Natural Tissue Composition & Cell-ECM Interaction

EunAh Lee Ph.D.

IIRC, Kyung Hee University

Current Life Science Topics

1. Fundamentals in Basic Biochemistry & Cell Biology
2. Introduction to Tissue Engineering & Regenerative Medicine
3. Developmental Tissue Reconstruction
4. Wound Healing & Regeneration
5. Natural Tissue Composition & Cell-ECM Interaction
6. Stem Cells & Cell-Based Therapy
7. Biomaterials
8. *Mid-Term Exam*
9. Mechano-transduction & Bioreactors
10. Discussions on Tissue Reconstruction
11. Regulation & Ethics
12. AI in Current Life Science
13. Machine Learning & Github
14. Deep Neural Network
15. Convolutional Neural Network
16. *Final Exam*

Study Materials

Natural Tissue Composition

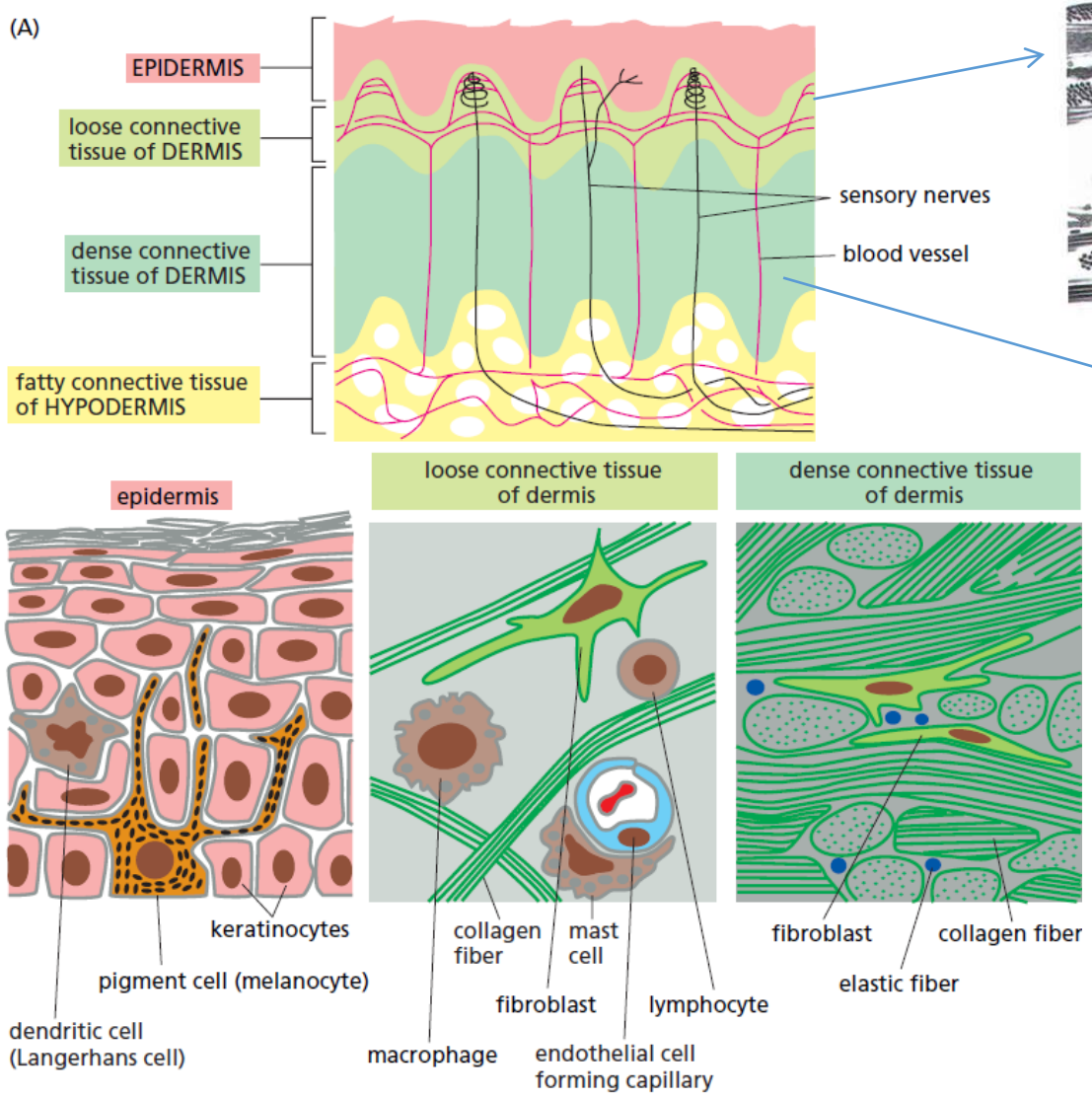
- MacLaren RE 2006 Nature
- Molecular Biology of the Cell (5th Ed) by Bruce Alberts

Cell-ECM Interaction

- Geiger B & Yamada KM, 2011 CSH Perspectives in Biology
- Principles of Regenerative Medicine by A. Atala A, Chapter 2 & 3
- Lee JK et al., 2018 Chemistry-A European Journal

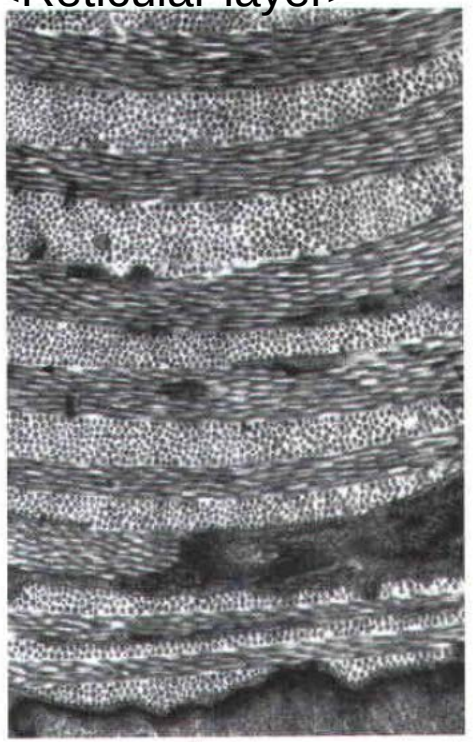
Natural Tissue Composition

Skin

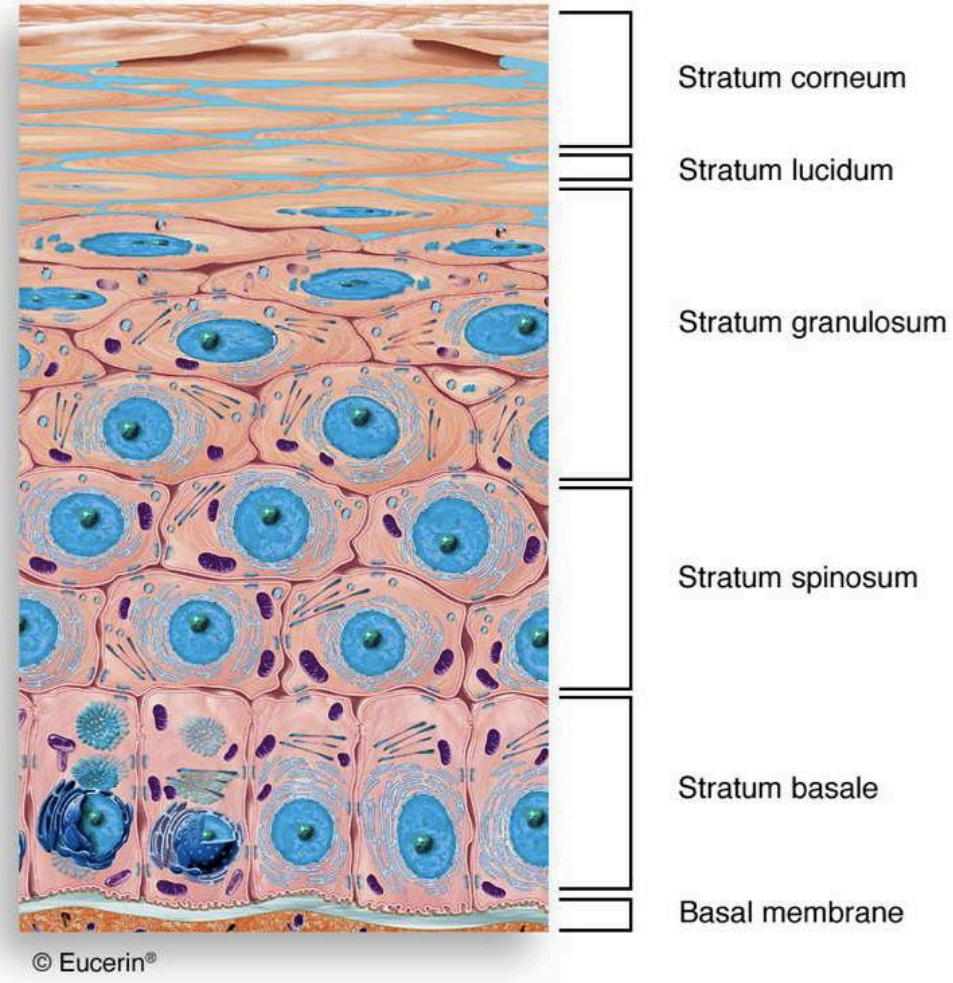


<Papillary layer>

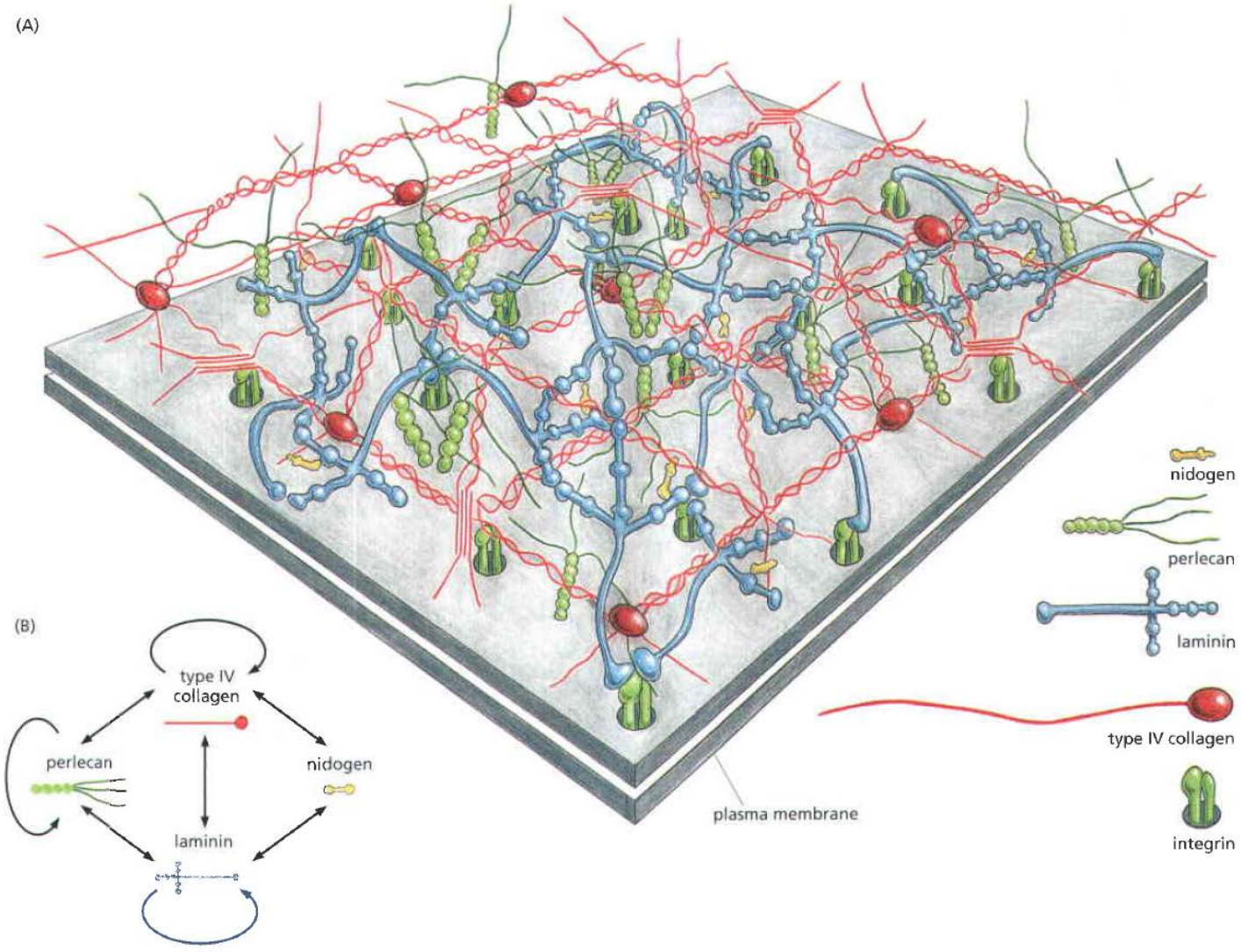
<Reticular layer>



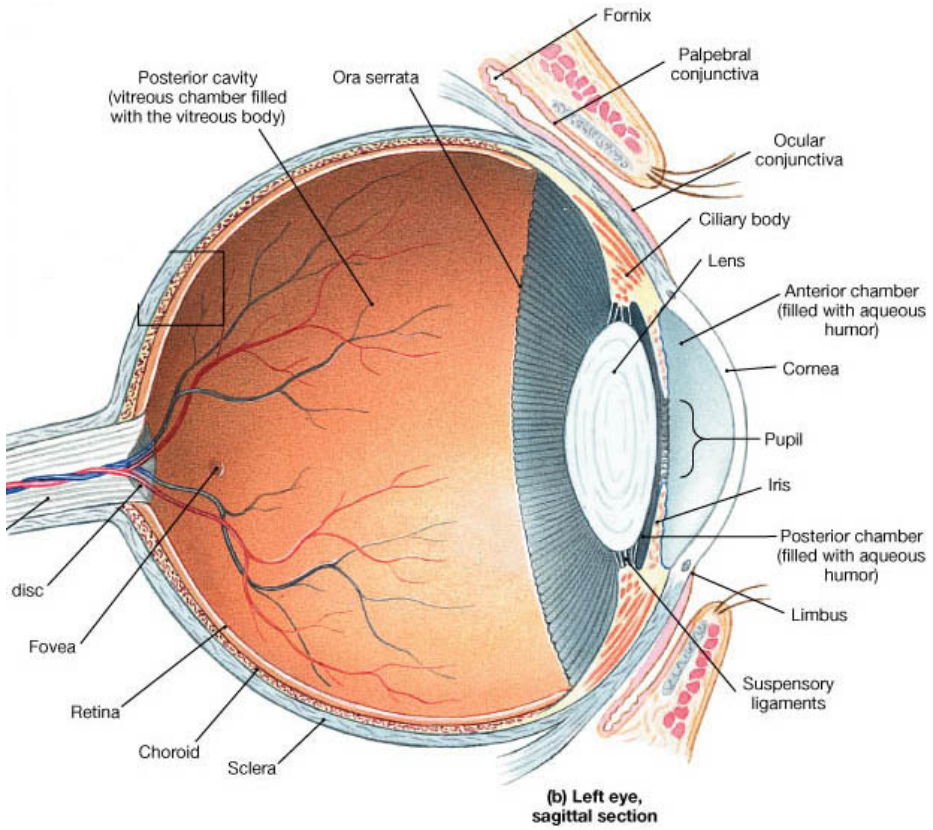

(B)
epidermis
dermis
100 μm

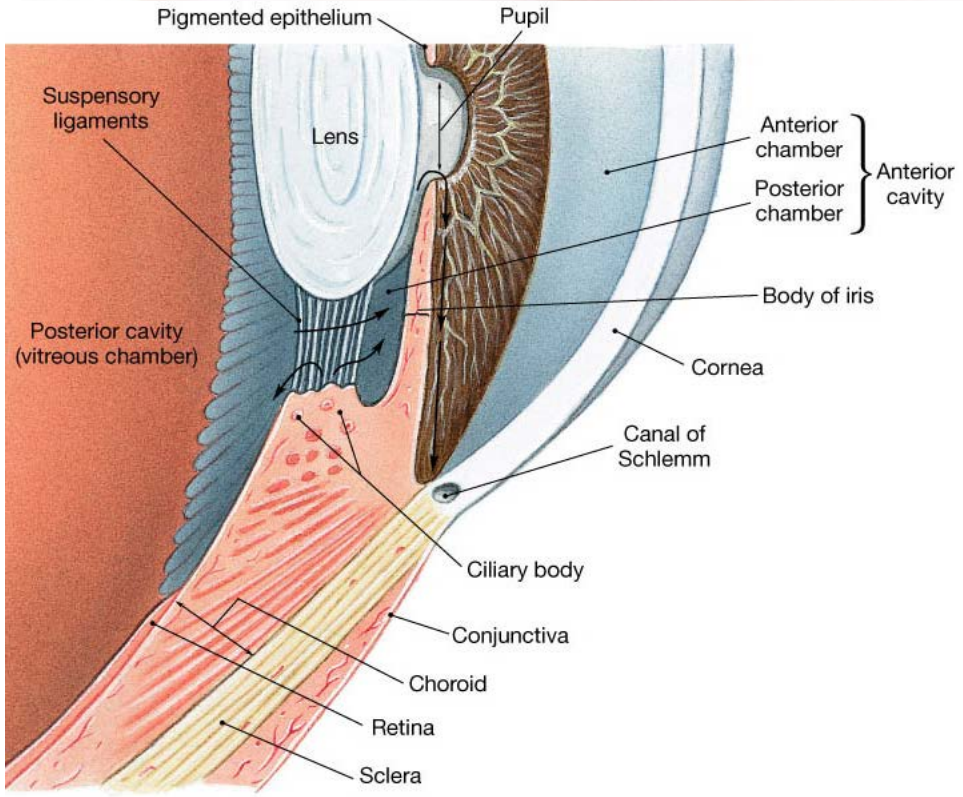

Stratum corneum
Stratum lucidum
Stratum granulosum
Stratum spinosum
Stratum basale
Basal membrane
© Eucerin®

Basement Membrane

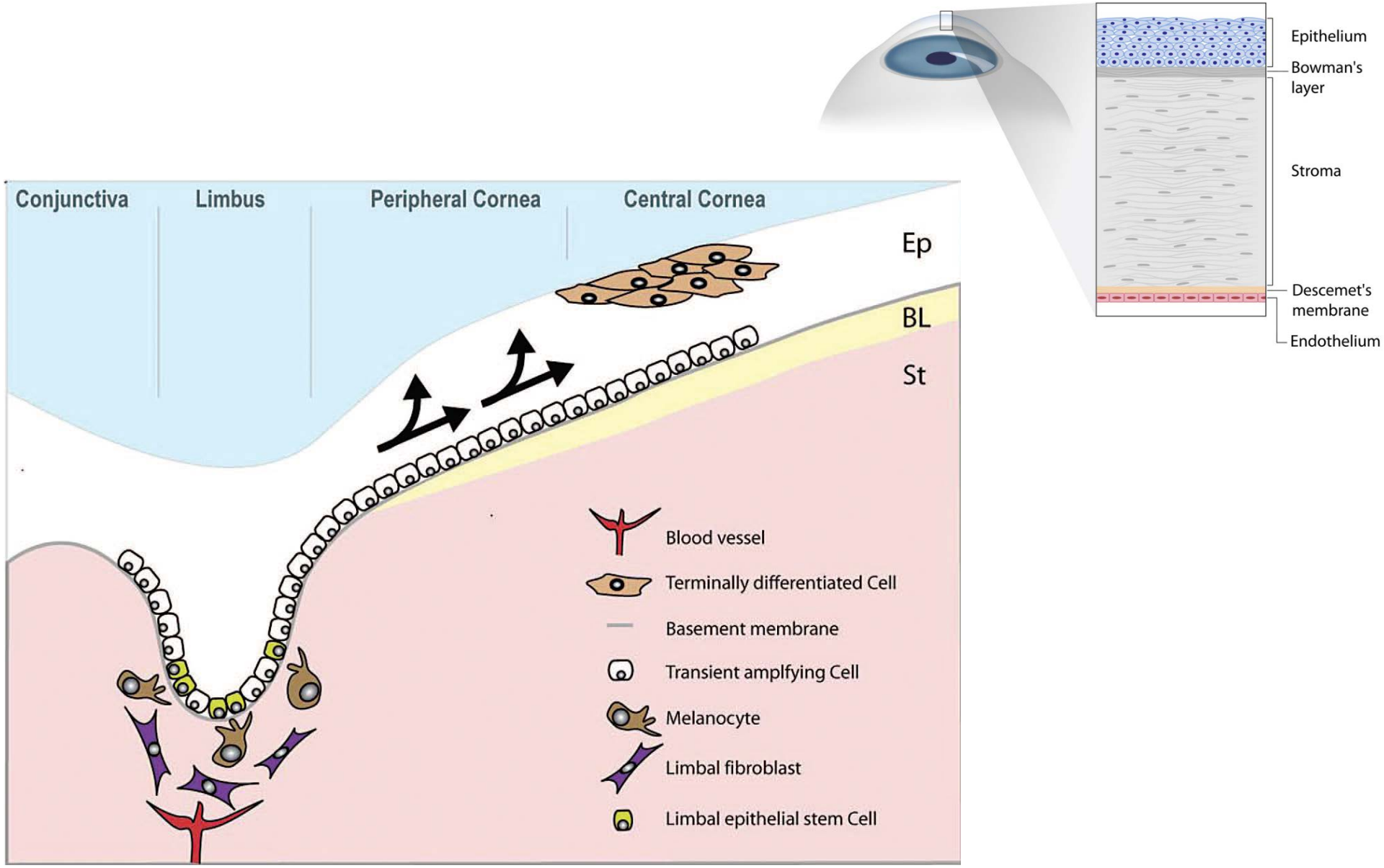


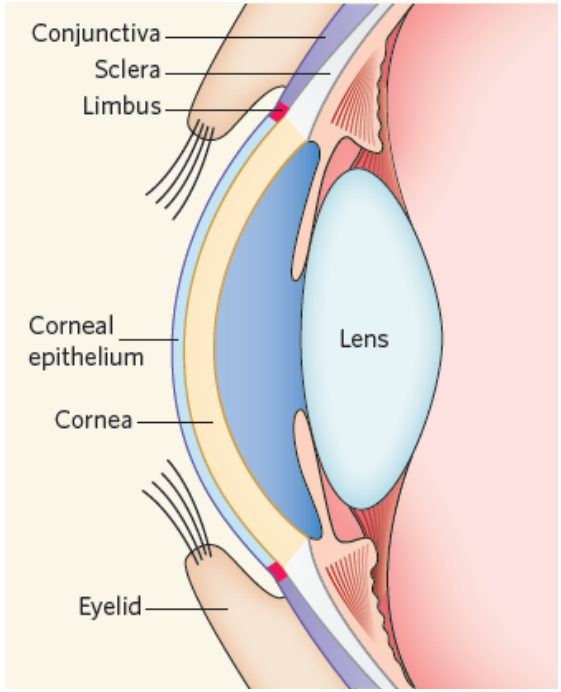
Eye

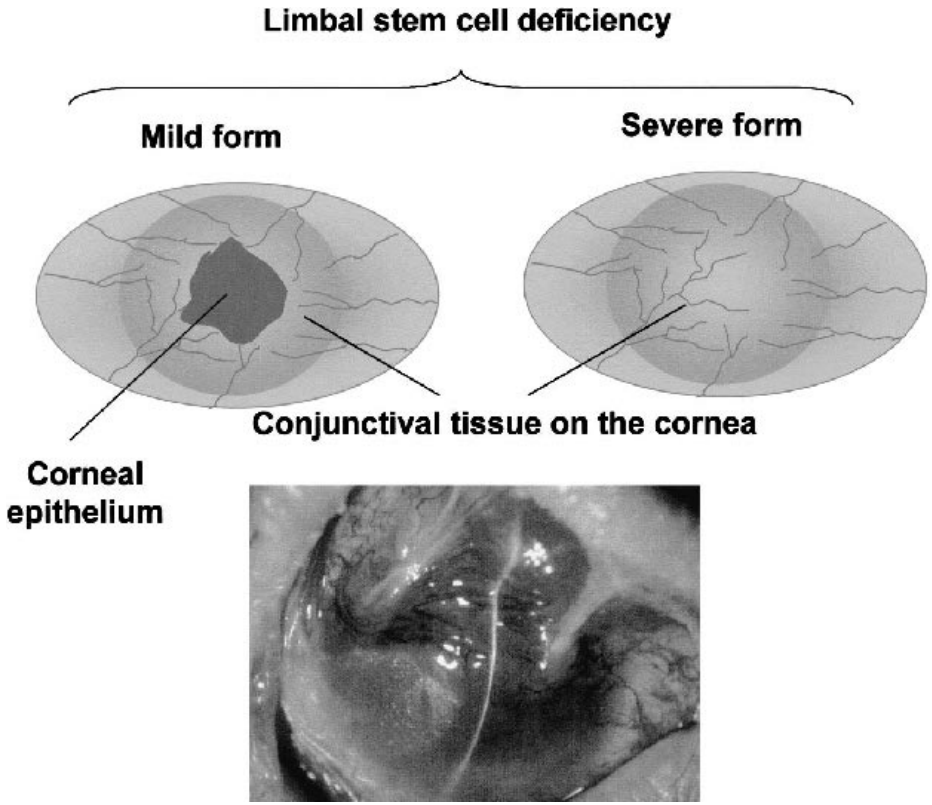

Fornix
Palpebral conjunctiva
Posterior cavity (vitreous chamber filled with the vitreous body)
Ora serrata
Ocular conjunctiva
Ciliary body
Lens
Anterior chamber (filled with aqueous humor)
Cornea
Pupil
Iris
Posterior chamber (filled with aqueous humor)
Limbus
Suspensory ligaments
disc
Fovea
Retina
Choroid
Sclera
(b) Left eye, sagittal section

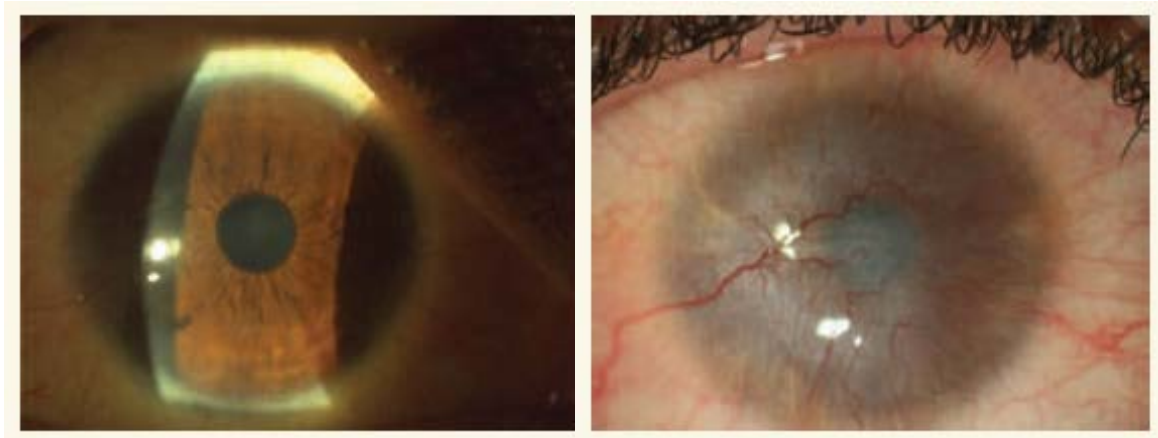

Pigmented epithelium
Pupil
Suspensory ligaments
Lens
Anterior chamber
Posterior chamber
Anterior cavity
Body of iris
Posterior cavity (vitreous chamber)
Cornea
Canal of Schlemm
Ciliary body
Conjunctiva
Choroid
Retina
Sclera

Cornea


Epithelium
Bowman's layer
Stroma
Descemet's membrane
Endothelium
Conjunctiva
Limbus
Peripheral Cornea
Central Cornea
Ep
BL
St
Blood vessel
Terminally differentiated Cell
Basement membrane
Transient amplfying Cell
Melanocyte
Limbal fibroblast
Limbal epithelial stem Cell


Conjunctiva
Sclera
Limbus
Corneal epithelium
Cornea
Lens
Eyelid


Limbal stem cell deficiency
Mild form
Severe form
Conjunctival tissue on the cornea
Corneal epithelium



