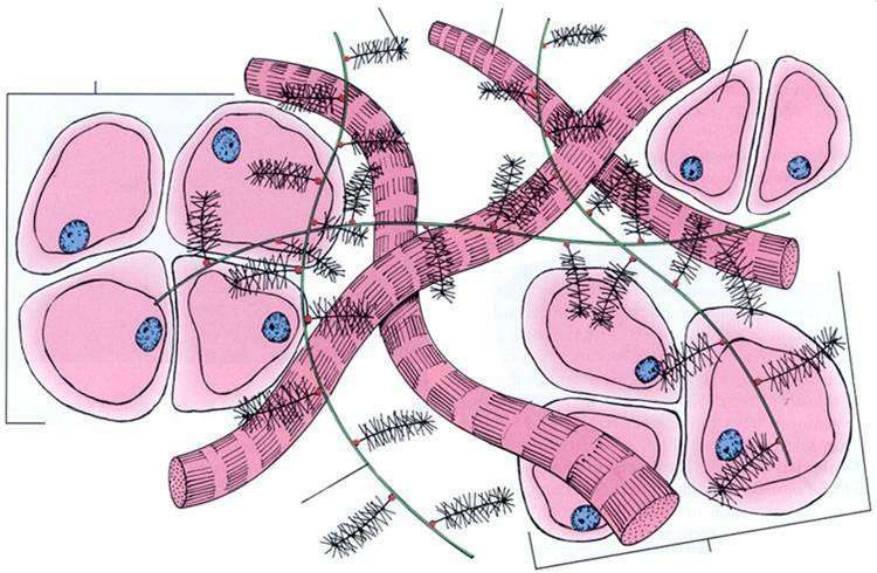

İSÜ İSTİNYE ÜNİVERSİTESİ İSTANBUL

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

NetwOArk The European Network on OsteoArthritis

COST Action 21110
Istanbul School

01TH - 03RD DECEMBER 2023

ARTICULAR CARTILAGE ENGINEERING TRAINING SCHOOL

Cartilage Histology and Stem Cells

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Histology ve Embryology Dept. İstanbul

Director of ISU3D

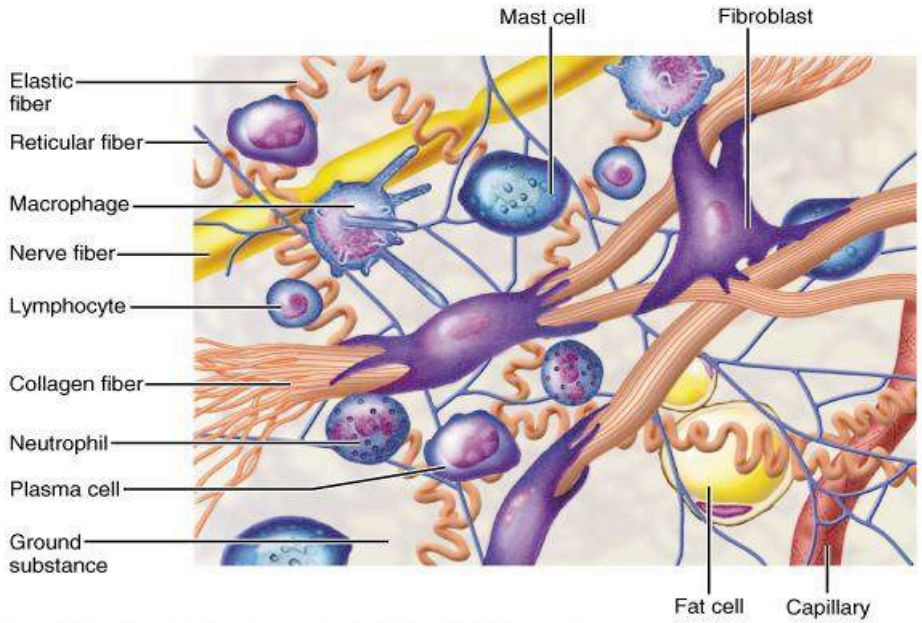
PI at ISUKOK

Founder & CEO of HD Bioink Corp.

Cartilage & Bone

There are 4 main tissues types;

1. Epithelial Tissue
2. Connective Tissue → Connective Tissue
3. Muscle Tissue
4. Nerve Tissue



Connective Tissue

1. Embryonic Connective Tissue
2. Adult Connective Tissue
   1. Loose connective tissue
   2. Dense connective tissue
3. Specialized connective tissue
   1. Cartilage tissue
   2. Bone tissue
   3. Blood tissue
   4. Hemopoietic tissue
   5. Lymphatic tissue
   6. Adipose tissue

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Cartilage & Bone as Connective Tissues

All connective tissues have the same basic components:

I. Cells

II. Extracellular matrix (ECM)

- Fibers:
- Ground substance:

Cartilage & Bone as Connective Tissues

All connective tissues have the same basic components:

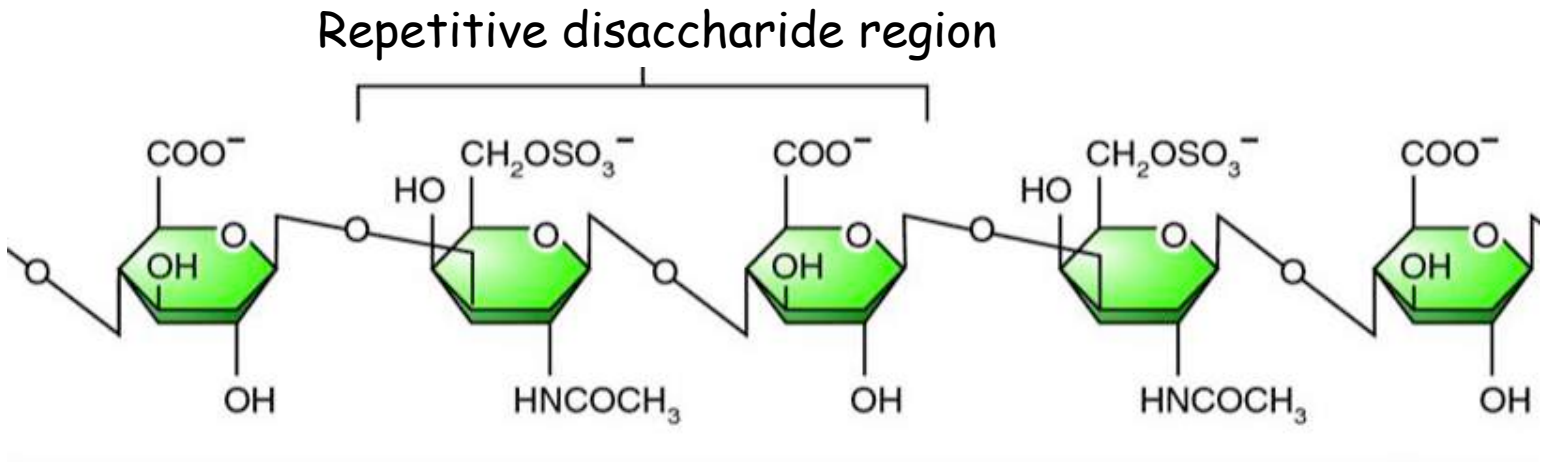
I. Cells

II. Extracellular matrix (ECM)

- **Fibers:** Collagen fibers, elastic fibers, reticular fibers
- **Ground substance:** Proteoglycans, Glycosaminoglycans (GAGs), multiadhesive glycoproteins

GROUND SUBSTANCE

1. Glycosaminoglycans (GAGs)
   - Heteropolysaccharide. Negatively charged. Attract water.



2. Proteoglycans

3. Multiadhesive glycoproteins

Glycosaminoglycans (GAGs)

Name	Molecular weight (kDa)	Localization	Function
Hyaluronan	100-10.000	Synovial fluid, ECM, vitreous humor (eye)	Lubricant, water absorbent, impact resistant
Chondroitin 4-sulfate, Chondroitin 4-sulfate	25	Cartilage, bone, cardiac valves	One of the components of Aggrecan In joint cartilage, shock absorber
Dermatan sulfate	35	Skin, blood vessels, cardiac valves	Have role in CVS diseases, cancer, infection, wound healing, fibrosis and cell behavior
Keratin sulfate	10	Cartilage, bone, cornea	Recognition of protein ligands, cell movement, implantation, axon guidance
Heparan sulfate	15	Basal lamina, cell surface	Facilitate the relationship between fibroblast growth factor (FGF) and its receptor
Heparin	40	Mast cells and granules of basophils	Anticoagulant. Interact with FGF

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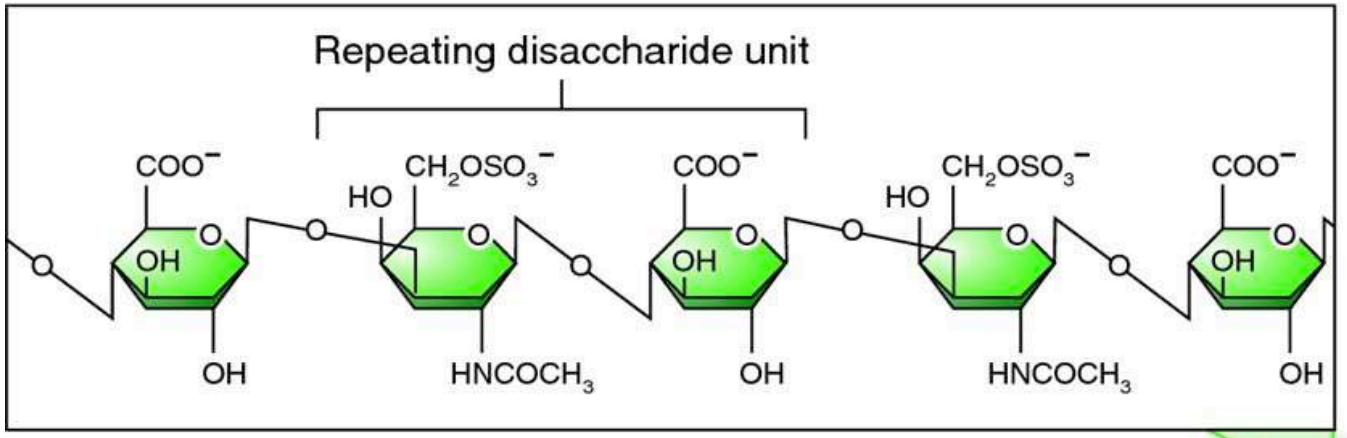
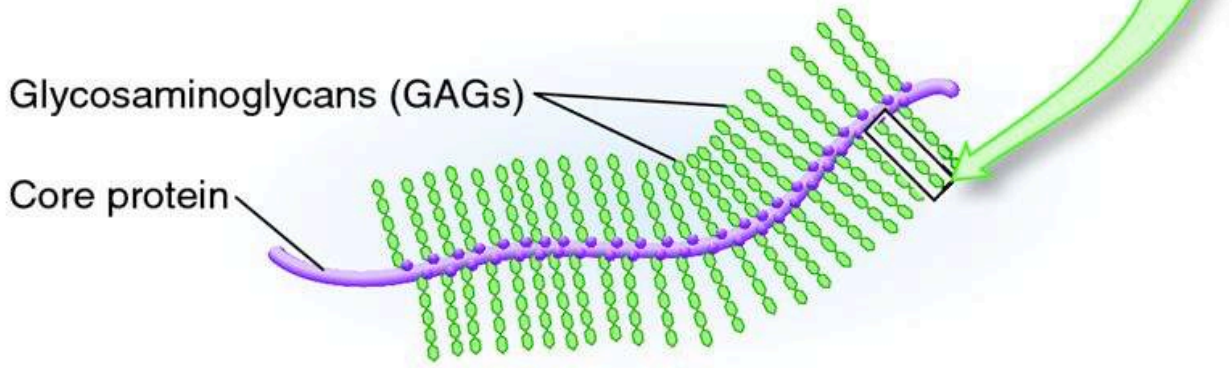
PROTEOGLYCANS

Formed by covalent binding of GAGs to core proteins.



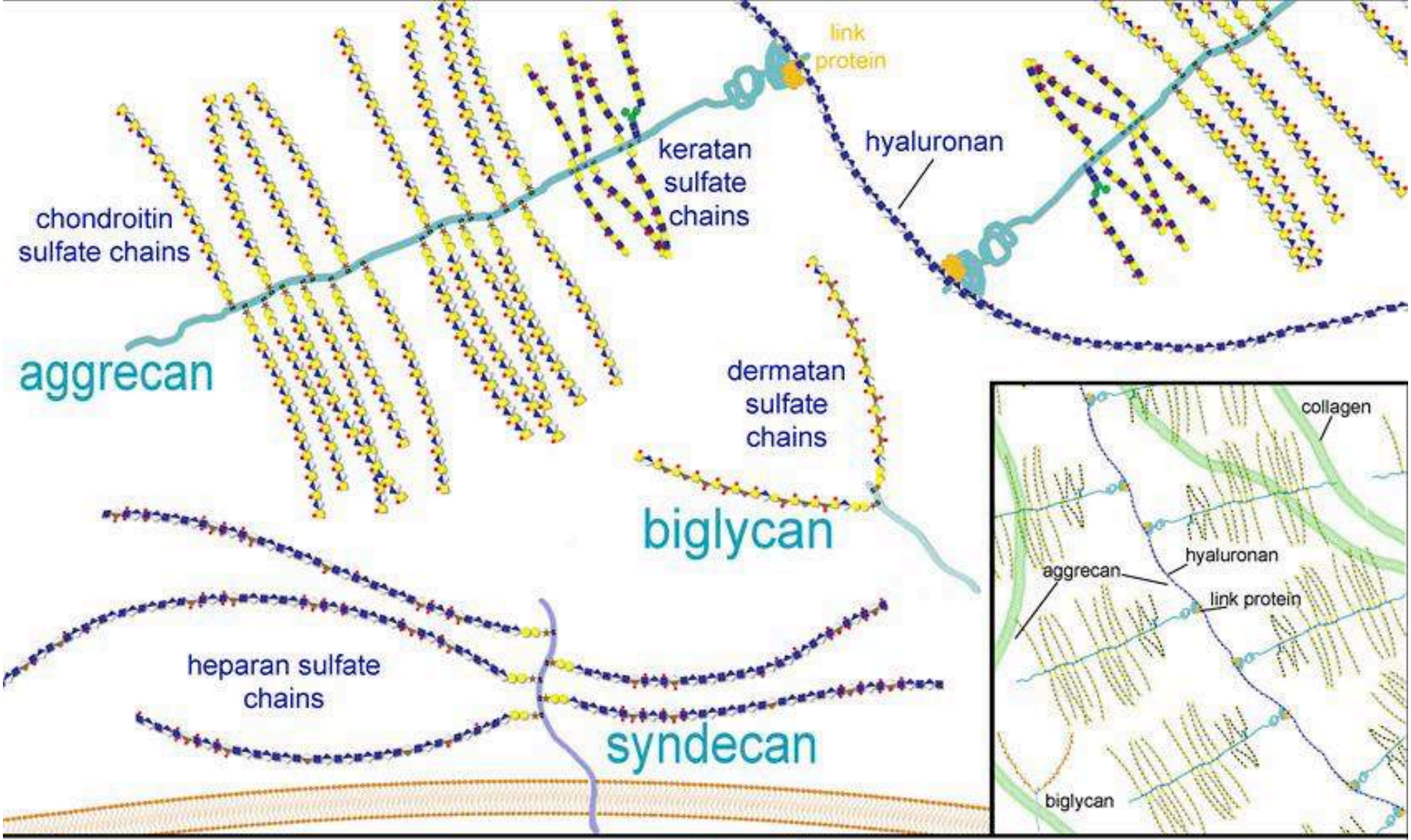
(a) Structure of chondroitin sulfate, a glycosaminoglycan



(b) General structure of a proteoglycan

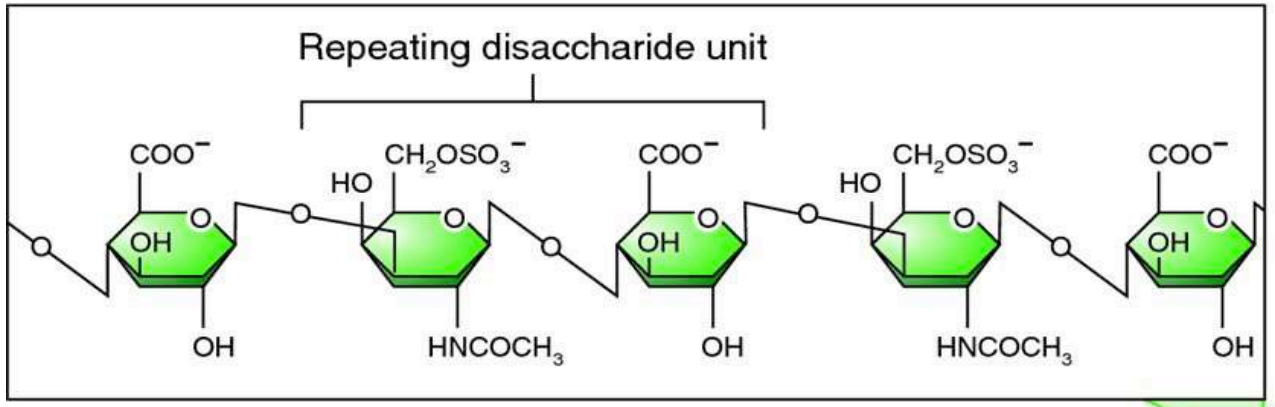
Proteoglycans

Name	Molecular weight (kDA)	Location	Function
Aggrecan	250	Cartilage, chondrocytes	Responsible for the hydration of ECM in cartilage
Decorin	38	Connective tissue, fibroblasts, cartilage, bone	Have role in collagen formation Regulate diameter of collagen fibers
Versican	260	Fibroblasts, skin, smooth muscle, brain, kidneys	Have role in cell-cell and cell-ECM interactions
Syndecan	33	Transmembrane proteins of cell membranes of embryonic epithelia, MSCs, lymphocytes	Extracellular part of it binds to collagen, fibronectin, tenascin, and Heparin. Intracellular part of it binds to cell skeleton through actin.

