«Αρθρώσεις-αρθρικός υμένας-Αρθρικός χόνδρος (Δομή και φυσιολογία)»

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ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΑΡΙΣΤΟΤΕΛΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΟΝΙΚΗΣ

Πανεπιστήμιο Ιωαννίνων

ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ

Πανεπιστήμιο Κρήτης

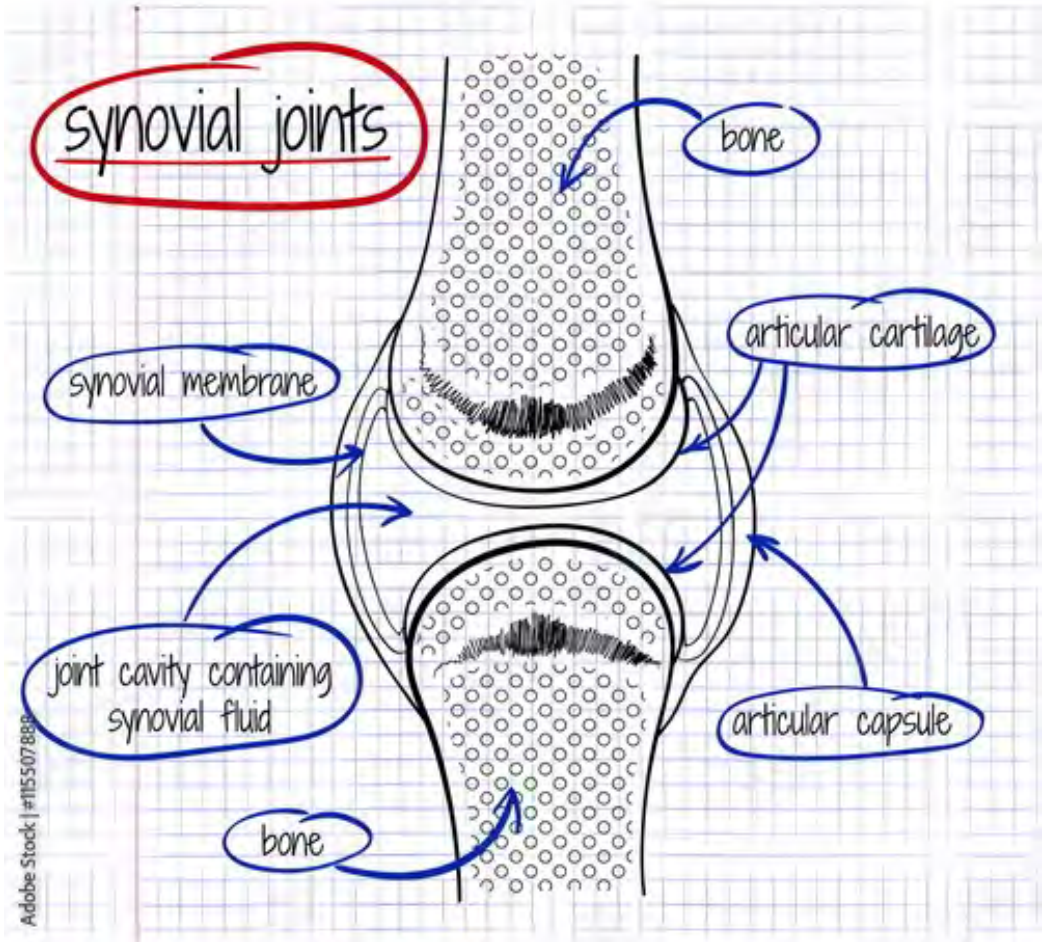
4ο ΔΙΑΠΑΝΕΠΙΣΤΗΜΙΑΚΟ ΠΡΟΓΡΑΜΜΑ ΕΚΠΑΙΔΕΥΣΗΣ ΣΤΗ ΡΕΥΜΑΤΟΛΟΓΙΑ 2022-24

Εκπαιδευτικοί στόχοι

- Βασικές γνώσεις: Αρθρικός υμένας- Αρθρικός χόνδρος
  - κύτταρα – matrix
  - λειτουργία – παθολογία

Normal synovial joint

- The synovial joint comprises opposing bones with articular surfaces that are covered by cartilage.
- The main protein in bone is **type I collagen**, whereas cartilage comprises mainly **type II collagen and proteoglycan molecules.**
- The non-articulating surfaces of synovial joints are lined by a thin adventitious layer known as the synovium.
- The entire structure is enclosed by **a fibrous capsule and, together with ligaments, muscles and tendons**, the fibrous capsule confers strength and stability to the joint.



Joint Remodeling - Homeostasis

- The joint is a dynamic environment that is subject to **minor trauma** continually — owing to **movement** and, in some joints, **compression** due to weight bearing — and is therefore subject to continued **wound healing and repair processes**.
- **Continual remodeling** of the articular cartilage and adjacent bone is therefore necessary, and this process requires a **balance of anabolic and catabolic** enzyme activity in both cartilage and bone.
- Carefully regulated **proteolytic enzymes** are responsible for maintaining the balance between anabolic and catabolic processes within the joint and cartilage.

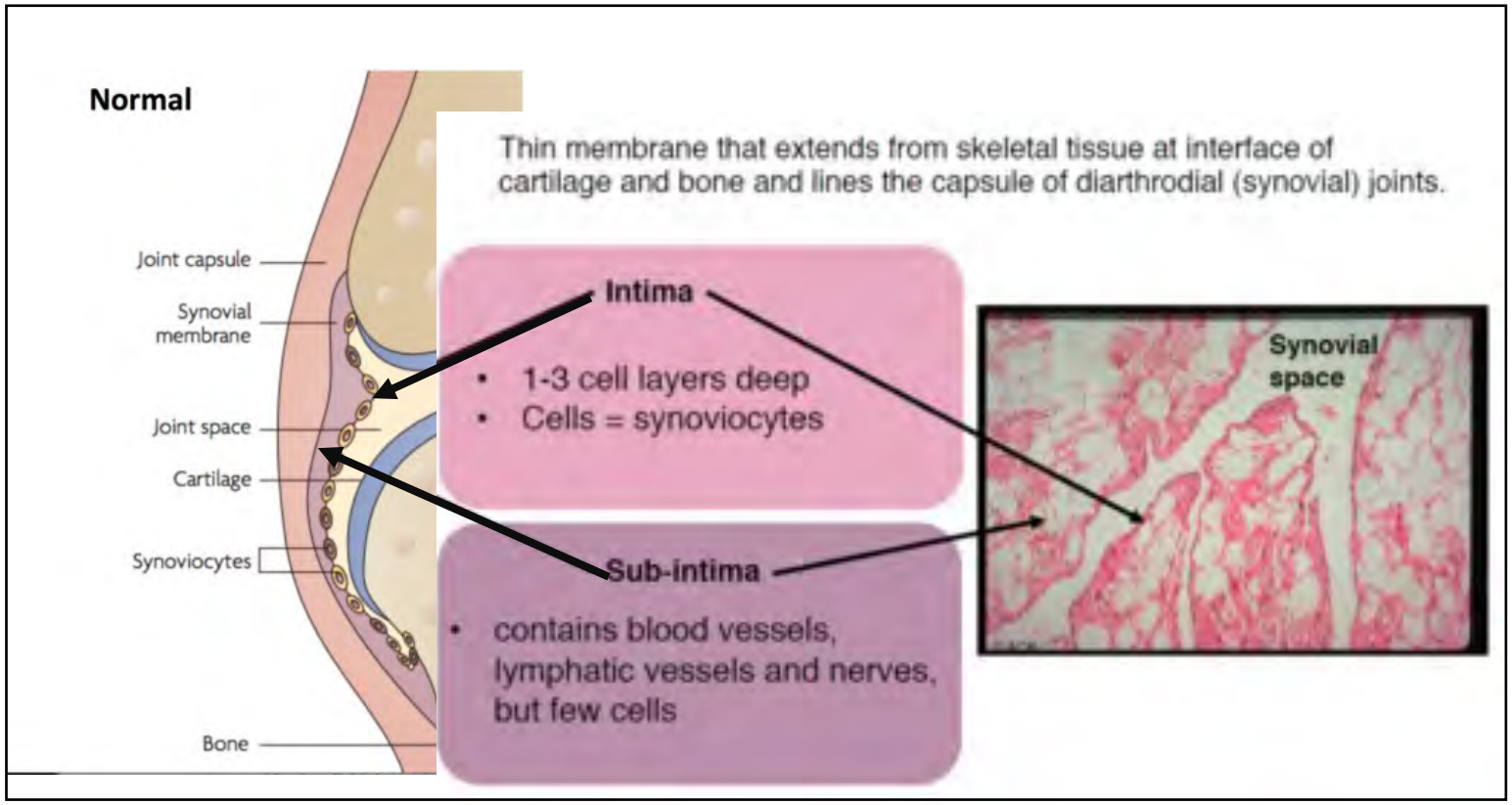
Nat Rev Rheumatol. 2017.