Retina

Neural tissue located at the back of the eye

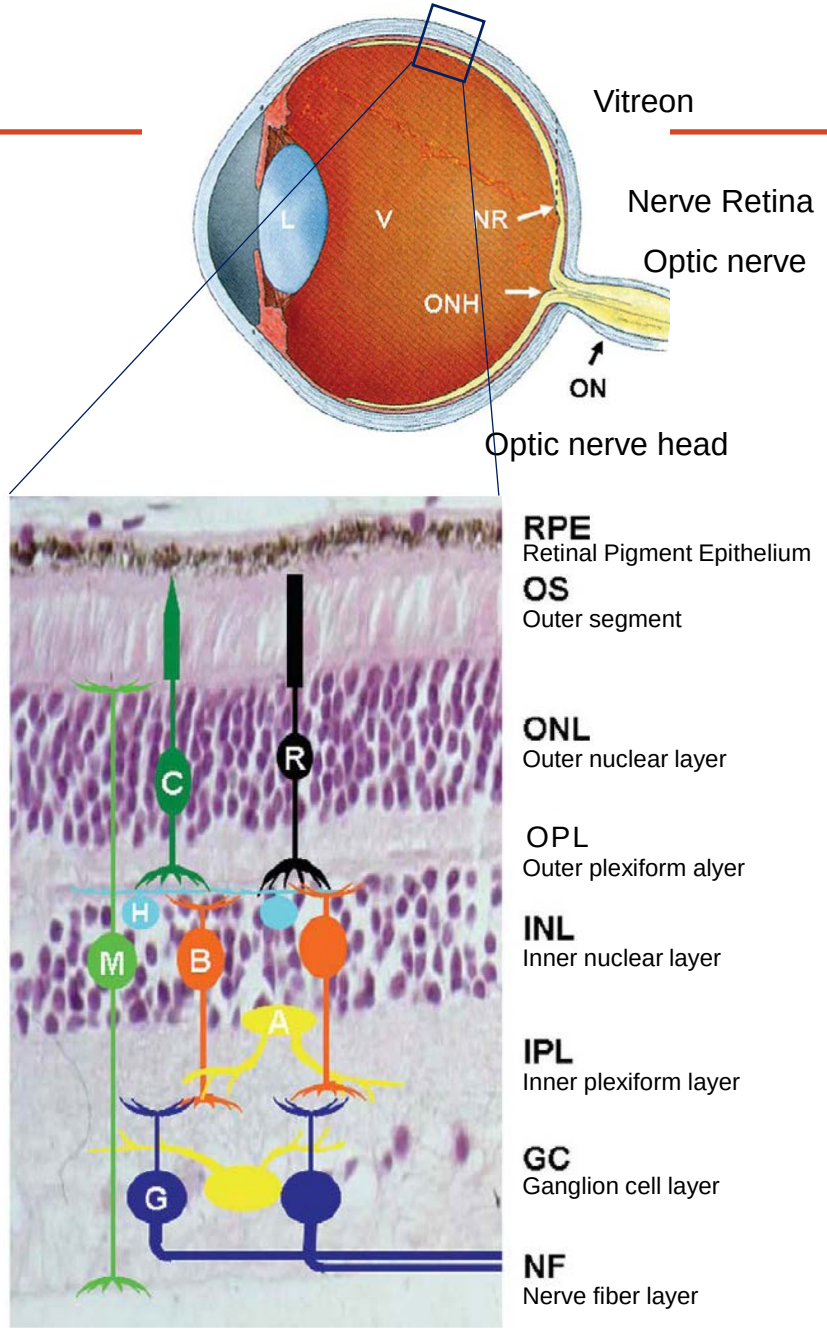
- Transmit information to visual processing centers in the brain

Recent studies showed that cell transplantation can restore visual function in mouse models of retinal degeneration

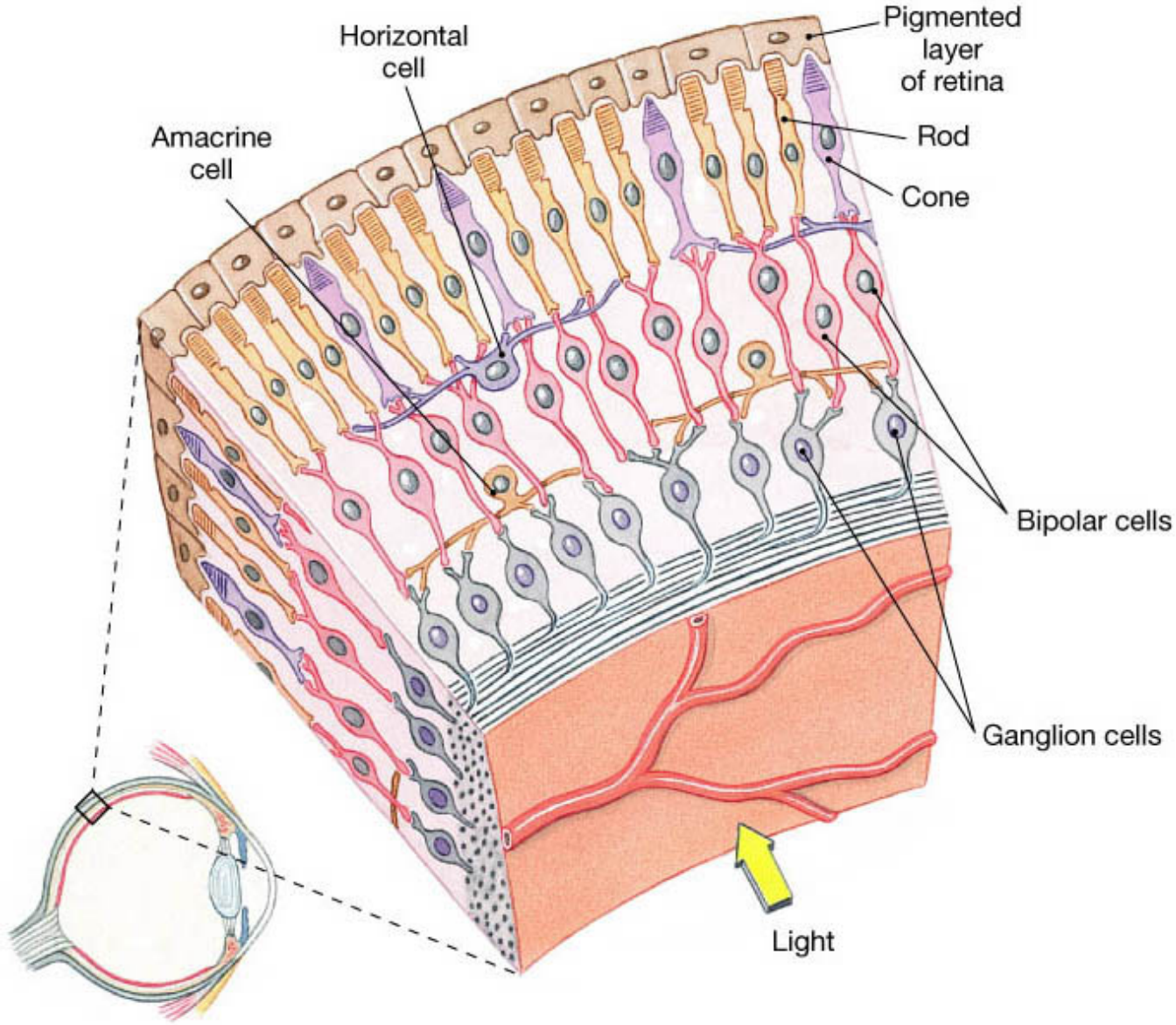
Laminated structure

Organized into three main layers

- Photoreceptors (Con, Rod)
- Bipolar ells
- Ganglion cells
- Amacrine cells
- Muller cells



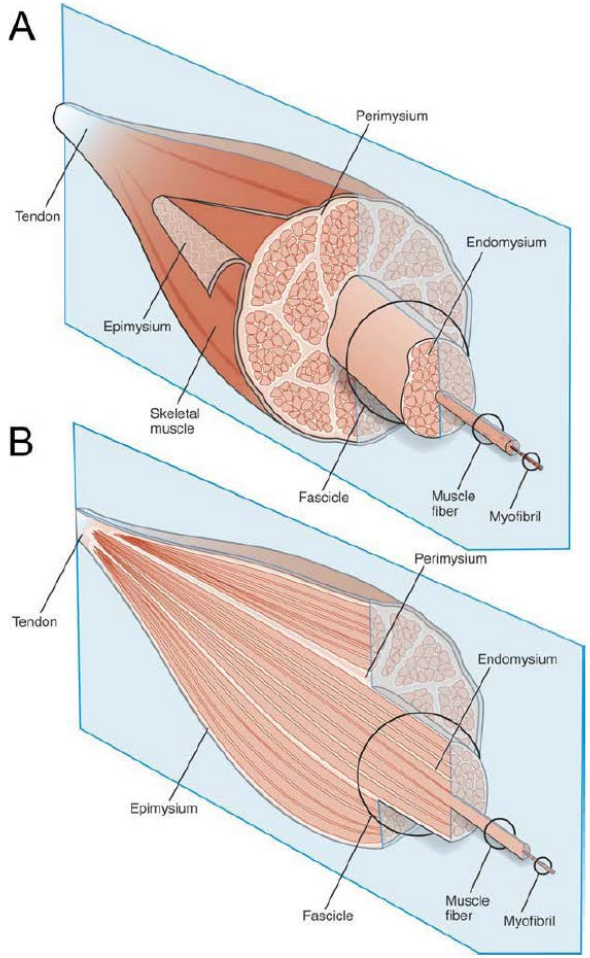
[Mouse Model of Retinal Regneration: MacLaren RE 2006 Nature]

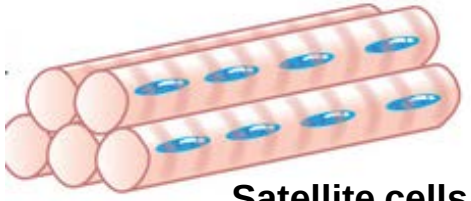

Horizontal cell
Pigmented layer of retina
Amacrine cell
Rod
Cone
Bipolar cells
Ganglion cells
Light

Muscle

Muscle is composite tissue of…

- Contractile material (sarcoplasmic reticulum)
- Blood vessels
- Nerves
- Connective tissue
  - Minor tissues strongly affects muscle´ s normal function
  - Ability to adapt
  - Reservoir of muscle stem cells
  - Recent biomechanical studies support the idea the ECM bears the majority of muscle passive load (motion, stiffness reflect their ECM property)
  - Nearly every pathologic change reported in muscle is also associated to some degree with ECM fibrosis

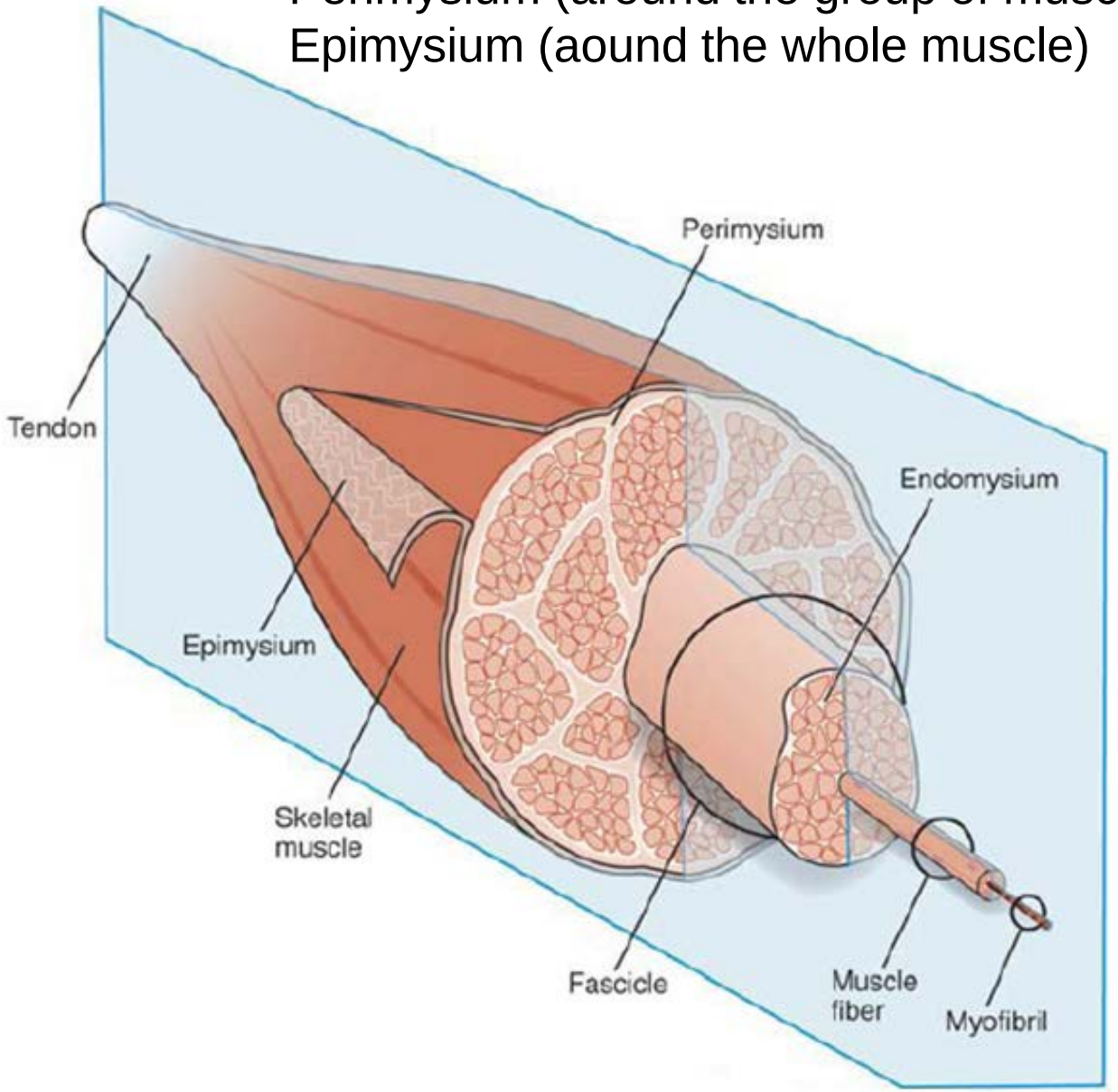


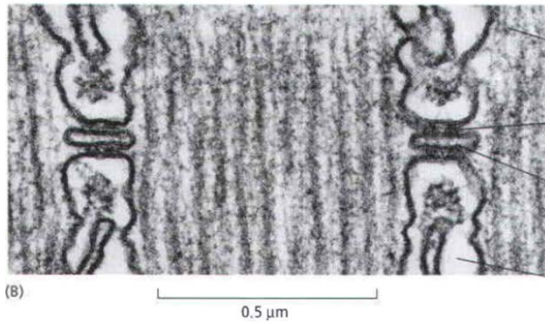


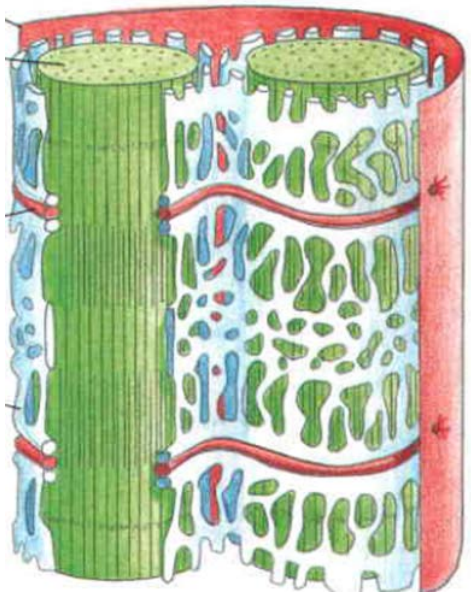
Satellite cells on muscle fiber

Connective tissues in muscle – arbitrary classification

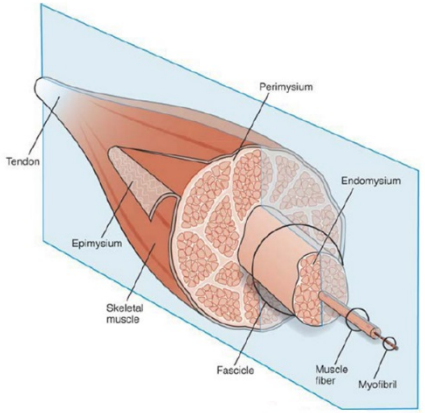
Endomysium (around the musle cells)
Perimysium (around the group of muscle cells)
Epimysium (aound the whole muscle)



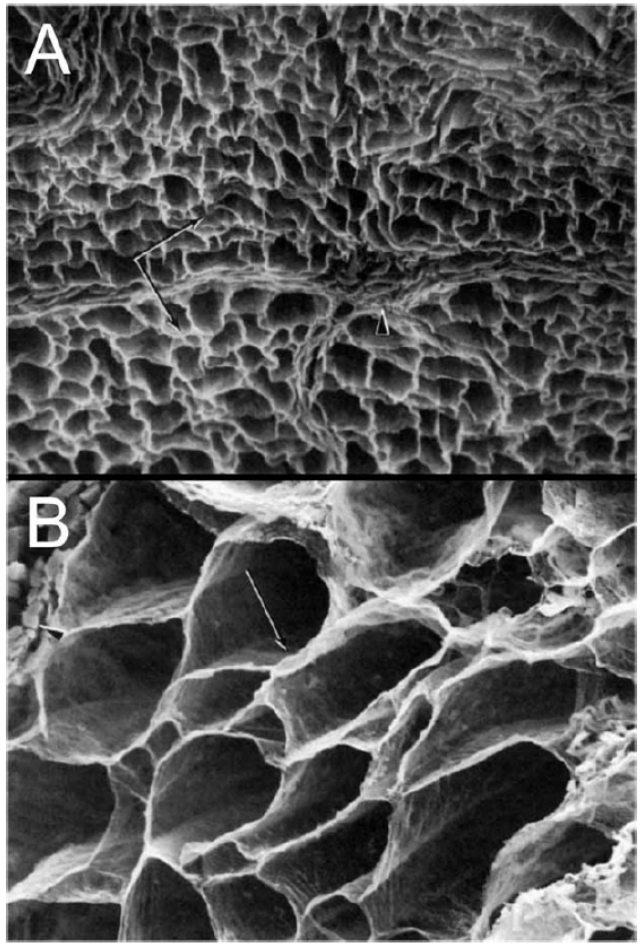


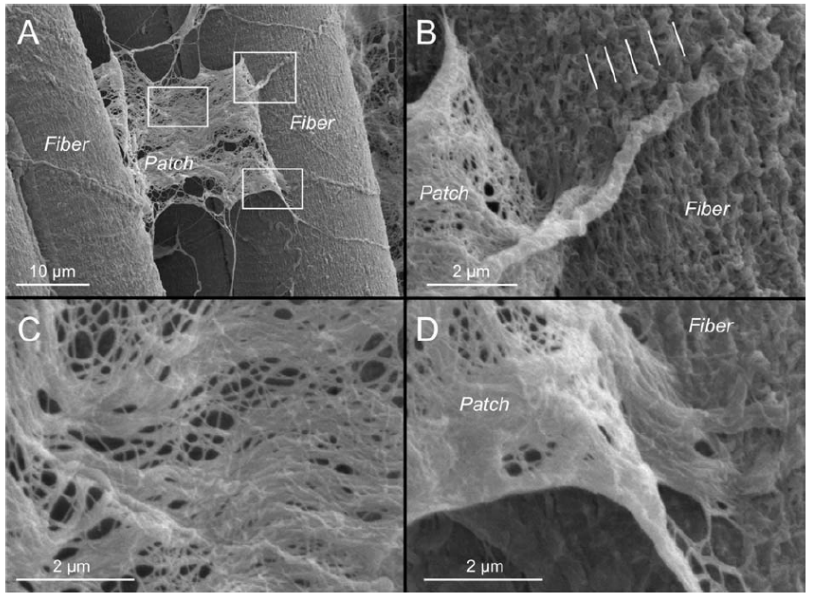


Muscle - Endomysium



Highly ordered network surrounds individual muscle fibers





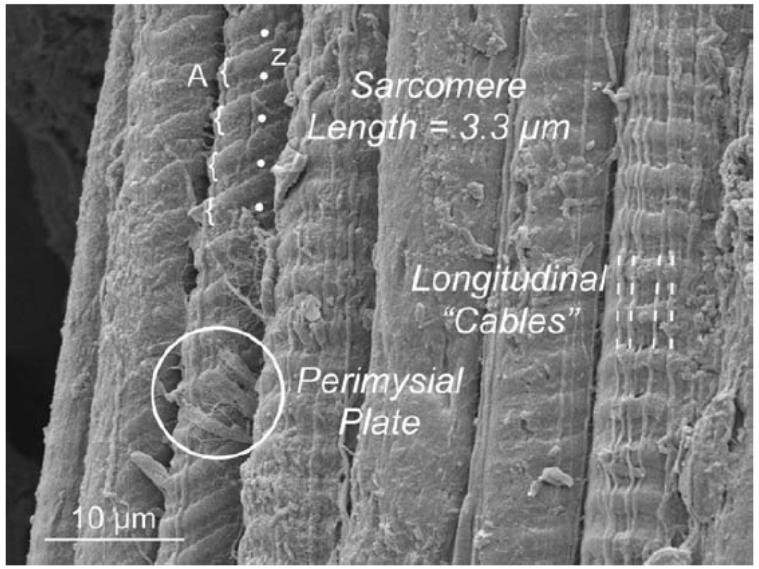
Extensor digitorum longus (EDL) muscle shows discrete patches of ECM, which doesn't seem like endomysium in a sense that they are lack of longitudinal periodicity and some are in tubular structure.

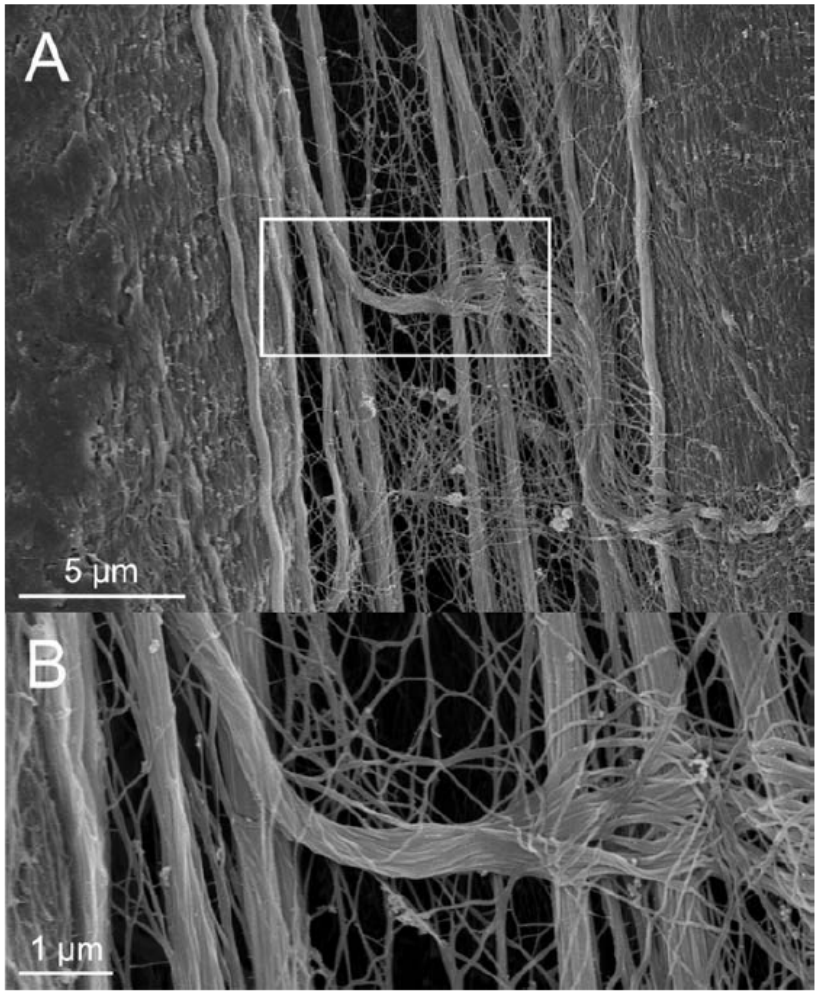
Muscle - Perimysium

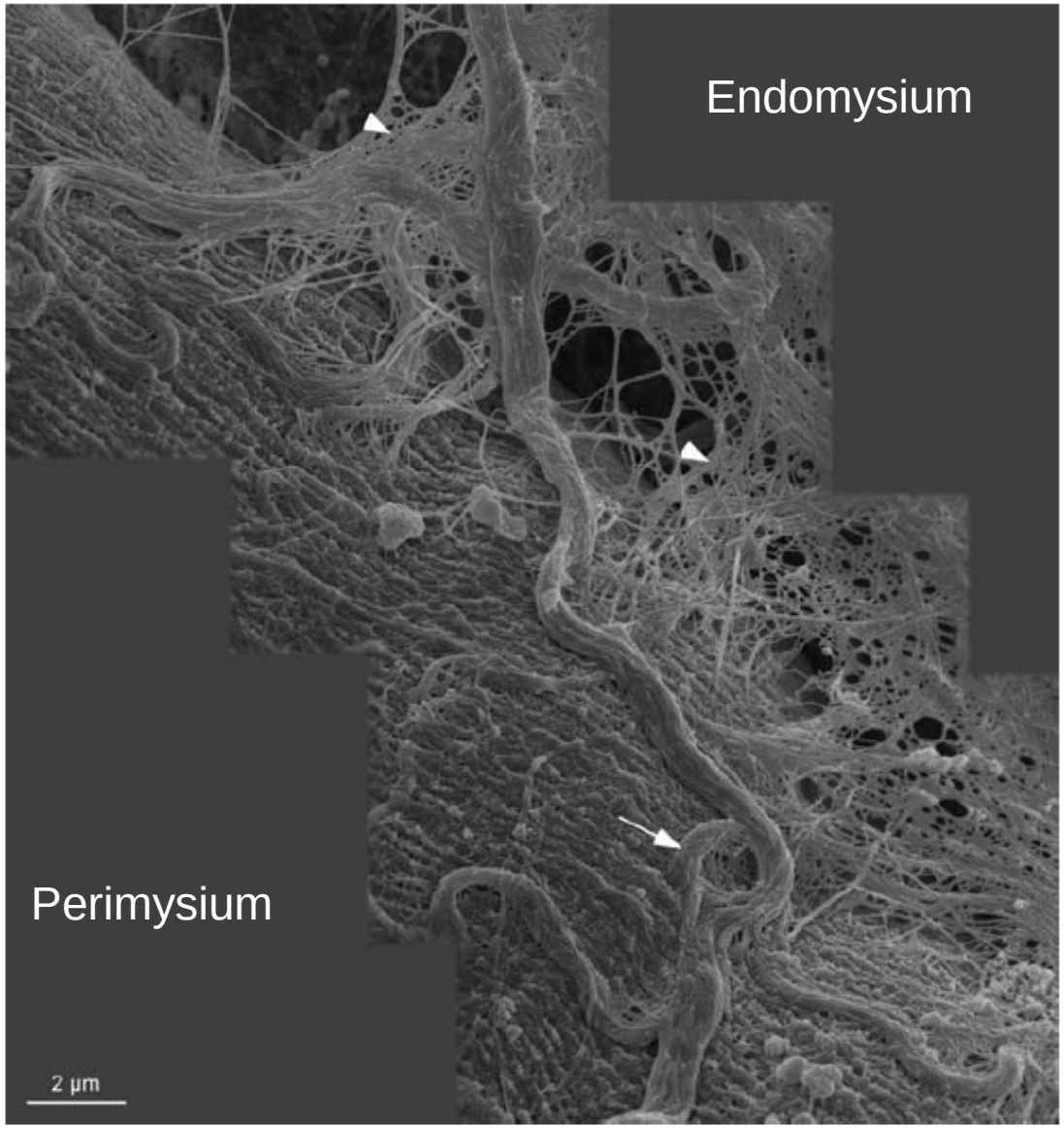
Thicker ECM region that presents a more visually obvious pattern

Longitudinally oriented fibers transverse collagen fibers:

- **interconnect muscle fibers**
- **Co-localized with focal adhesions and intracellular subdomains**
  (perimysium may be involved in cell signaling)