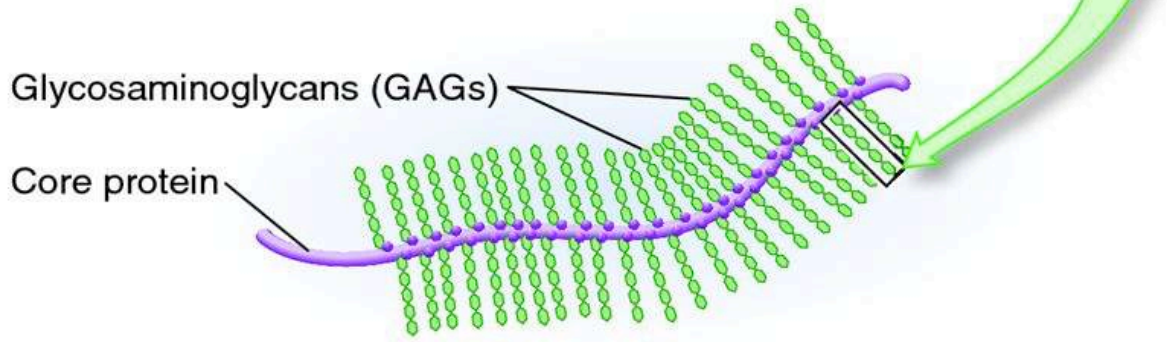

link protein
keratan sulfate chains
hyaluronan
chondroitin sulfate chains
aggrecan
dermatan sulfate chains
biglycan
heparan sulfate chains
syndecan
collagen
hyaluronan
aggrecan
link protein
biglycan

PROTEOGLYCANS

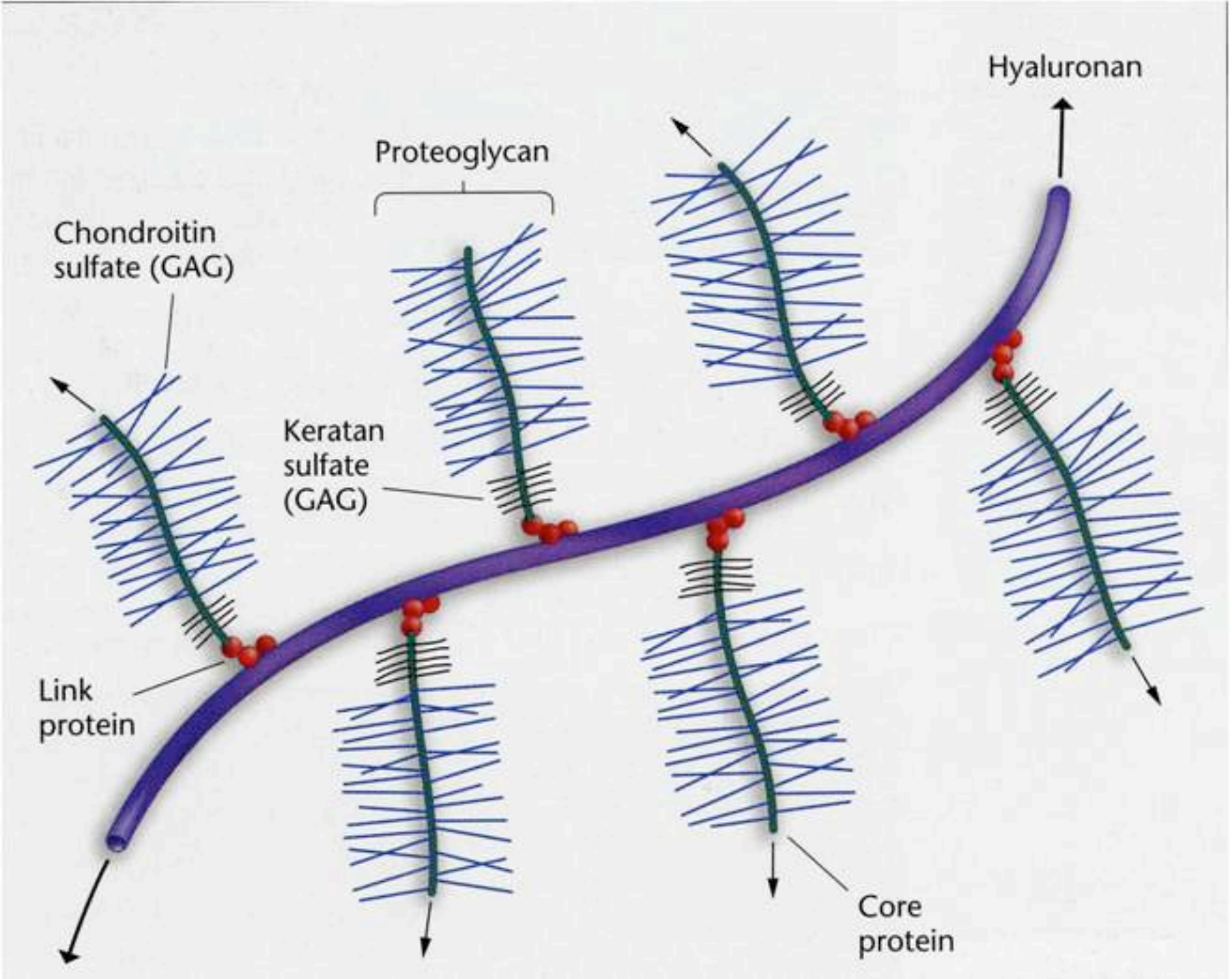
Aggrecan gp. = Core protein + ~100 chondroitin sulfate + ~60 keratan sulfate



(a) Structure of chondroitin sulfate, a glycosaminoglycan



(b) General structure of a proteoglycan



<u>Aggrecan gp. =</u>
Core protein +
~100 chondroitin sulfate + ~60 keratan sulfate

300+ aggrecans can bind to a single hyaluronan fiber.

Multiadhesive Glycoproteins

- Proteins that have binding site for collagens, proteoglycans, and GAGs.

Functions:

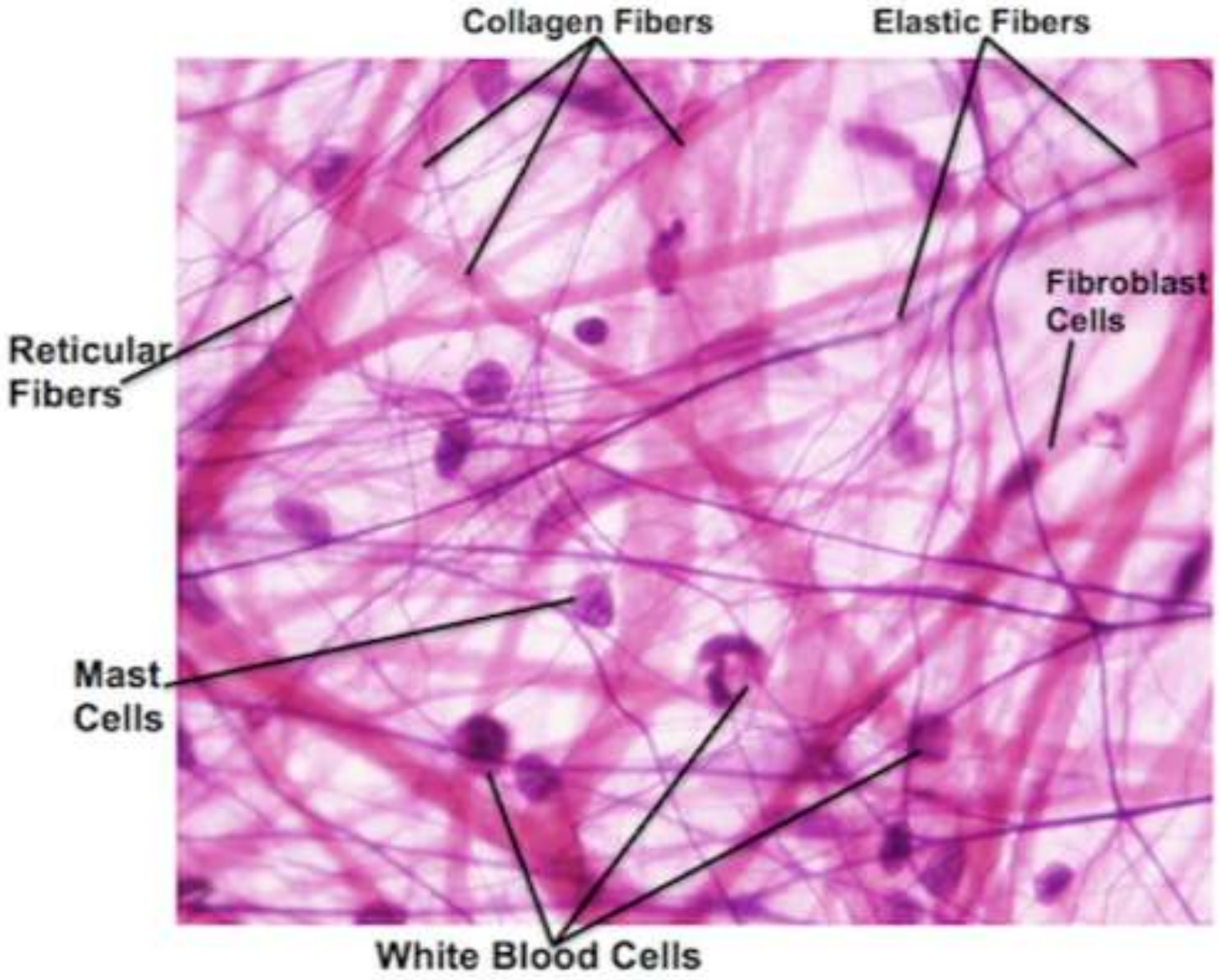
- Stabilization of ECM
- Binding cells surface to ECM
- Interaction with integrin and laminin receptors on the cell surface
- Role in cell movement, proliferation, differentiation

Multiadhesive Glycoproteins

Name	Molecular weight (kDa)	Location	Function
Fibronectin	250-280	ECM	Cell adhesion and migration. Includes binding sites for integrins, collagen type IV, heparin and fibrin.
Laminin	140-400	Basal lamina of epithelia and external lamina of cartilage, Schwann cells and adipocytes	Binds the cell to the basal lamina. Contains binding sites for integrin receptors, collagen type IV, heparan sulfate, heparin, entactin and other laminins.
Tenascin	**1680**	Periosteum, perichondrium, muscle-tendon connections, wounds, cancers	Regulates attachment of ECM to cells by binding to fibronectin, heparin, integrins, adhesion molecules and various growth factors.
Osteopontin	**44**	Bone	Binds to osteoclasts. Osteopontin can bind calcium to hydroxyapatite crystals, and integrin receptors.
Entactin / Nidogen	150	Specific to basal lamina	Connects laminin and type IV collagen. Also attaches to perlecan and fibronectin.

FIBERS

1. Collagen fibers
2. Reticular fibers
3. Elastic fibers

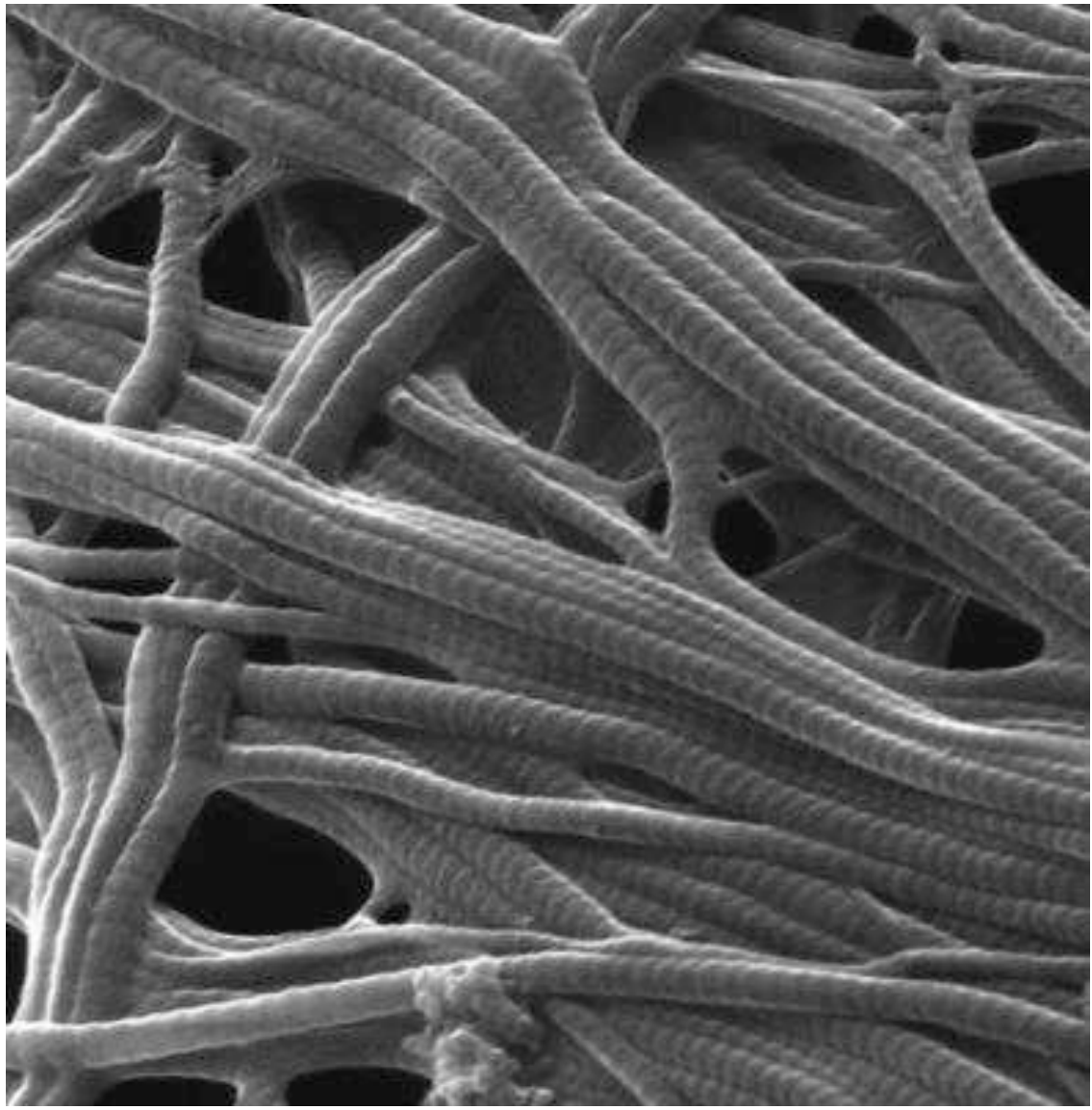


1. COLLAGEN FIBERS

- The most common fiber in the connective tissue.
- Flexible but quite strong.

- Vitamin C is required for synthesis. Deficiency ?

- Discovered 42 types
- The most common type is collagen type I (%90).