Function of the synovium

1. Maintenance of intact, non-adherent surface of the joint
    - Hyaluronan
        - Produced by FLS
        - Inhibits adhesion
        - “Trapped” in the synovium”

2. Cartilage lubrication
    - Lubricin

3. Chondrocyte nutrition



Function of the synovium

4. Control of synovial fluid (SF) volume - composition

SF= plasma + HA

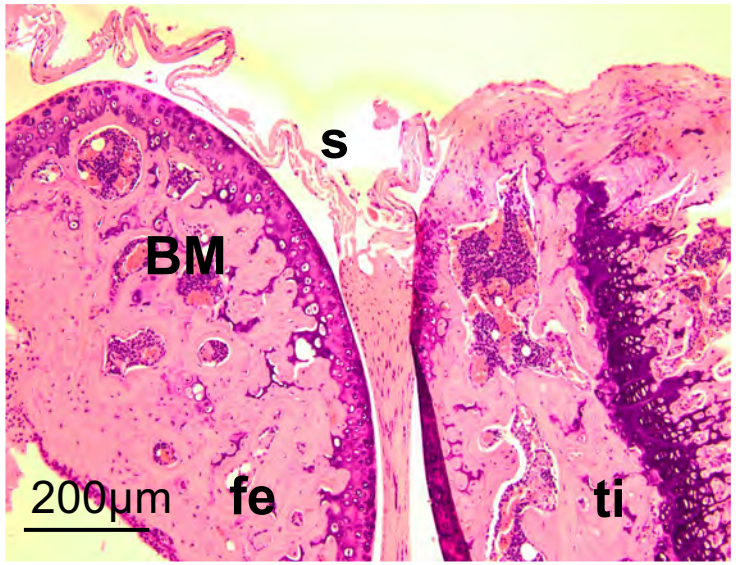
- SF Volume:
  - increased by hyaluronan
  - decreased by (–)ve hydrostatic pressure

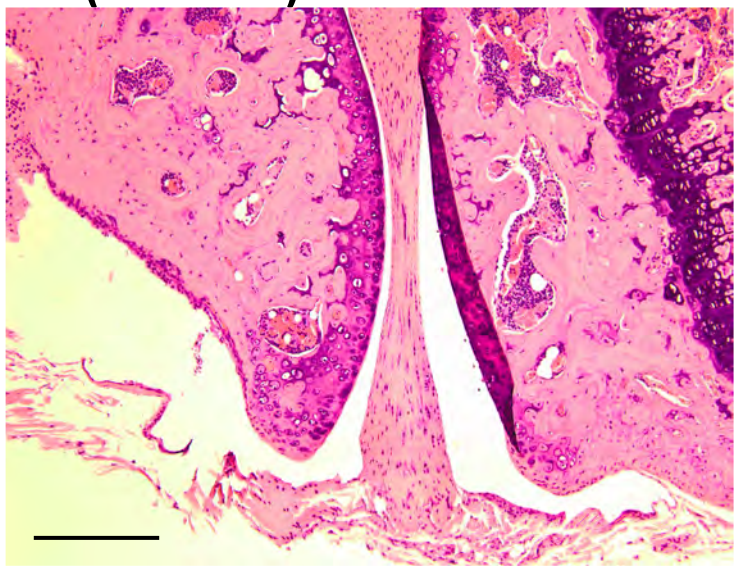
✓Mechanism of effusions

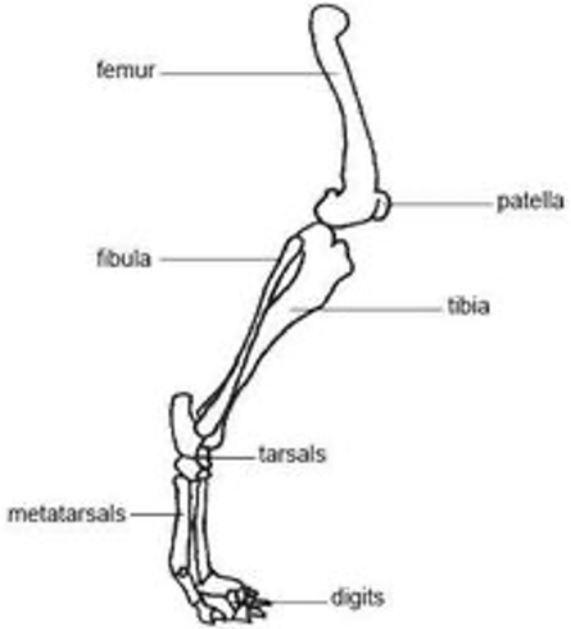
1. Mechanical irritation of FLS (*friction forces or debris of cartilage*) → ↑HA → ↑volume
2. Inflammation → increase vascular permeability → ↑ volume

H&E in FFPE mouse joint sections

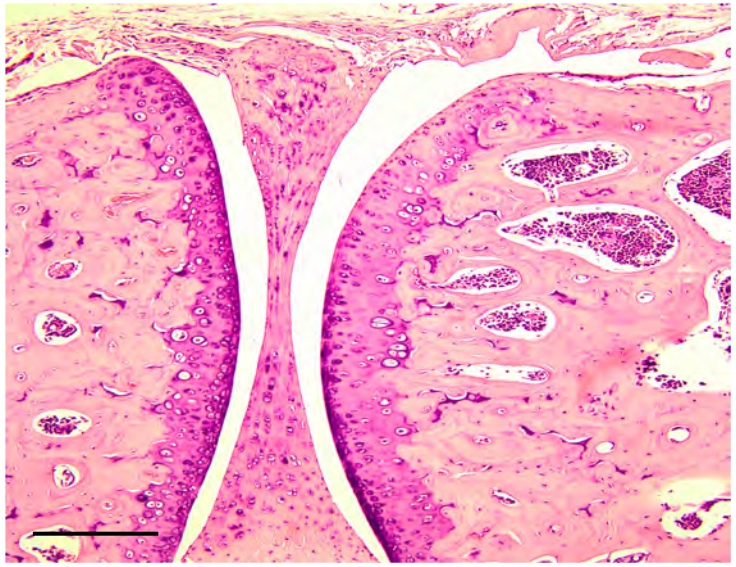
Normal limb (Score = 0)

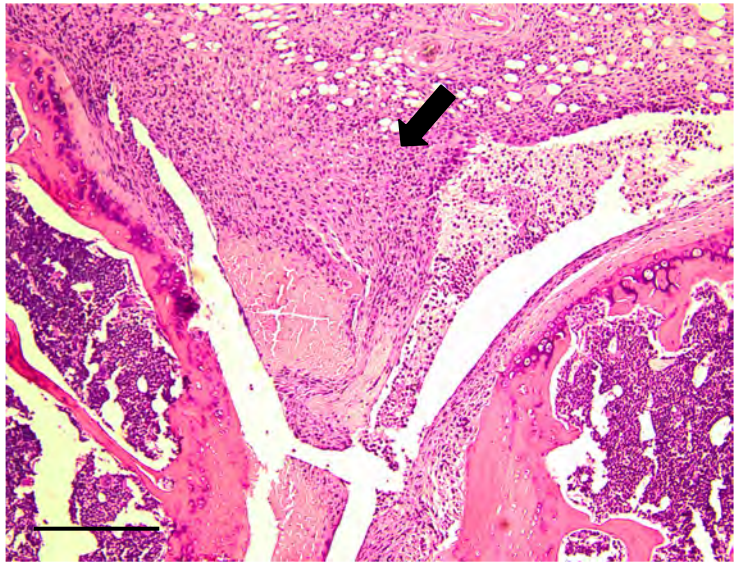






Arthritic limb (Score = 25)