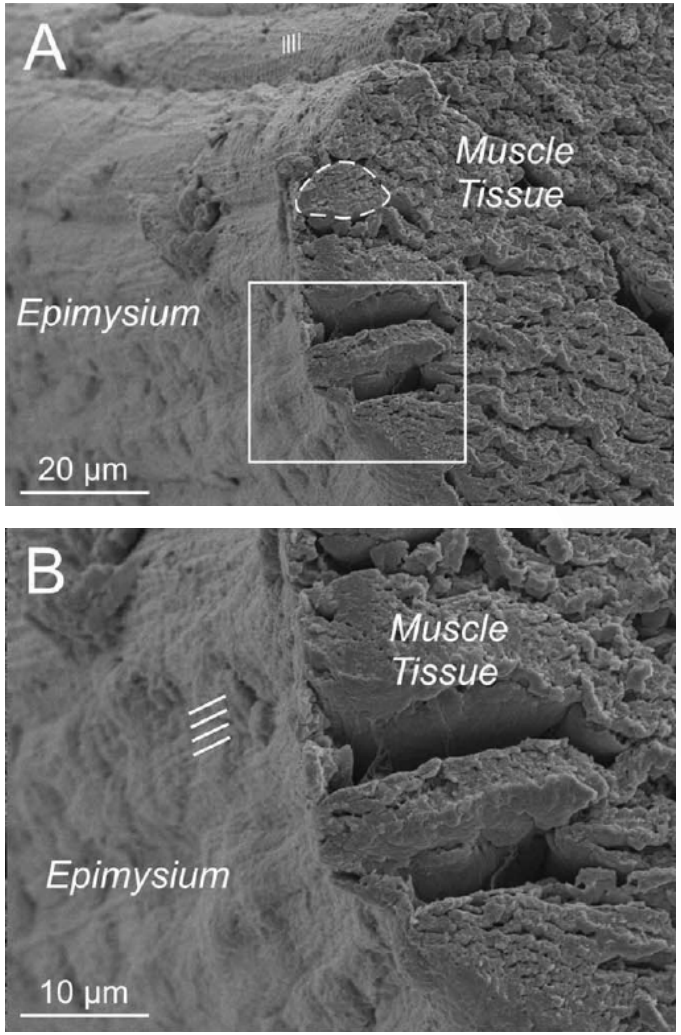

Endomysium
Perimysium
2 µm

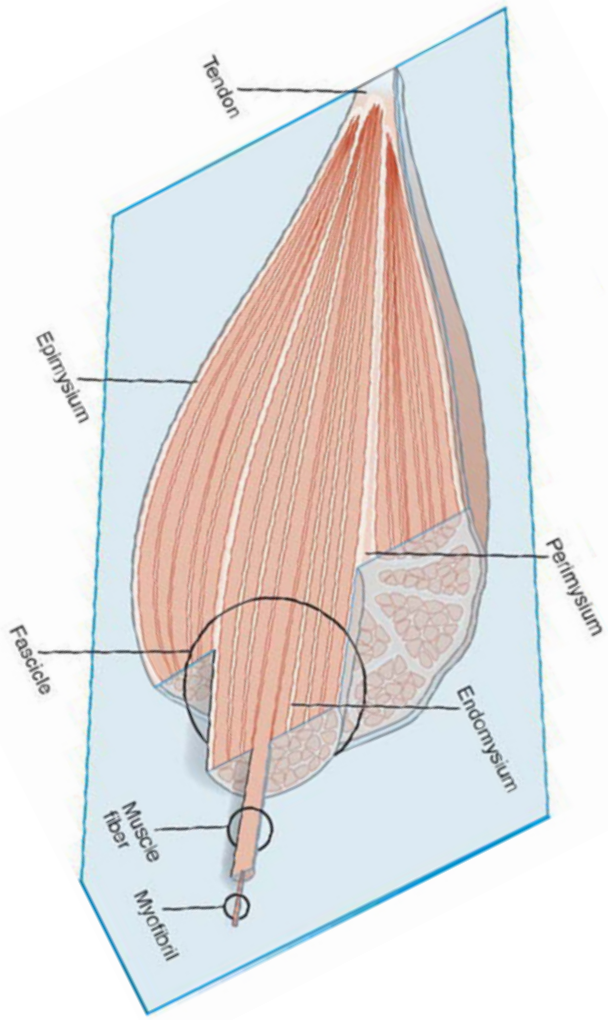
Muscle - Epimysium

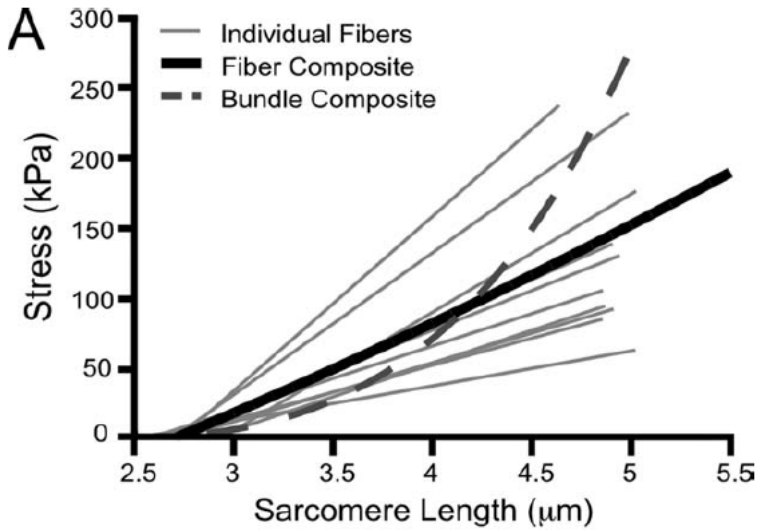
Primarily composed of very large collagen bundles with a familiar crimp pattern seen in tendon.

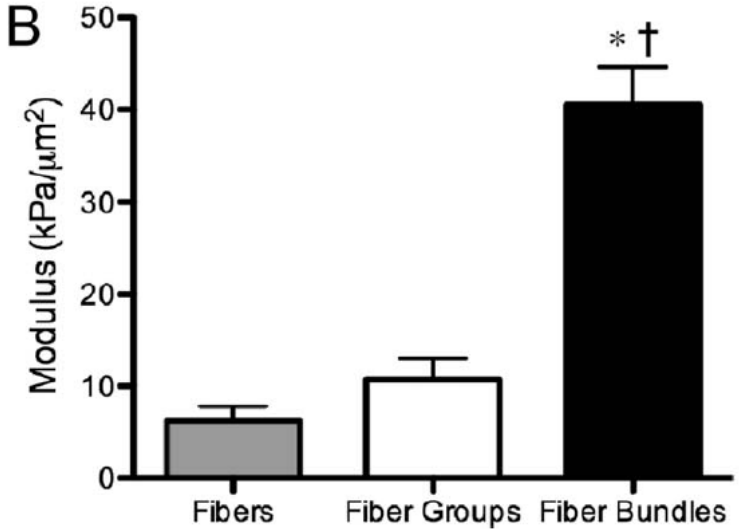
Sheets of perimysial collagen join and become continuous with tendon

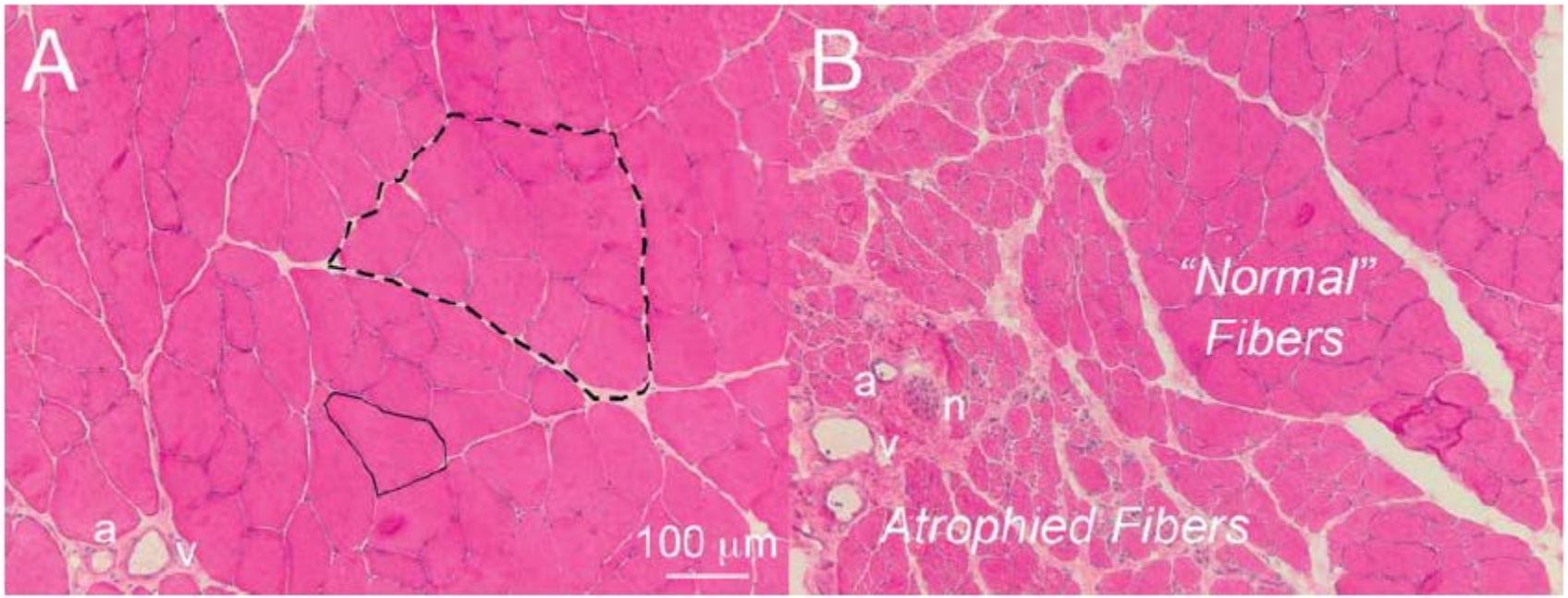
- **Both contain…**
  - **Primarily Col I**
  - **Primary PG: decorin**
- **Perimysium is continuous with tendon**
- **(CF) epimysium and endomysium contains equal amount of Col I & Col III**






A
Individual Fibers
Fiber Composite
Bundle Composite
Stress (kPa)
300
250
200
150
100
50
0
2.5
3
3.5
4
4.5
5
5.5
Sarcomere Length (μm)


B
Modulus (kPa/μm²)
50
40
30
20
10
0
* †
Fibers
Fiber Groups
Fiber Bundles


A
a
v
100 μm
B
"Normal" Fibers
a
n
v
Atrophied Fibers

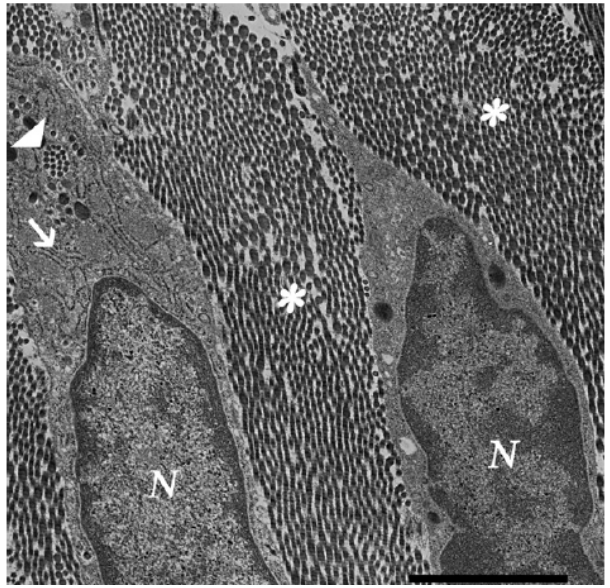
Tendon

Main function of tendon

- Transmit contraction force produced by the muscle to the bone
- Promote movement
- Articular stability

Factors inducing alterations or lesions

- Excess or absence of stress (Degenerative tendinopathies, Lacerations or ruptures)
- Age
- Diabetes



Aging & Disuse

- Increase in the amount of inter/intramolecular crosslinking -> results in an increase in cristalinity
- Decreases in proteoglycans & water content

Gross Anatomy & Histology of Tendons

Paratenon
- Allows free movement of tendon aginst proximal tissues
- Supports blood vessels (reach to the endotenon & epitenon)

Epitenon
- Find sheath of connective tissue located underneath the paratenon
- Links several fascicles to comprise tissue
- Inner surface is continuous with the endotenon

Endotenon
- Coats and links each tertiary bundle, secondary bundle (fascicle), and primary bundle (sub-fascicle) of collagen fibers
- Vessels: arterioles and capillaries are present in endotenon
- >these structures maintain the fibroblast of the tendon (intense synthesis of ECM)

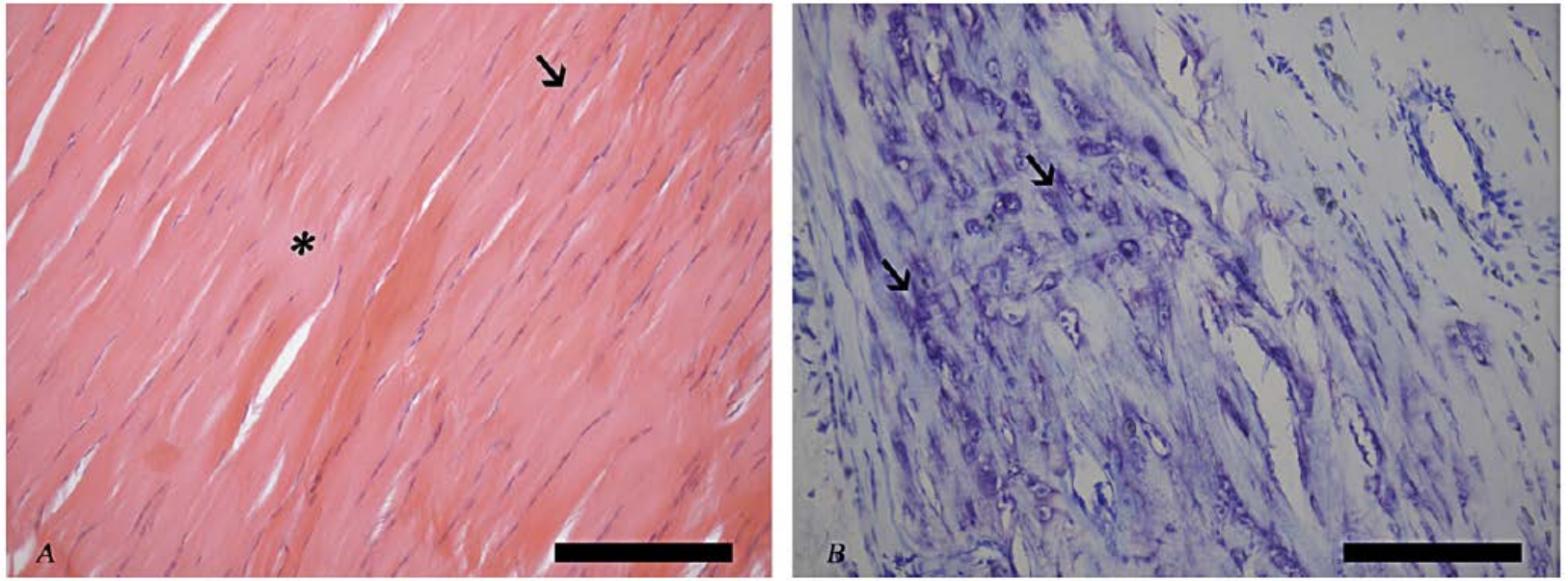
Structure and Biochemical Compositions

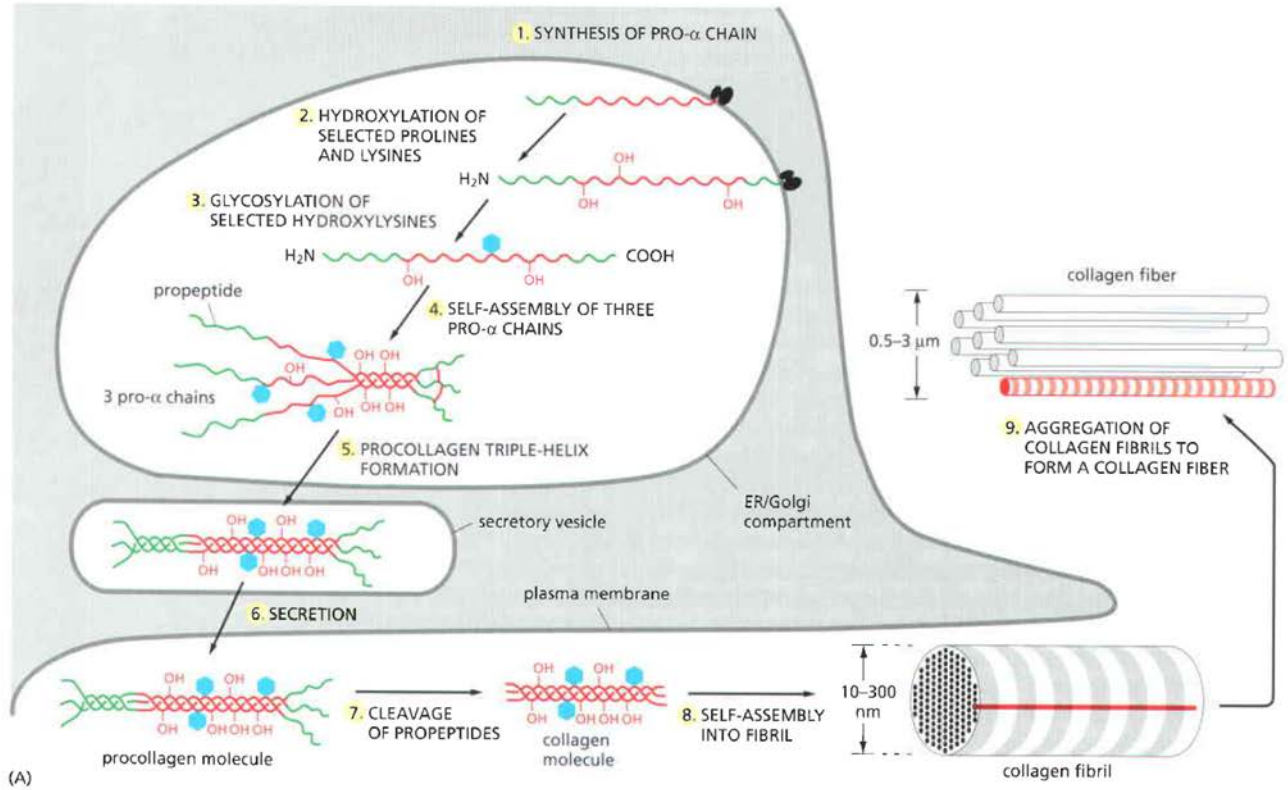
Structure and Biochemical Compositions

- Collagen
- Elastin fibers
- PGs

Type I collagen in tendon

- fibrils -> fibers -> bundles -> fascicles
- 270 nm in length of 1 molecule -> several cm in length
- 300 nm in diameter





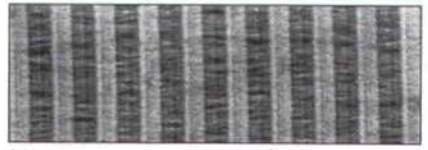
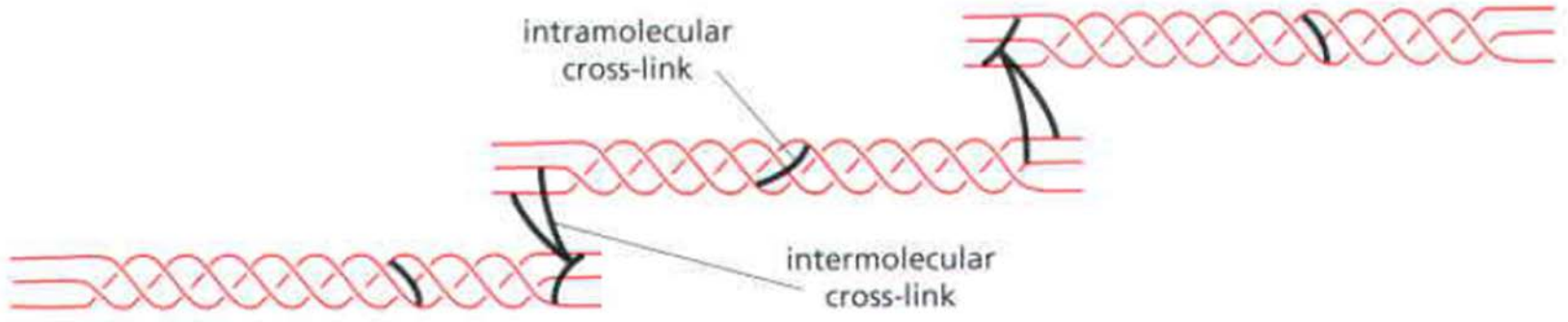


Figure 19–66 The intracellular and extracellular events in the formation of a collagen fibril. (A) Note that procollagen assembles into collagen fibrils in the extracellular space, often within large infoldings in the plasma membrane (not shown). As one example of how collagen fibrils can form ordered arrays in the extracellular space, they are shown further assembling into large collagen fibers, which are visible in the light microscope. The covalent cross-links that stabilize the extracellular



Biochemical Composition of Tendon

Collagen

3 polypeptide chain – triple helix

Characteristic sequence of amino acid

- Gly-X-Y
- 1/3 of X positions are proline
- 1/3 of Y positions are hydroxyproline

Glycine

- Smallest R group (Hydrogen)
- Occupy innermost position along the polypeptide chain

Lysine-modified by…

- Lysyl hydroxylase -> hydroxylation
- Lysyl oxidase -> formation of aldehyde group
  -> Enable formation of intramolecular & intermolecular cross-link

- The number of crosslinks varies among the tendons depending on the biomechanical load of each tendon

Elastic fibers

- Low proportion of elastin allows for the transmission of the tension force to the bone and prevent excessive stretching of the tendon during muscle contraction, enabling articular movements

Non-fibrillary components

<PGs>
- Central protein skeleton + At least one chain of GAGs
- Strong negative charge (due to the presence of sulfated GAGs)
- Highly hydrated -> slimy characteristics

<Small PGs>
- Small PG: fibromodulin, decorin

<Non-collagenous ECM protein>
- COMP (Cartilage Oligomeric Matrix Protein)
  - member of thrombospondin family
  - mutation in its gene cause disease (skeletal dysplasia, pseudoachondroplasia)

Crimp: characteristic feature of tendon

- Characteristic of structures consisting of fibrillary collagens, especially type I, that are present in connective tissues submitted to tension loads
- The basic undulated pattern of the fiber is altered in such a way as to adapt to the mechanical stimulation
- There are variations in the organization of the crimp in different regions of the same tendon
- Stress & deformation require large areas of cramp
  - Benefit the collagen bundles in adapting to tension

Cells

- Fibrocytes (flat nuclei)
- Fibroblasts (enlarged nuclei)

} 90~95%

-> intense synthesis of macromolecules of ECM

- Chondrocytes (bone insertion area)
- Vascular cells (endotenon, paratenon)

} 5~10%

Biomechanical behavior

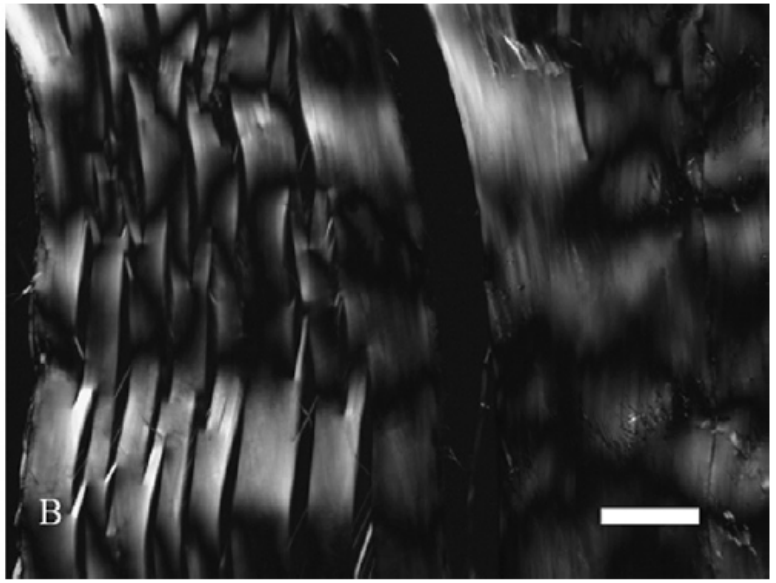
- The ECM adapts to different biomechanical forces and presents local differences in terms of composition and organization along the same tendon

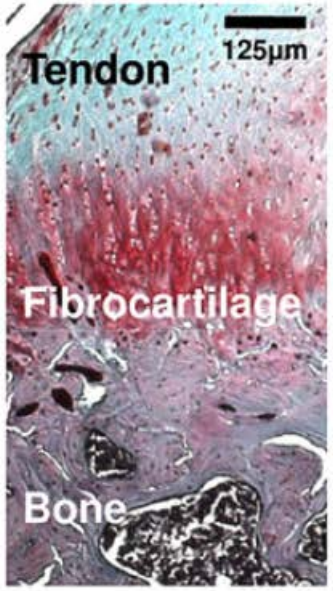
- Tendons that surround any articulation or run along any bone tuberosity are subjected to tension forces and compression forces
  -> causing the accumulation of PGs
  -> a structure similar to fibrocartilage is formed (bone insertion area)

- Mechanical charge (mechanical stimulation)
  - Leads to a physiological adaptation of the tissue
  - Important for the ECM homeostasis in connective tissue because it affects the expression of specific proteins of the ECM
  - Interaction between ECM-Cell
    (feedback mechanism of the Cell-ECM interaction)
    - PGs can bind to GFs and then present those to their respective receptors
    - ECM can also release GFs under mechanical stimulation

<Linear dichroism>

45° **Parallel**





Tissue Region	Cell Type	Major Matrix Component
Tendon	Fibroblasts	Collagen types I, III (Diameter: 40-400 nm)*
Non-mineralized Fibrocartilage	Fibrochondrocytes	Collagen types I, II, III
Mineralized Fibrocartilage	Hypertrophic Fibrochondrocytes	Collagen types I, II, X
Bone	Osteoblasts Osteocytes Osteoclasts	Collagen type I (Diameter: 34.5-39.5nm)^

Bone

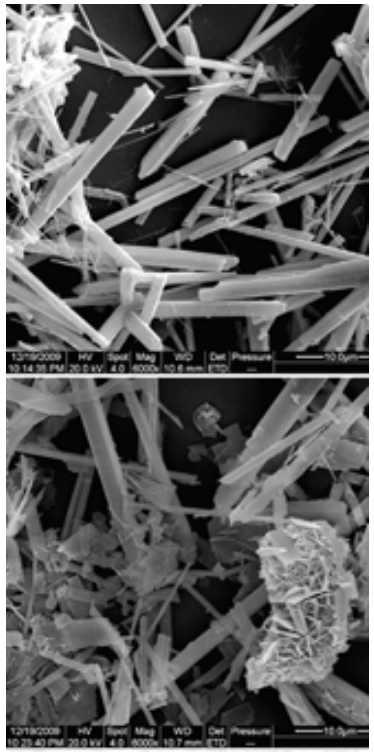
- Basic building block of bone: mineralized collagen fibril
- Collagen acts as a frame work
- Bone fulfills two major functions
  - Mechanical function
  - Metabolic function

    (a reservoir for calcium and phosphate needed for metabolism)

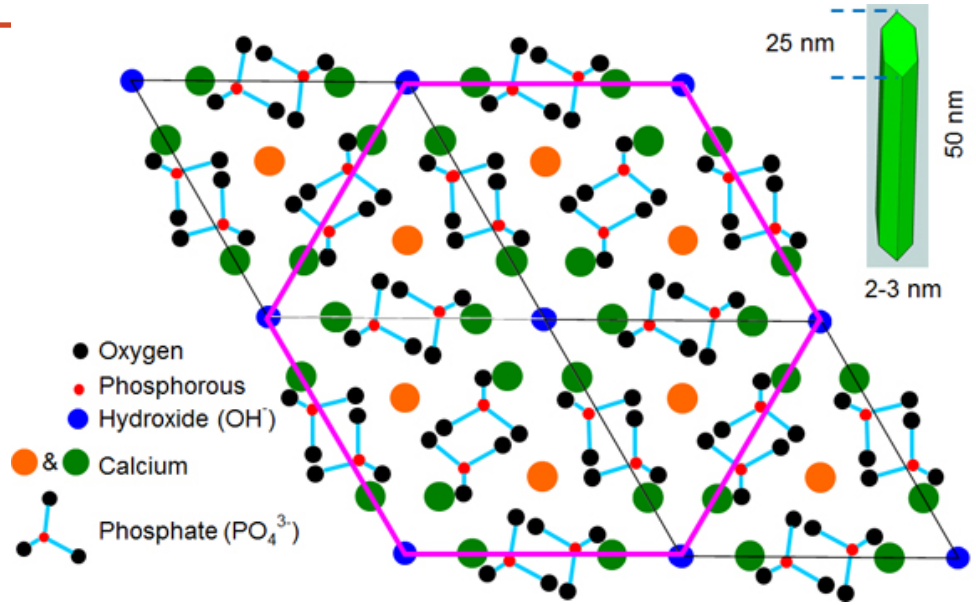
Lamellar bone structure

- SEM of fracture surface
  - Adjacent to periosteum: new bone, sharp fracture surface
  - Internal area: old bone, relatively blunt fracture surface (fibrillar nature is not apparent)
  - Plywood-like organizational motif