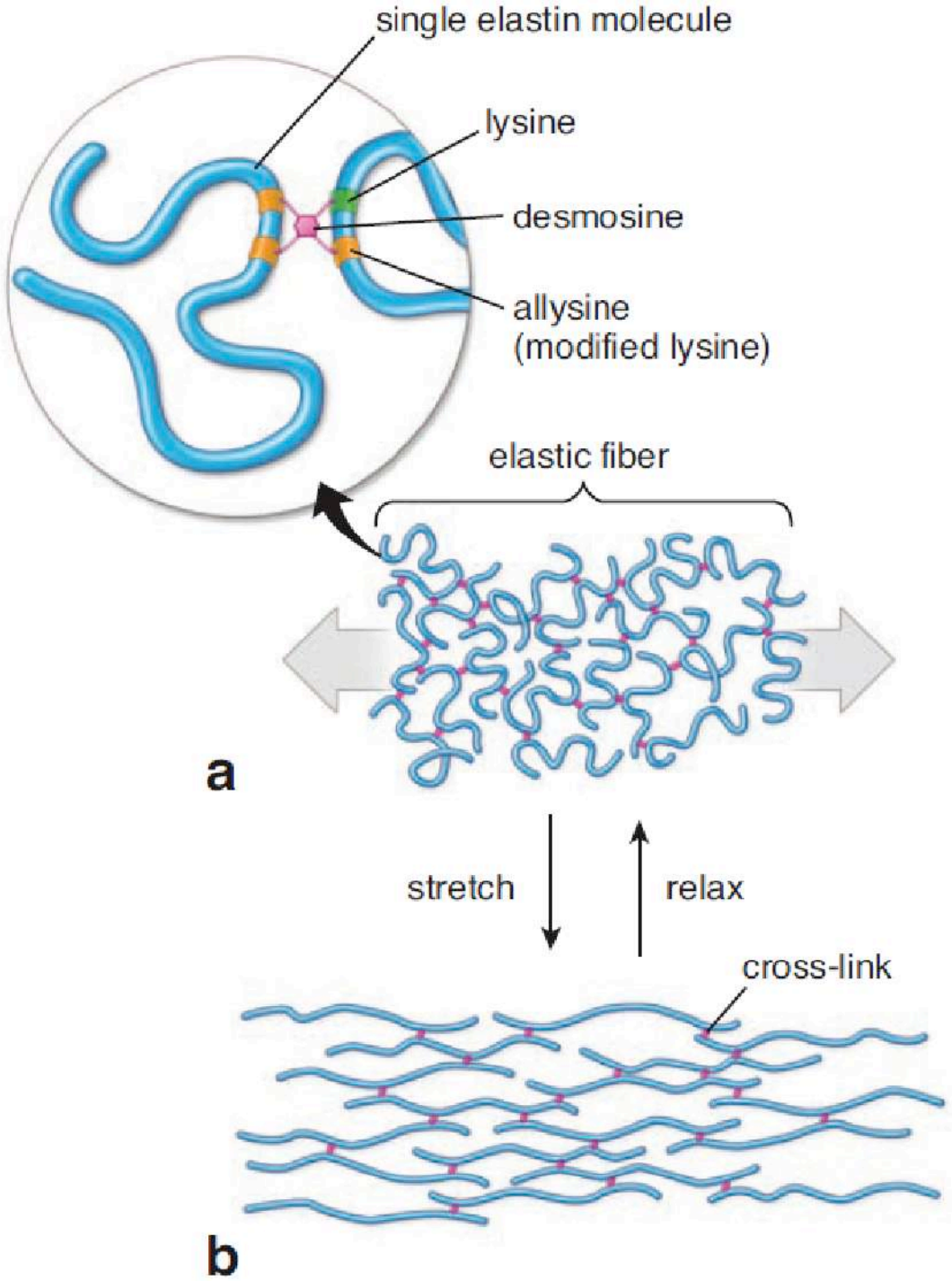


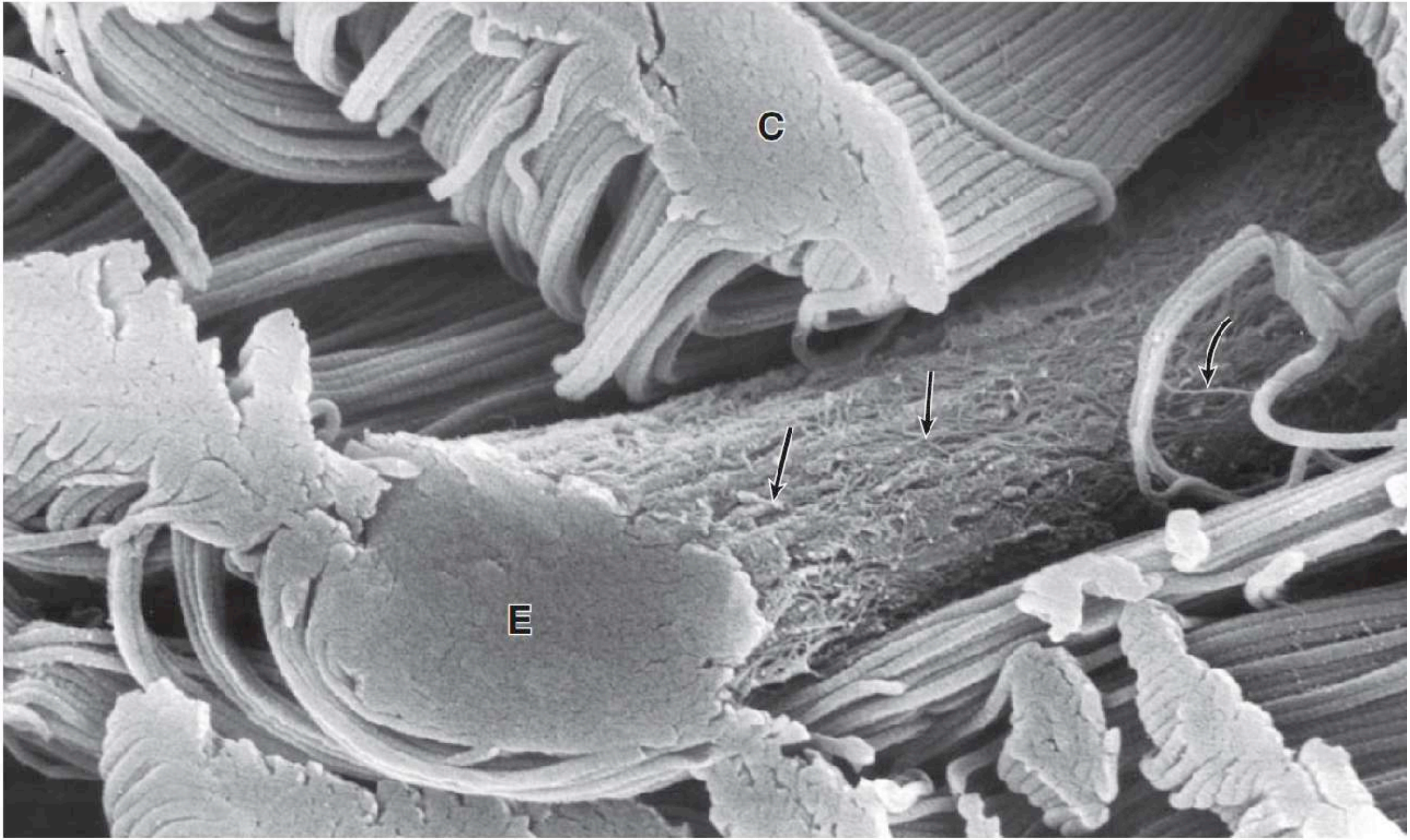
Some Collagen Types

Related Collagen	Place	Function
I	**Connective tissue in skin, bone, tendon, ligaments,** dentine, sclera, fascia, organ capsules	Provides strength aganist force and tension
II	**Cartilage** (hyaline and elastic), notocord, intervertebral discs	Provides resistance to applied pressure intermittently
III	Loose connective tissue, smooth muscle, blood vessels, fetal skin	Provides support for various organs and vessels by forming **Reticular Fibers**
IV	Basal lamina, kidney glomeruli, lens	Support and filtration
IX	Cartilage, with collagen type II	Has role in attaching collagen type II with proteoglycans
X	Hypertrophy zone of prolonging bone	Contributes to bone mineralization

3. ELASTIC FIBRES

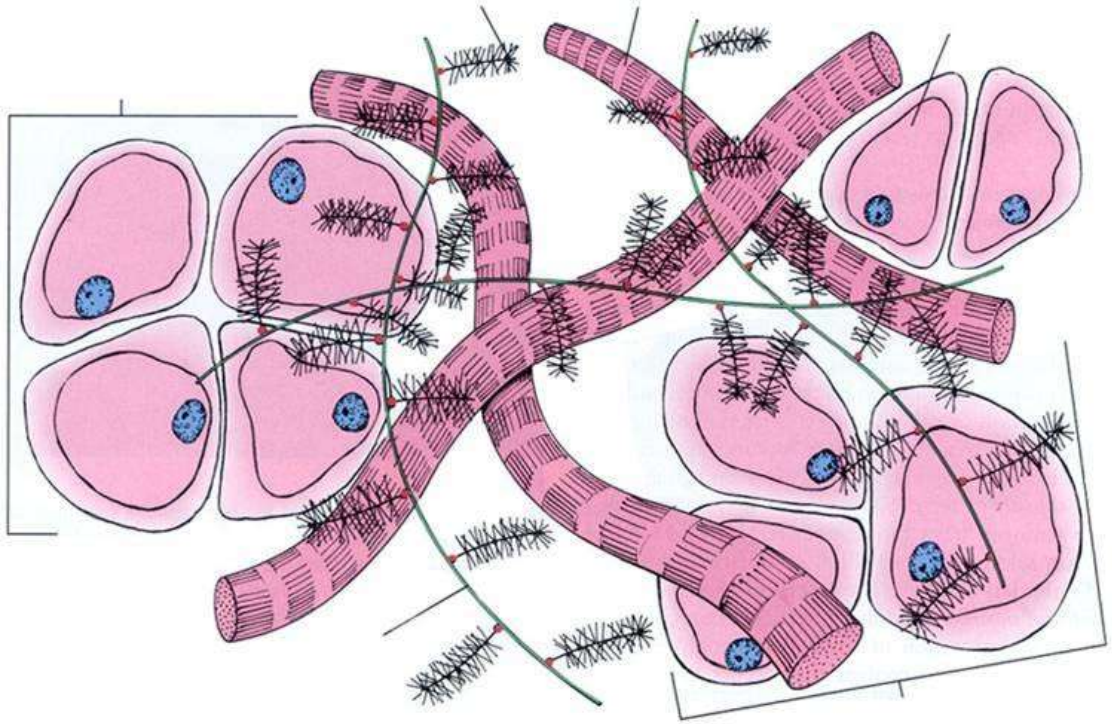
- Thinner than collagen and it branches.
- Composed of elastin and fibrilin proteins.
- Unlike collagen, it's hydrophobic.
- Synthesized by fibroblasts and smooth muscle cells of blood vessels.
- Can be found as fibrils or layers.




C
E

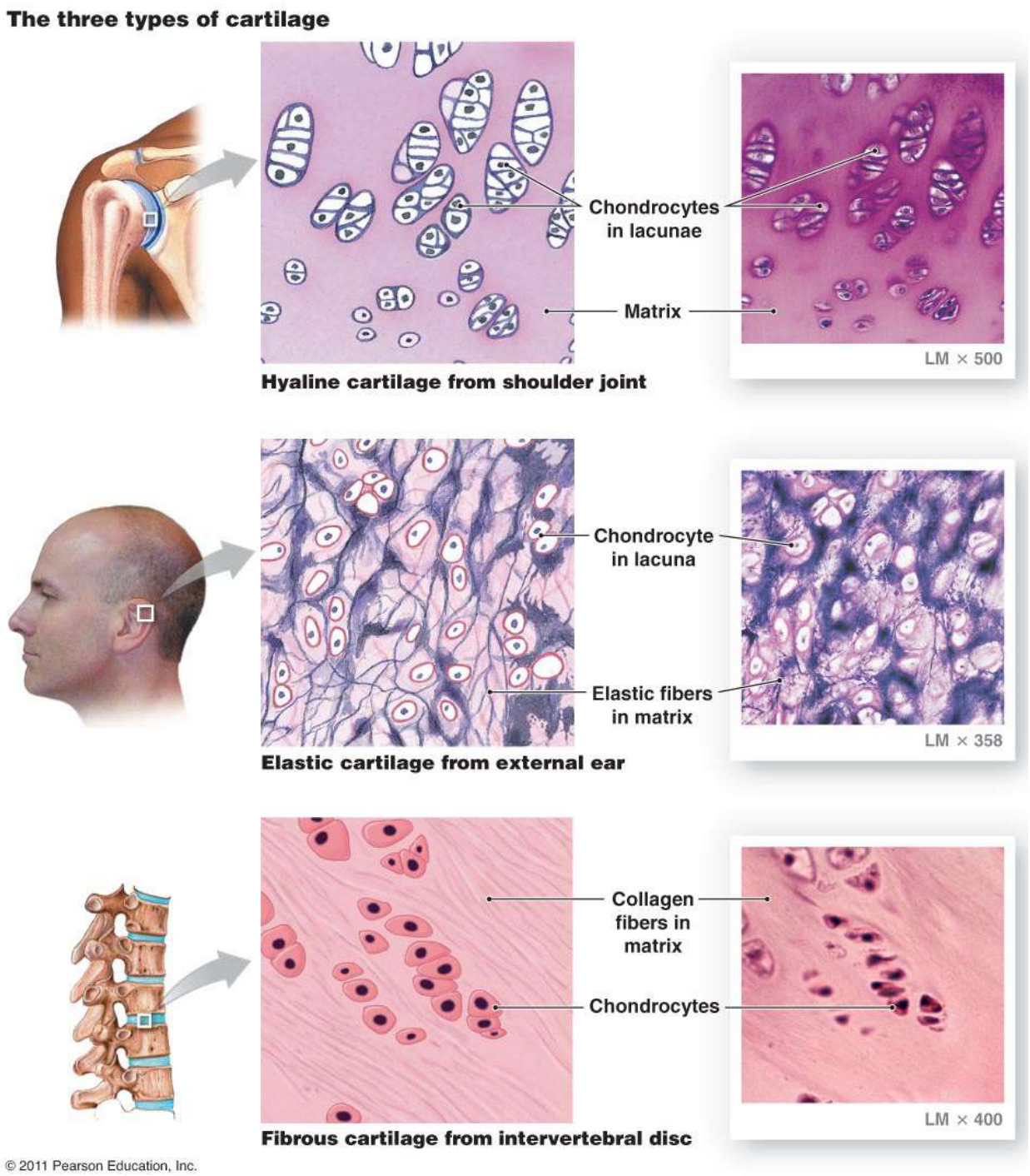
Components of Cartilage Tissue

1) **Cells** → % 5 (Chondrocytes)
2) **Extracellular matrix** → % 95
    1) **Fibres:** Collagen (**Type II**), Elastic*
    2) **Ground substance:**
        1) GAGs
        2) Proteoglycans
        3) Multiadhesive glycoproteins