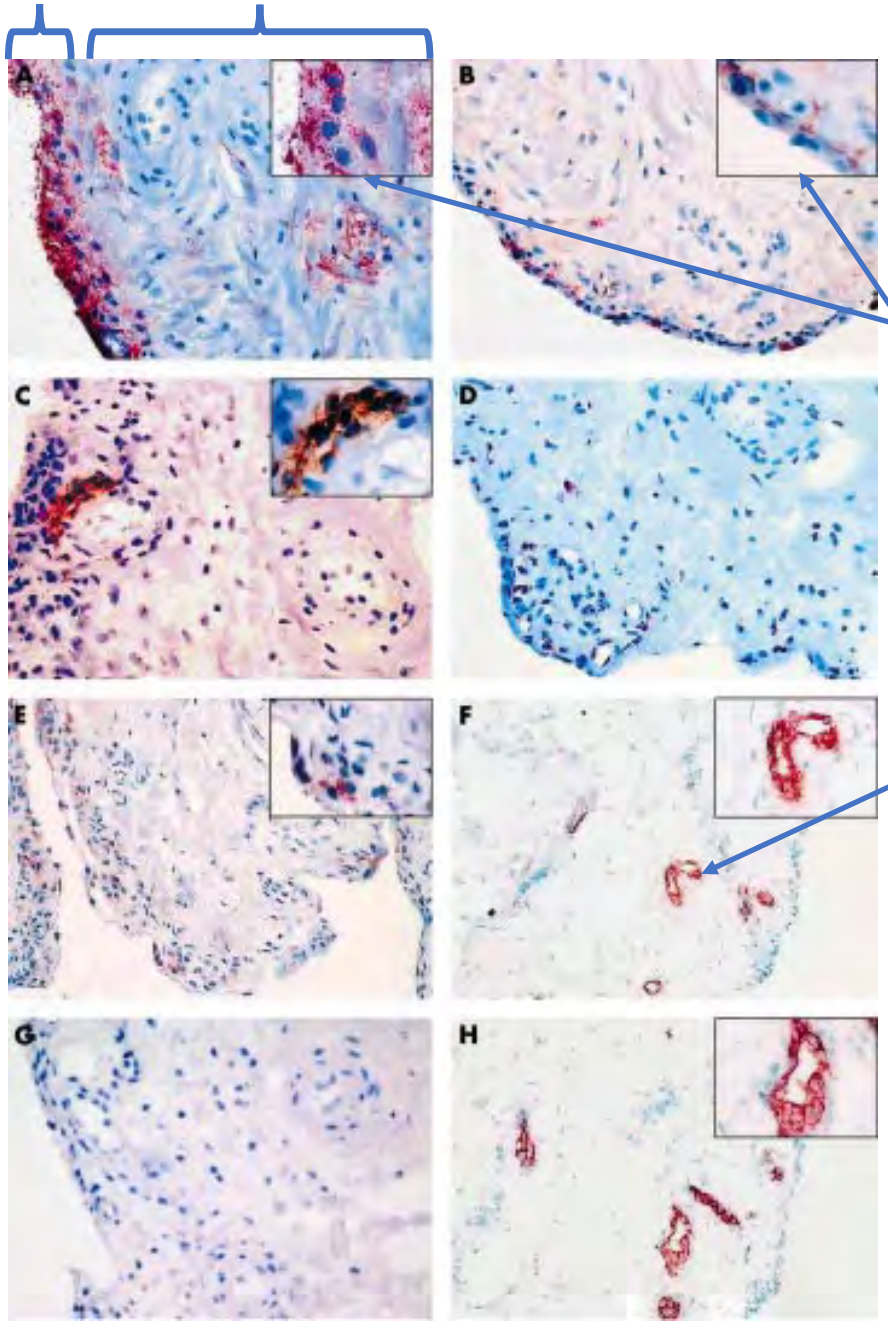




fe: femur
ti: tibia
BM: bone marrow
s: synovial membrane

Courtesy: Elpida Neofotistou

Normal synovium - Synovial cells

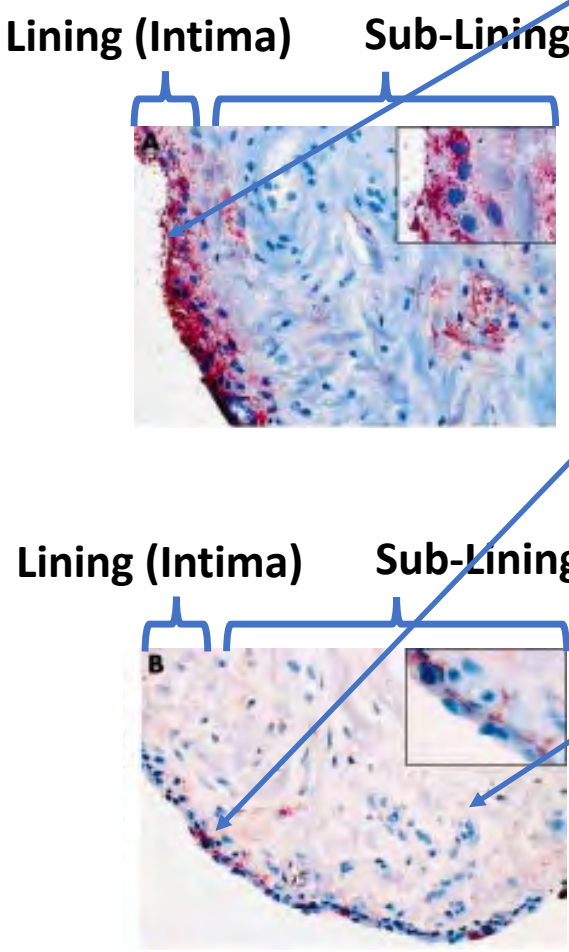


Immunohistochemical labelling of a representative **normal synovial** membrane stained with:

- **(A) anti-CD55 (fibroblast-like synoviocytes - FLS)**
- **(B) anti-CD68 (macrophages)**
- (C) anti-CD3 (T cells)
- (D) anti-IL1b
- (E) anti-interleukin 1 receptor antagonist (anti-IL1Ra)
- (F) anti-factor VIII (endothelial cells)
- (G) anti-RANKL
- (H) monoclonal antibody for OPG.