Synthetic hydroxyapatite



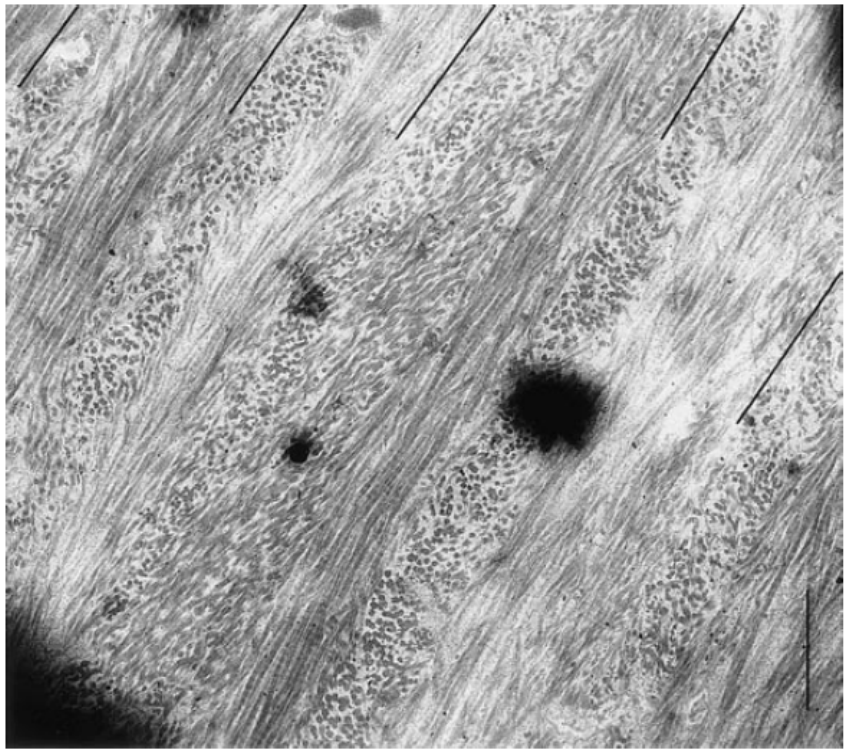
Bone tissue

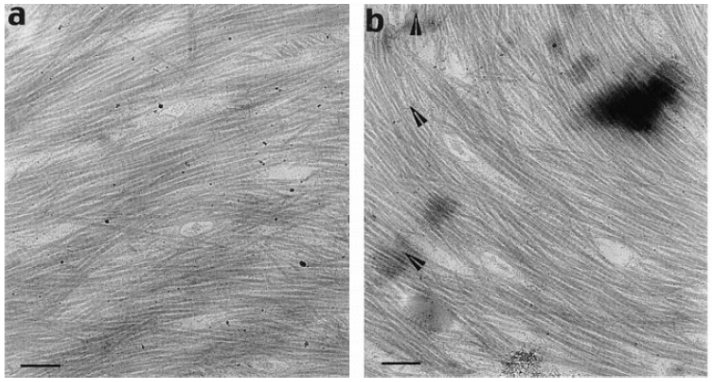

25 nm
50 nm
2-3 nm
Oxygen
Phosphorous
Hydroxide (OH-)
& Calcium
Phosphate (PO4 3-)

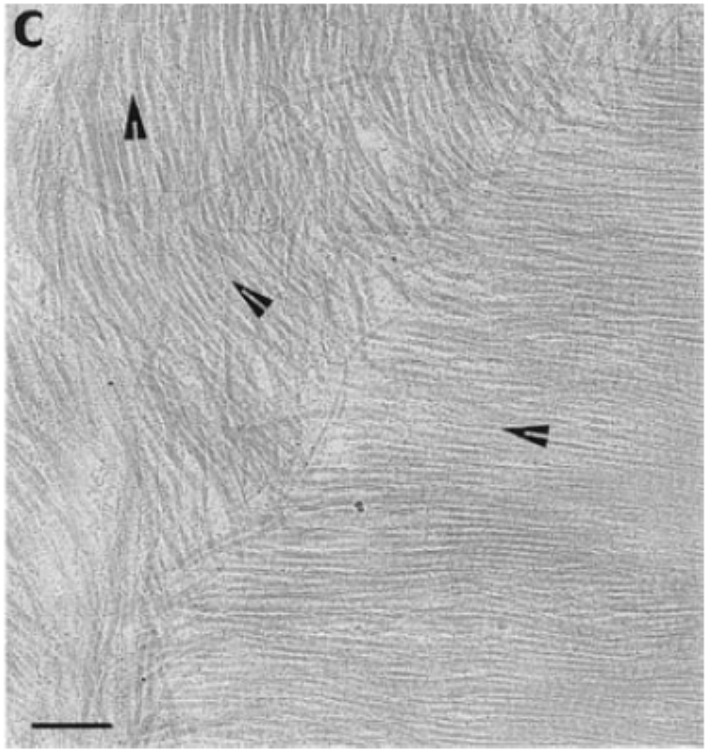

Collagen fibers
Hydroxyapatite containing calcium and phosphate

Lamellar bone structure

- TEM image revealing collagen organizaiton





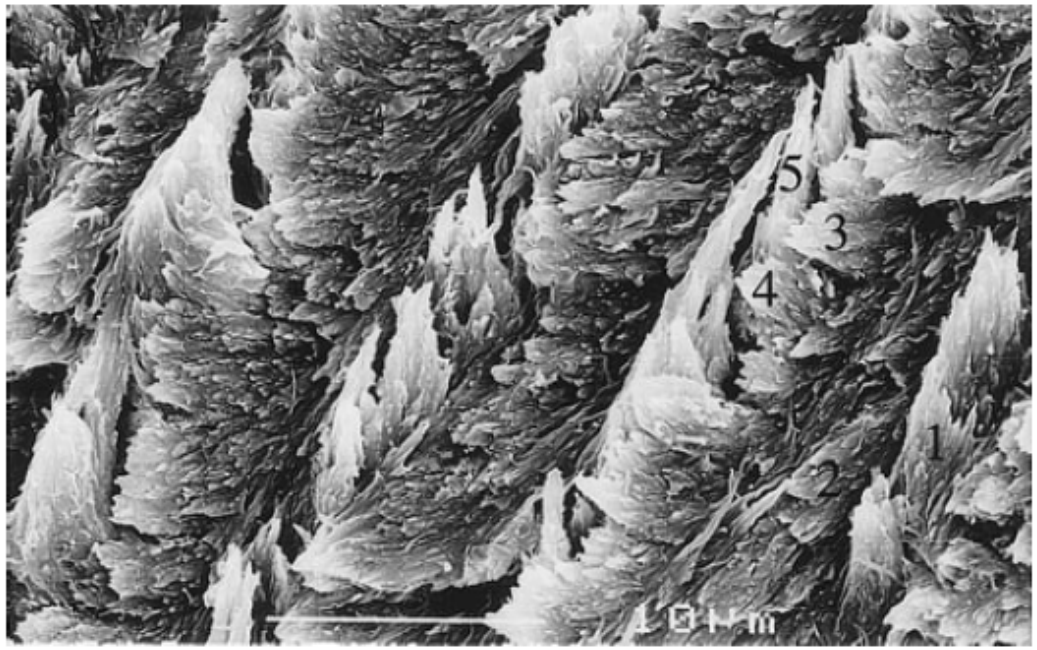


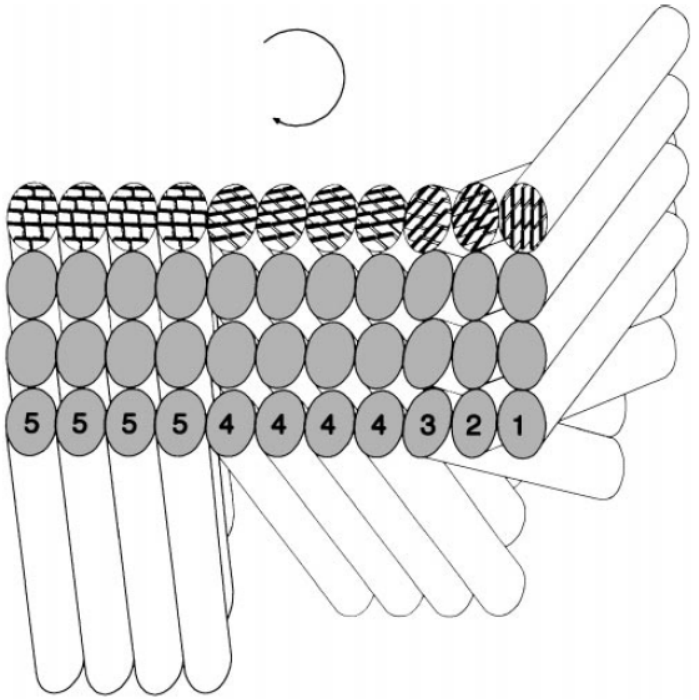
Lamellar Units

- 5 sub-layer model
  - Fibrils in successive sublayers progressively rotate by about 30^{o} in one direction
  - Successive sublayers are oriented at 0^{o}, 30^{o}, 60^{o}, 90^{o}, and 120^{o}

Lamellar Unit Structure

- Structure of a lamellar unit is asymmetric
- 4th and 5th sublayers are much thicker than the 1st, 2nd, 3rd sub-layers
- Differences in relative thicknesses of each of the five sub-layers in a lamellar unit are apparent in each different genera of mammals.



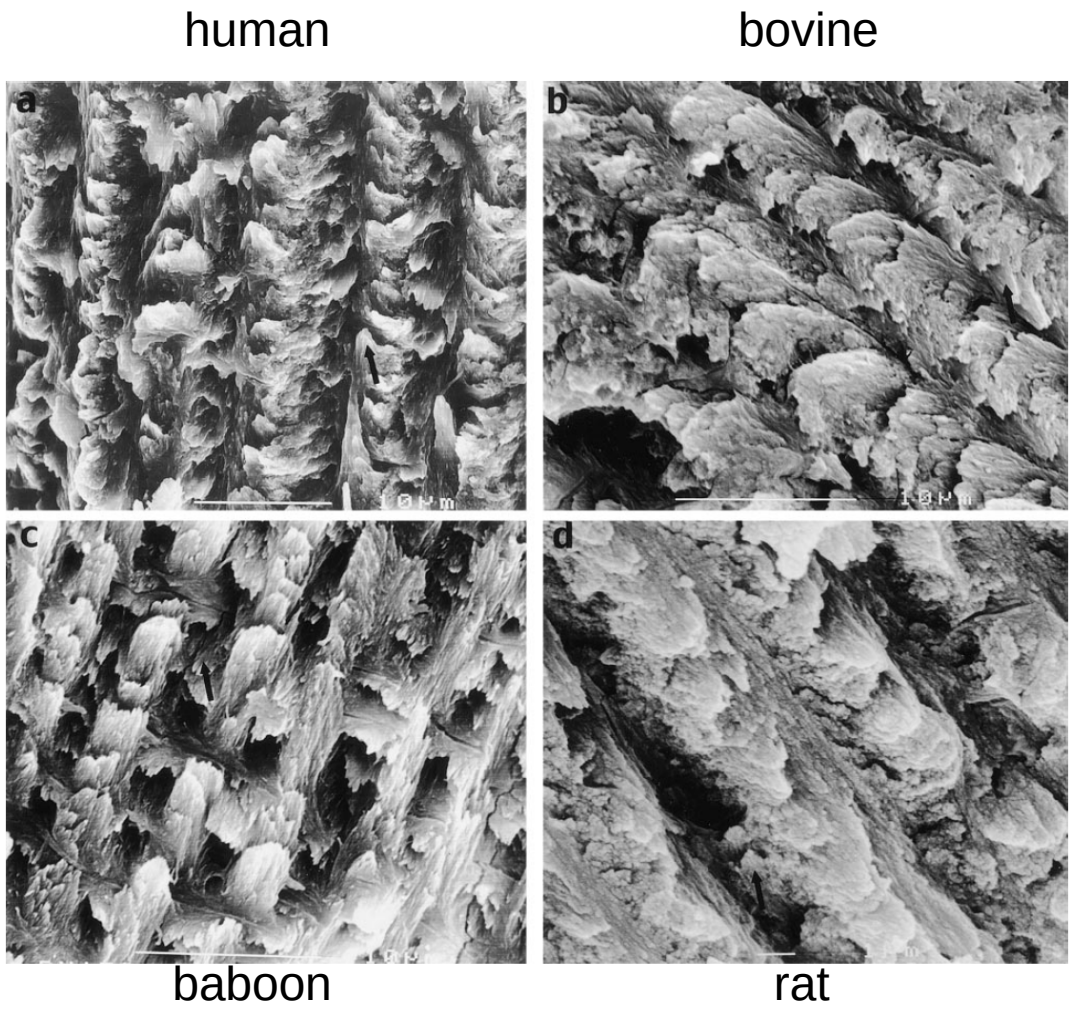


<EX: in baboon>

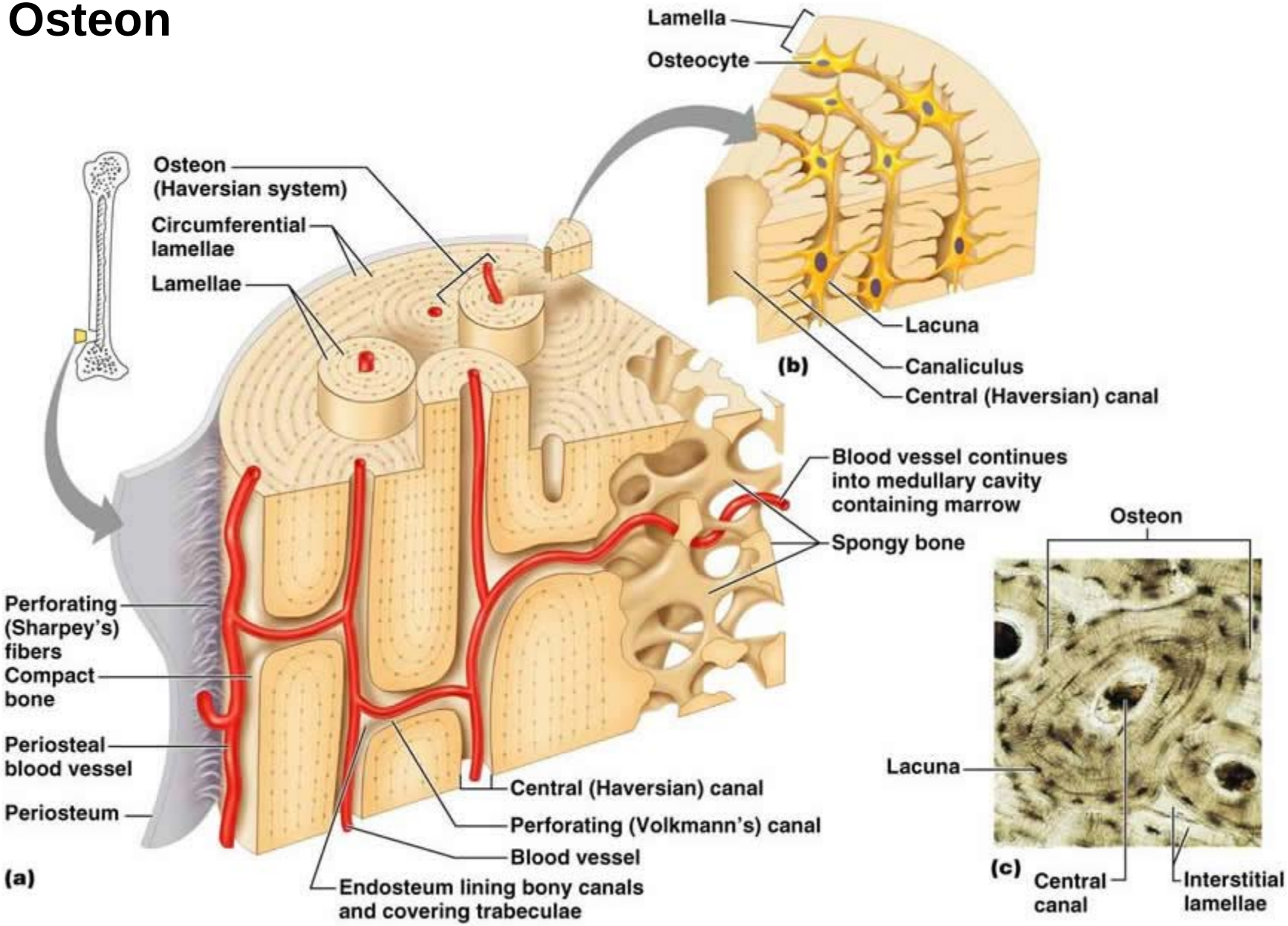
- Average thickness of a lamellar unit: 4.8 um
- A mineralized collagen fibril has diameter of 0.1 um
- Number of layers of collagen is around 50.
  - 4^{th} – 20 layers
  - 5^{th} – 20 layers
  - 1^{st}, 2^{nd}, 3^{rd} – 10 layers

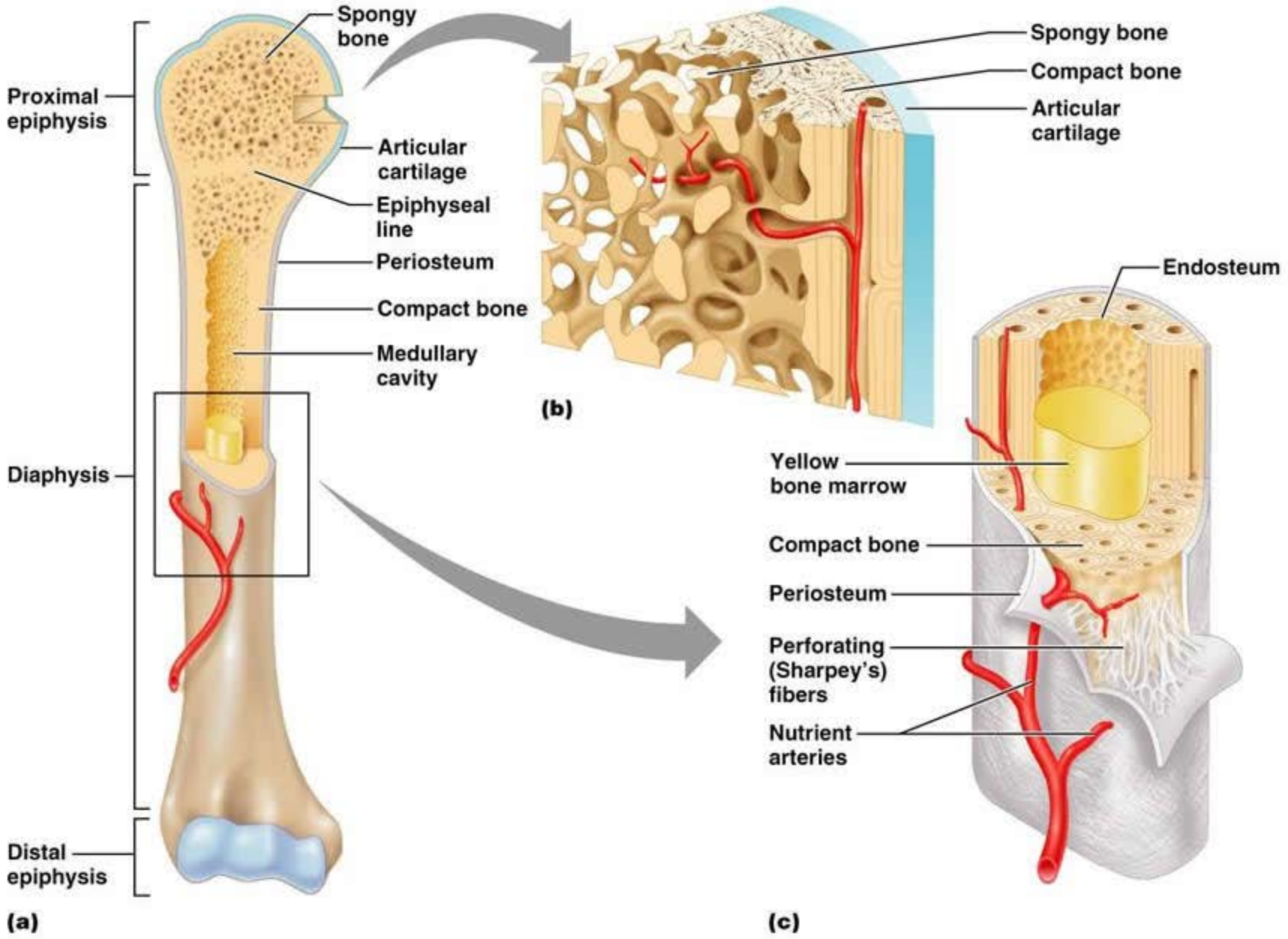
Structure – function relationship

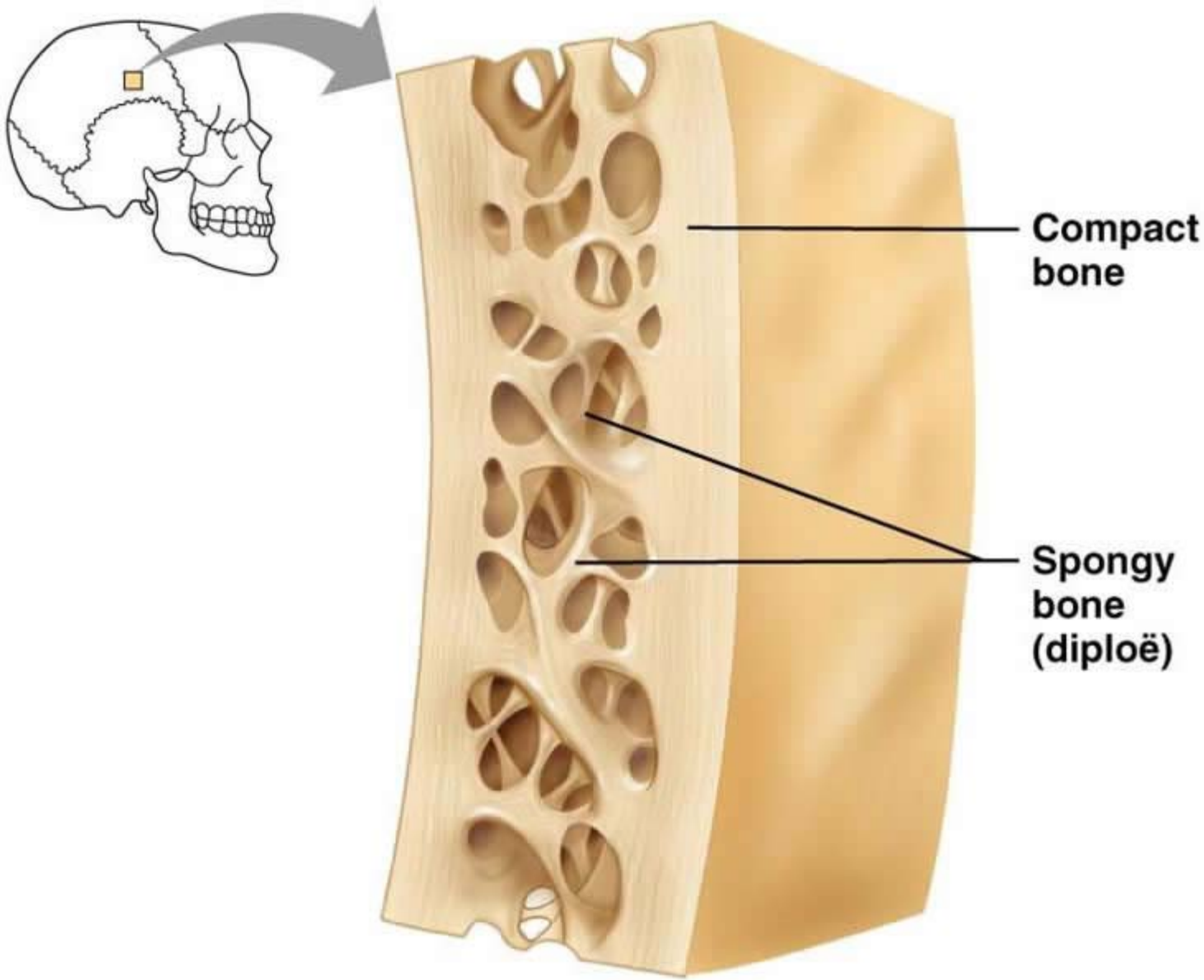
- Relative thickness of the sublayers are adapted to specific functions
  (In areas of tension and compression)
- Lamellar bone is present in two basic forms
  - Lamellae (parallel arrays)
  - Osteon (cylinders)



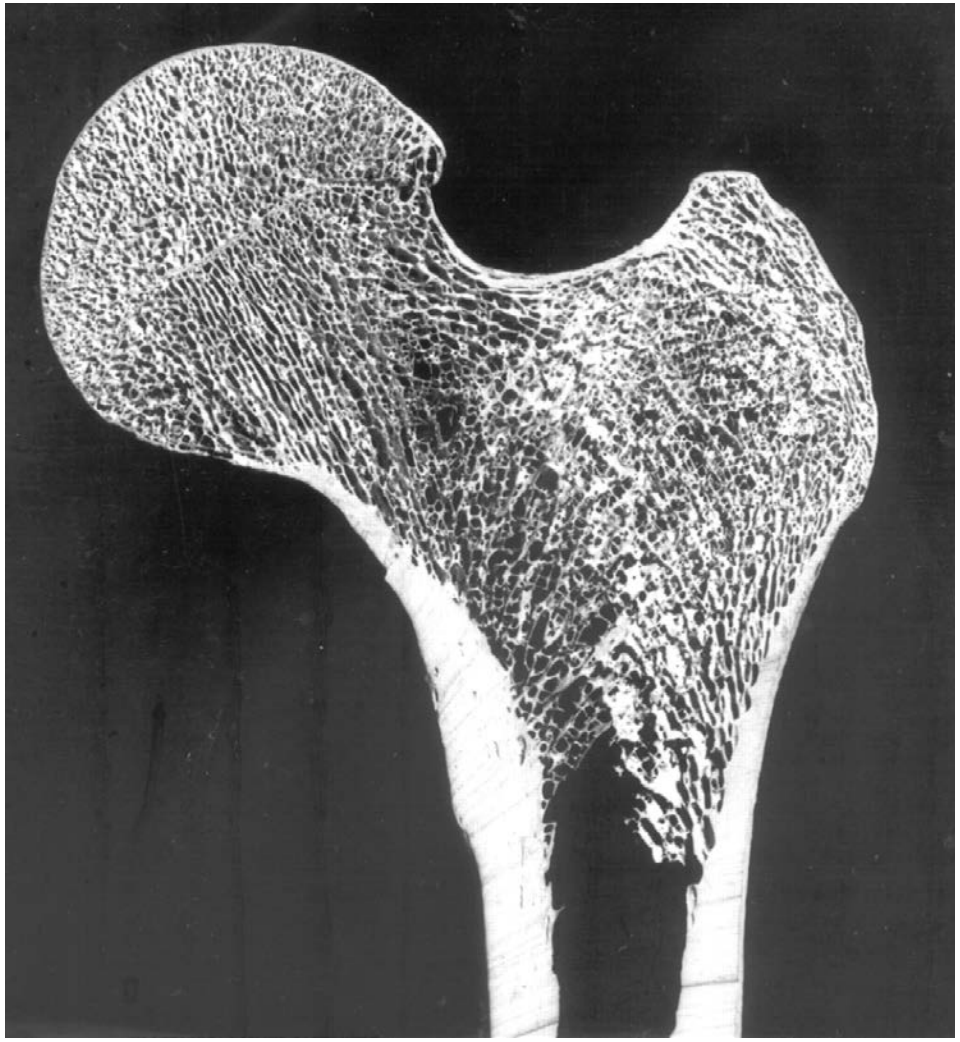
Osteon

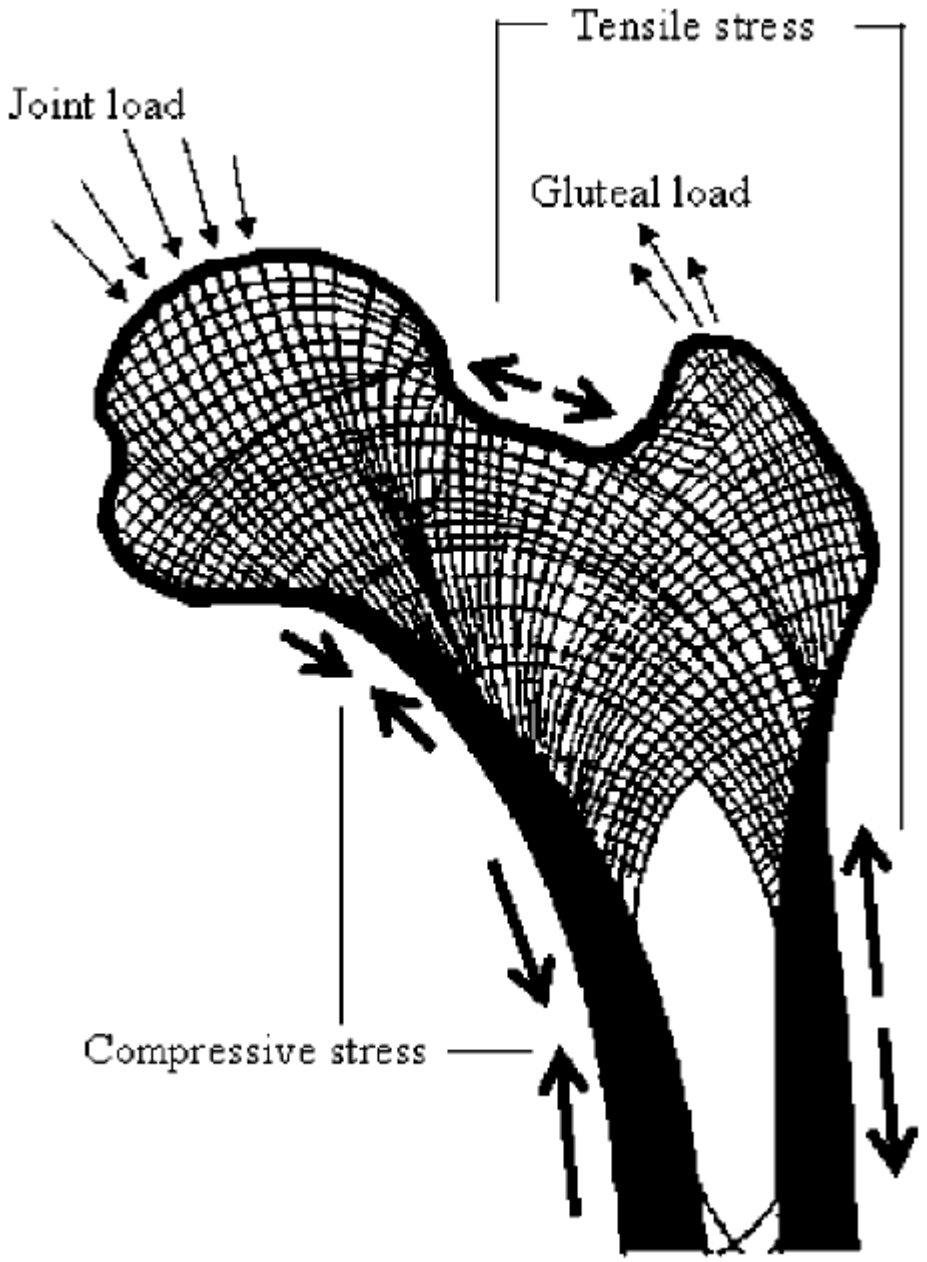

Lamella
Osteocyte
Osteon
(Haversian system)
Circumferential
lamellae
Lamellae
Lacuna
Canaliculus
Central (Haversian) canal
(b)
Blood vessel continues
into medullary cavity
containing marrow
Spongy bone
Perforating
(Sharpey's)
fibers
Compact
bone
Periosteal
blood vessel
Periosteum
Central (Haversian) canal
Perforating (Volkmann's) canal
Blood vessel
Endosteum lining bony canals
and covering trabeculae
(a)
Osteon
Lacuna
Central
canal
Interstitial
lamellae
(c)