Cartilage Tissue Types

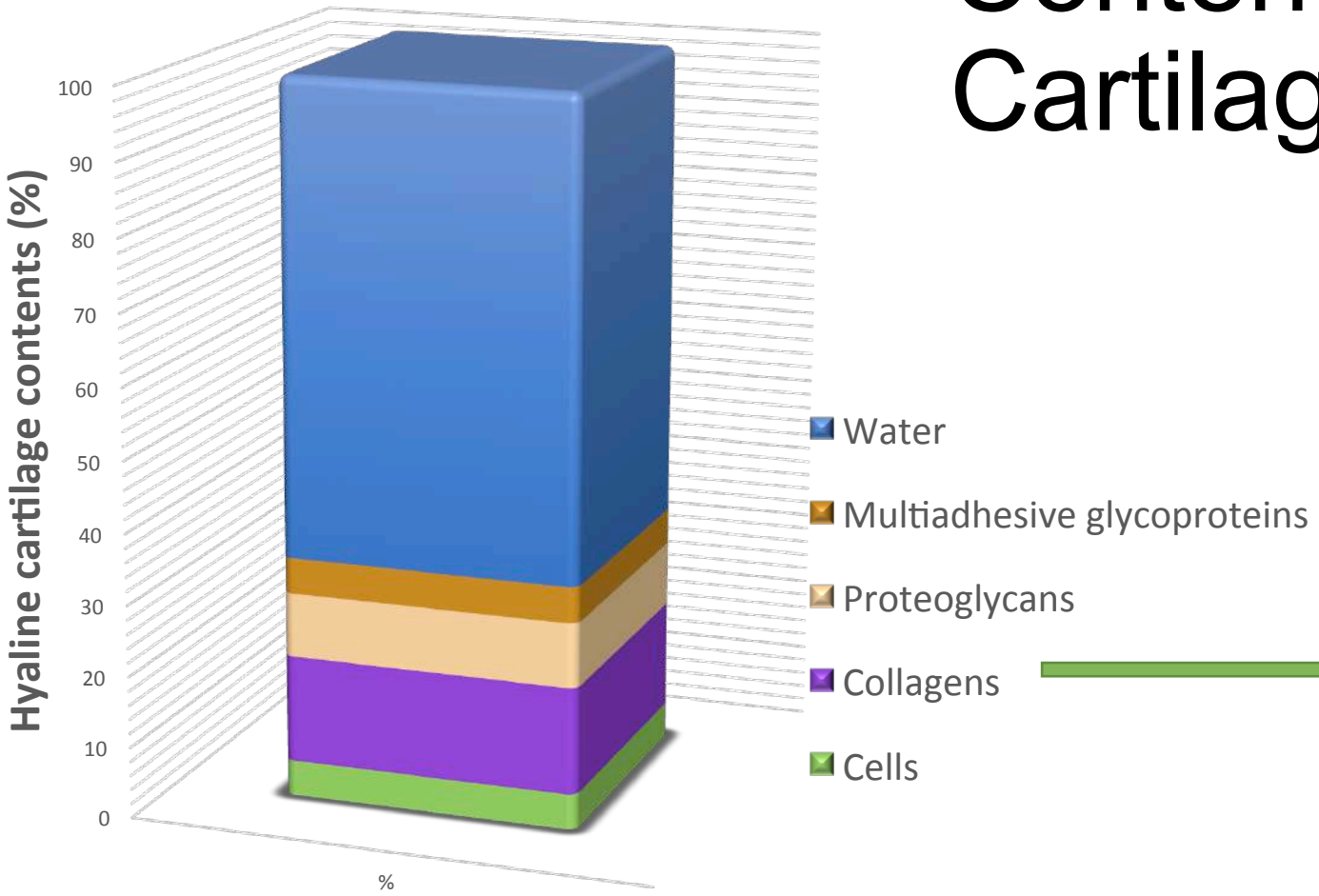
1. Hyaline cartilage
2. Elastic cartilage
3. Fibrous cartilage

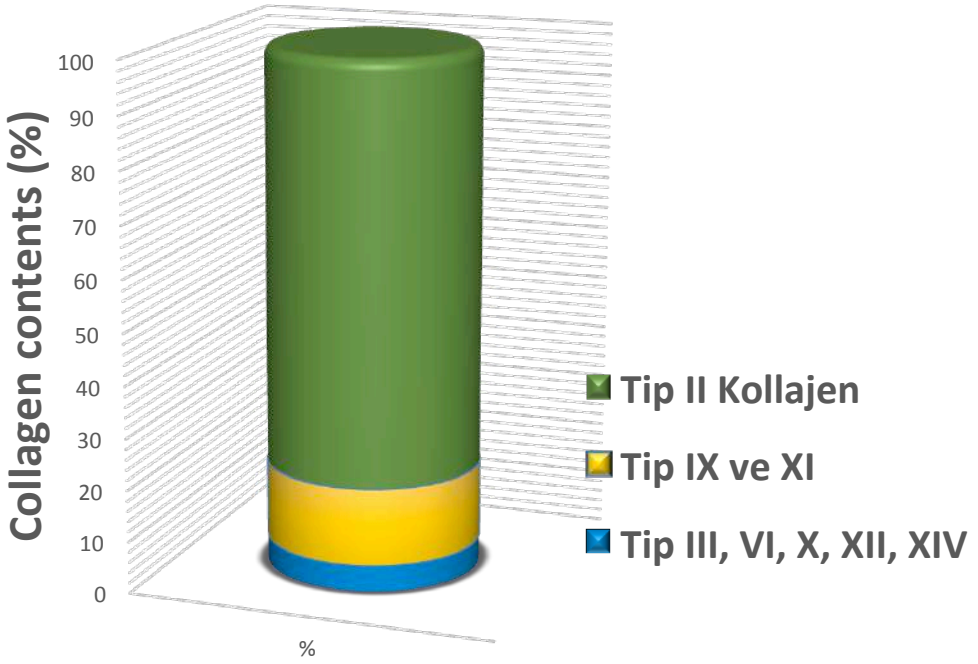


Cartilage Tissue Types

1. Hyaline cartilage
2. Elastic cartilage → Hyaline cartilage + Elastic fibers
3. Fibrous cartilage → Hyaline cartilage + Collagen type I