1. **Lining (Intima)** → **FLS & Mϕ**
2. **Sublining layers**

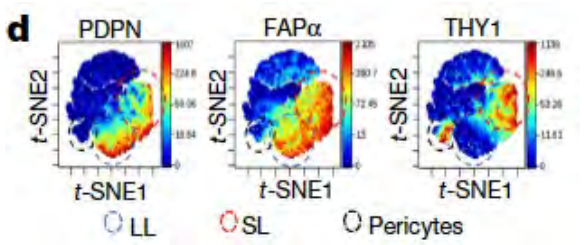
Normal synovium - Synovial cells
Lining (Intima)



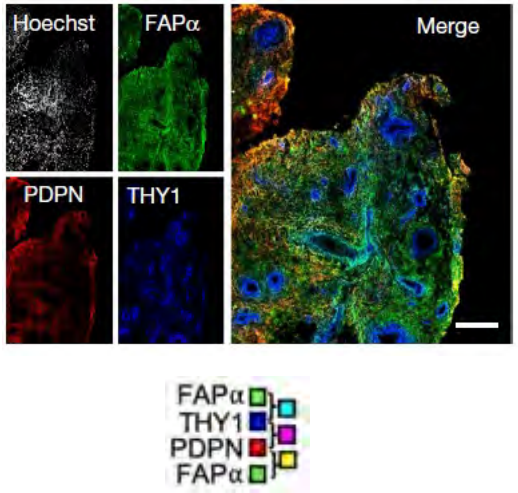
- **Fibroblast-like synoviocytes – FLS**
  - UDPGD (UDP-glucose to UDP-glucuronate **Hyaluronan production**)
  - CD55
  - ICAM1
  - VCAM1 **(allow PMNs to traffic to SF BUT NOT monocytes**)
- **Macrophages**
  - CD163
  - CD68
  - CD14
  - FcγRI (Ig receptor (ACPA/RF) **local inflammation**)
- **Matrix**
  - No basement membrane
  - **Rich in hyaluronan**
  - **Lubricin (glycoprotein)**

Distinct FLS subsets drive inflammation & damage in RA: Lining & sub-lining FLS

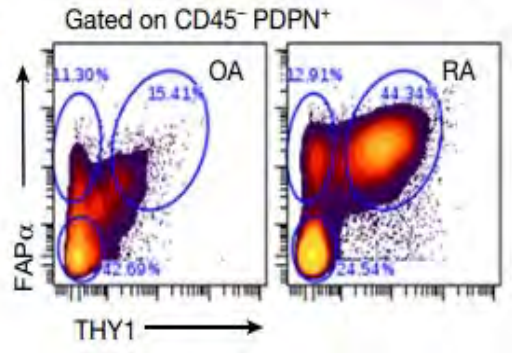
Human RA synovium: 3 sybtypes of FLS



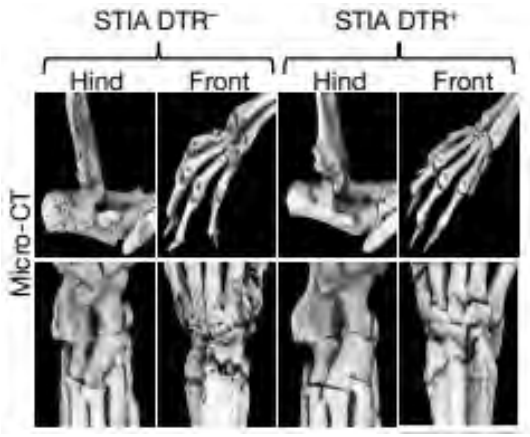
CyToF



Expansion of THY1+ FLS in human RA synovium



FLS depletion ameliorates synovitis in RA mouse models



Adoptive FLS transfer experiments documented differential function of sub-lining (SL) & lining (LL)

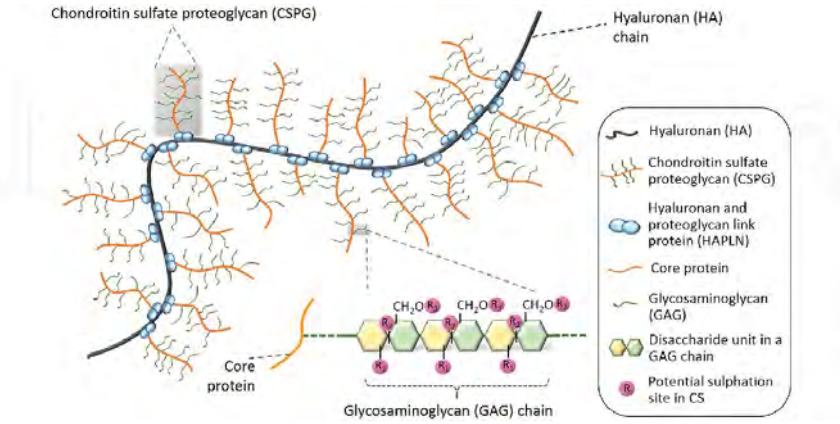
- ✓ THY1 (CD90) + (SL): INFLAMMATION
- ✓ THY1 (CD90) – (LL): DAMAGE

Croft et al. Nature 2019, 570: 246–251

Major molecules of SF-synovium: Hyaluronan (HA) & Lubricin (PRG4)

- ✓ **Hyaluronan** (HA) [simplest glycosaminoglycan (negatively charged polysaccharides] produced predominantly by fibroblast-like synoviocytes
  - **Inhibits synovial adhesion**
  - Lubrication
  - It protects joint tissues inhibiting the stimulatory effect of pro-inflammatory cytokines on the production of matrix metalloproteinases

- ✓ **Lubricin [proteoglycan 4 (PRG4)]** (glycoprotein) is secreted by synoviocytes as well as chondrocytes in the superficial layer of articular cartilage.



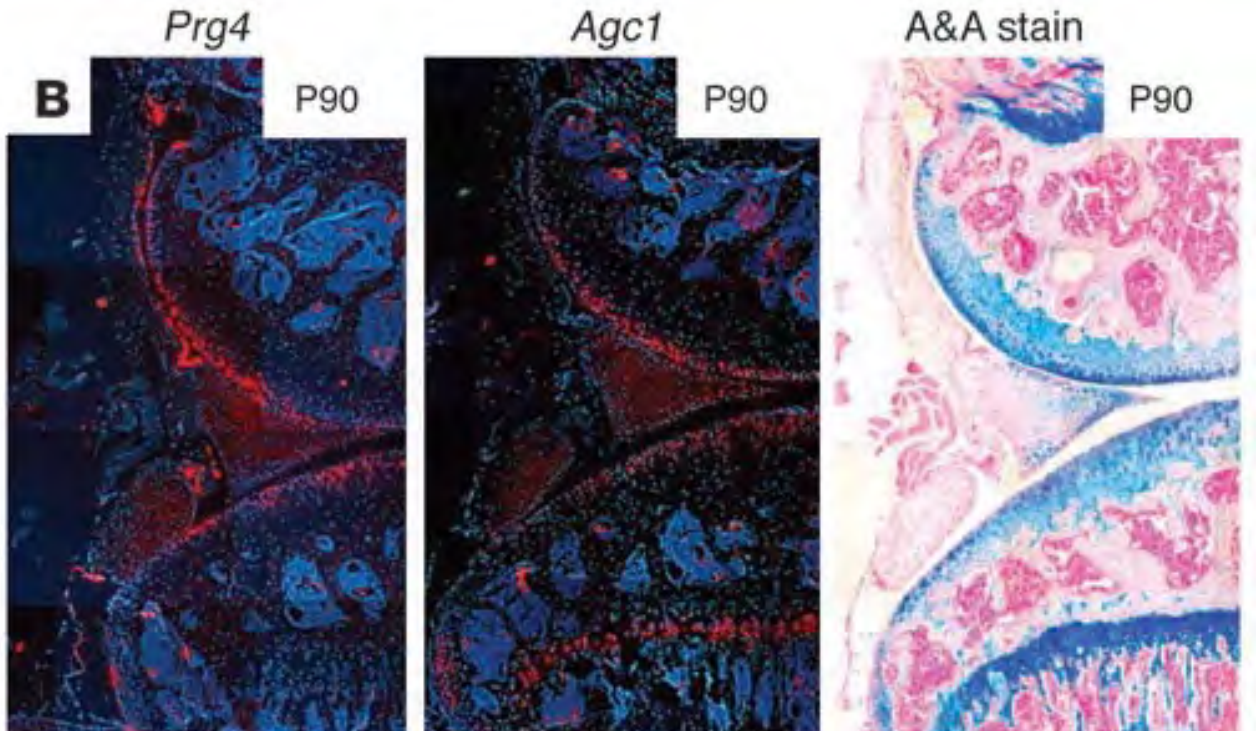
TISSUE ENGINEERING: Part A
Volume 16, Number 4, 2010

Lubricin

- Lubricin, a product of the gene proteoglycan 4 (PRG4) is a major component of synovial fluid
- It is an O-linked glycosylated protein that is highly expressed by synoviocytes
- Participates in the boundary lubrication of synovial joints

