism of hyaline cartilage formation during embryogenesis is called histogenesis of cartilage.



SLIDE 19: Histogenesis of hyaline cartilage.
A: The mesodermal mesenchyme is the precursor tissue of all types of cartilage.
B: Mitotic proliferation of mesenchymal cells gives rise to a highly cellular tissue.
C: Chondroblasts are separated from one another by the formation of a great amount of matrix.
D: Mitotic division of cartilage cells gives rise to isogenous groups (interstitial growth), each surrounded by a condensation of territorial matrix.



SLIDE 20: This slide is an important summary slide for hyaline cartilage, since it emphasizes vascularization, presence or absence of a perichondrium, as well as cell and collagen type and locations. We should emphasize that the lack of vascularization limits the size of cartilage, because diffusion processes become rate limiting (recall that gases such as oxygen and carbon dioxide do not diffuse well in liquids found in the extracellular substances of cartilage).



SLIDE 21: A section of hyaline cartilage stained with hematoxylin and eosin (H&E). Note the 1) lacunae containing one or more (isogenous groups) cells with relatively large nuclei with prominent nucleoli; 2) territorial matrix immediately surrounding the lacunae; 3) a hallmark histological feature of hyaline cartilage: the homogeneous almost translucent appearance of the interterritorial matrix. The term hyaline means “glass-like”.

SLIDE 22: This glass-like appearance can be appreciated even at the gross anatomical level. Here is an example of hyaline cartilage covering the head of the femur from a 16-year-old patient. Note the bluish-white translucent appearance of this healthy cartilage.

SLIDE 23: ELASTIC CARTILAGE - Now let's describe the histological features of Elastic cartilage.

Elastic Cartilage

- Avascular
- Surrounded by a perichondrium
- Matrix has type II collagen plus elastic fibers that can be stained with orcein.
- This type of cartilage is found in the external ear, epiglottis and auditory tube.

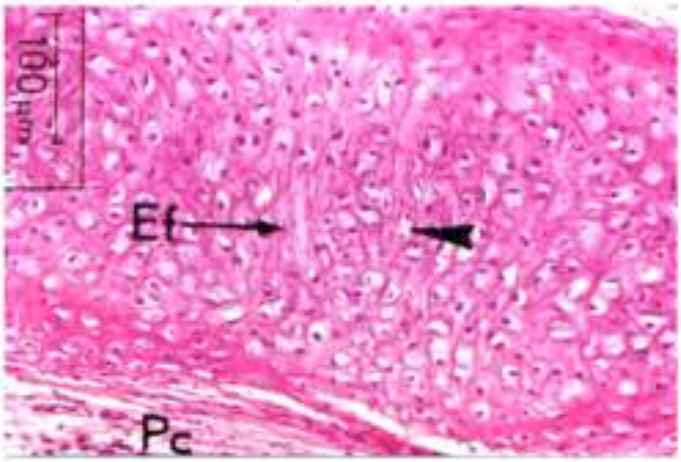
SLIDE 24: Elastic cartilage is:
1) avascular
2) surrounded by a perichondrium
3) matrix has type II collagen plus elastic fibers

This type of cartilage is found in the external ear, epiglottis, and auditory tube.

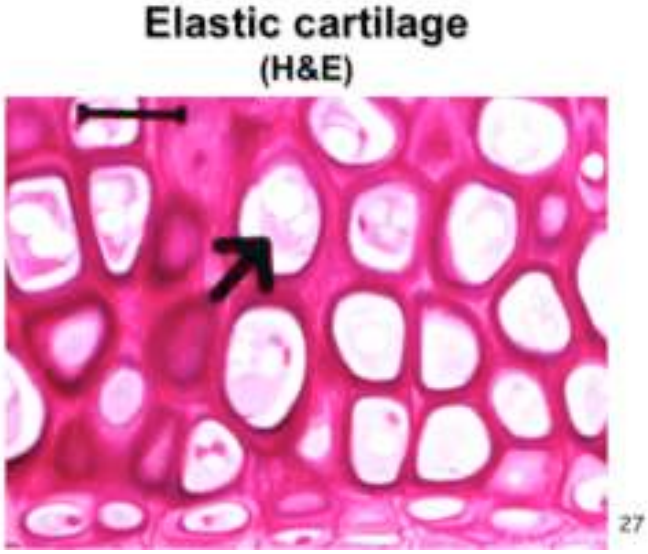
Elastic cartilage in the ear
(H&E)

SLIDE 25: A low magnification "survey" image showing the elastic cartilage plate in the pinna of the ear. (Note the characteristics of the stratified squamous epithelium of the skin covering the periphery of the of the ear). Higher magnification (next slide) is required to discern the characteristic histological details of elastic cartilage.