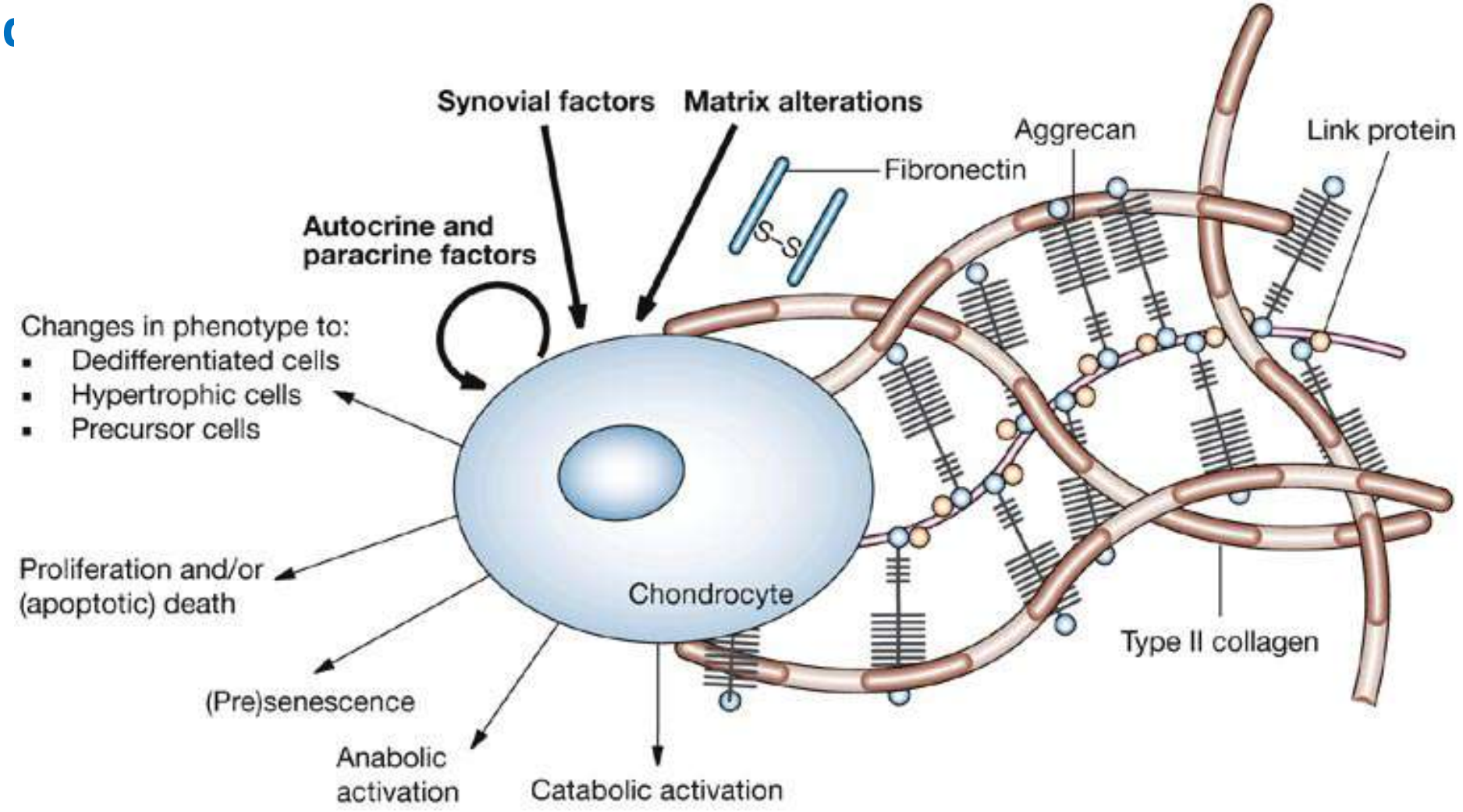
Contents of Hyaline Cartilage

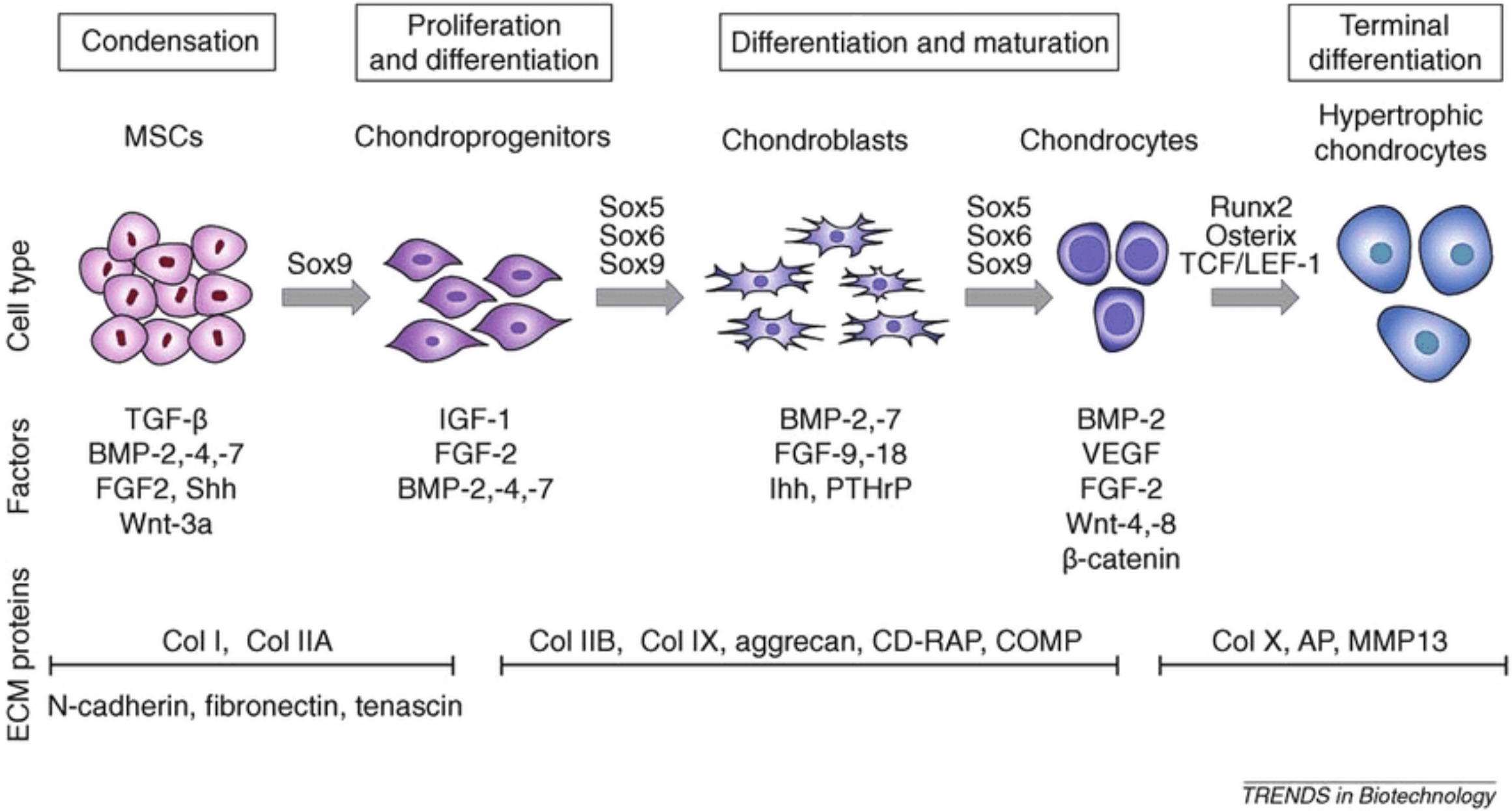


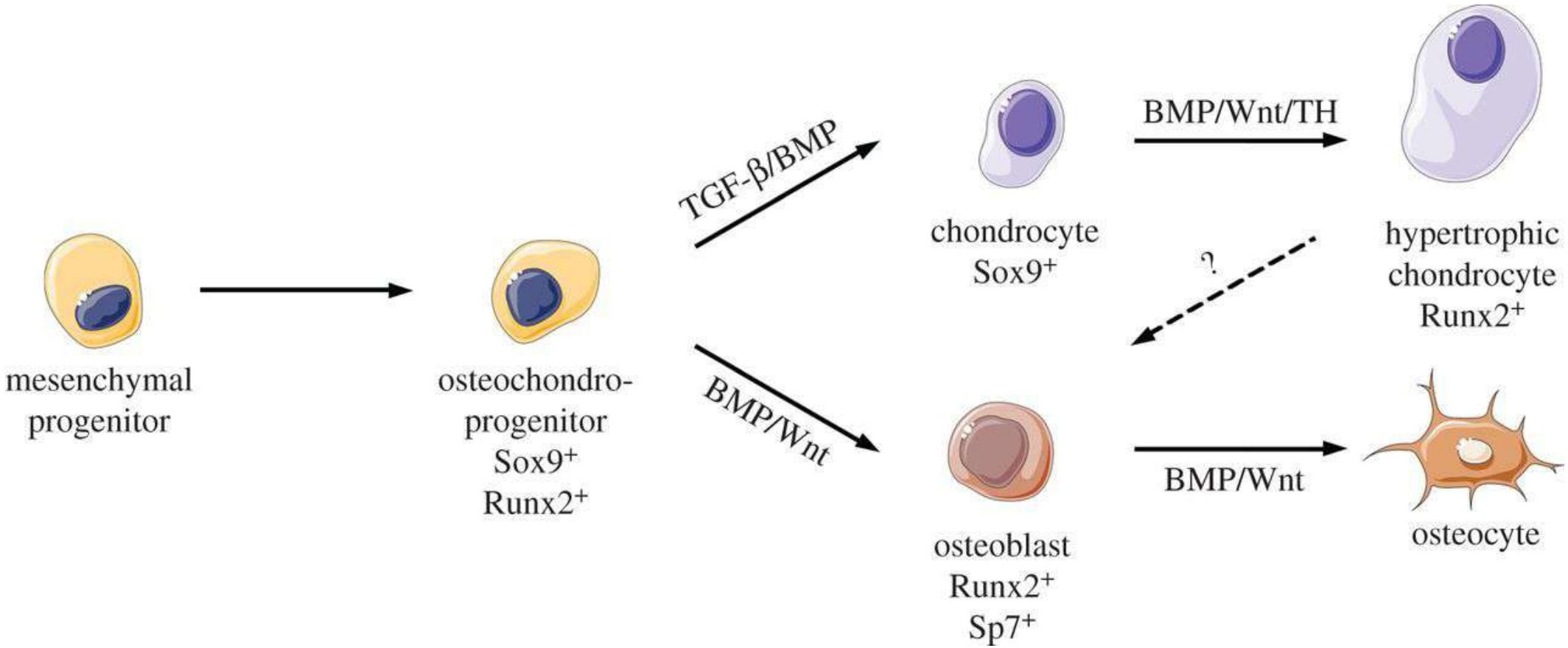


Cells of the Cartilage Tissue

Mesenchymal stem cells / Fibroblasts → **Chondroblasts** → **Chon**

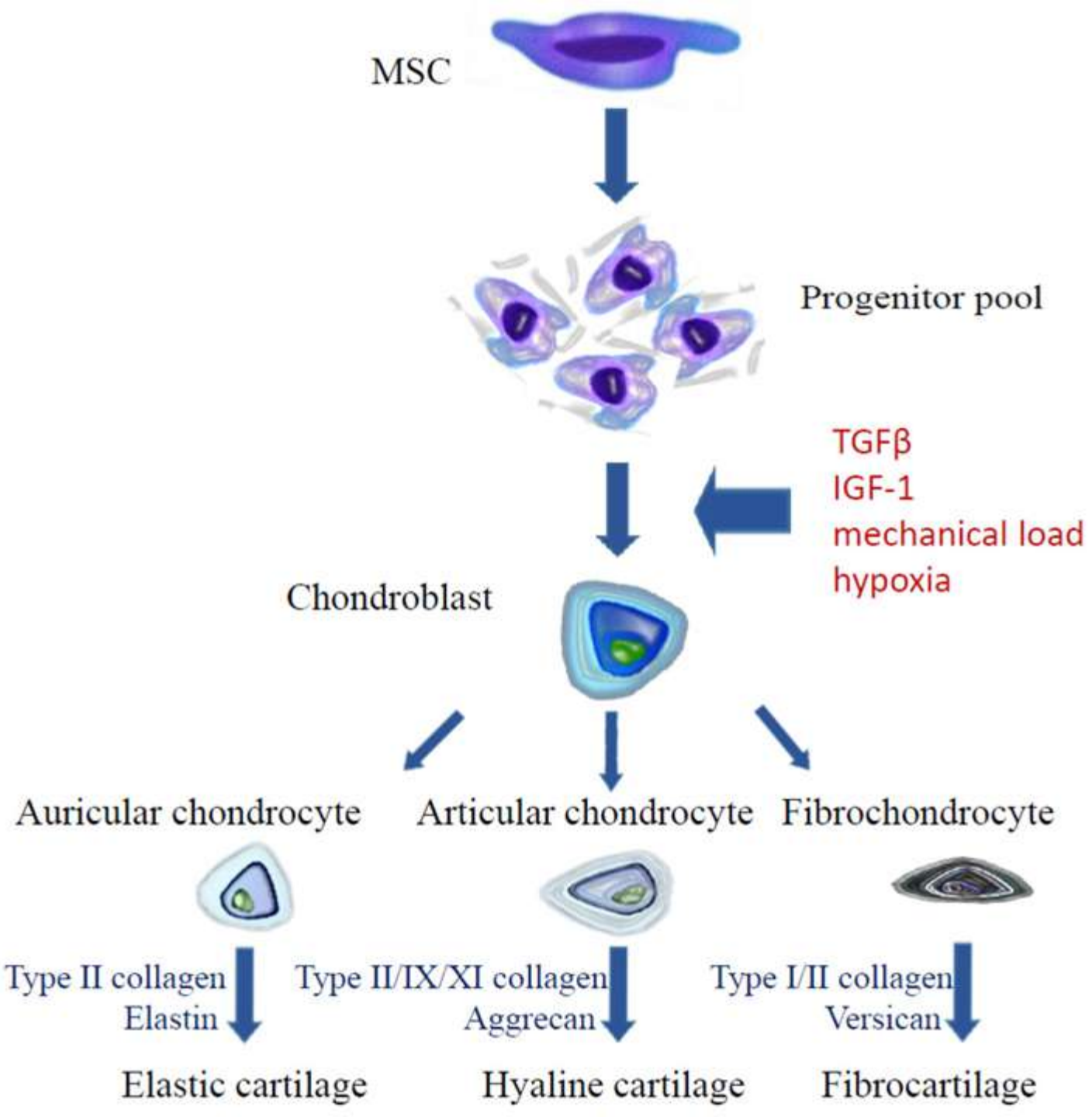


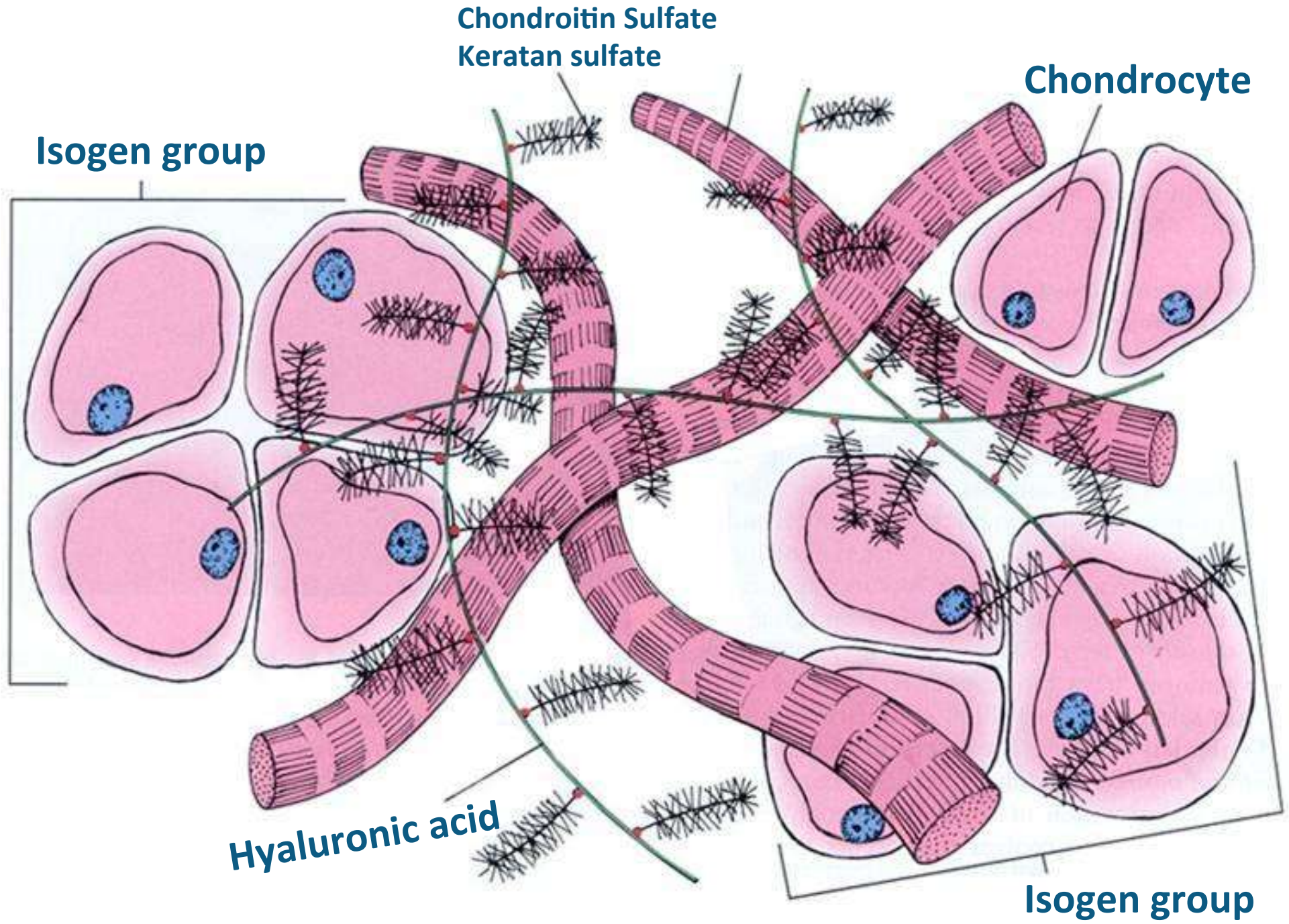

Condensation
Proliferation and differentiation
Differentiation and maturation
Terminal differentiation
MSCs
Chondroprogenitors
Chondroblasts
Chondrocytes
Hypertrophic chondrocytes
Cell type
Sox9
Sox5
Sox6
Sox9
Sox5
Sox6
Sox9
Runx2
Osterix
TCF/LEF-1
Factors
TGF-β
BMP-2,-4,-7
FGF2, Shh
Wnt-3a
IGF-1
FGF-2
BMP-2,-4,-7
BMP-2,-7
FGF-9,-18
Ihh, PTHrP
BMP-2
VEGF
FGF-2
Wnt-4,-8
β-catenin
ECM proteins
Col I, Col IIA
Col IIB, Col IX, aggrecan, CD-RAP, COMP
Col X, AP, MMP13
N-cadherin, fibronectin, tenascin


mesenchymal progenitor
osteochondro-progenitor Sox9+ Runx2+
TGF-β/BMP
BMP/Wnt
chondrocyte Sox9+
BMP/Wnt/TH
?
hypertrophic chondrocyte Runx2+
osteoblast Runx2+ Sp7+
BMP/Wnt
osteocyte

Cartilage type specific subtypes

1. Hyaline cartilage
2. Elastic cartilage
3. Fibrous cartilage




Chondroitin Sulfate
Keratan sulfate
Chondrocyte
Isogen group
Hyaluronic acid
Isogen group

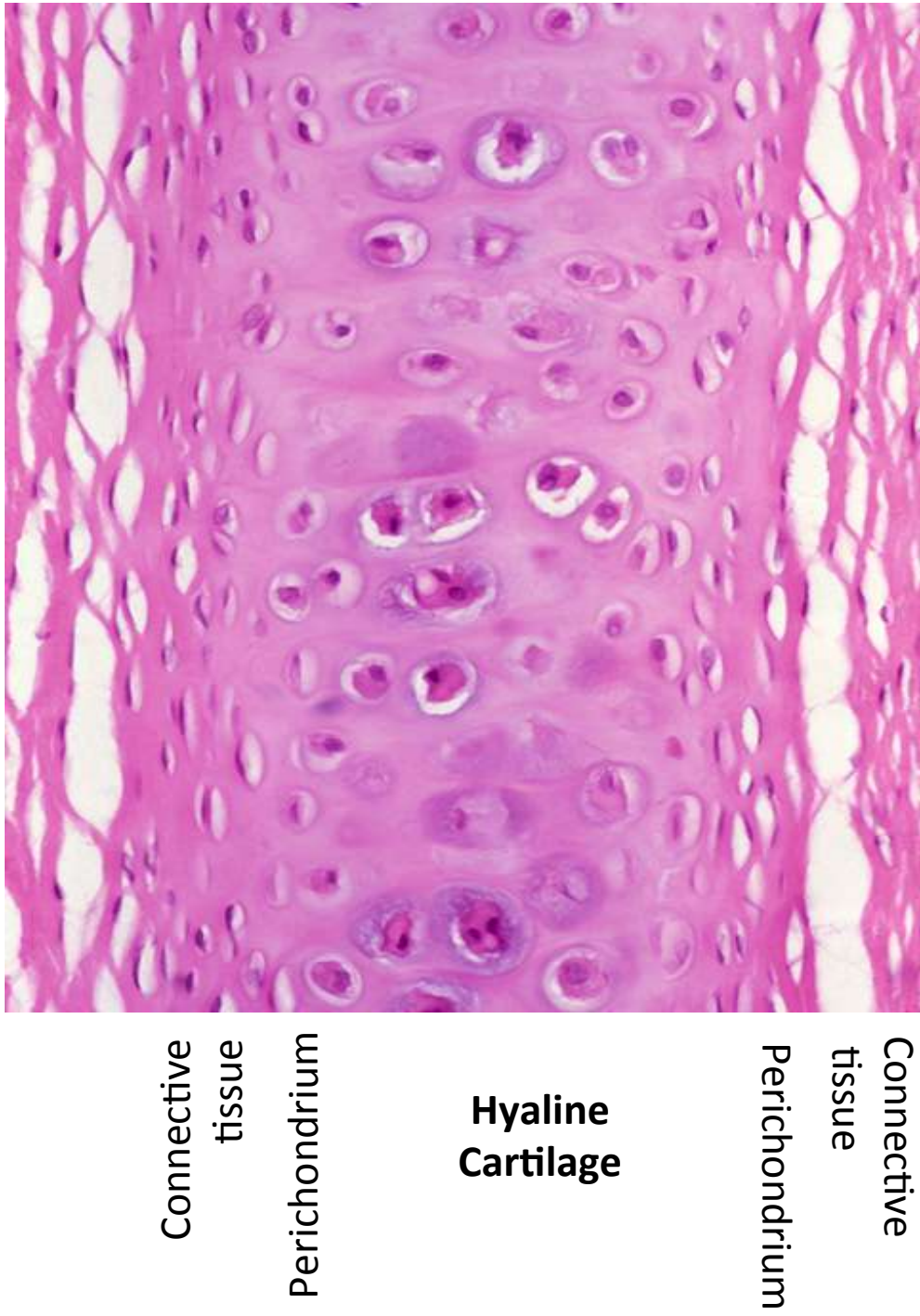

Trachea H&E
territorial matrix
isogenous group
interterritorial matrix

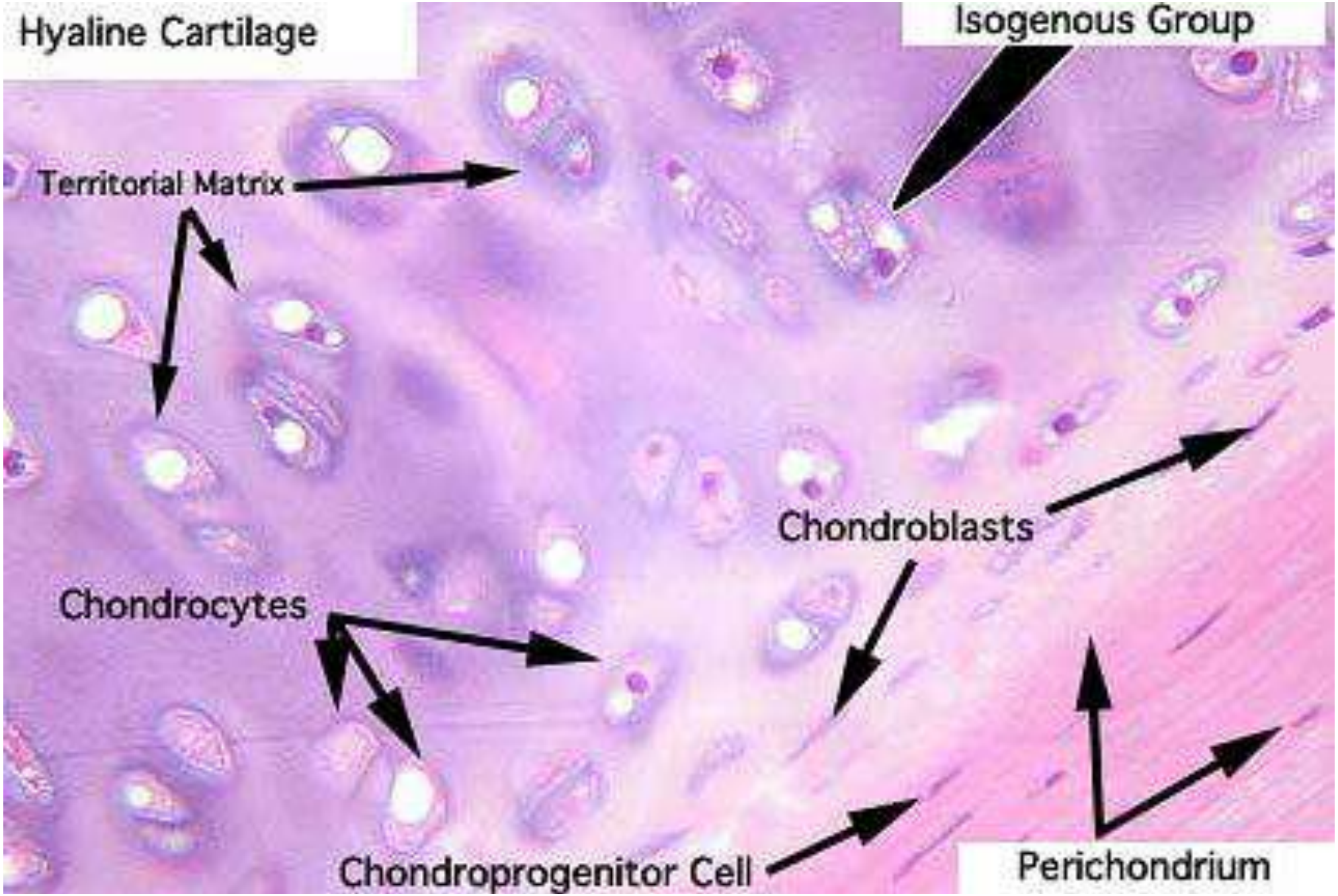
Perichondrium

- Irregular dense connective tissue capsule
- Surrounds the hyalin cartilage.
- Also contains fibroblasts at the outer layer.

* Hyaline cartilage in the joints does not have perichondrium.