3-month-old (P90) mouse

Alizarin red and Alcian blue–stained (A&A-stained)



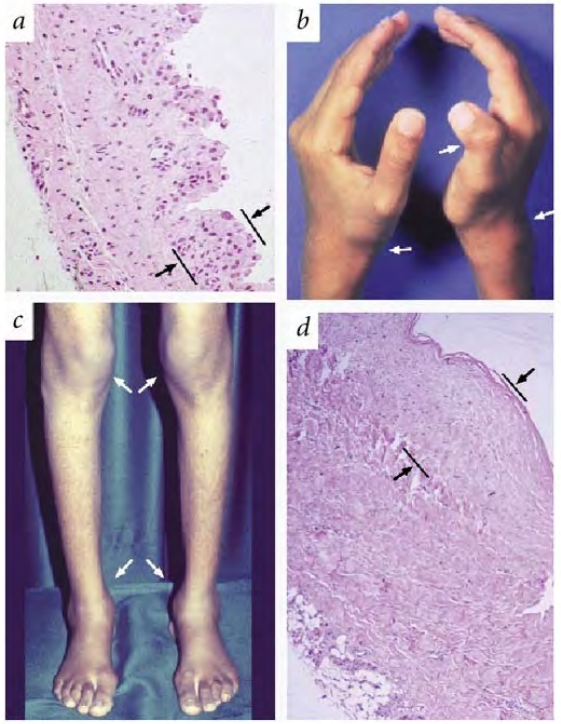
J. Clin. Invest. 115:622–631 (2005).

Lubricin mutations

HUMAN

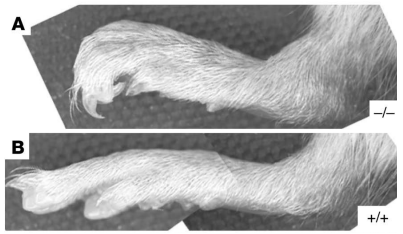
Congenital or early-onset camptodactyly (CAMP)

- ✓ loss-of-function mutations in PRG4
- ✓ childhood-onset noninflammatory arthropathy
- ✓ synovial hyperplasia.
- ✓ pro-gressive coxa vara deformity
- ✓ noninflammatory pericardial effusion.

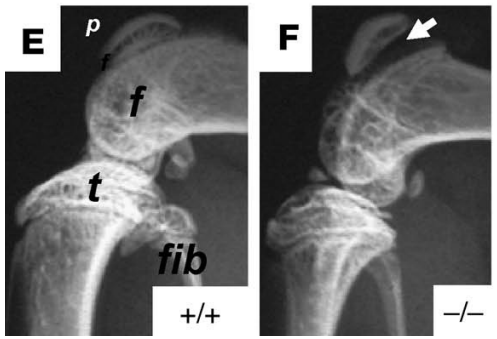


Prg4–/– mice.

Hind paws of 6-monthold Prg4–/– (A) and wild-type (B) mice.



- Lateral knee x-ray of a 4-month-old wild-type mouse (E) or Prg4–/– mouse.
- Increased joint space between the patella and femur (arrowheads) and osteopenia of the patella, femoral condyles, and tibial plateau.

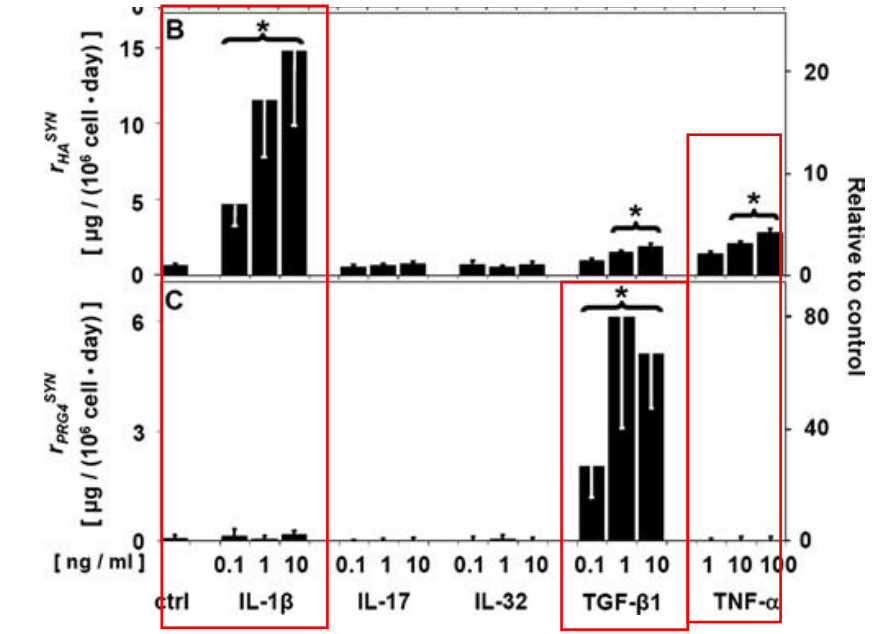


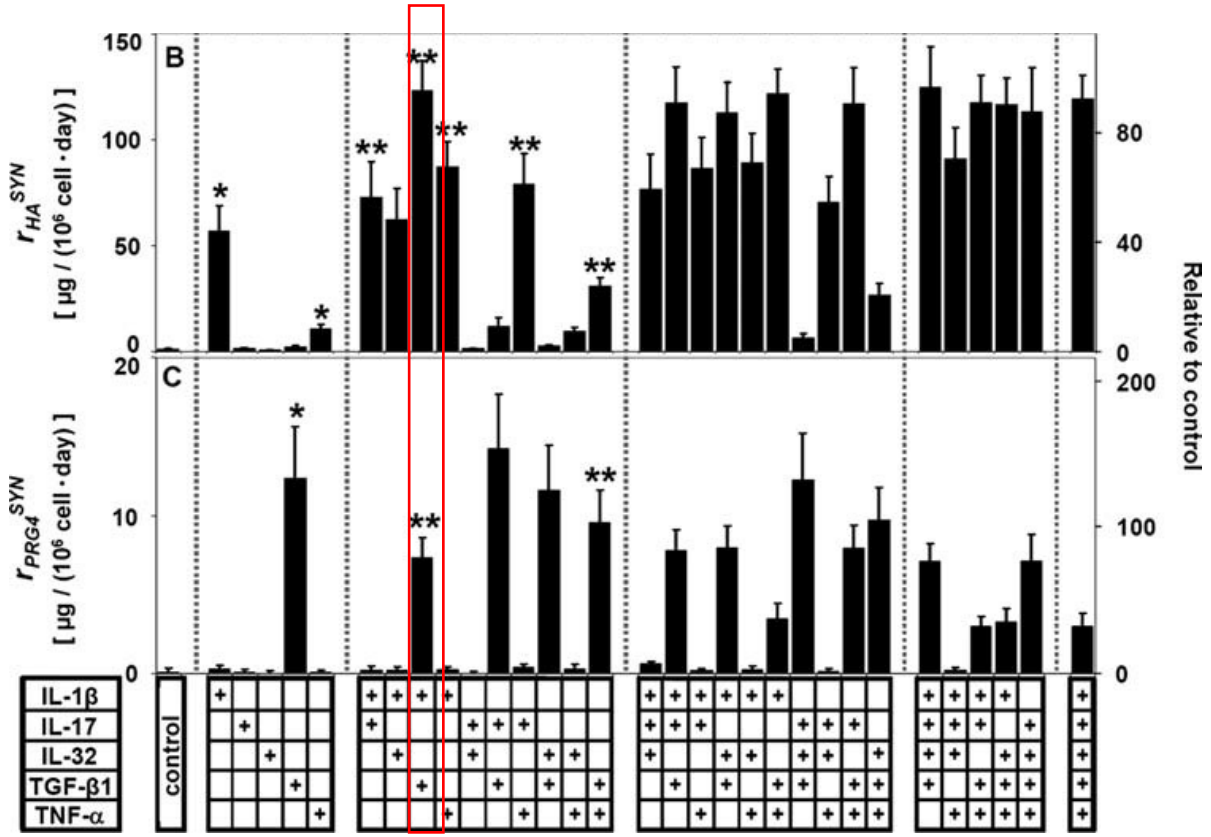
patella (p), femoral condyle (f), tibial plateau (t), and fibula (fib) (F)

J. Clin. Invest. 115:622–631 (2005).

Cytokines affects synthesis and molecular weight of HA and PRG4

- HA secretion rates were increased:
  - *40-fold by IL-1b
  - *80-fold by the combination of IL-1b + TGF-b1 or TNF-a + IL-17.
- PRG4 secretion rates were increased
  - *80-fold by TGF-b1
  - this effect was counterbalanced by IL-1b and TNF-a.