Spongy bone
Proximal epiphysis
Articular cartilage
Epiphyseal line
Periosteum
Compact bone
Medullary cavity
Diaphysis
Distal epiphysis
(a)
Spongy bone
Compact bone
Articular cartilage
(b)
Endosteum
Yellow bone marrow
Compact bone
Periosteum
Perforating (Sharpey's) fibers
Nutrient arteries
(c)


Compact bone
Spongy bone (diploë)

Femoral Trabecular Pattern





Cartilage

Hyaline cartilage

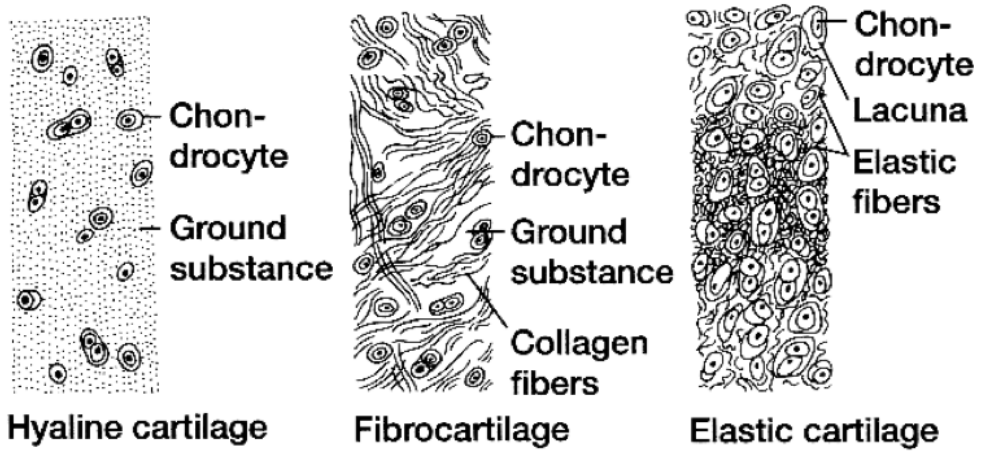
- Abundant inter-terretorial matrix

Fibro cartilage

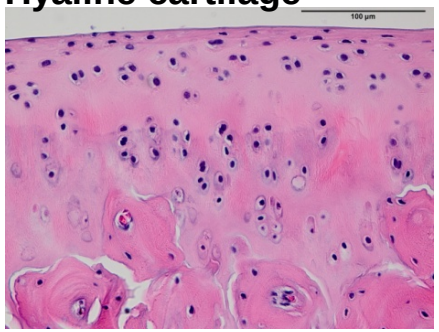
- Col I expression

Elastic cartilage

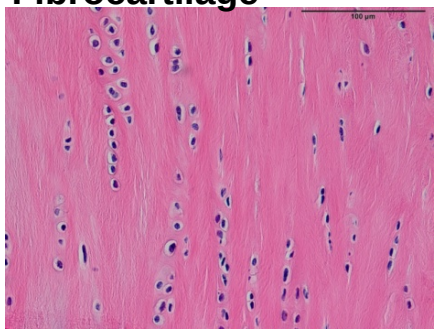
- Large lacunae
- Elastin expression



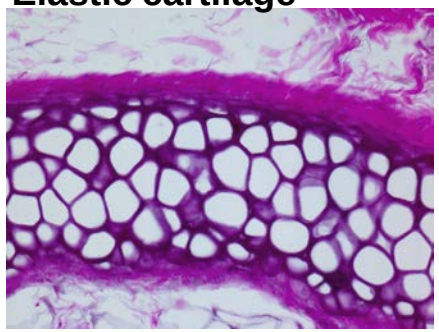
Hyaline cartilage



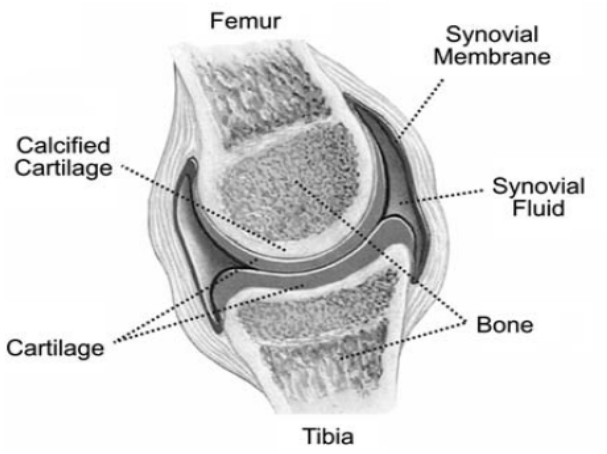
Fibrocartilage

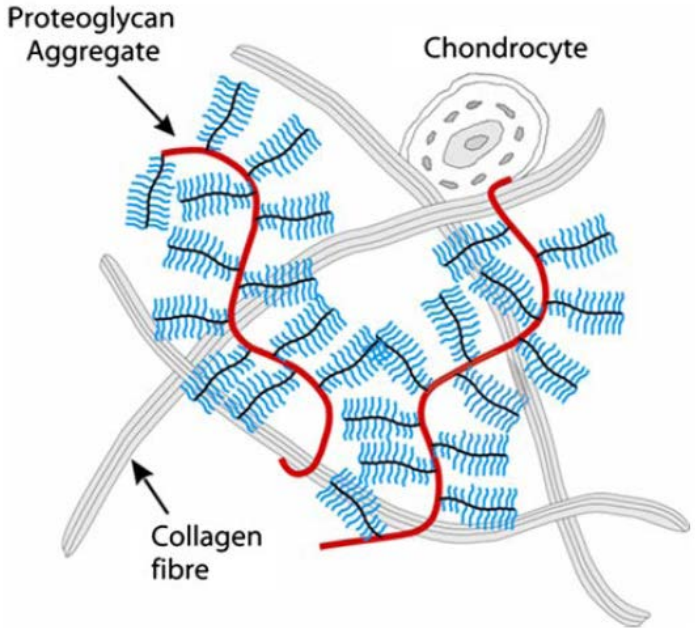


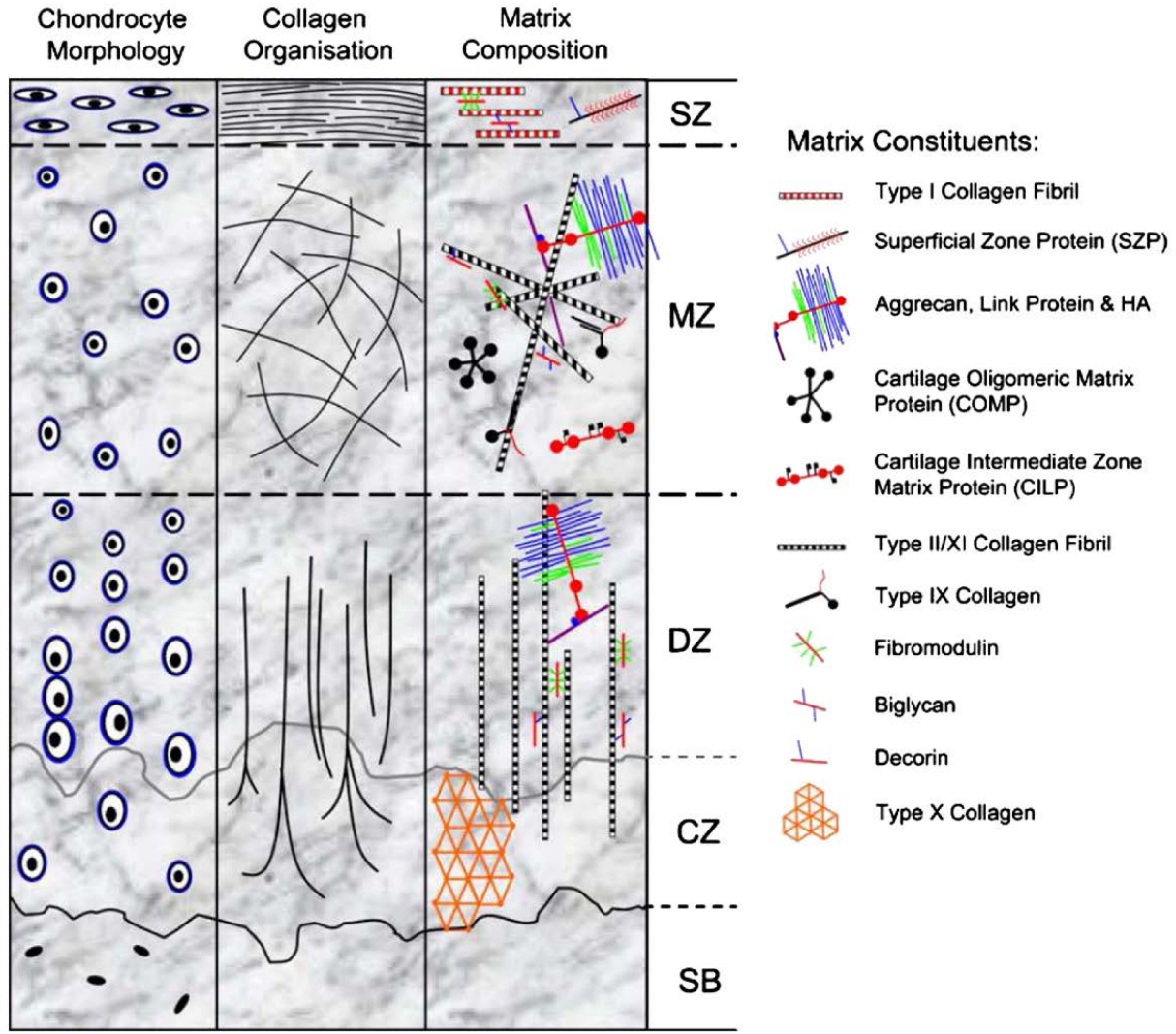
Elastic cartilage



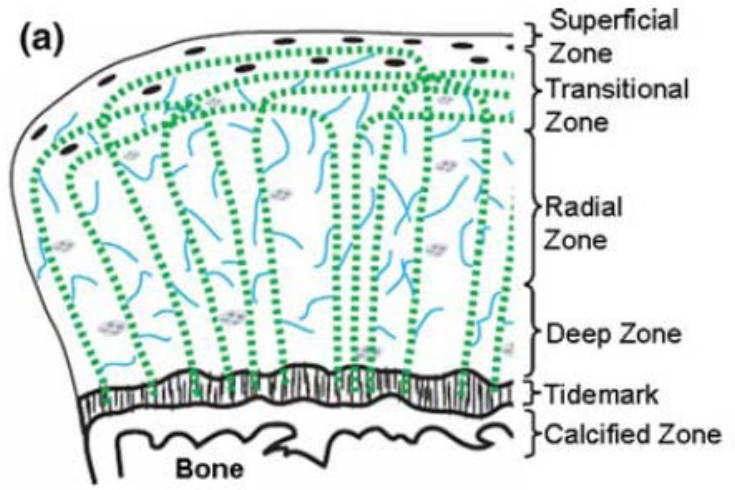
Structure & ECM organization of Articular Cartilage

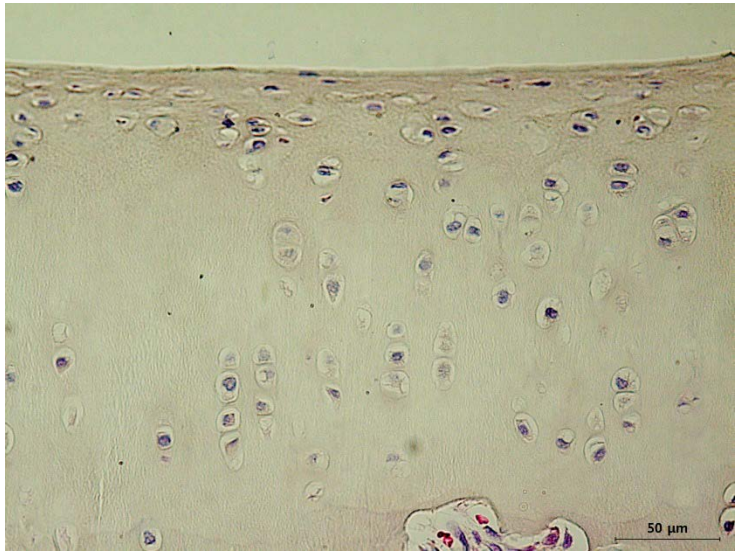




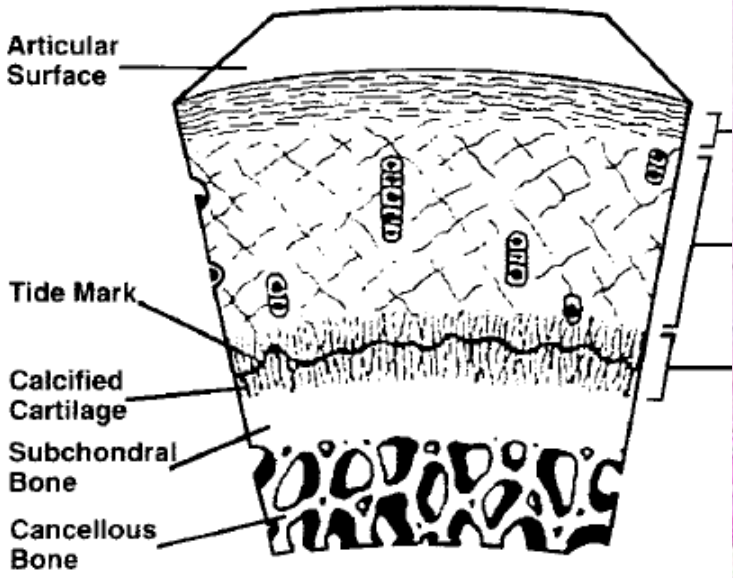


Schematic drawing of articular cartilage demonstrating zonal arrangement & macromolecular organization

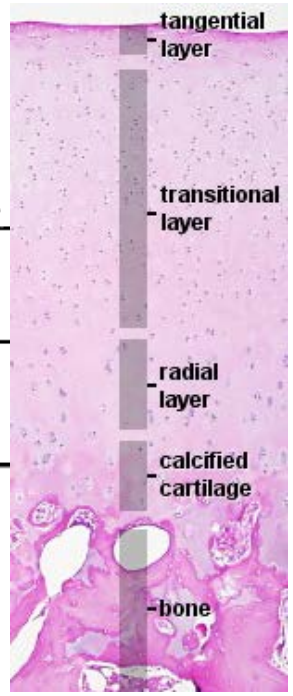




H&E Stained section. CC: Calcified Cartilage(X 400)

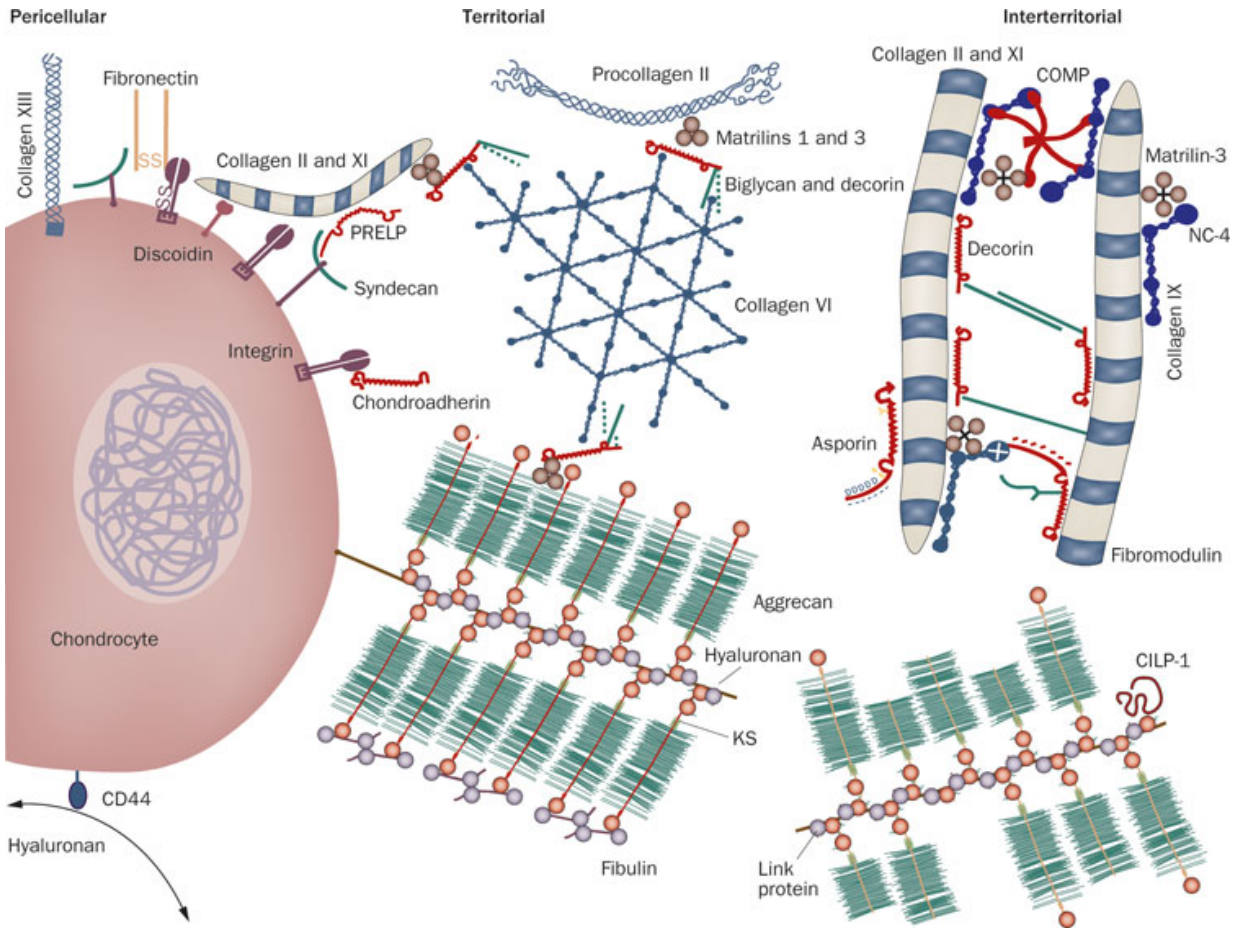


Mandelbaum BR et al., 1998



UWA Blue Histology

Figure 2 The molecular organization of normal articular cartilage



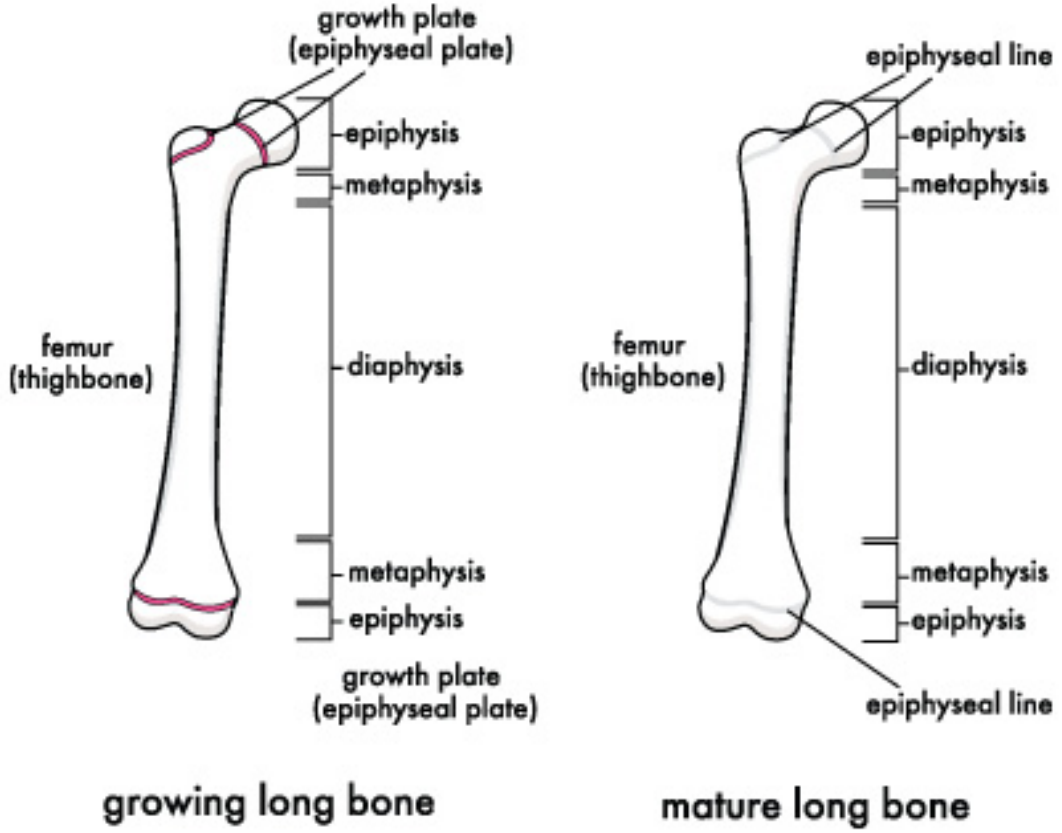
Heinegård, D. & Saxne, T. (2010) The role of the cartilage matrix in osteoarthritis
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2010.198

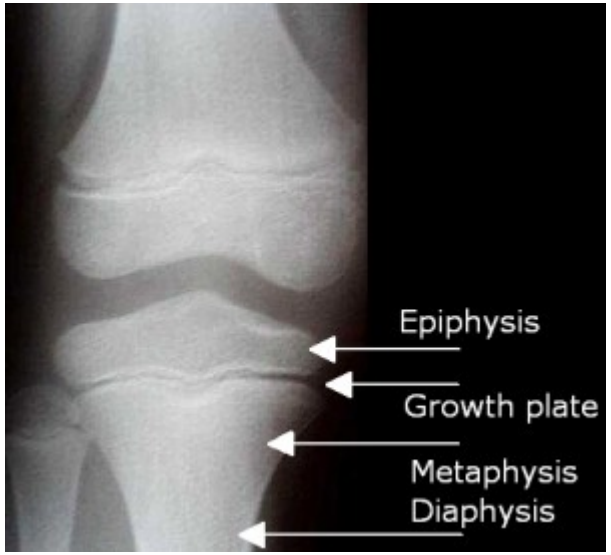
nature REVIEWS RHEUMATOLOGY

Growth Plate

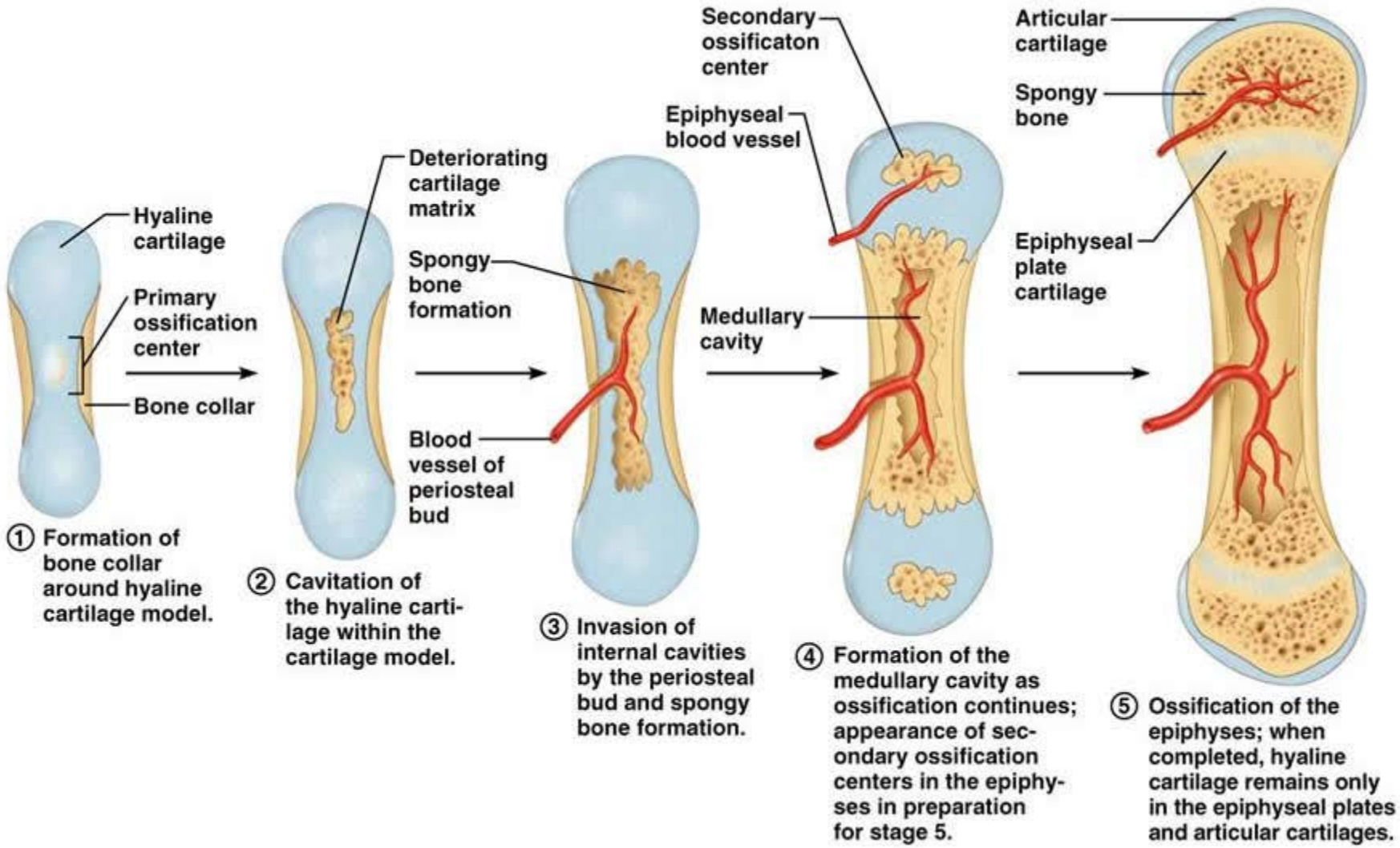
Bone formation

- Intramembranous ossification
- Endochondral ossification





[Engineering growing tissues: Alsberg E 2002 PNAS]



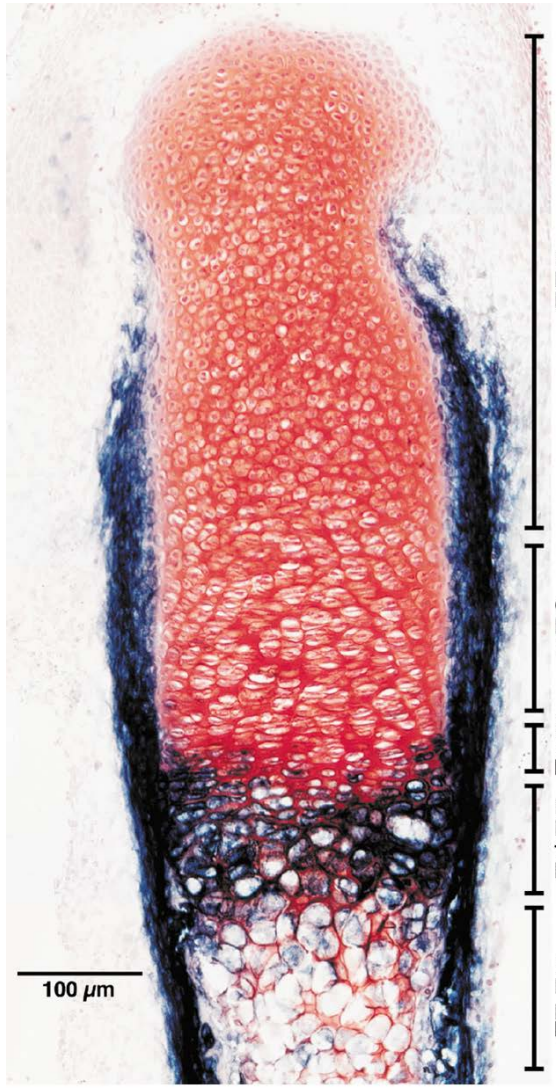
① Formation of bone collar around hyaline cartilage model.