** There is no perichondrium in fibrous cartilage.

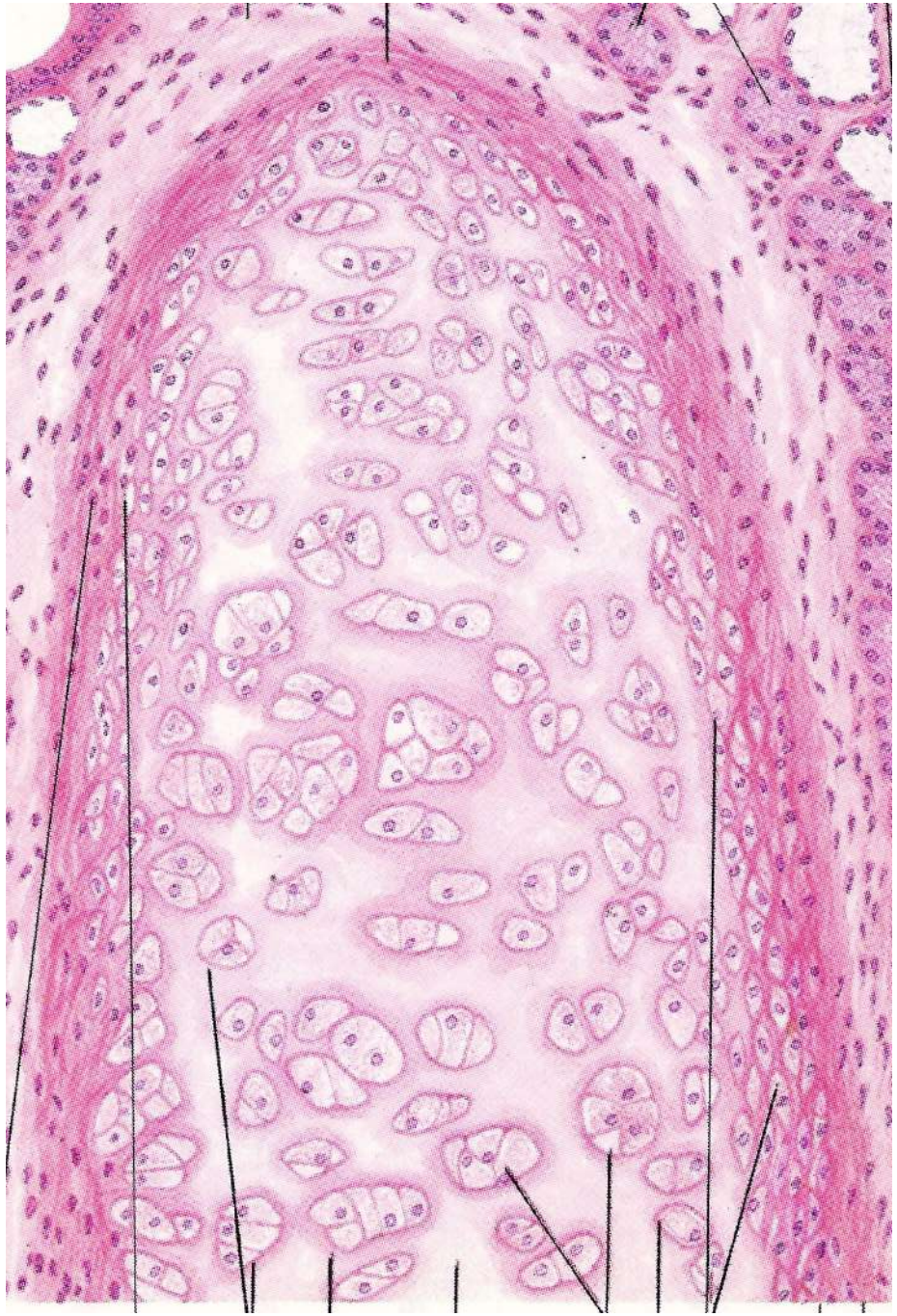



Hyaline Cartilage
Isogenous Group
Territorial Matrix
Chondroblasts
Chondrocytes
Chondroprogenitor Cell
Perichondrium

Growth of Cartilage

1. **Appositional growth:**
   - At the perichondrium.
   - New cells are formed by differentiation of stem cells into chondroblasts.

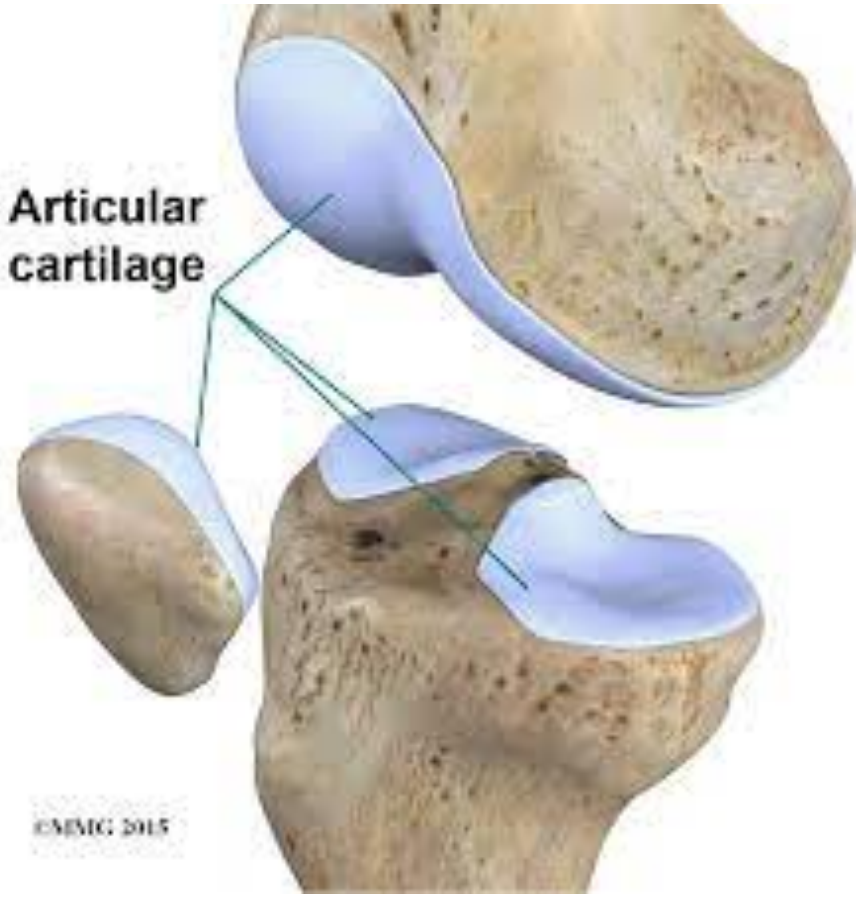
2. **Interstitial growth:**
   - By mitotic division of inner chondrocytes.

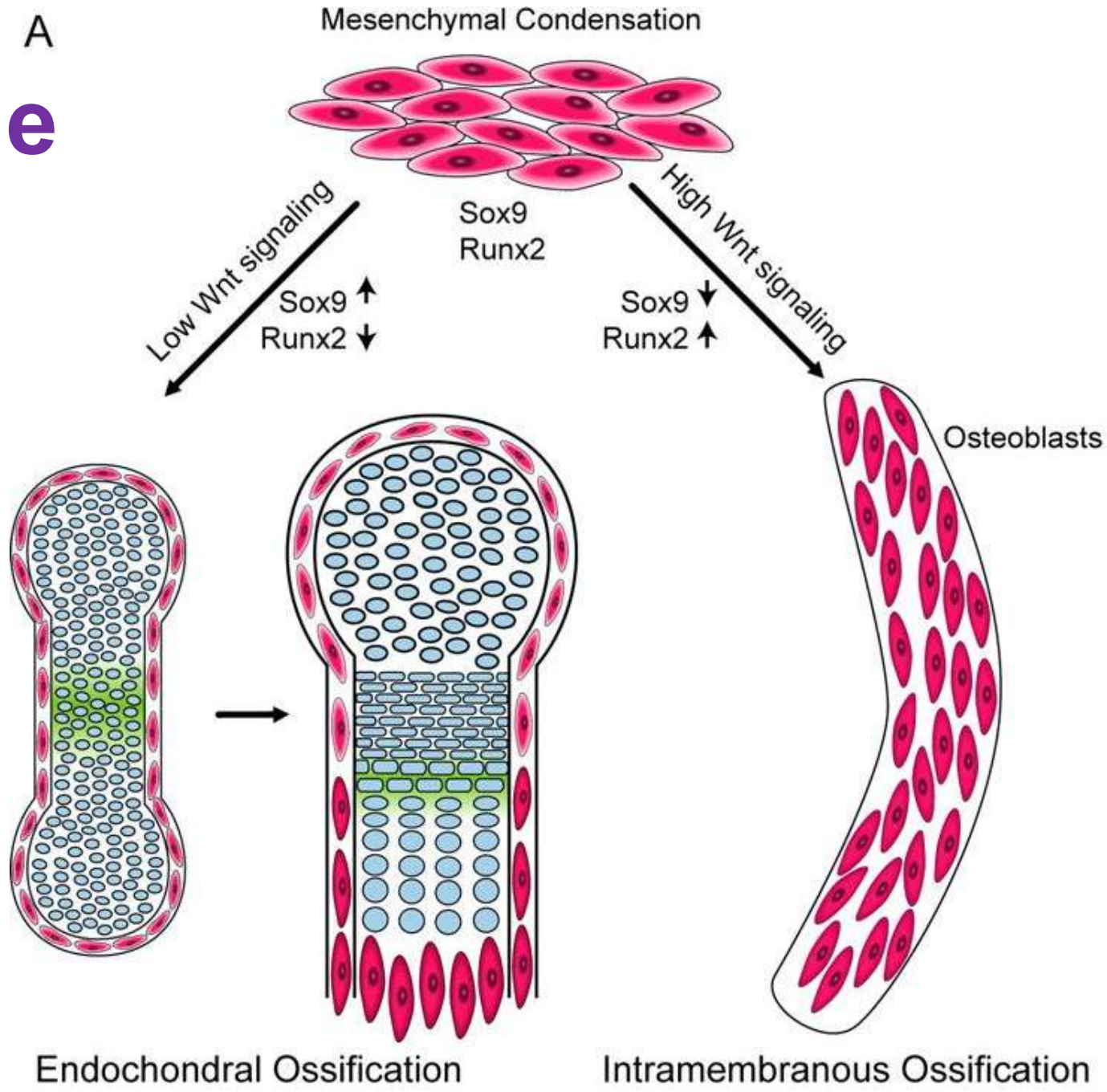


Regeneration of Cartilage

- Quite slow!
- In general, the connective tissue (scar tissue) remains at the site of damage instead of cartilage.

Articular Cartilage





Articular (Joint) Cartilage

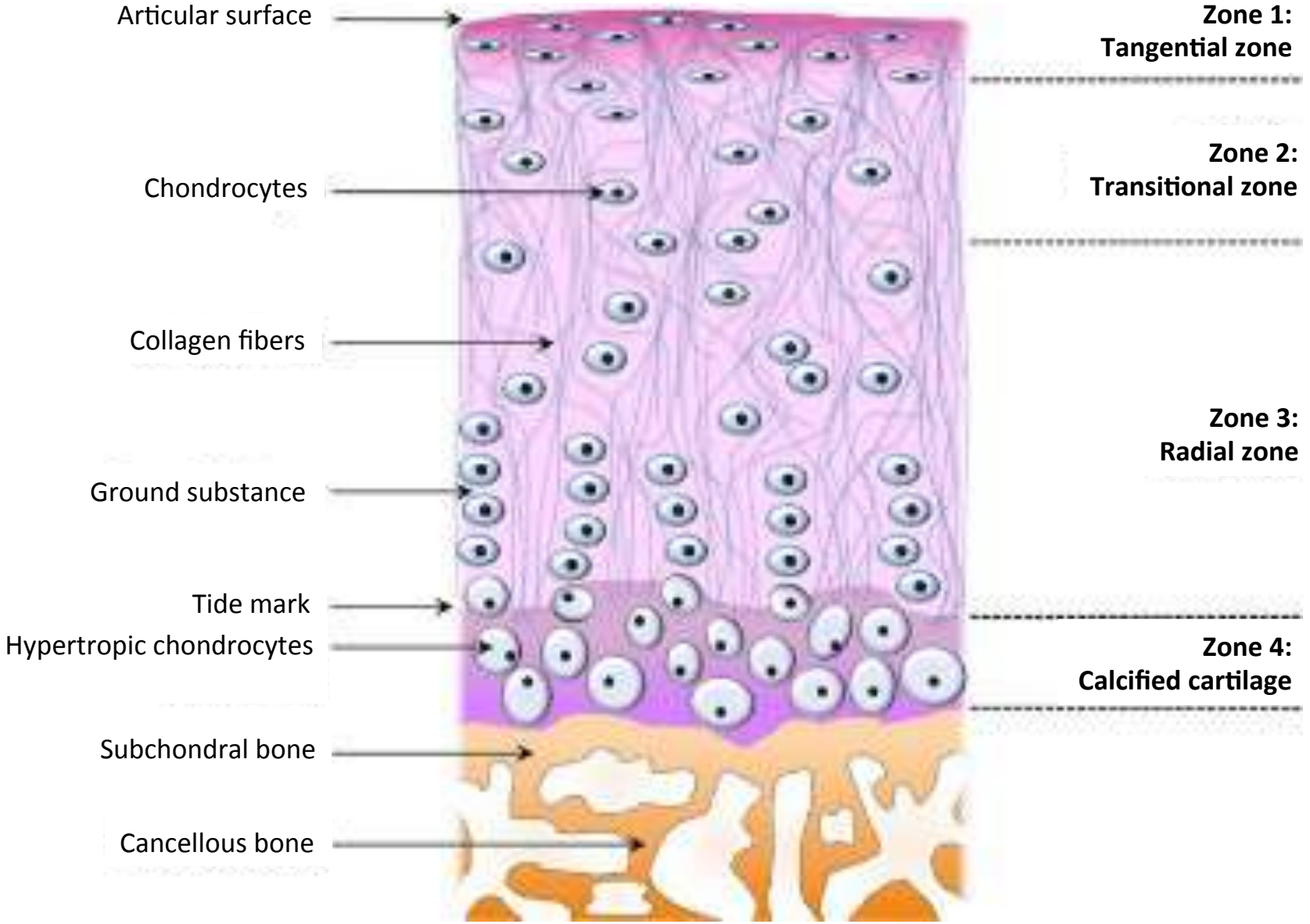
Articular cartilage is basically hyaline cartilage without perichondrium.