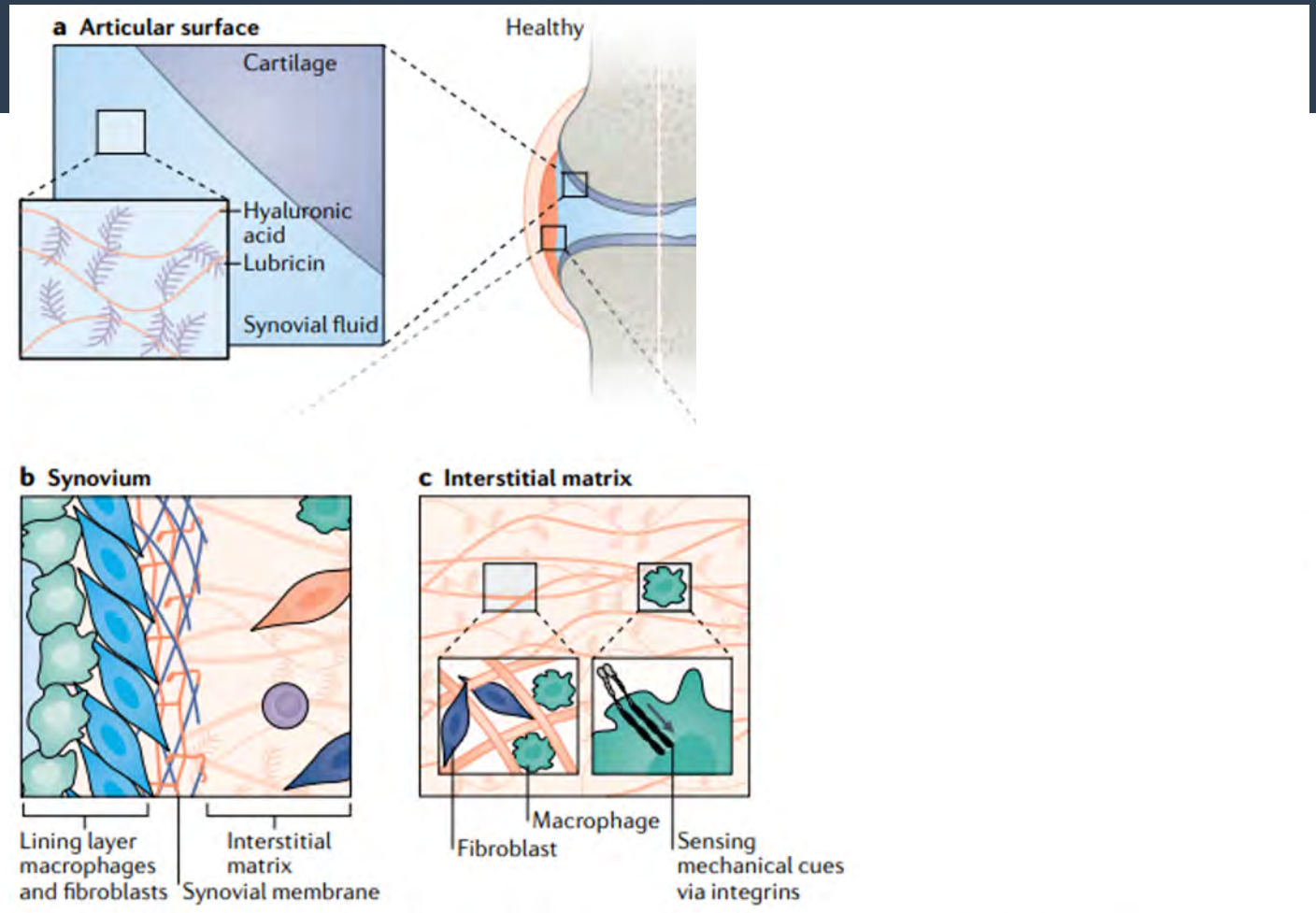




Role of Synovium in the maintenance of normal homeostasis in the synovial joint

- Expression of the protective **lubricin**
- Secretion of **matrix metalloproteinases (MMPs**) by FLSs
- **Immune sentinel roles** of resident **macrophages** and **FLSs**
- **Regulated entry and exit of leukocytes** involved in immune surveillance
- **Local regulati**on by cytokines and growth factors.

The tissue microenvironment: cartilage, synovium, matrix



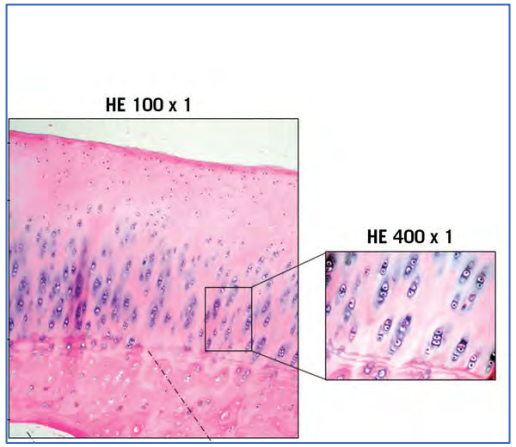
Physical properties, mechanical cues
Patterning of soluble factors
Direct cell signalling

Buckley et al. Nature Reviews Rheumatology 2021, 17:195-

Cartilage: generics

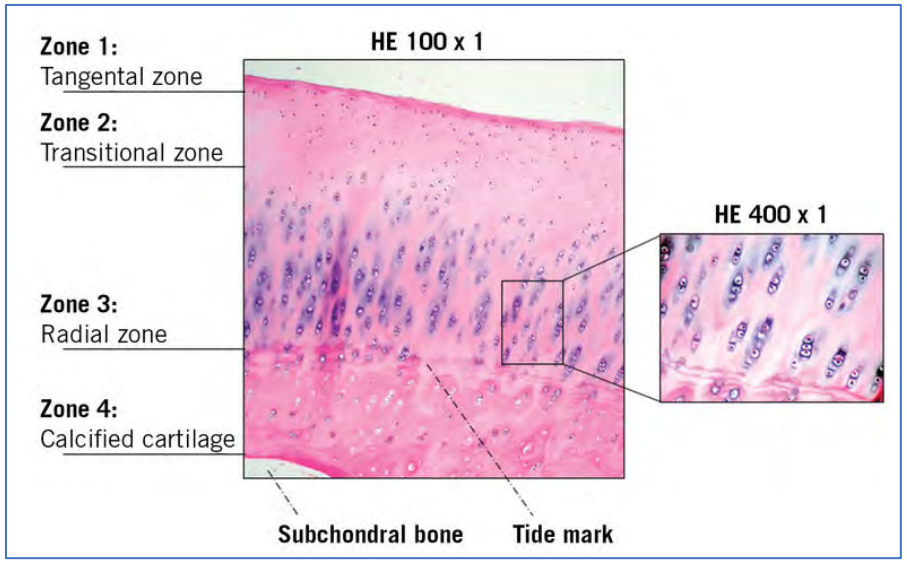
- In an articulating joint cartilage can be subjected to compressive and frictional forces several million times annually
- Cartilage is **avascular tissue** deriving its nourishment from **synovial fluid**, a filtrate of blood that has components added and removed by the joint capsule's intimal lining cells, the synoviocytes
- Adult **chondrocytes do not normally divide** in vivo and defects in cartilage surfaces are not self-repairing
- Therefore, for a joint to maintain its function throughout a lifetime of use, there must be biologic **mechanisms that help minimize damage** resulting from activities of daily living.