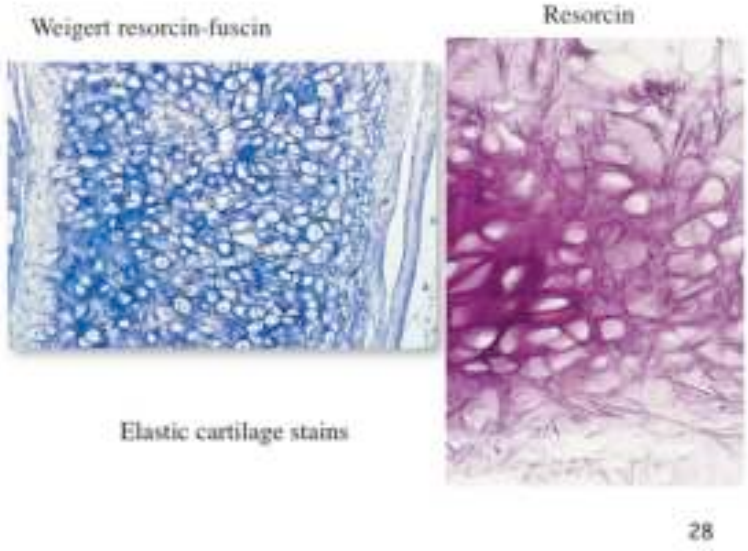
Elastic cartilage
(H&E)



SLIDE 26: A medium magnification of the elastic cartilage showing the definitive linear substructure in the ear elastic cartilage caused by the presence of elastic fibers (Ef).



SLIDE 27: This is a high magnification image of elastic cartilage showing the characteristic morphology of the lacunae reminiscent of a “plate of oysters” (arrow).



SLIDE 28: Left panel: Low magnification survey view of elastic cartilage (epiglottis) stained for elastin fibers (Weigert resorcin-fuscin) in the matrix (P = perichondrium).

Right panel: Photomicrograph of elastic cartilage, stained for elastic fibers. Cells are not stained by simple resorcin stain. This flexible cartilage is present in the auricle of the ear and in the epiglottis. Medium magnification. These stains contain orcein to stain the elastic fibers. Orcein is derived from lichens and is called orchilla. It is converted to orcein with ammonia and air, or urine.

Fibrocartilage

- Avascular
- Lacks a perichondrium
- Chondrocytes (type II collagen) and fibroblasts (type I collagen)
- Intermediate between hyaline cartilage and dense fibrous CT
- Intervertebral discs, menisci of the knee joint and articular disc of the temporal mandibular joint.

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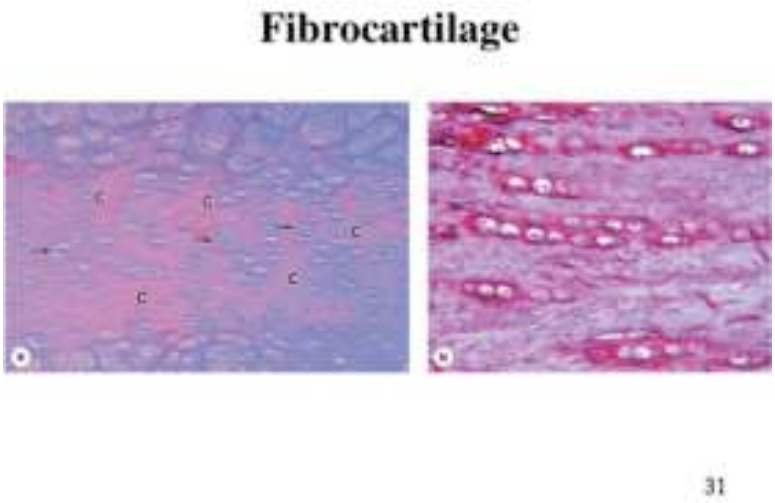
SLIDE 29: Fibrocartilage:
1) avascular
2) lacks a perichondrium
3) consists of chondrocytes and fibroblasts surrounded by type I collagen leading to a less rigid ECM than is found in hyaline cartilage.

Fibrocartilage is considered an intermediate tissue between hyaline cartilage and dense fibrous CT. It predominates in intervertebral disks, articular disks of the knee, the mandible, sternoclavicular joints, and pubic symphysis.