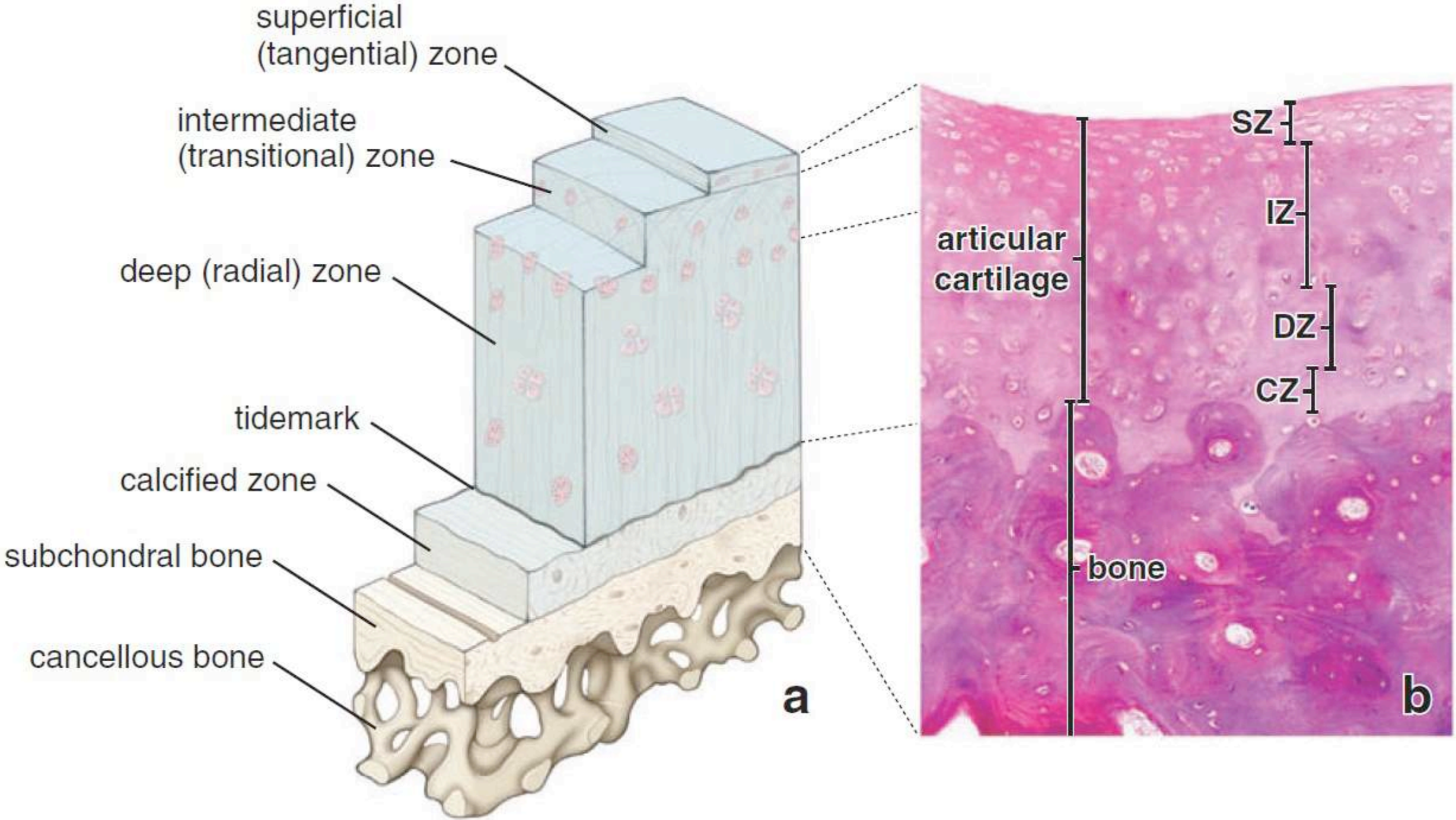
4 zones of articular cartilage :

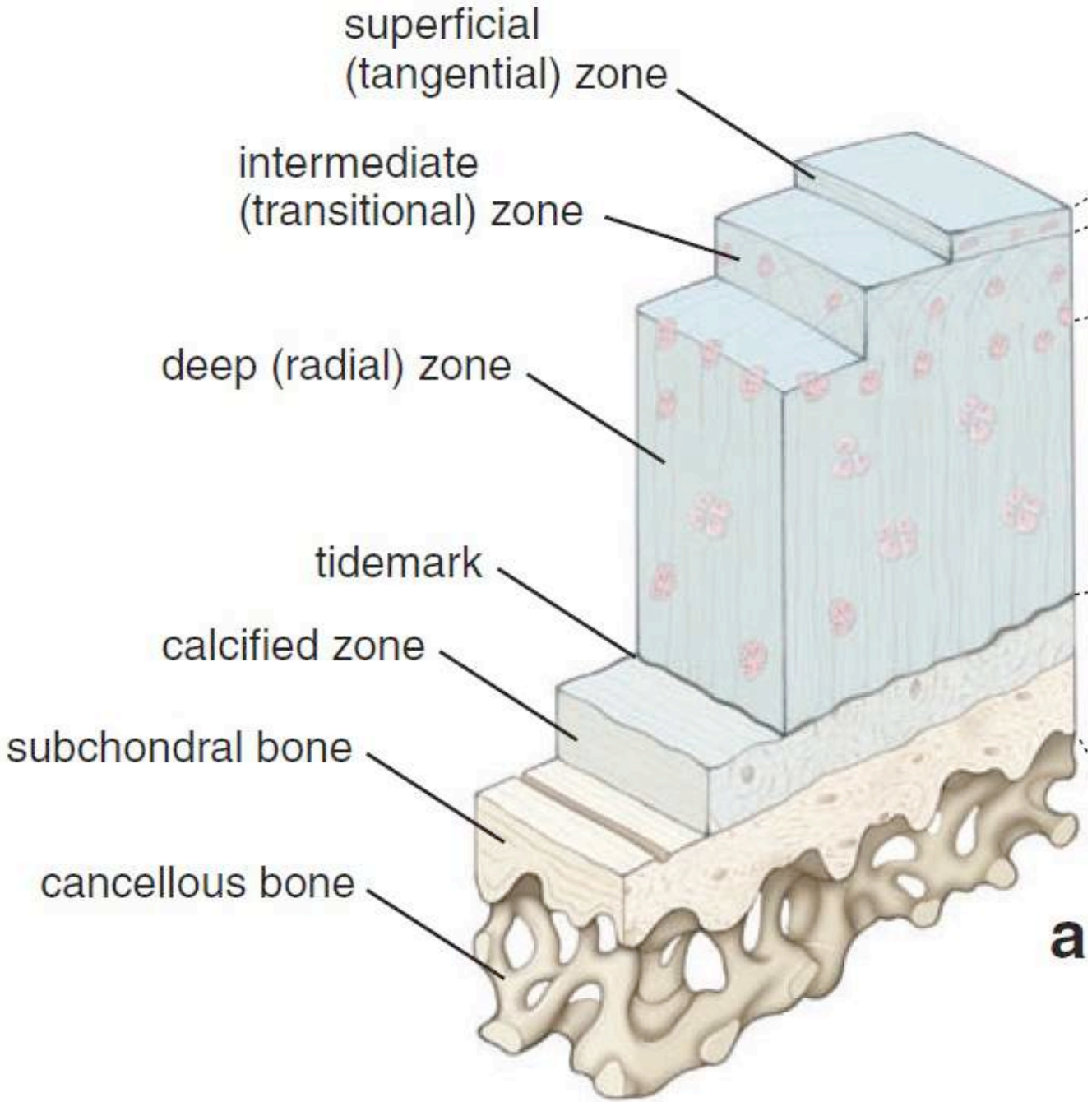
1. **Superficial (Tangential) zone:** Flat chondrocytes + Surface-parallel type II collagen bundles
2. **Intermediate (Transitional) zone:** Round chondrocytes + Randomly distributed fibers
3. **Deep (Radial) zone:** Small round chondrocytes + arms parallel to the long axis of the bone
4. **Calcified zone:** Small chondrocytes + Calcified matrix

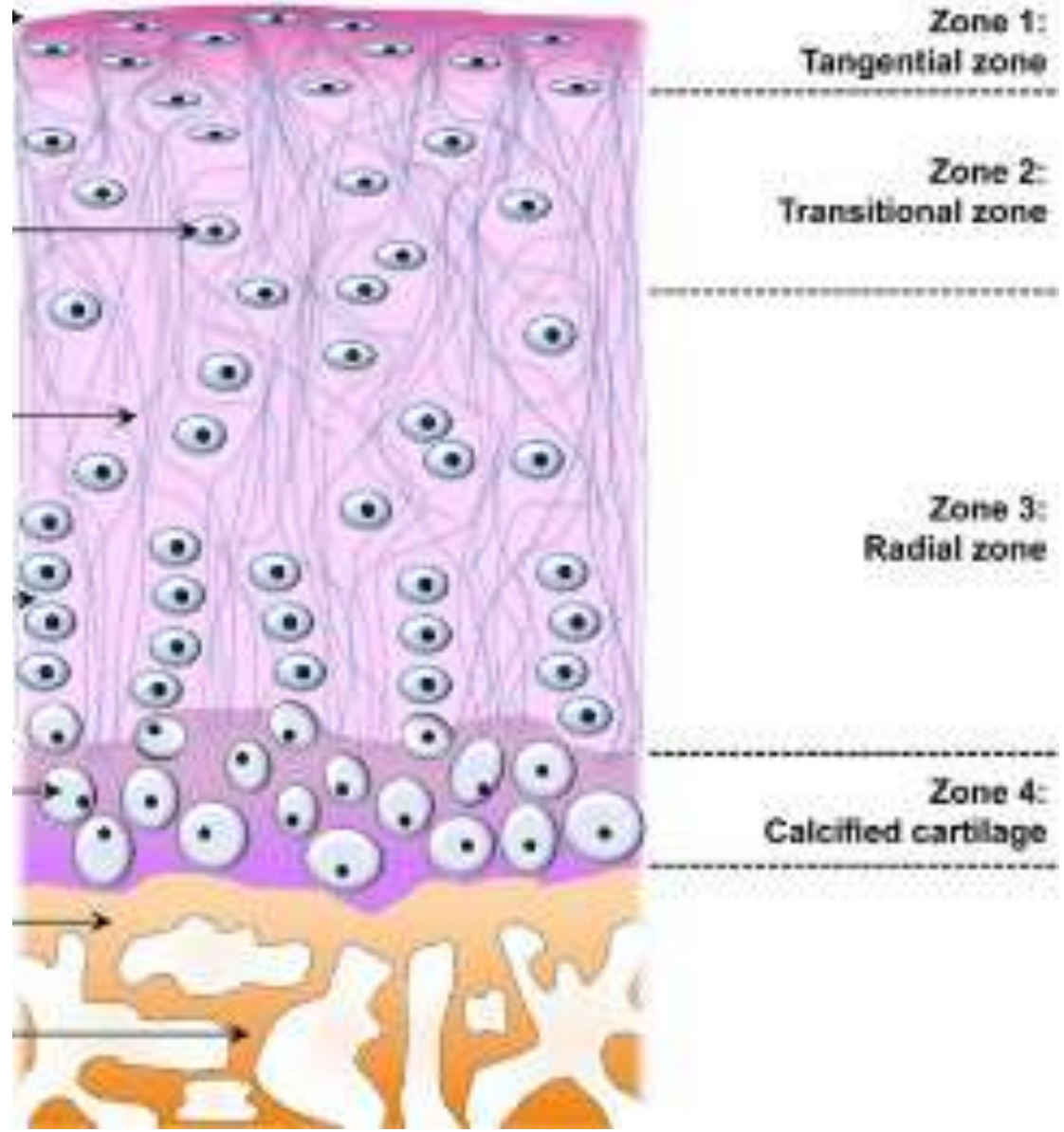
Regeneration → From deep (radial) zone to upwards

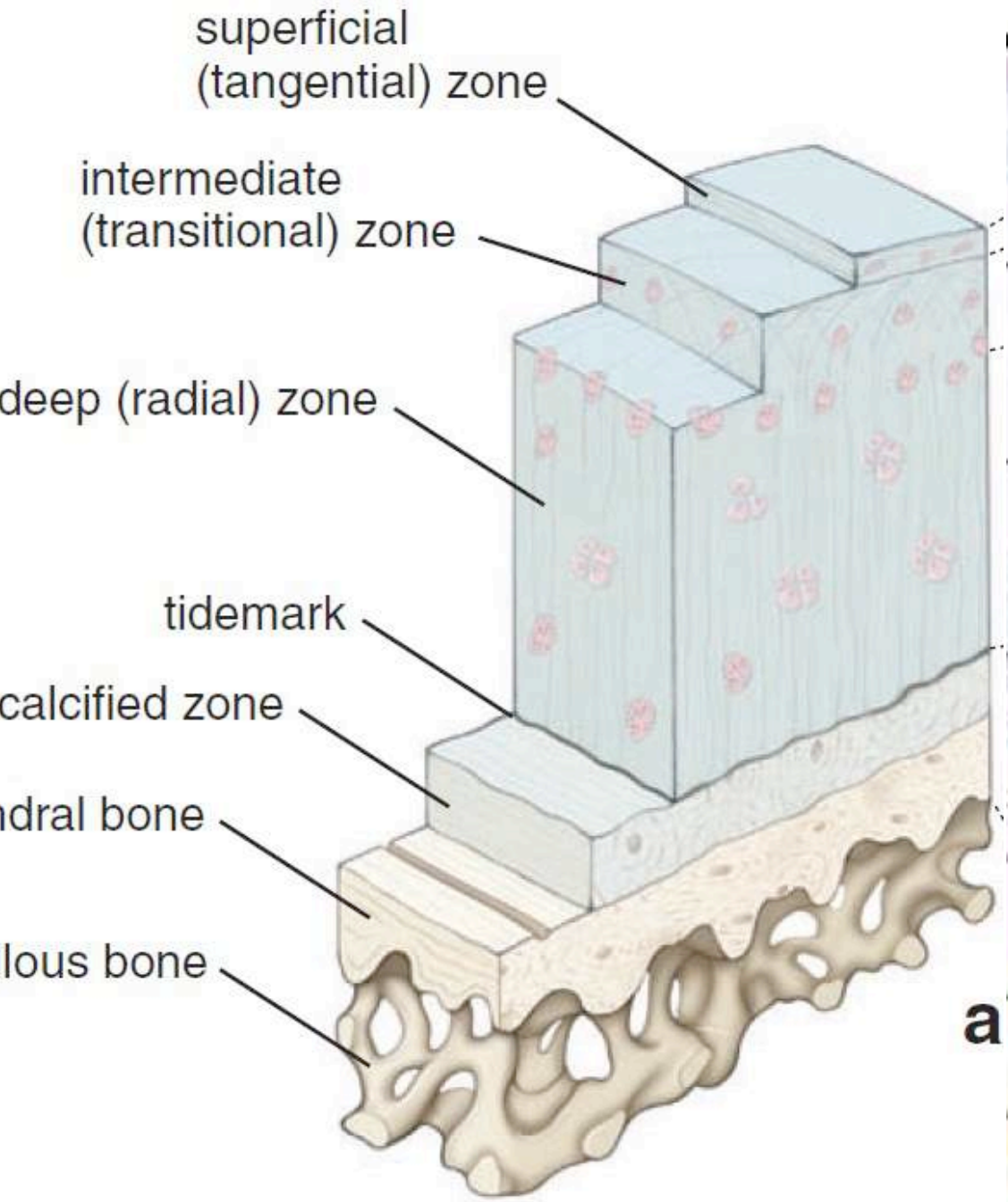
Articular Cartilage

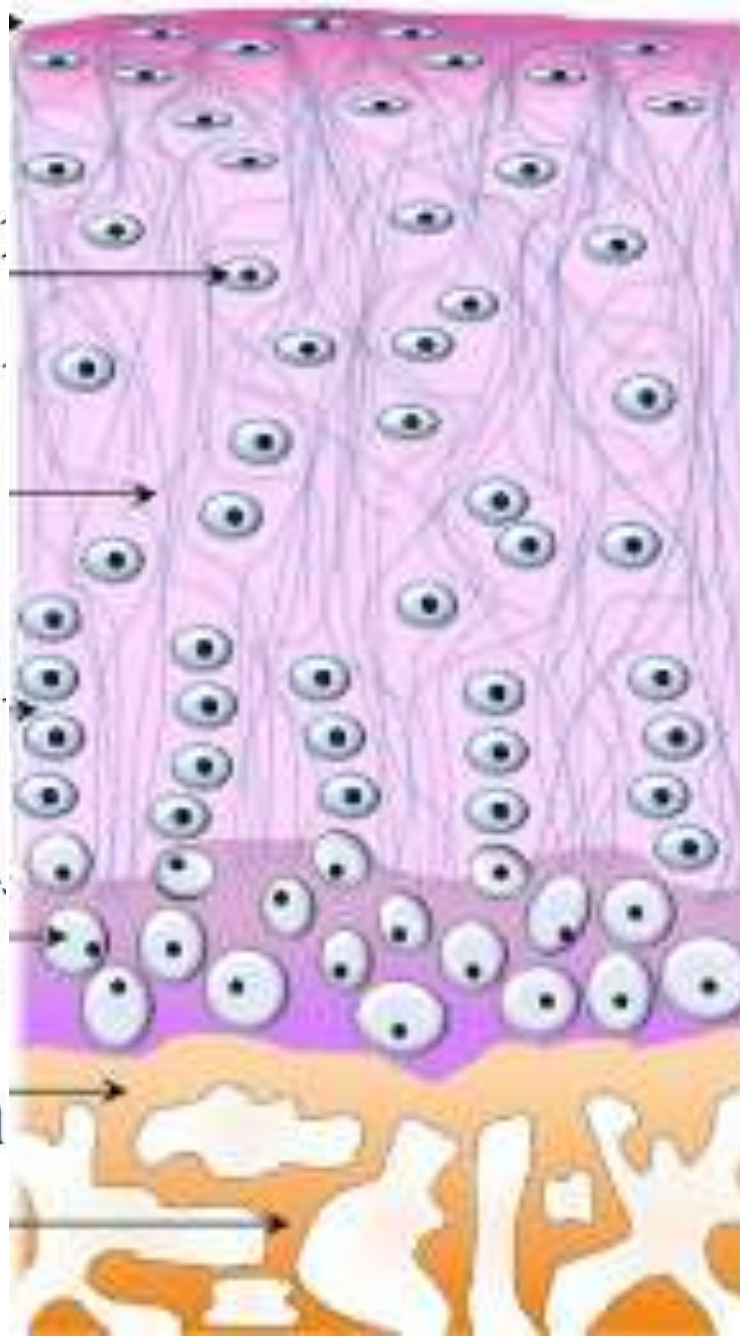



superficial
(tangential) zone
intermediate
(transitional) zone
deep (radial) zone
tidemark
calcified zone
subchondral bone
cancellous bone
a
articular
cartilage
SZ
IZ
DZ
CZ
bone
b


superficial
(tangential) zone
intermediate
(transitional) zone
deep (radial) zone
tidemark
calcified zone
subchondral bone
cancellous bone
a