② Cavitation of the hyaline cartilage within the cartilage model.

③ Invasion of internal cavities by the periosteal bud and spongy bone formation.

④ Formation of the medullary cavity as ossification continues; appearance of secondary ossification centers in the epiphyses in preparation for stage 5.

⑤ Ossification of the epiphyses; when completed, hyaline cartilage remains only in the epiphyseal plates and articular cartilages.



Resting chondrocytes
Markers: type II collagen
Moderate mitotic index

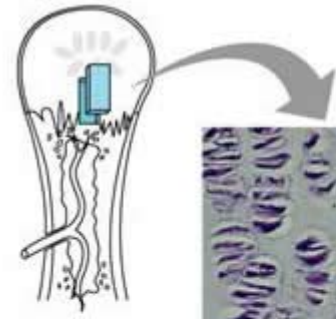
Columnar chondrocytes
Markers: type II collagen
High mitotic index

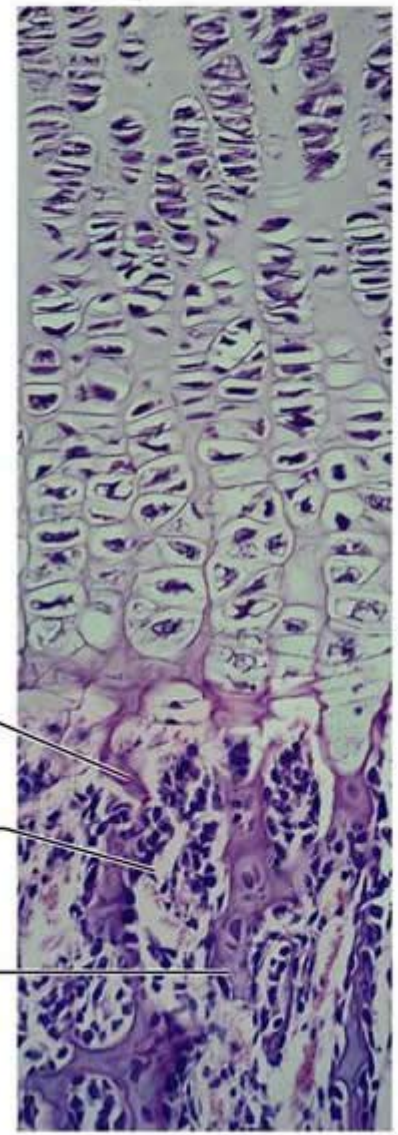
Prehypertrophic chondrocytes
Markers: type II collagen, Ihh
Post-mitotic

Hypertrophic chondrocytes
Markers: alkaline phosphatase, type X collagen, VEGF
Post-mitotic

Primary spongiosum (bone)
Markers: osteocalcin, alkaline phosphatase, osteopontin
High mitotic index

Arthritis Research





Calcified cartilage spicule

Osteoblast depositing bone matrix

Osseous tissue (bone) covering cartilage spicules

Resting (quiescent) zone

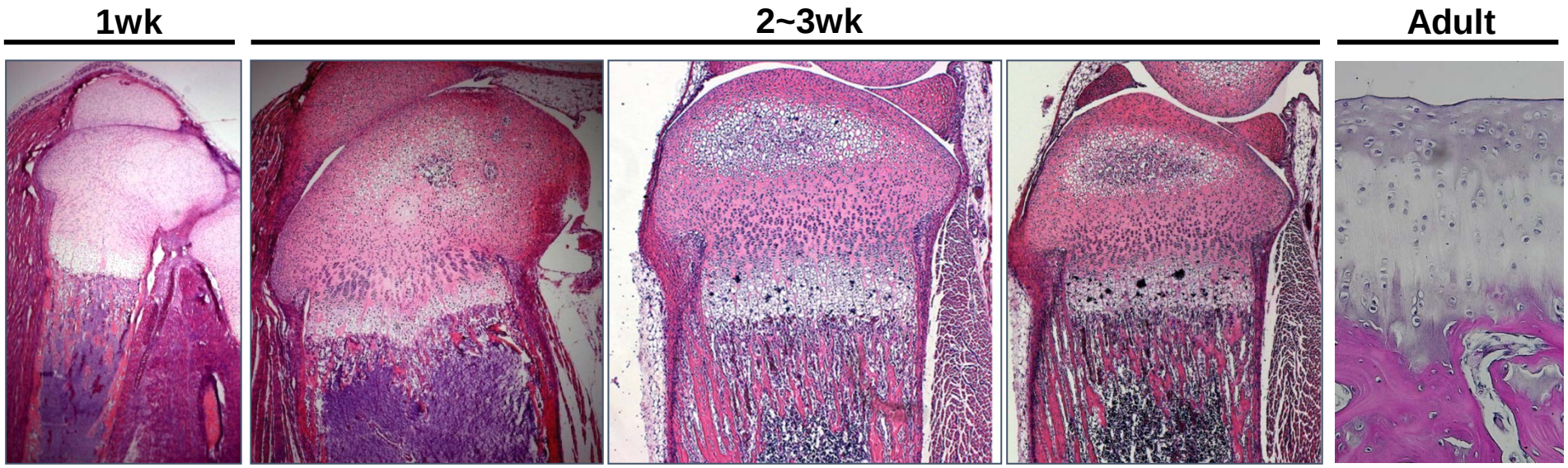
Growth (proliferation) zone
Cartilage cells undergo mitosis

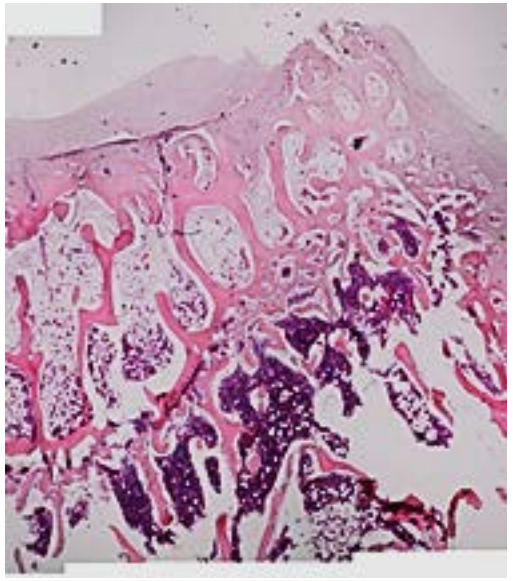
Hypertrophic zone
Older cartilage cells enlarge

Calcification zone
Matrix becomes calcified; cartilage cells die; matrix begins deteriorating

Ossification (osteogenic) zone
New bone formation is occurring

Cartilage in patellar joint





Bone	Estimated age of fusion	
	Proximal/Medial end	Distal/Lateral end
Humerus (upper arm)	10 – 15 years	9 – 15 years
Radius (lower arm)	14 – 19 years	16 – 22 years
Femur (upper leg)	15.5 – 19.5 years	14.5 – 22 years
Tibia (lower leg)	15.5 – 22 years	14.5 – 19.5 years
Clavicle (collarbone)	19 – 30 years	19 – 20 years

Cell-ECM Interaction

For many years, the ECM was thought to serve only as a structural support for tissues…

1966, Hauschka & Konigsberg
: interstitial collagen promoted conversion of myoblasts to myotubes

1968, Wessells & Cohen
: collagen play a crucial role in salivary gland morphogenesis

1972, Bernfield
: GAGs play a crucial role in salivary gland morphogenesis

1977, Hay
: ECM is an important component in embryonic inductions

1982, Mina J Bissell "Dynamic Reciprocity"
: the ECM is postulated to exert an influence on gene expression via transmembrane proteins and cytoskeletal components.

1991, Ingber, 1995, Boudrean
: cell-ECM interactions can regulate…

- **Cell adhesion, migration, growth, differentiation, apoptosis**
- **Modulate cytokine & growth factor activities**
- **Activate intracellular signaling**

ECM's Function on Cells & Tissues

Physical scaffolding

Cell adhesion to comprise tissues

Defining boundary between different tissues

Determines cell morphology & mediate cell movement

Adjust cellular activity through cell surface receptors

Repository for various cell signaling factors

ECM Components

Fibrous matrix proteins

- Collagen
  - Comprise 25% of total proteins
  - Skin, bone, ligament, tendon, cartilage
  - Physical support and protection of cells
  - 27 isoforms
    - fibrous: I, II, III, V, XI
    - Linker proteins: IX, XII, XIV, XVI

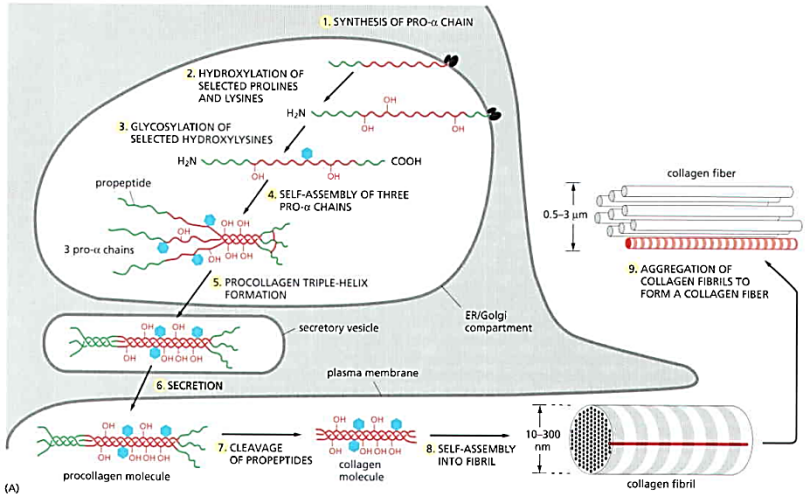
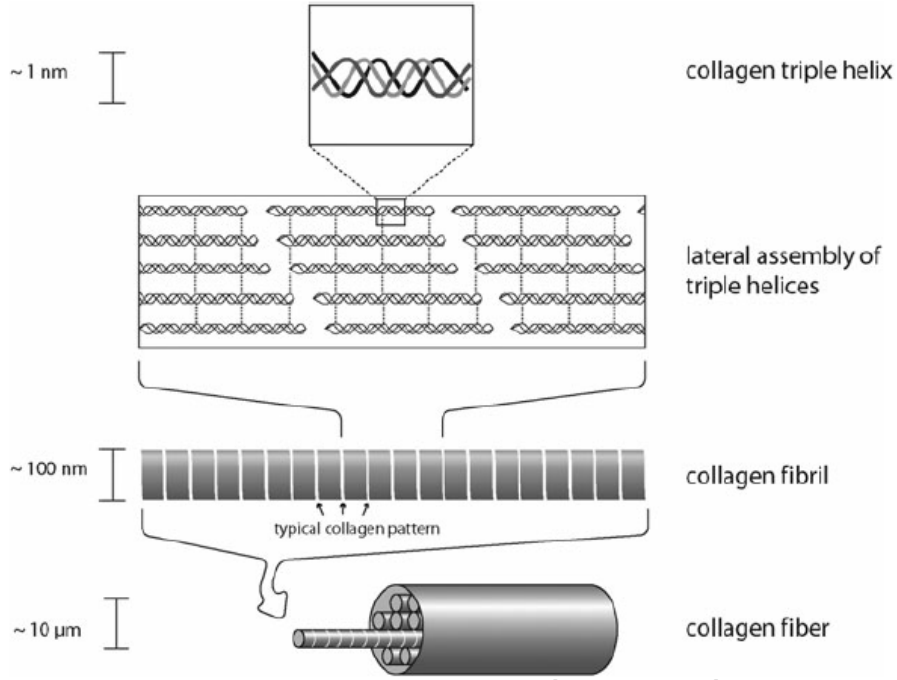
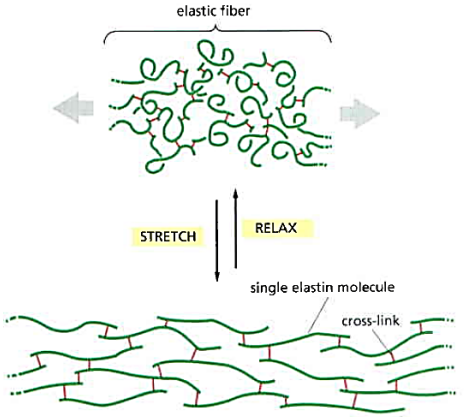


Figure 19–66 **The intracellular and extracellular events in the formation of a collagen fibril.** (A) Note that procollagen assembles into collagen fibrils in the extracellular space, often within large infoldings in the plasma membrane (not shown). As one example of how collagen fibrils can form ordered arrays in the extracellular space, they are shown further assembling into large collagen fibers, which are visible in the light microscope. The covalent cross-links that stabilize the extracellular assemblies are omitted in this picture. (B) Electron micrograph of a negatively stained collagen fibril reveals its typical striated appearance. (B, courtesy of Robert Horne.)

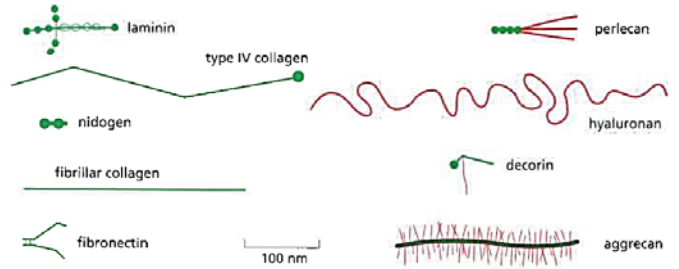


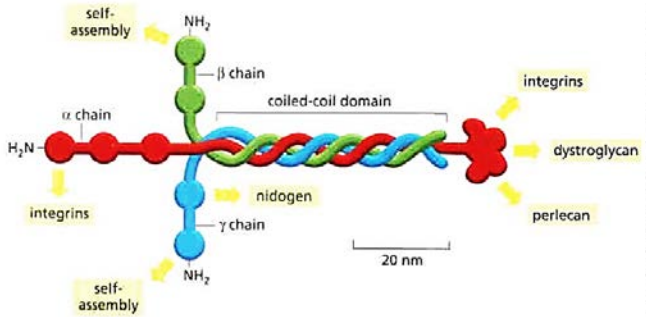
Heim, M 2010 Chem Soc Rev

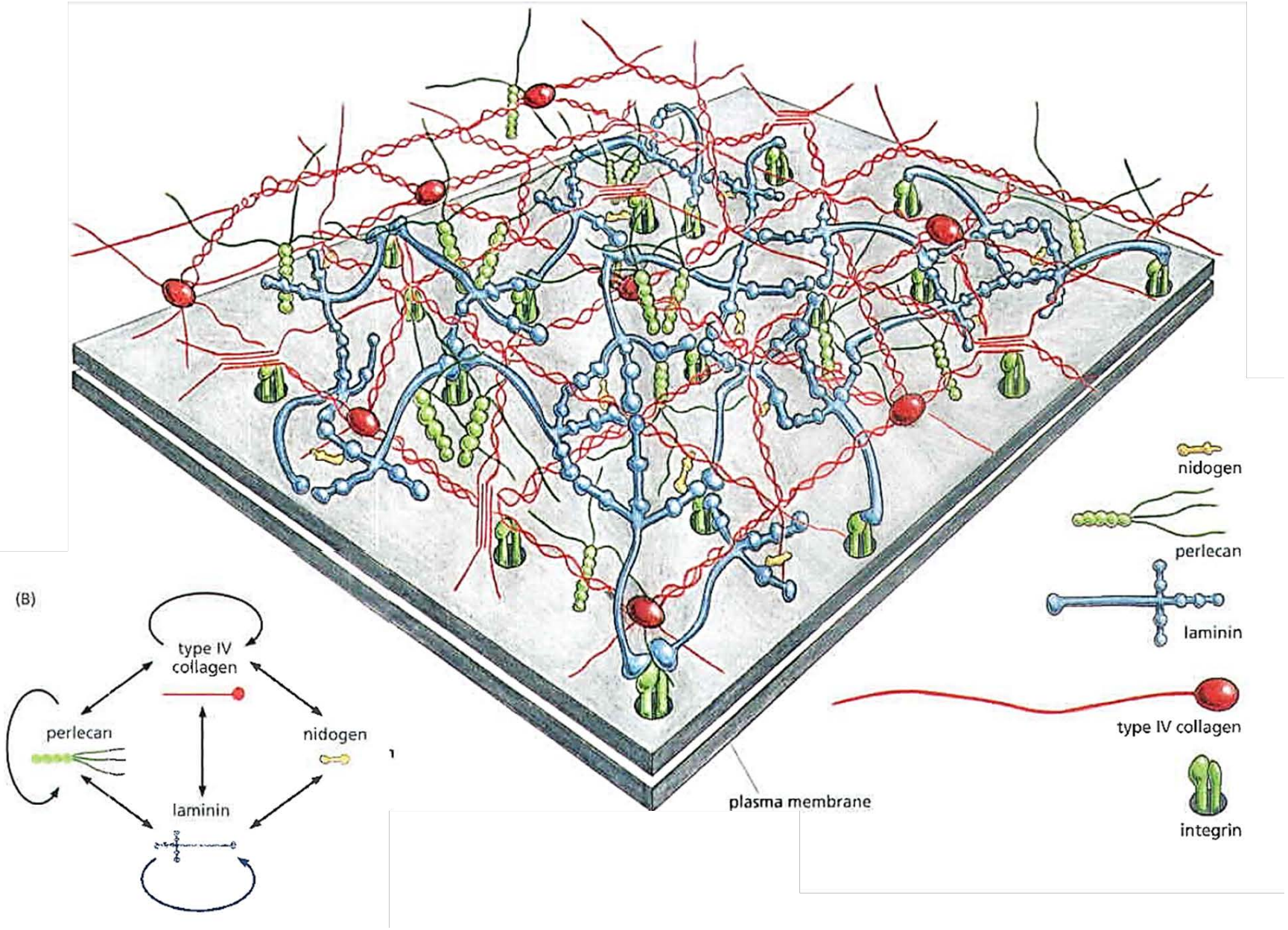
- **Elastin**
  - Low tensile strength, high plasticity and elasticity
  - Produced by fibroblast and smooth muscle cells
  - Blood vessel, lung, skin



- Adhesive glycoproteins
  - Links ECM to cells
  - Bound to proteoglycan, fibrous protein, and ECM receptors such as Integrin on cell surface
  - Fibronectin, Laminin, & non-fibrous collagen
  - **Fibronectin**
    - 250kDa, homo-dimer
    - Binds to heparin, collagen, fibrin..
    - Cell attachment & cell migration, blood coagulation
  - **Laminin**
    - Present in basement membrane in conjunction with collagen IV, heparin, heparan sulfate..
    - Forms spiral mesh
  - **Non-fibrous collagen**
    - Membrane type: IV, VIII, XV, XVIII
    - Attached to cell plasma membrane: XIII, XV, XVII XXIII
    - Basement membrane: IV, XV, XVIII
    - Endothelium: VIII
    - Ubiquitous presence in most tissues: XIII
    - Skin: XV

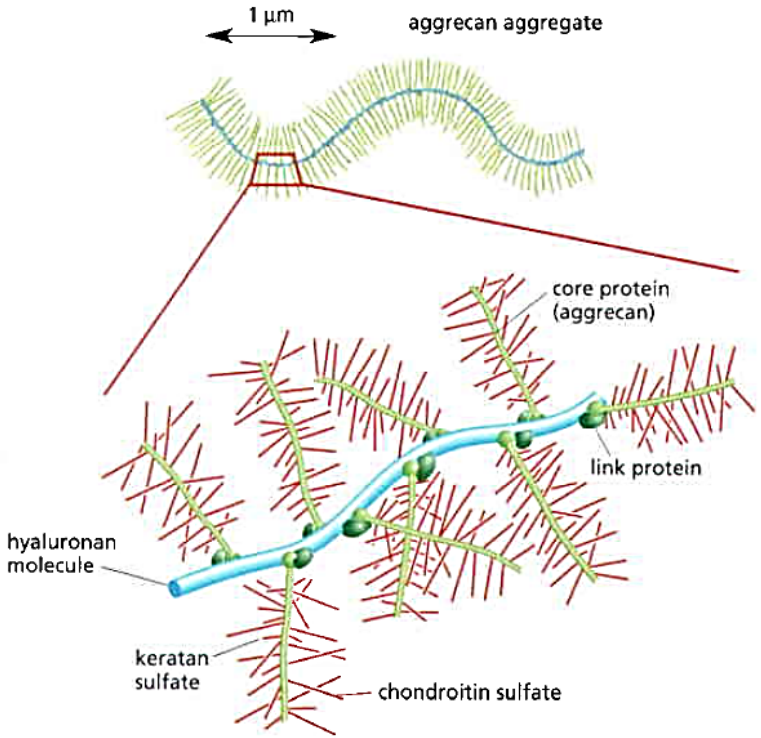


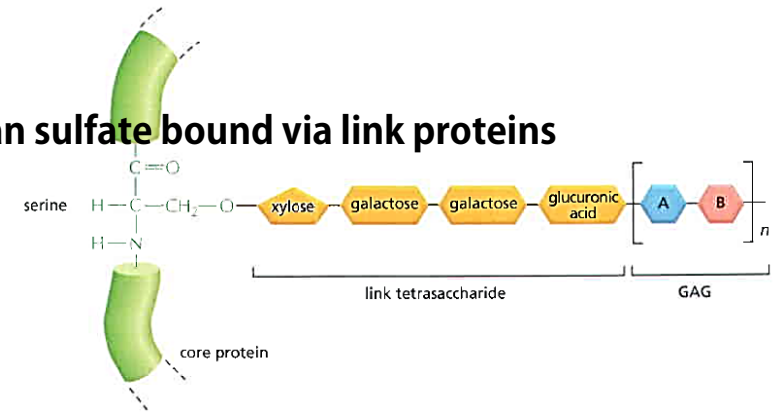



(B)
type IV collagen
perlecan
nidogen
laminin
plasma membrane
nidogen
perlecan
laminin
type IV collagen
integrin

Proteoglycan

- Protein is linked to glycosaminoglycans (GAGs) via covalent bonding
- GAGs show negative charge
  - Repulsion between GAGs strands
  - Cations or H2O bound to the GAGs surface
  - High water content & high volume
  - Resistance to compression
- GAGs:
  - Hyaluronan or hyaluronic acid (high water content -> turgor force/ Receptor: CD44, RHAMM)
  - Chondroitin sulfate (Cartilage, ligament, tendon, artery/ Tensile strength)
  - Heparan sulfate (found in almost all animal tissues/ Involved in development, vasculogenesks, blood coagulation, cancer development etc…)
  - Keratan sulfate (Cornea, cartilage, & bone)
- Aggrecan
  - Composed of hyaluronan backbone and chondroitin sulfate or keratan sulfate bound via link proteins



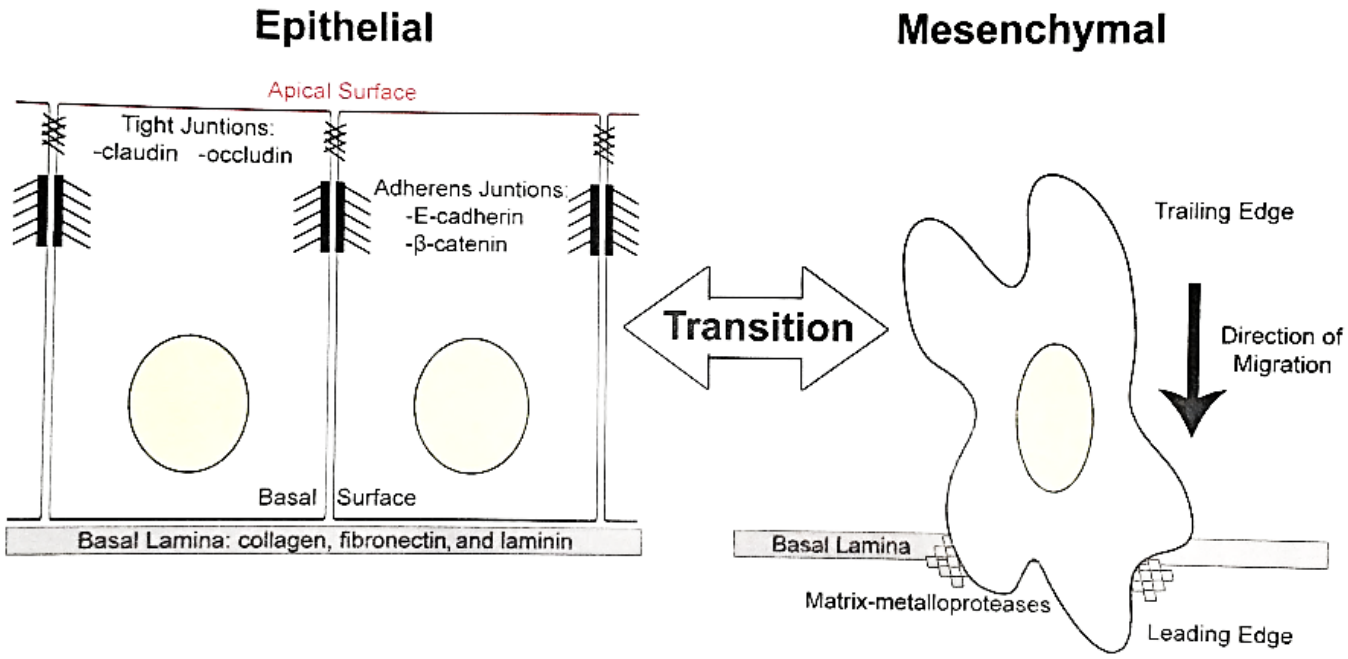


Mesenchymal cells

- Immersed in an interstitial matrix

Epithelial & endothelial cells

- Contact a basement membrane



ECM-Cytokine Interaction

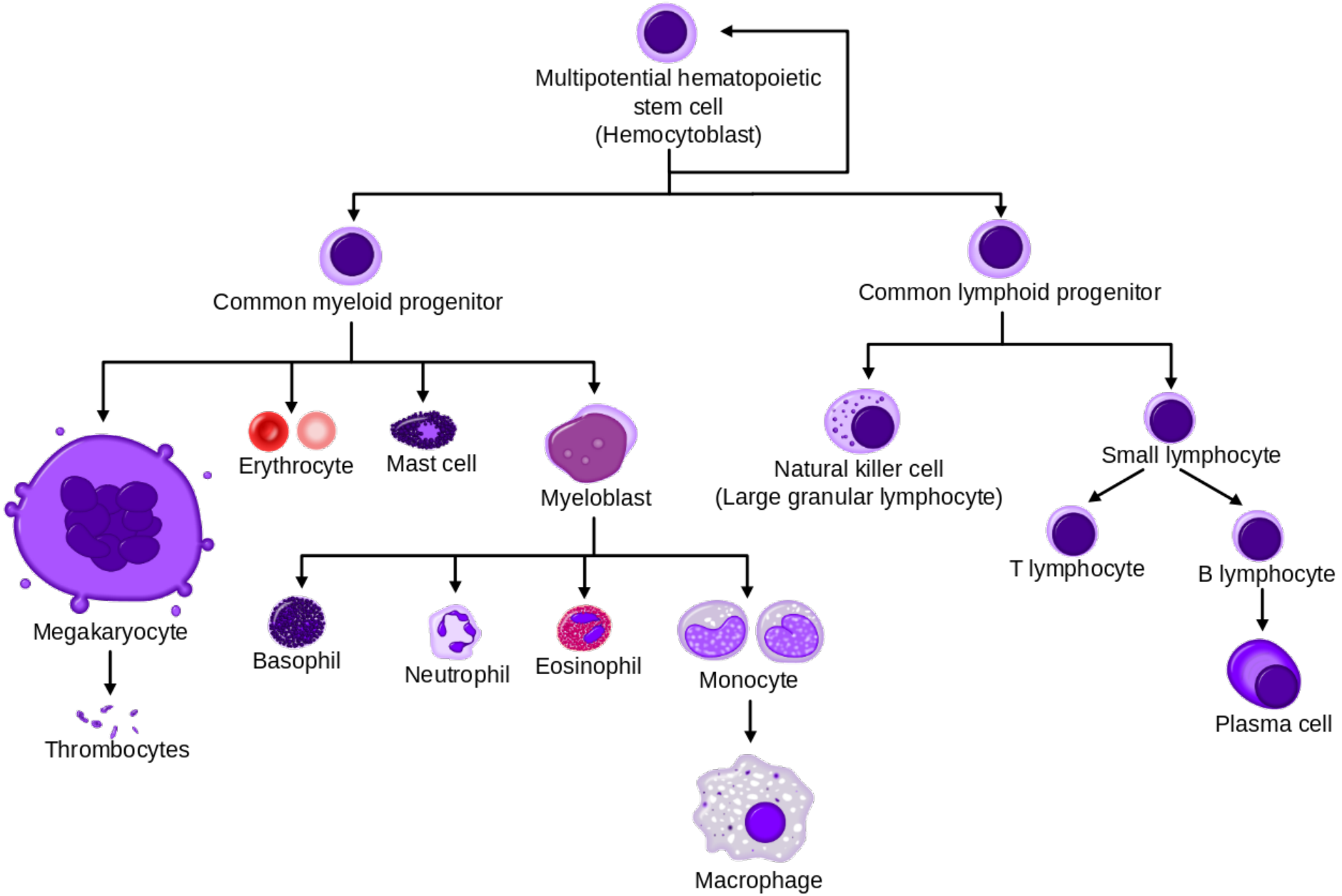
GF & cytokines interact with ECM

ECM serve as a GF reservoir

- Binds to GF or cytokine
- Protect from degradation
- Present GFs more efficiently to their receptors