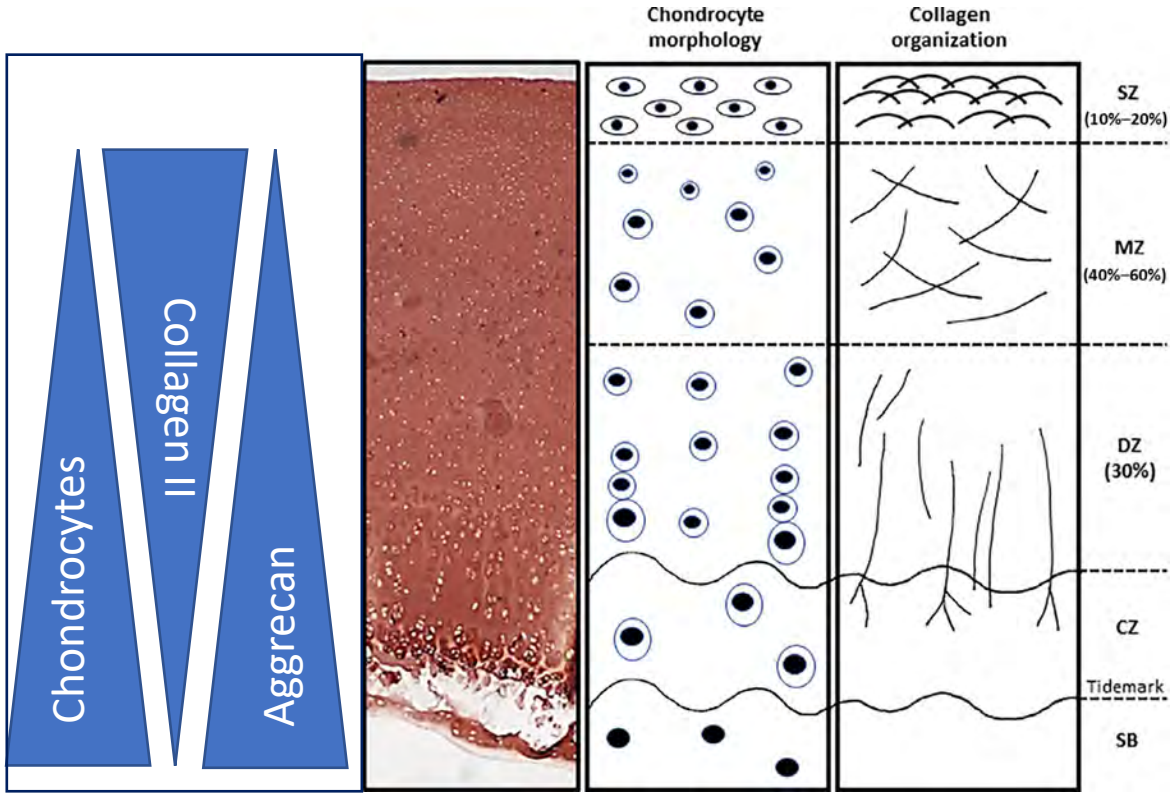
- Composition:
  - Water 70%
  - 90% of cartilage dry weight: collagen II & aggrecan
  - Chondrocytes:1-2% of the volume



Cartilage: Function

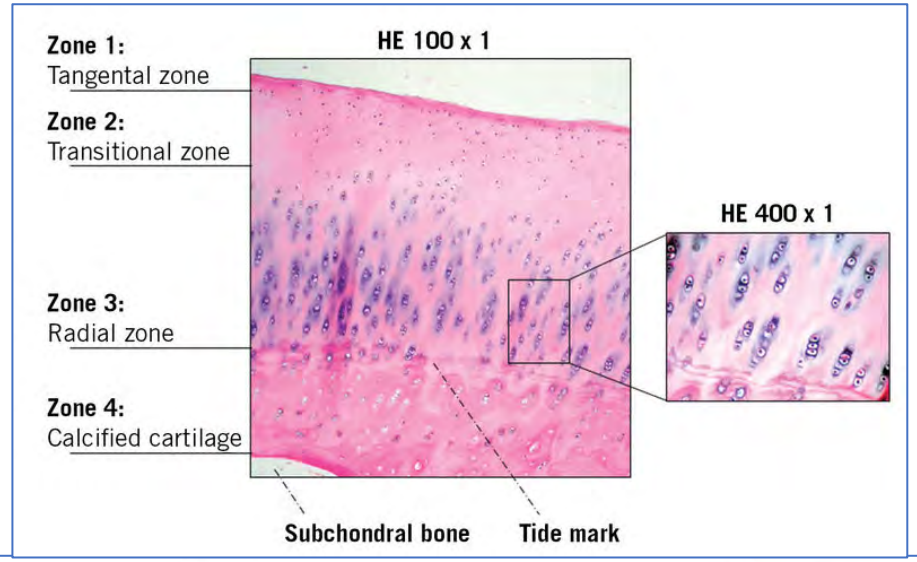
- Function
  - ✓ Distribute the load to protect the underlying bone
    - ✓ Mechanism: high content of water which moves out and back into the cartilage
  - ✓ Provide low friction movements

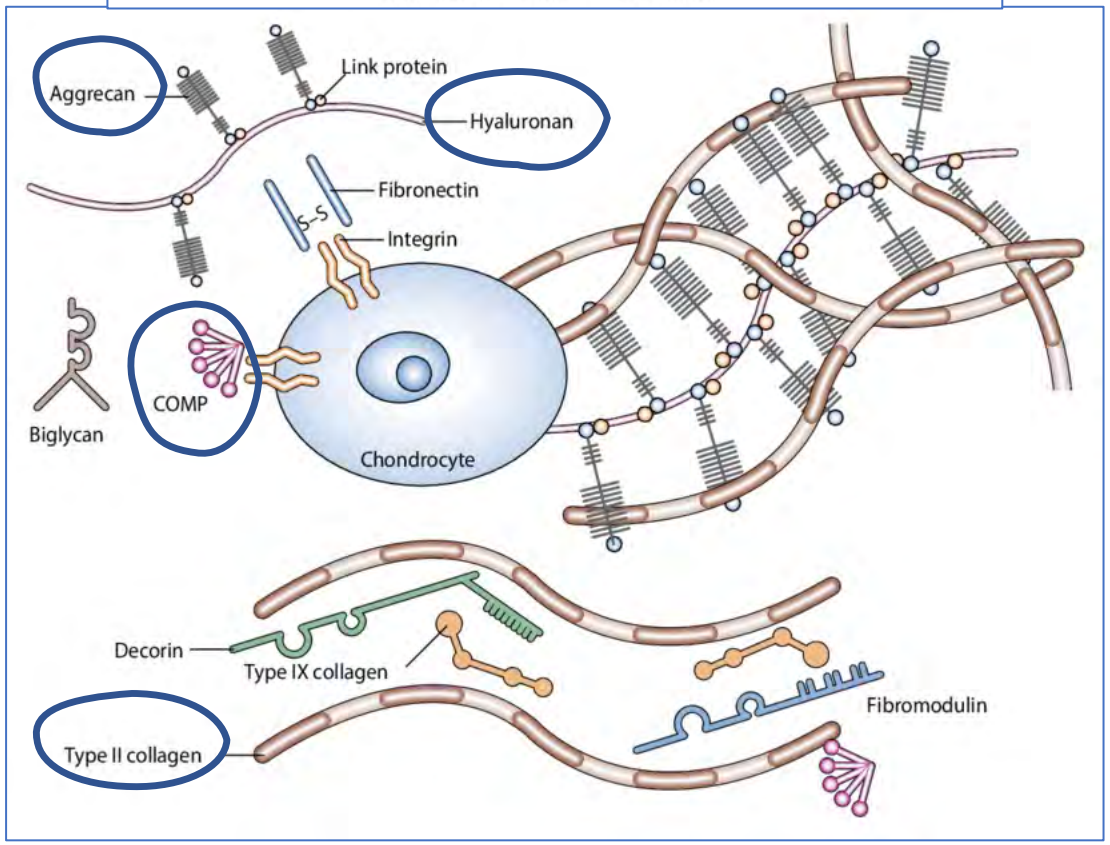




Cartilage: structure

- Tissue organization
  - ✓ Cells: chondrocytes
  - ✓ Matrix
    - Collagen II: 50% of dry weight
    - Aggrecan (proteoglycan): 25 % of dry weight
    - COMP (Cartilage Oligomeric Matrix Protein)
    - Other organic and inorganic