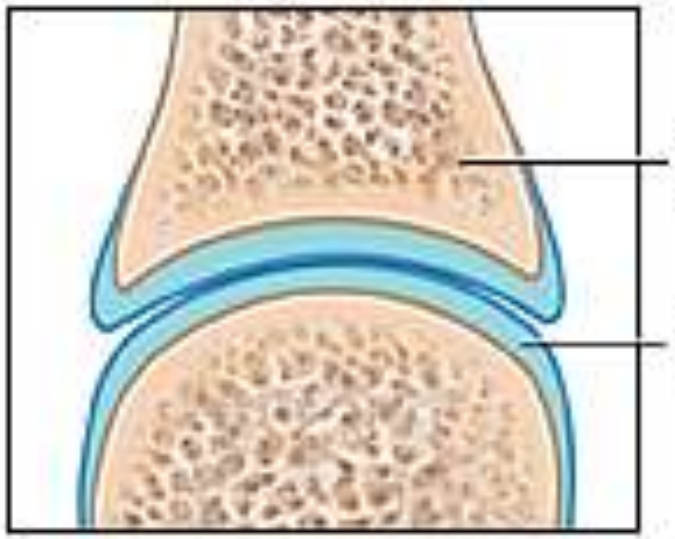

Zone 1:
Tangential zone
Zone 2:
Transitional zone
Zone 3:
Radial zone
Zone 4:
Calcified cartilage



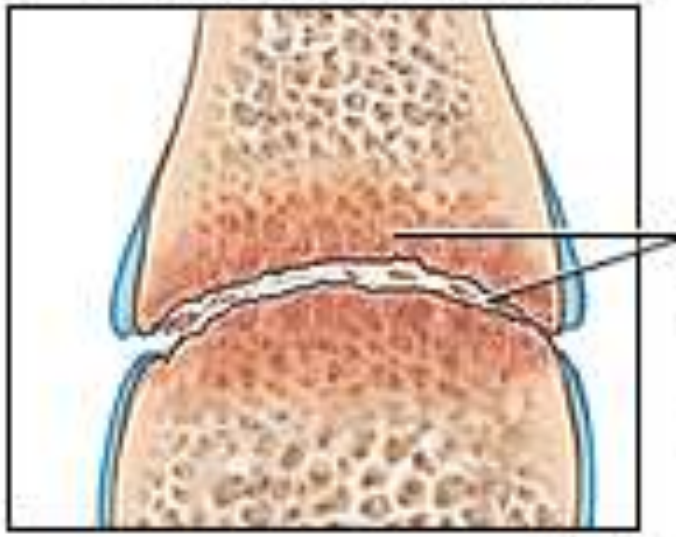


a

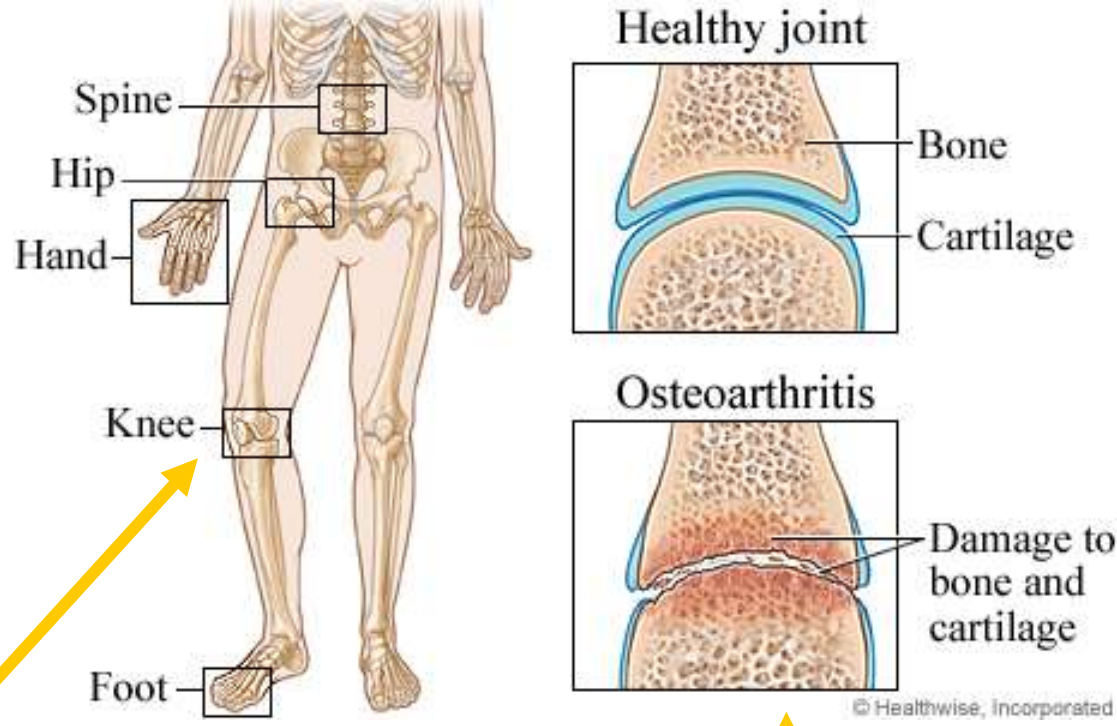
Healthy joint



Osteoarthritis

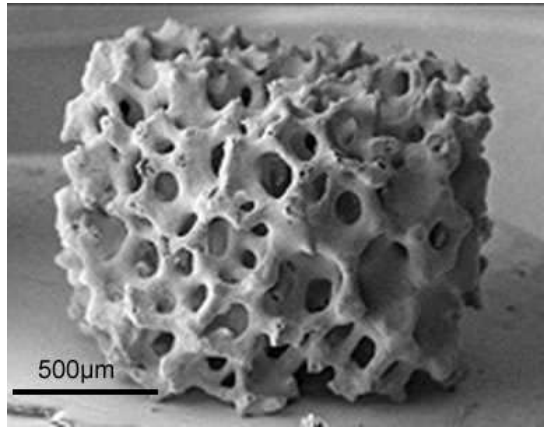


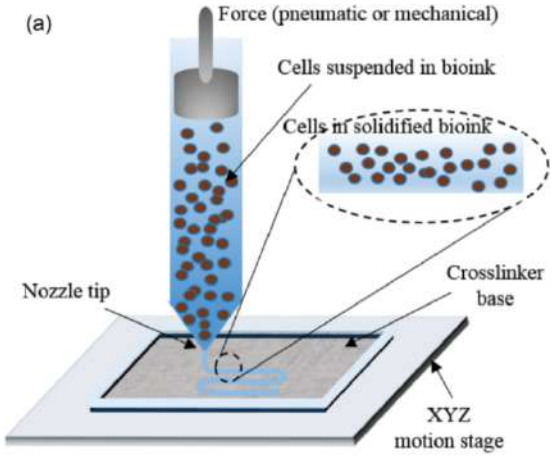
Osteoarthritis



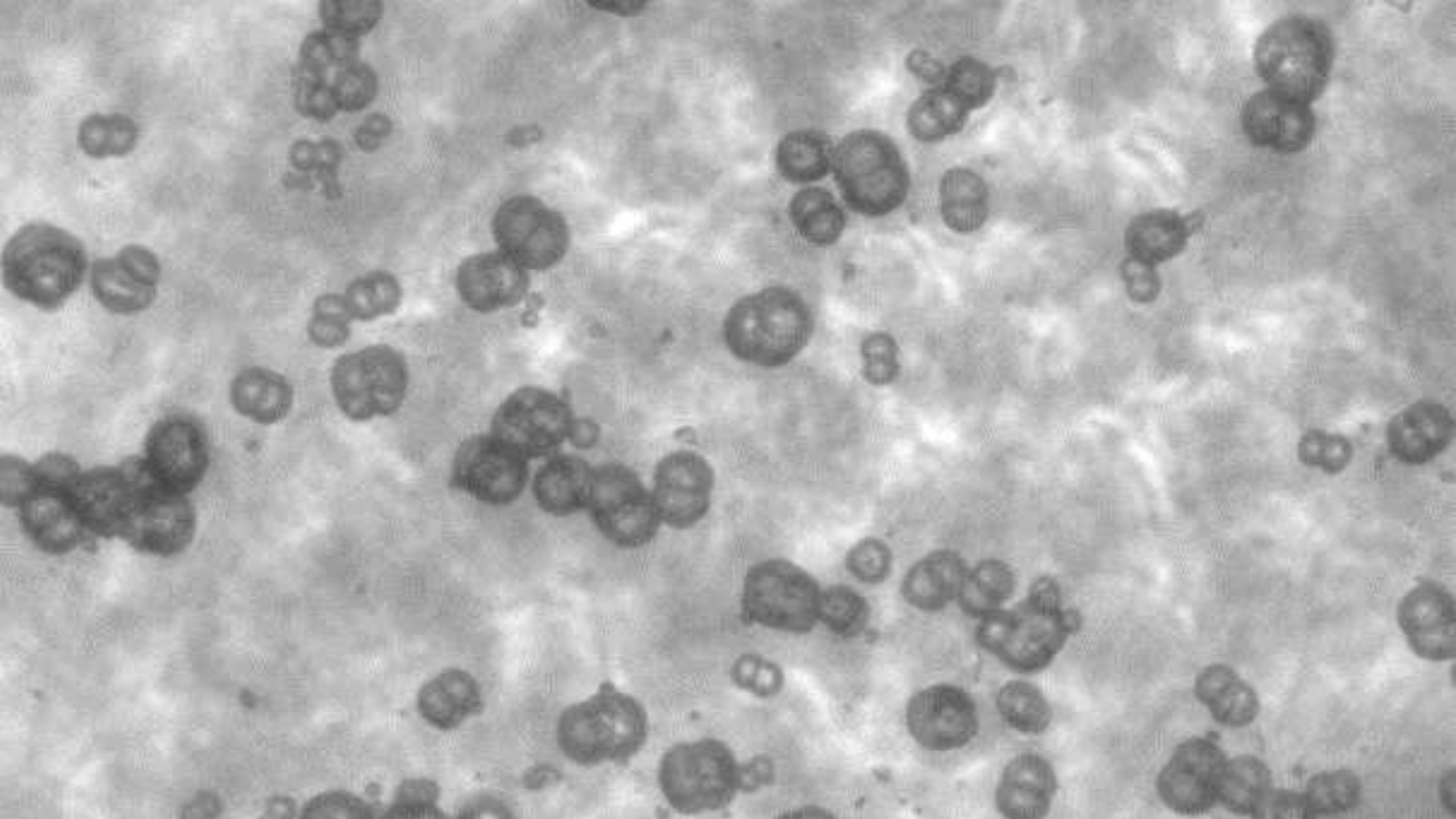
- **Pathogenesis:** Aging
- Common among ppl. over 65 years old. Progressive.
- Pain and movement loss, especially in load-bearing joints.
- Reduced amount of proteoglycan in **ECM** → Reduced amount of water → Decreased lubricity
- **Chondrocytes** → Synthesize IL-1 and tumor necrosis factor (TNF-alpha), the synthesis of metalloproteinases increases, collagen and proteoglycan synthesis decreases.
- The surface of the cartilage is eroded. The erosion continues until the lower bone.
- **Result:** Total function loss in joint
- **Treatment:** No?. Painkillers (Symptomatic treatment).

Tissue Engineering - Artificial Cartilage





After post-printing cultue for 3 mounths,
Artificial ear cartilage was implanted to the patient



Cartilage developed within an organoid in our labs

İstinye Üniversitesi

Kök Hücre ve Doku Mühendisliği AUM

3B Tasarım ve Prototipleme AUM

DARICI LA

Thank you

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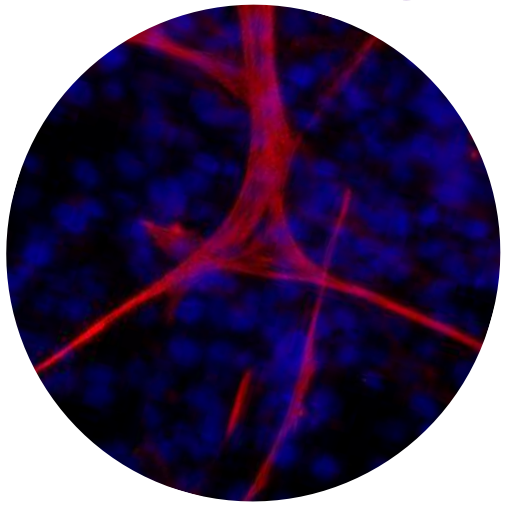
High-Definition Biological Solutions

Since 2018



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Bone Matrix

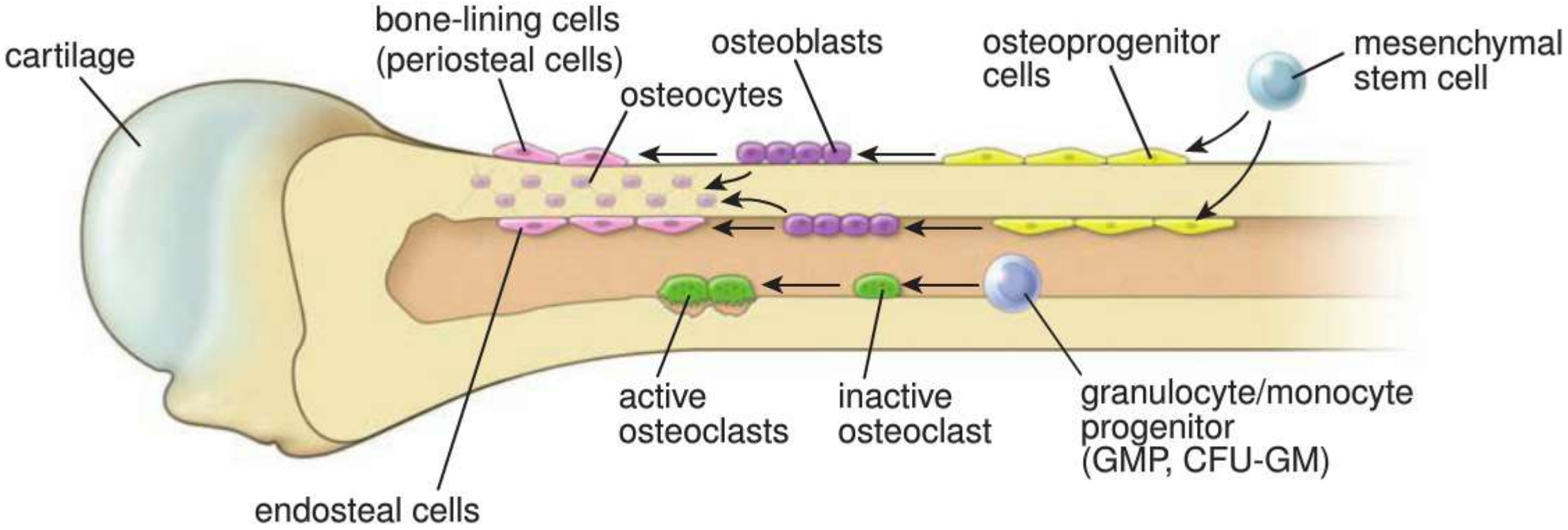
1. **Proteoglycans, GAGs**
2. **Multiadhesive Glycoproteins**
    - **Osteonectin:** Connects collagens to hydroxyapatite crystals
    - **Sialoproteins (Osteopontin/BSP1):** Cell-bone matrix connection
    - **Bone Sialoprotein 2 (BSP2):** Initiates calcium phosphate formation
3. **Bone-specific vitamin K-dependent proteins**
    - **Osteocalcin:** Grabs calsium at the blood
    - Protein S, matrix Gla-protein….
4. **Growth factors and Cytokines**
    - IGF, TGF-b, PDGF
    - **Bone Morfogenic Proteins (BMP) (Esp. BMP-7): Trigger bone formation**

Bone Cells

- Osteoprogenitor cells
- Osteoblasts / osteocytes
- Bone-lining cells (periosteal/endosteal cells)
- Osteoclasts

Mesenchymal Stem Cells

Osteoprogenitor Cells



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