Bind & activate GF receptors

- Examples
- Neutrophile binds to FN -> up-regulates TNF-α
- HGF-mediated hepatocytes proliferation depends on heparan sulfate
- Endothelial cell bound to FN -> proliferation & migration up-regulated by VEGF
- Laminin-5, tenascin-C, and Decorin has EGF-like repeats
  -> binds & activate EGFR


Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Erythrocyte
Mast cell
Myeloblast
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
Megakaryocyte
Thrombocytes
Basophil
Neutrophil
Eosinophil
Monocyte
T lymphocyte
B lymphocyte
Plasma cell
Macrophage

ECM – Cell binding

1991 Humphries, 1992 Hynes, 1995 Cullberg & Ekblom

- Various ECM molecules contain specific amino acid motifs that allow them to bind directly to cell surface receptors

Tripeptide RGD: best characterized motif (first found in FN)

Other amino acid adhesive motifs have been found in

- Laminin, entactin, thrombin, tenascin, fibrinogen, vitronectin, collagen I, collagen VI, bone sialoprotein, osteopontin

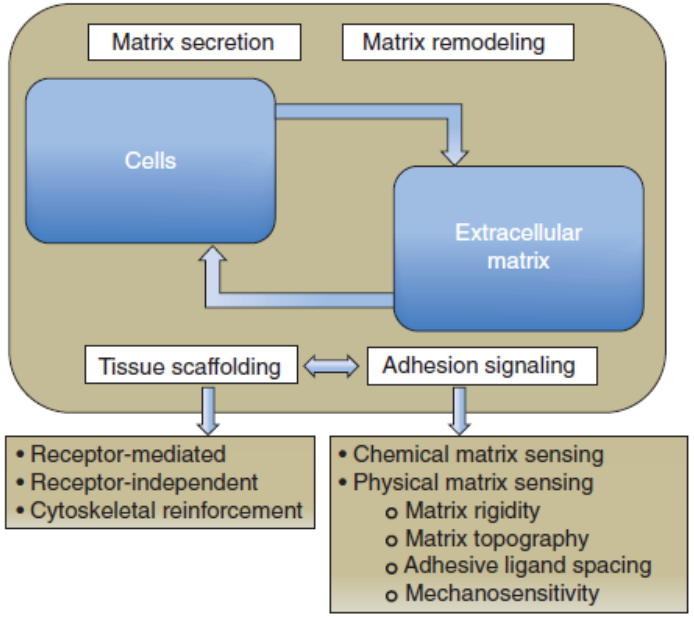
Cell-ECM Interaction

ECMs link cells together into functional tissue and organs

Convey accurate positional information (cellular & extracellular environment)

Cell-ECM interaction

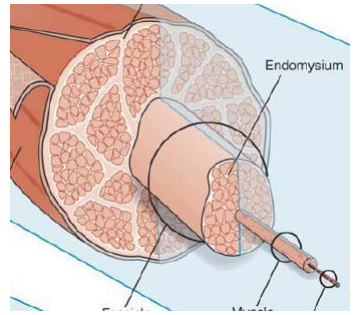
- **Reciprocal, bidirectional interaction**
- **Cells responding to matrix**
- **Remodeling & reconstruction of ECM by cells**



Molecular & Structural Diversity of ECM

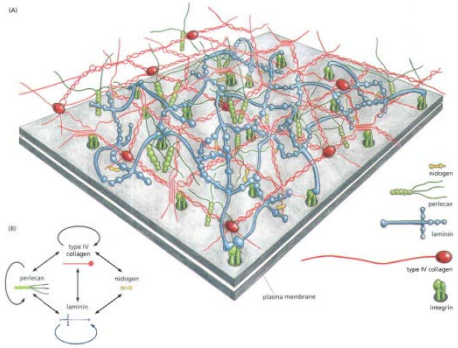
1. Chemical composition: customized to tissue type

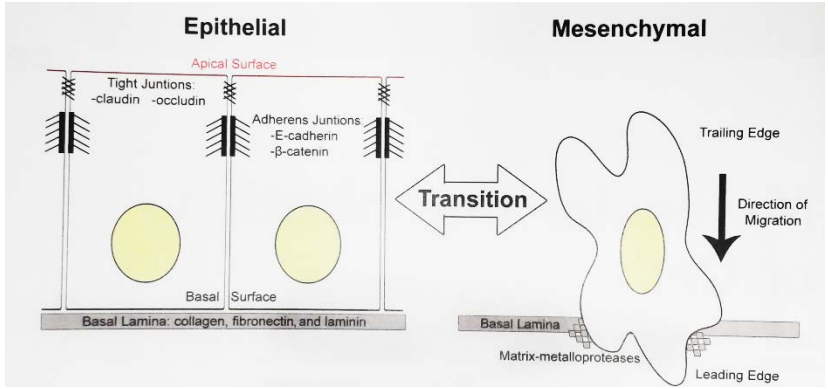
- Fascia & tendon: high level of collagen I
- Basement membrane: Col IV, LN, FN…
- Cartilage: Col II, GAGs…

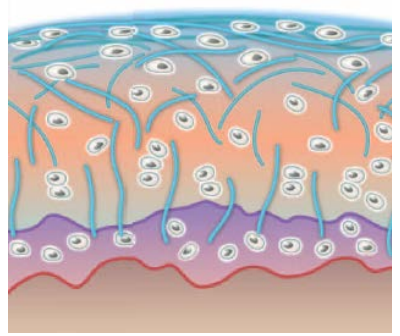


2. Dimensionality

- 2D: Cells on basement membrane or culture dish
- 3D: Cells in connective tissue (embedded in 3D environment)
- 1D: fibrils or linear patterns of ECM







3. Mechanical properties

- Rigidity (elasticity or compliance)
- Mechanical heterogeneity (local porosity & cross-linking)
- Overal anisotropy

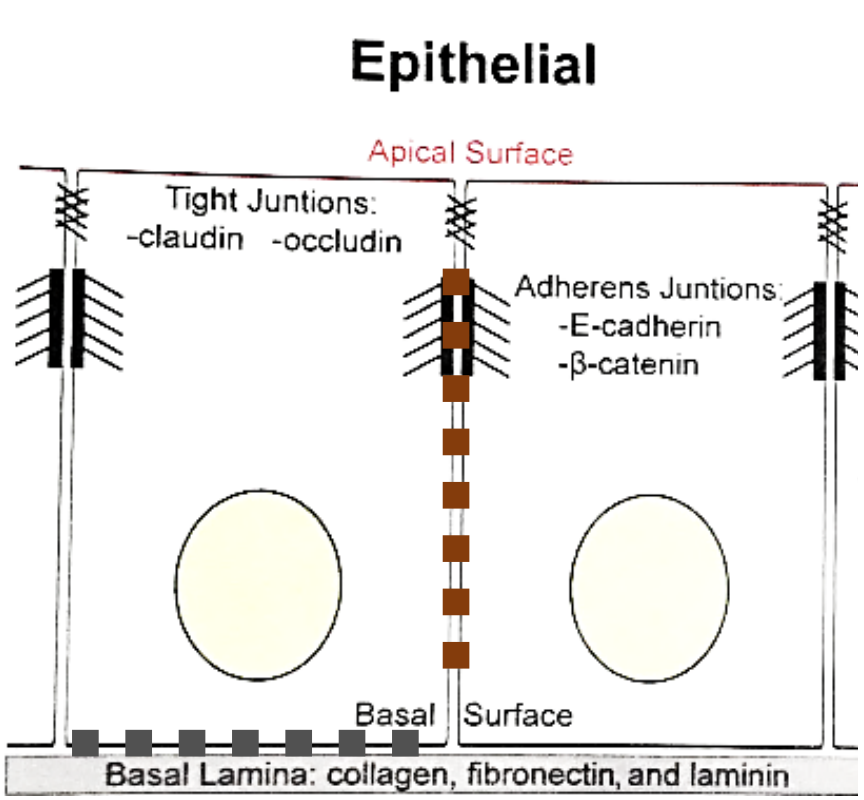
- Rigidity sensing can be a cell-type specific property
  - Cells grow optimally on adherent surfaces that match the cell′ s intrinsic elasticity

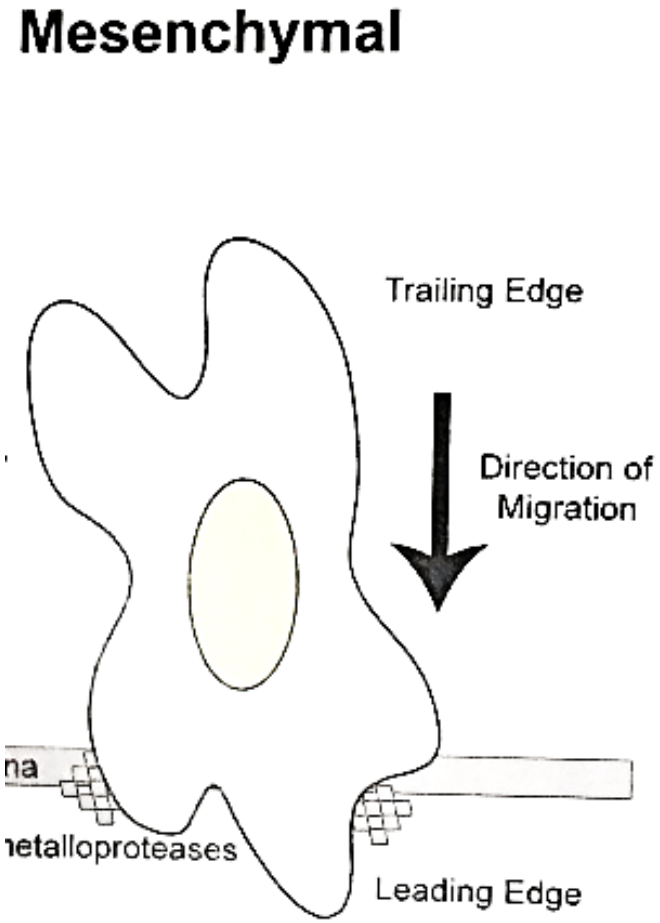
Cells can respond to the micro-topology or even nano-topography of a surface to which they adhere

Physical dimension play critical role in….

- **Biological activities**
- **Cell morphology**
- **Rate of adhesion**
- **Migration**
- **Proliferation**
- **biosynthesis**

Contacts made by cells..





Focal contact

Cell-cell contact

Distinct type of cell adhesion

Pericellular matrix

- Cell-bound matrix
- Can be quite thick (up to several μm)
- Covering the entire cell surface
- Transparent & high water content

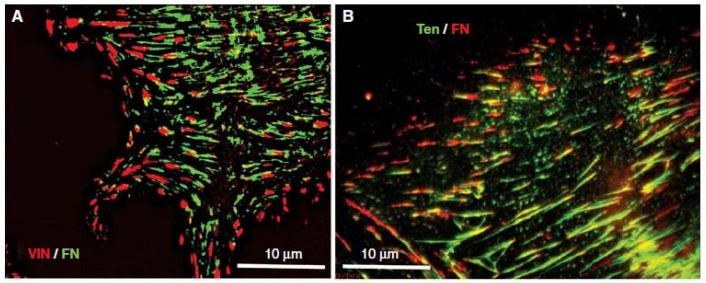
Focal adhesions (FAs)

- Generated by interaction with flat, rigid surface
- Several square micrometers in size
- Located at the ends of actin stress fibers
- Stimulated by small GTPase RhoA
- Actin stress fiber at these sites were shown to be colinear with extracellular FN fibrils -> suggests that the two are tightly linked mechanically

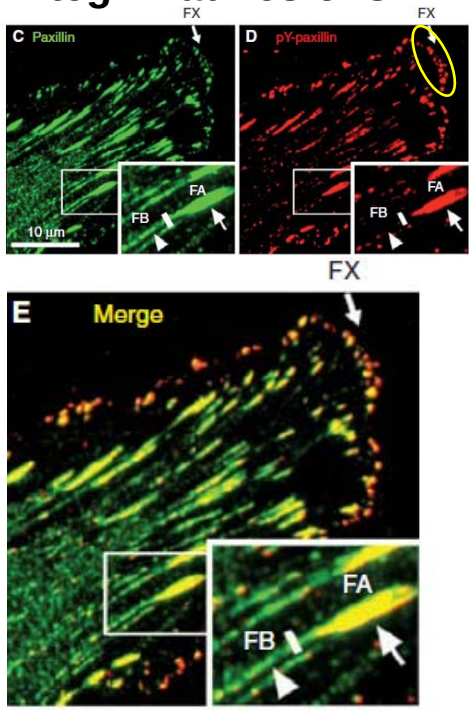
Focal complexes (FX: dot-like nascent adhesion)

- Short-lived adhesions
- Transform into focal adhesions
- Formed along lamellipodial protrusions (induced by Rac1)

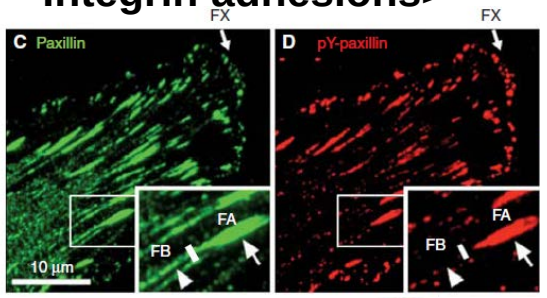
<FAs by Vinculin, Tenascin>

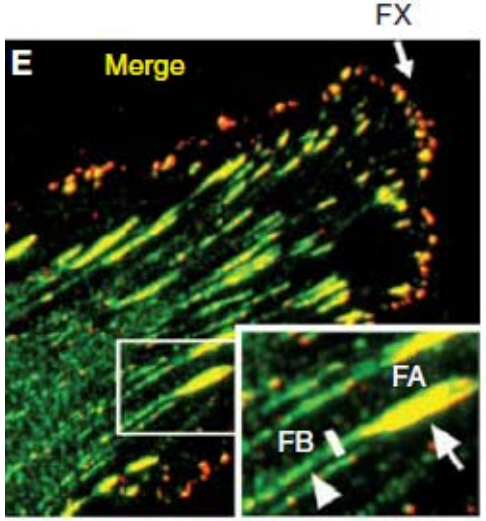


<Major forms of Integrin adhesions>



<Major forms of Integrin adhesions>





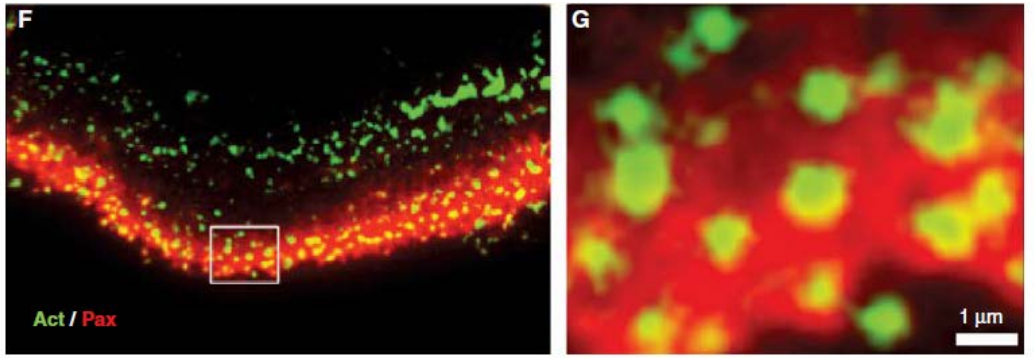
Elongated fibrillar adhesions (FB)

- Enriched under the central areas of cells
- Formed mainly along the matrix fibrils (FN)

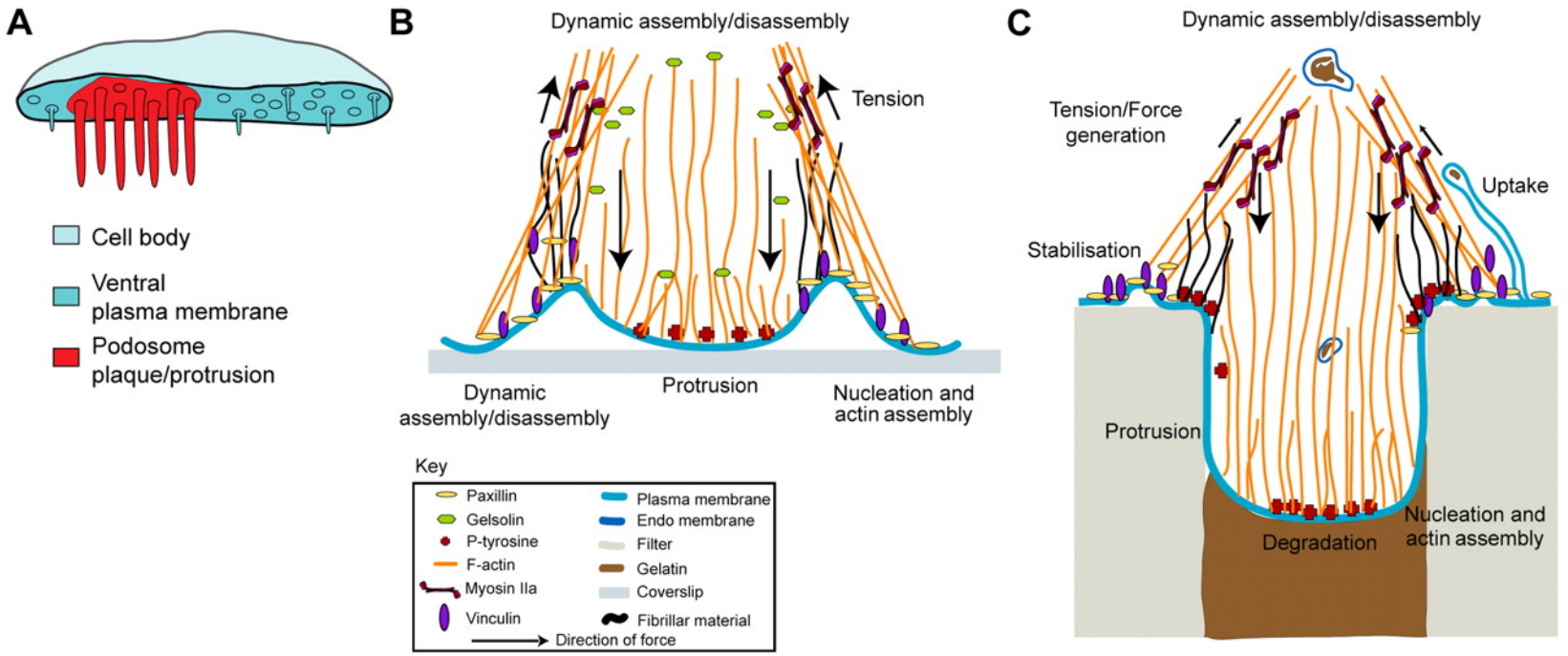
Podosomes or invadopodia

- Small, ring-like adhesions formed around actin bundle (apparently anchoring it to the membrane or thin membranous protrusions associated with actin-cortactin core, respectively)
- Podosomes are prominent in different monocytic derivatives (osteoclasts, macrophages, dendritic cells)
- Invadopodia are partially characteristic of invasive, transformed cells that invade by degrading the ECM

<Podosomes>



Models of protrusive podosomes from quantitative EM data.



Christian Gawden-Bone et al. J Cell Sci 2010;123:1427-1437

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Receptors for ECM

Integrins

Syndecan family

CD44

RHAMM

EBP

Integrins

Heterodimeric transmembrane protein

Composed of α and β subunits

18 α & 8 β subunits have been identified so far

Pair each other in a variety of combinations (Form heterodimers with one α & one β subunit)

- **Give rise to a large family that recognizes specific sequences on the ECM molecules**

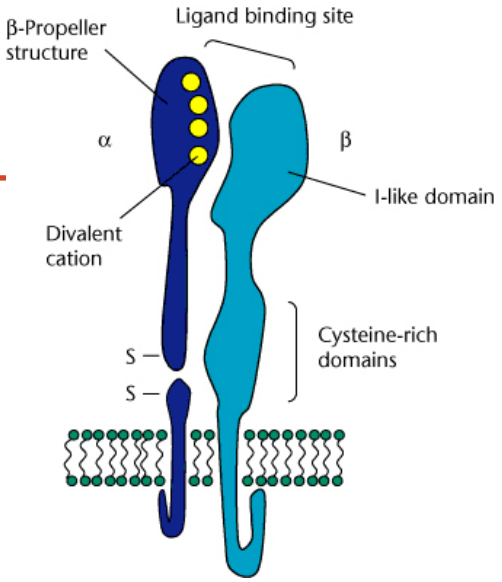
The extracellular domain of the α subunits contains four regions that serve as binding sites for divalent cations.
-> appear to augment ligand binding and increase the strength of the ligand-integrin interactions

Dual physiological function

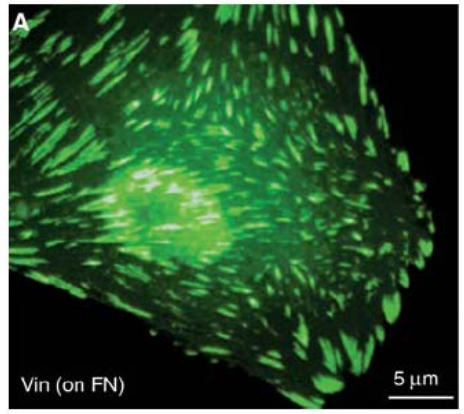
- **Physical & structural role**
- **Sensing & signaling role**

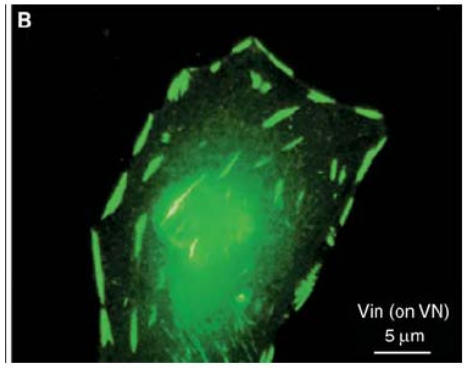
Central role in the formation, maturation, and function of variety of cell adhesions

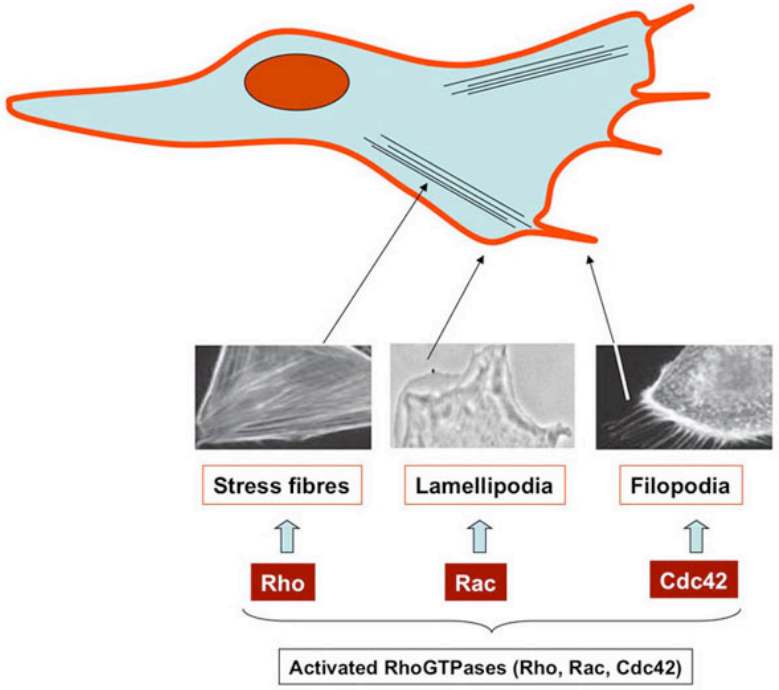
Different cell response to ECM
: cells adhering to FN spread much more than those adhering to VN and their Fas (Focal Adhesions) are located throughout the ventral membrane.
: more peripheral distribution of FA on VN

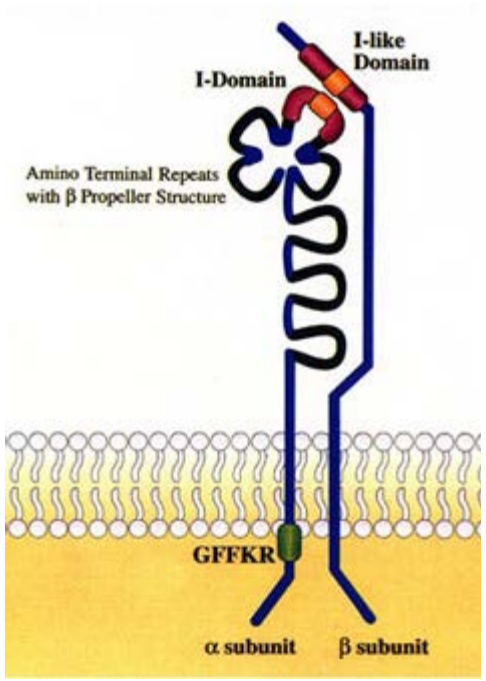


Vinculin on FN (A) and VN(B)

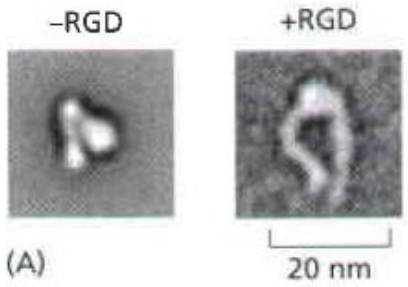


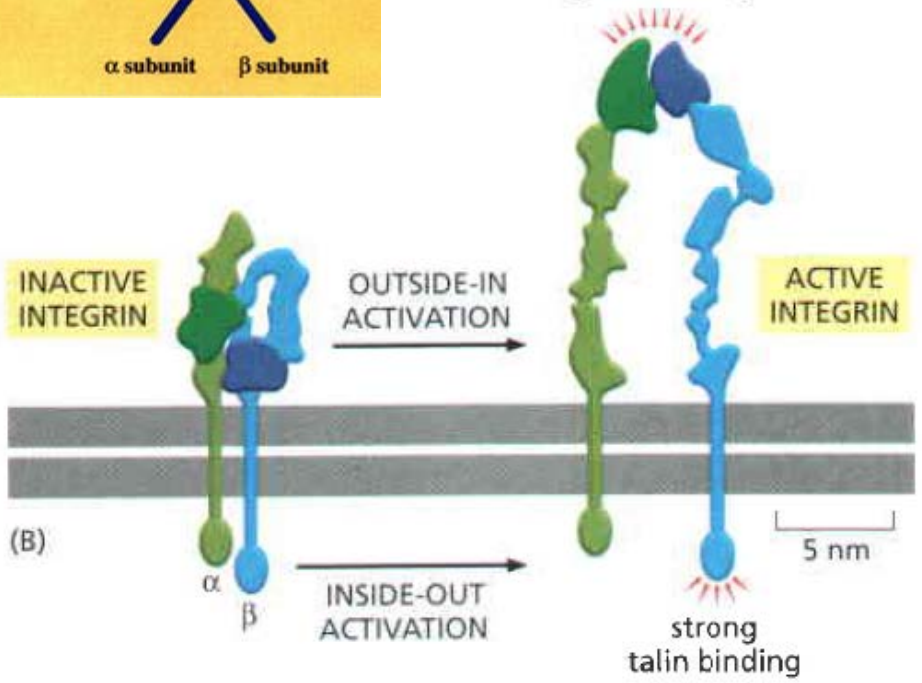


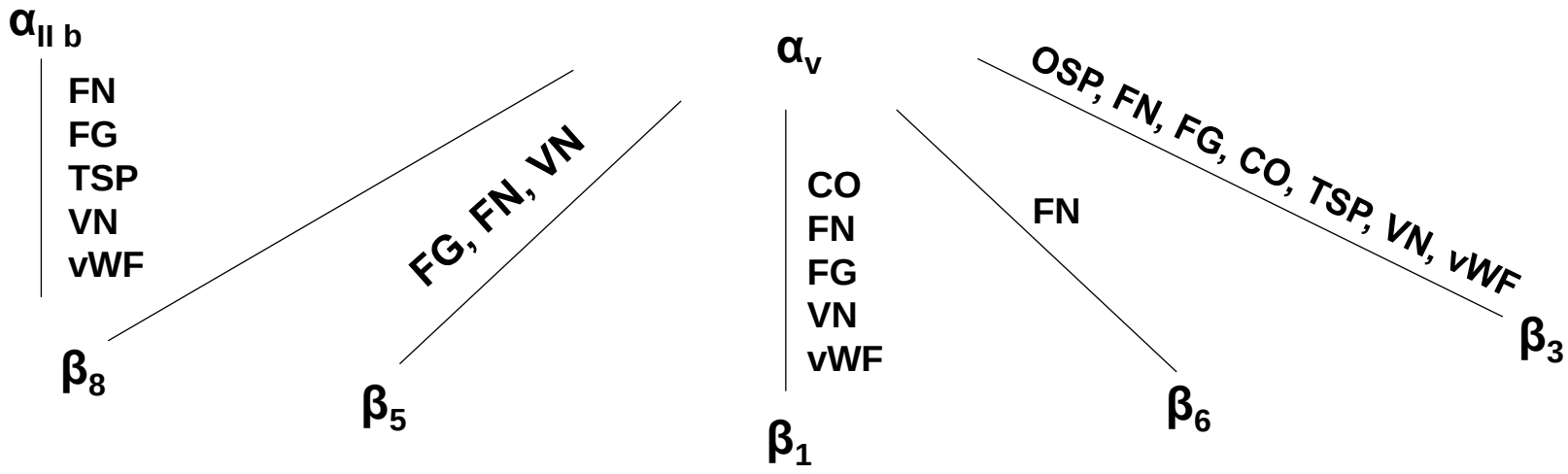


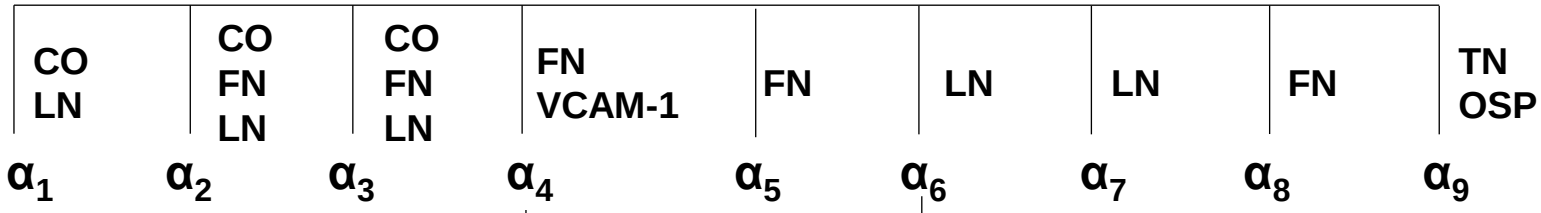


If the traction force is increased, the attached adhesion grows: relaxation of tension leads to its dissociation.