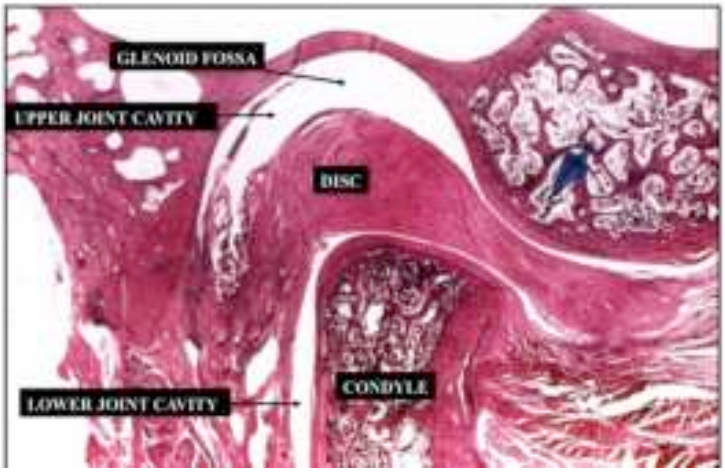
SLIDE 30: Example of fibrocartilage at low magnification.

A distinguishing histological feature of fibrocartilage is that the lacunae containing chrondrocytes are found aligned along the mechanical lines of stress within the cartilage. They have the appearance of boxcars in a train of chrondrocytes along tracks of stress.

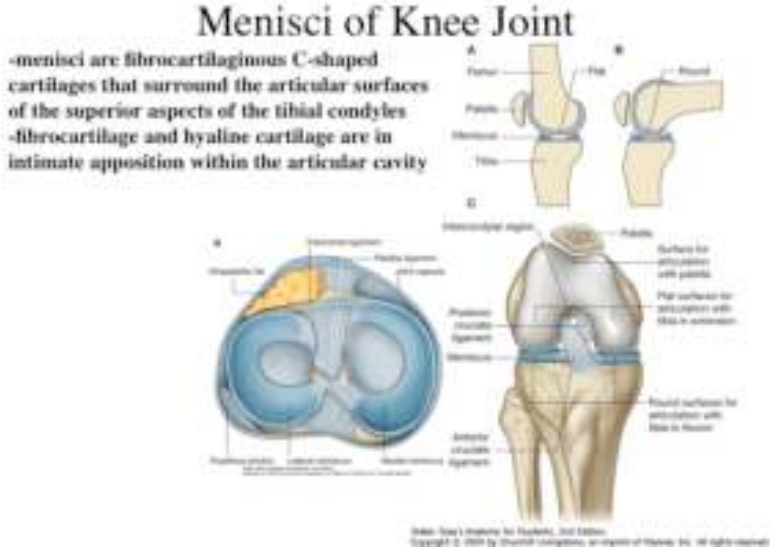


SLIDE 31: Left panel, pubic symphysis (Masson Trichrome): arrows show the directionality of alignment of both cells in lacunae & stress. C represents differing concentrations of collagen type I which in turn exhibit a differing staining pattern.
Right panel, intervertebral disc (Picrosirius-hemotoxylin): chondrocytes in lacunae show the orientation of stress. This representation would be found at insertion points of tendons & ligaments (aponeurosis).

SLIDE 32: The intervertebral discs function as cushions and shock absorbers between adjacent vertebrae to dampen injury by mechanical forces. They also permit limited mobility with the vertebral column, as they are anchored in place by the anterior and posterior longitudinal ligaments. The 2 components of the intervertebral discs include 1) the peripheral annulus fibrosis, a fibrocartilagenous ring arranged in concentric layers, with the collagen bundles in each layer oriented orthogonally to those in the adjacent layer; and 2) the central nucleus pulposus, derived from the embryonic notochord, and composed of a few type II collagen fibers embedded within an HA-rich ground substance, With age, the nucleus pulposus loses water and is replaced by fibrocartilage, thereby accounting for the phenomenon of age-related height loss.



SLIDE 33: Another site of fibrocartilage is the articular disc of the temporomandibular joint.



SLIDE 34: The menisci of the knee joint are additional anatomic locations of fibrocartilage. They are the C-shaped cartilages that envelop the articular surfaces of the superior aspects of the tibial condyles, which articulate with the corresponding articular surfaces of the medial and lateral condyles of the femur.

SLIDE 35: THE END