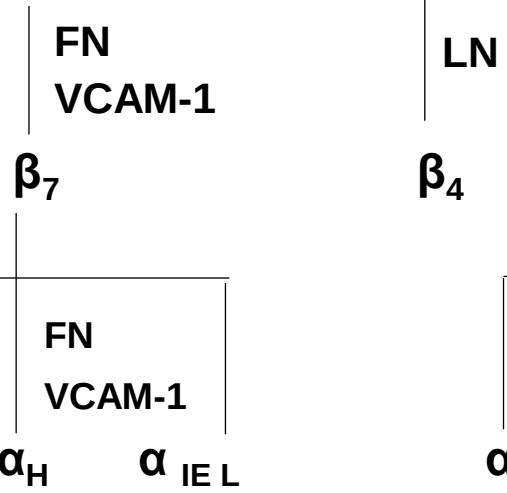


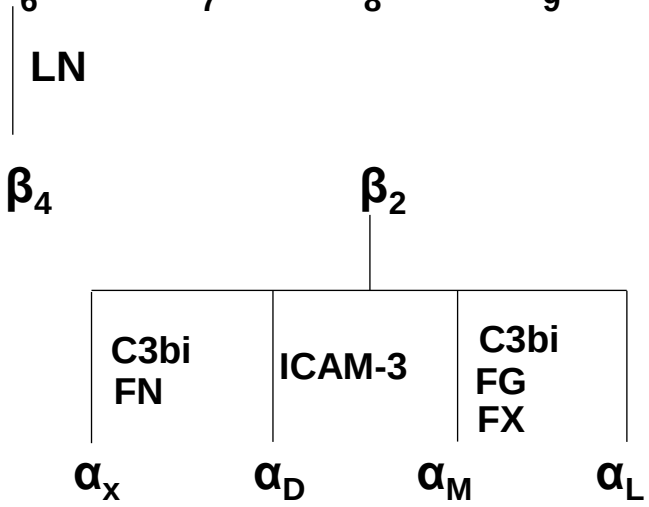






Fibronectin
Fibrinogen
Thrombospondin
Vitronectin
von Willebrandt factor
Collagen
Osteopontin
Laminin





Syndecan family

Proteoglycan receptor for ECM

Interact with the matrix via chondroitin-, dermatan-, and heparan- sulfate glycosaminoglycans

Also associate with the cytoskeleton promoting intracellular signaling events -> cytoskeletal reorganization through activation of Rho GTPases

CD44

Carries chondroitin sulfate and heparan sulfate chain on ECD.

Undergo tissue-specific splicing & glycosylation
-> yield multiple isoforms

Primary ligand- hyaluronan

Interact with other ECM matrix molecules

- FN, LN, Col IV, and Col XIV

Bind to the widely distributed GAG hyaluronan

Stimulate…

- Cell adhesion
- Proliferation
- Migration
- Invasion
- Matrix assembly

CD44 transmembrane adhesion receptor that can be cleaved from the cell surface to become a component of ECM

RHAMM (Receptor for hyaluronate mediated motility)

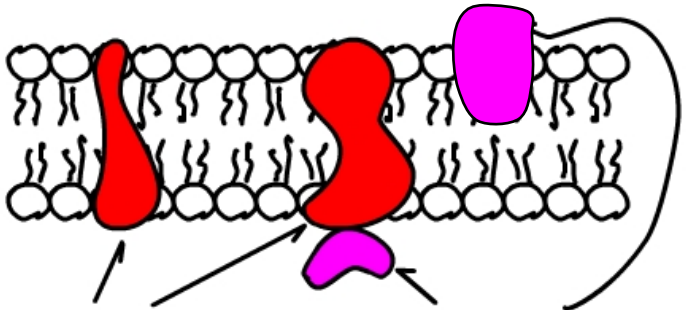
Cell-associated, non-integral proteoglycan

Lack transmembrane domain
-> indirectly activate intracellular signaling through interactions with transmembrane ECM receptors (such as integrins or CD44)

Bind to the widely distributed GAG hyaluronan

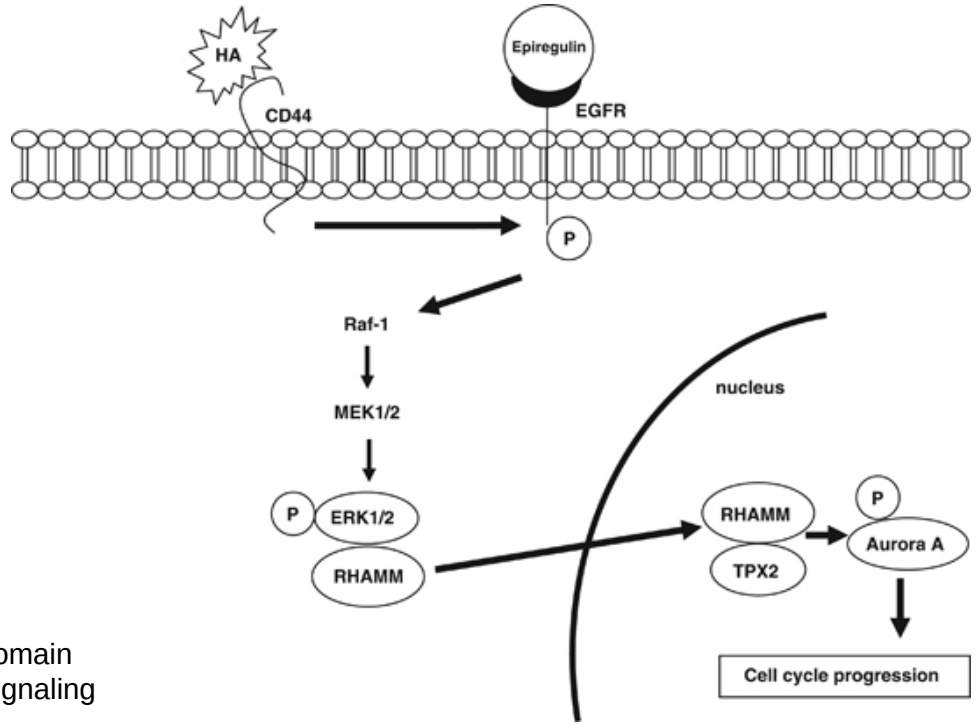
Stimulate…

- **Cell adhesion**
- **Proliferation**
- **Migration**
- **Invasion**
- **Matrix asse**



Integral Protein
- Transmembrane domain
- Direct intracellular siganling

Peripheral Protein
- No transmembrane domain
- Indirect intracellular signaling



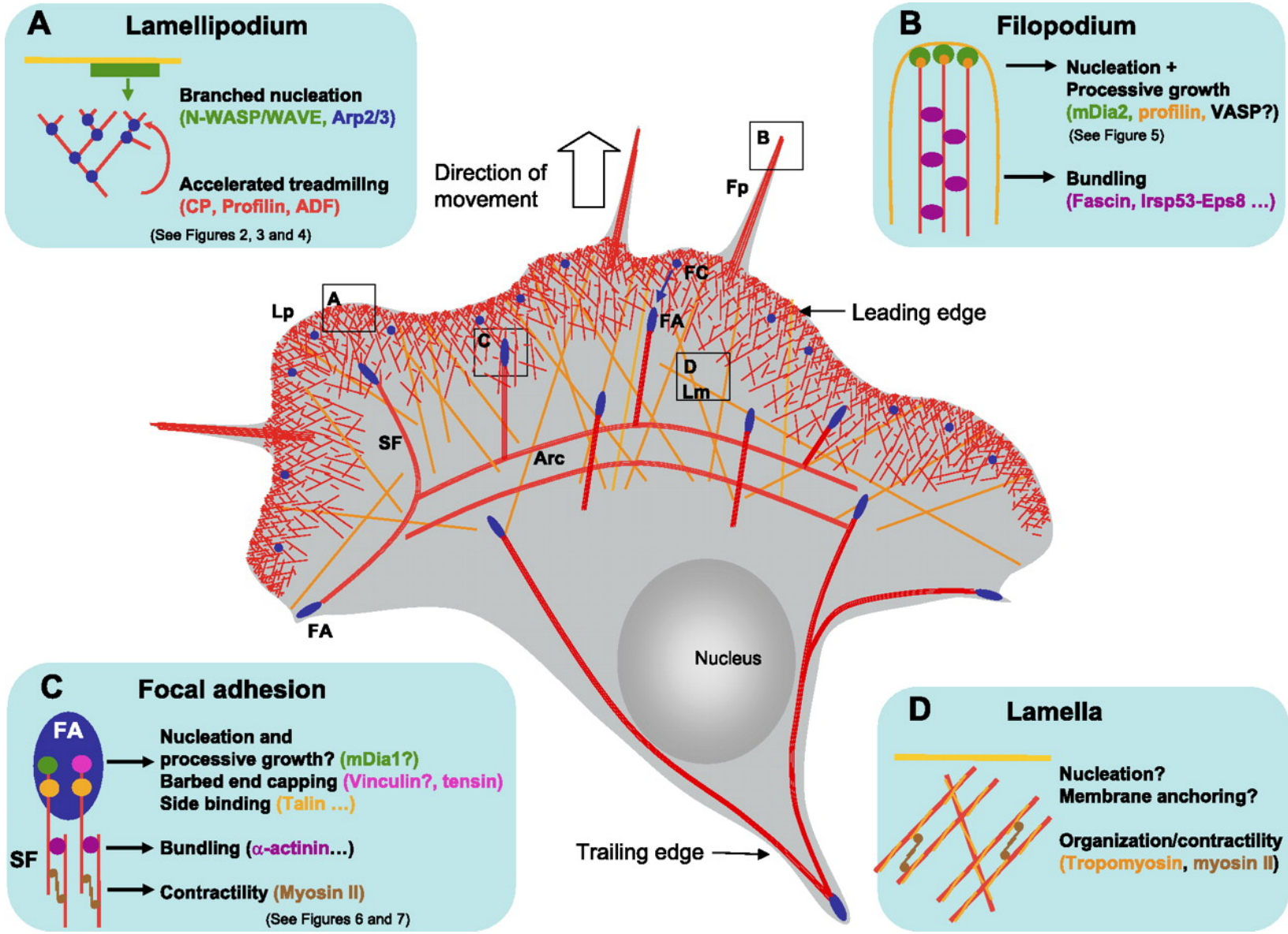
EBP (Elastin-binding protein)

Recognizes GXXPG sequence of elastin, laminin, fibrillin, and peptides derived from these ECM

Forms ELR (Elastin/Laminin Receptor) complex with cathepsin A, neuraminidase 1
-> implicated in mechano-transduction

ELR binding to proteolytically cleaved peptides
-> induce migration and/or proliferation of keratinocytes, fibroblasts, endothelial cells, and monocytes.

Cell Movement



Assembly & Remodeling of Integrin Adhesions

Lamellipodia

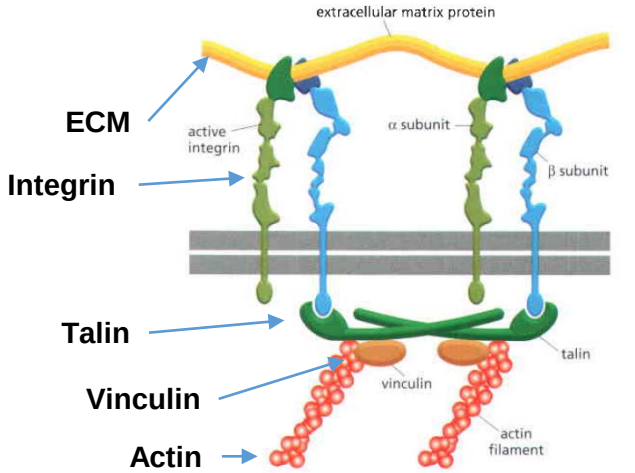
Local complexes

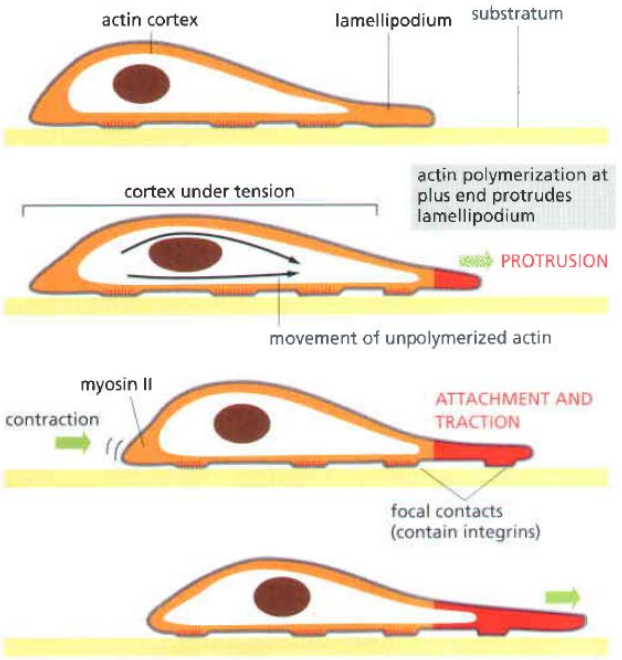
- Earliest integrin-containing structures
- 100nm in diameter
- Including integrin, talin, paxillin
- Binding of vinculin -> talin
  - Triggers clustering of activated integrin
  - Associate with actin through the vinculin tail
  - Strengthening actin-integrin link
  - Drive growth into larger focal complexes

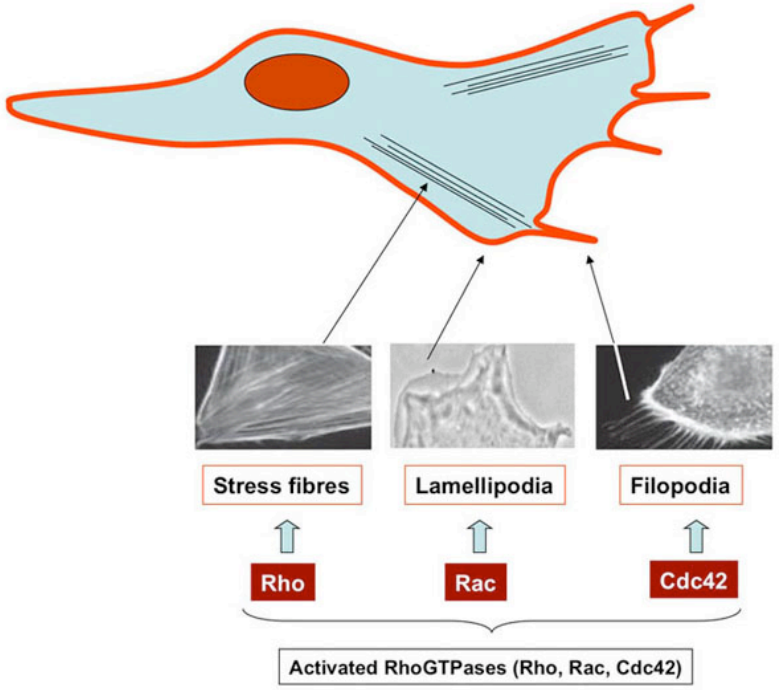
Lamella

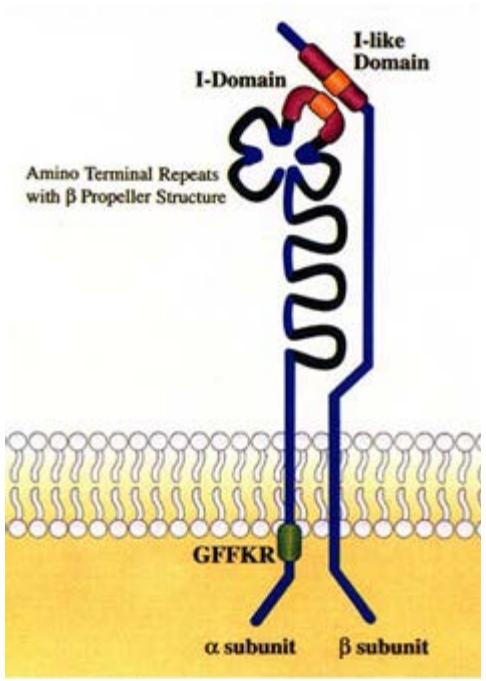
Tropomyosin & myosin II are prominent

Located ~2-4um from the leading edge









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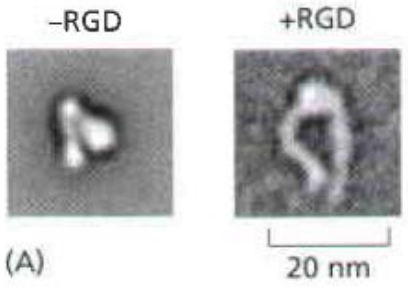
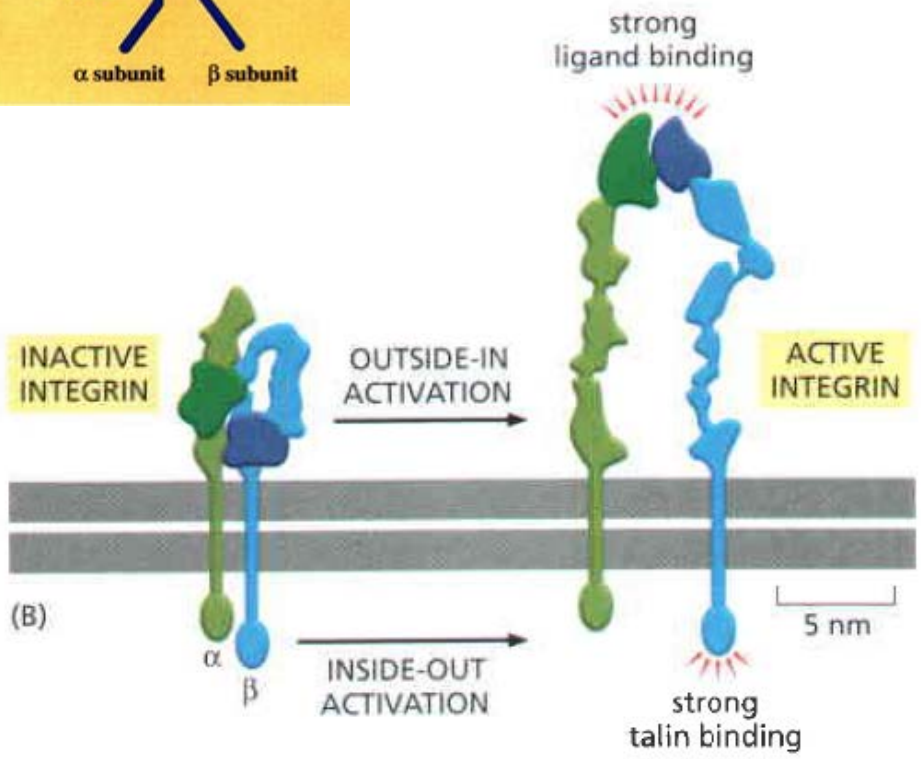




Two-way Dialogue between Cell-ECM

Matrix itself can reciprocally regulate cell behavior

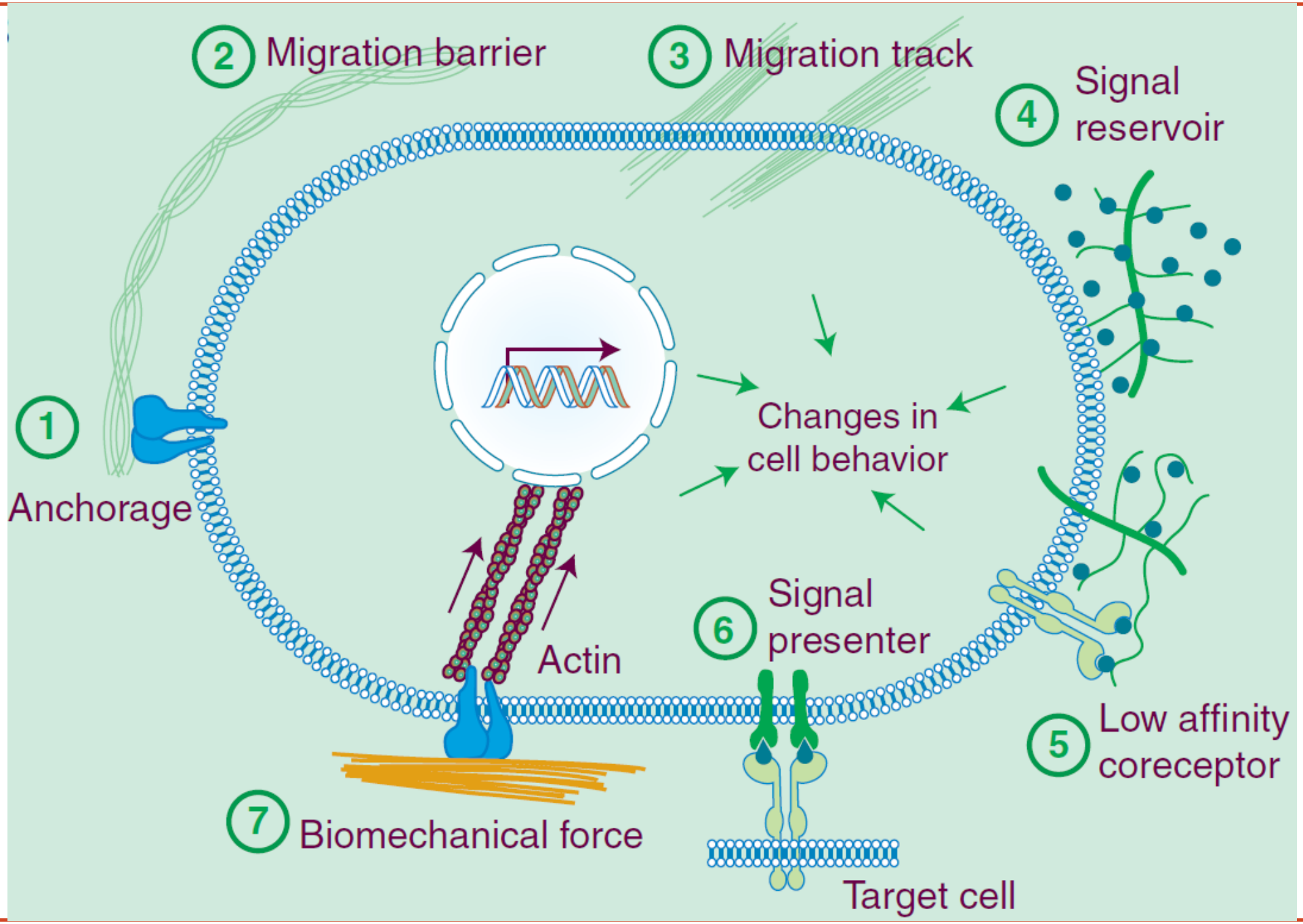
Role as a reservoir for GF, cytokines

Stiffer matrices evoke..

- **Larger focal adhesion**
- **Increased intracellular contractility**
- **Durotaxis: cells move toward areas of greater substrate stiffness**

Cells can reorganize a random meshwork of collagen fibrills into oriented fibrils
-> promote their migration along the reorganized fibrils

- **Contact guidance: the propensity to migrate along oriented patterns sush as fibrils or grooves**


2 Migration barrier
3 Migration track
4 Signal reservoir
1 Anchorage
Changes in cell behavior
Actin
6 Signal presenter
5 Low affinity coreceptor
7 Biomechanical force
Target cell

Anoikis (Redding & Juliano 2005)

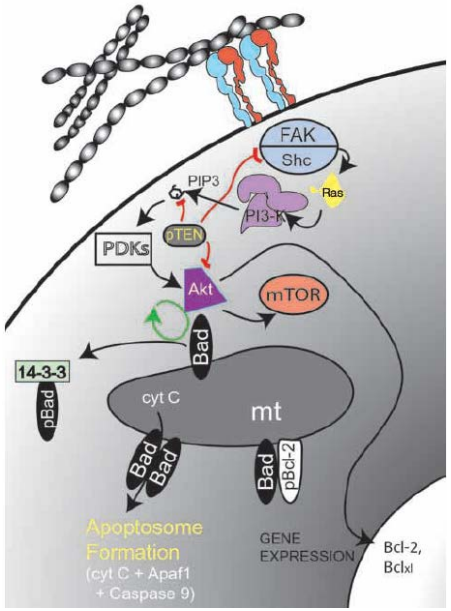
Apoptosis promoted by cell detachment from the matrix

No adhesion -> FAK inactivation
-> Loss of FAK-mediated PI-3K & Src
-> BAX activation & translocation -> apoptosis

- Loss of integrin-mediated adhesion
  :movement of pro-apoptotic protein BAX
  from cytoplasm -> mitrochondria

Role of ECM on cell survival:

- ECM is reservoir of GFs
- Laminin-5, tenascin-C, & Decorin has EGF-like repeats
  -> binds & activate EGFR -> Survival signal



EOD