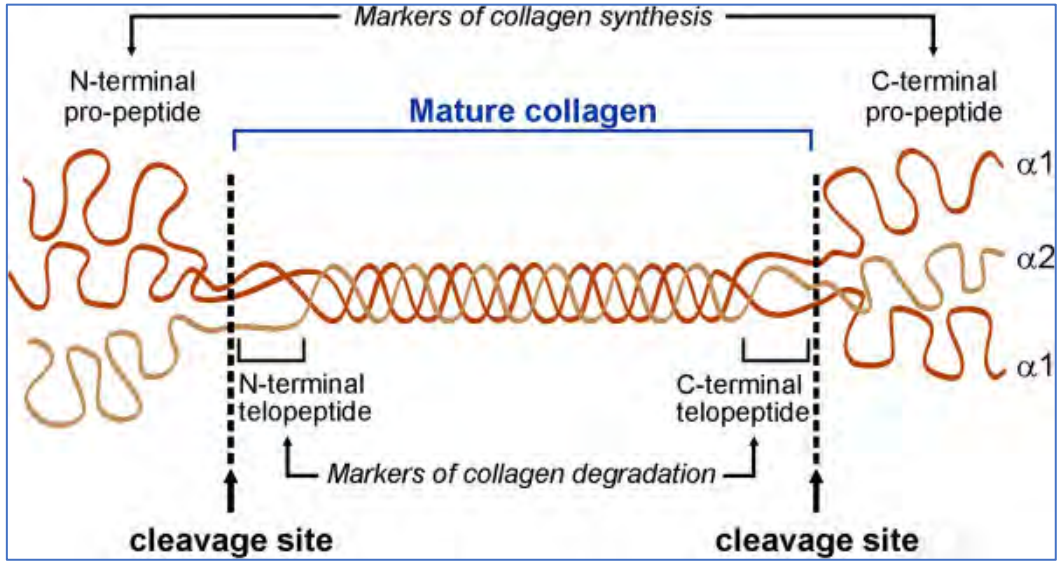


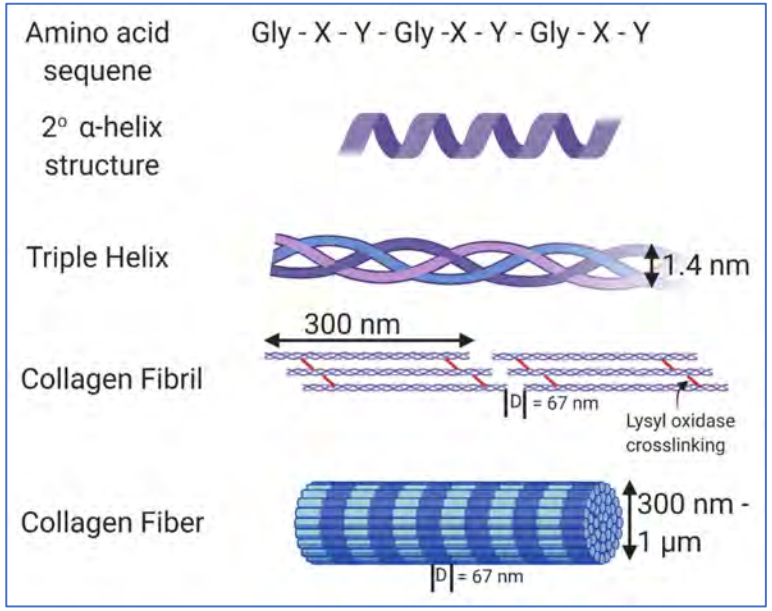
Cartilage tissue (Matrix) structure: collagens

Collagen II *(COL2A1):*

- ✓ Procollagen
- ✓ Collagen 3ple helix
- ✓ Collagen linked to other matrix molecules
  - COMP: role to stabilize collagen network
  - Decorin: binds to fibrin forming collagens
  - Fibromodulin: LRR protein
  - Collagen IX
- **Half-time:>100 years**

Other Collagens: IX/XI, VI, XII, XIV





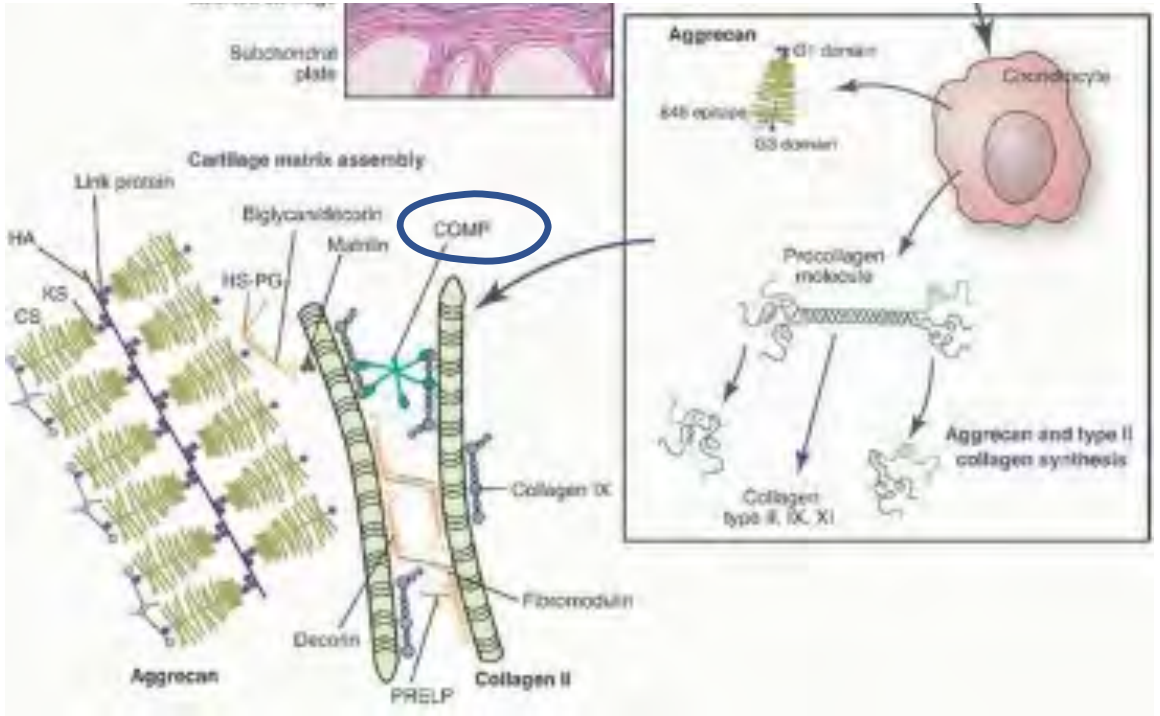
Pathologies associated with matrix proteins

- ✓ OA Pathology associated with:
  - ✓ Collagen :
    - ✓ ↑ destruction of IX = accelerated cartilage degradation
    - ✓ ↑ Coll III & IV
    - ✓ COMP: ↑ in early OA (repair mechanism)
- ✓ Pseudoachondroplasia, multiple epiphyseal dysplasia
  - ✓ COMP mutations

Cartilage tissue structure: COMP

- Protein that links collagen I/II/IX
- Binds 5 collagen molecules
- in OA is upregulated (compensatory mechanism) and may be cleaved-released=measured

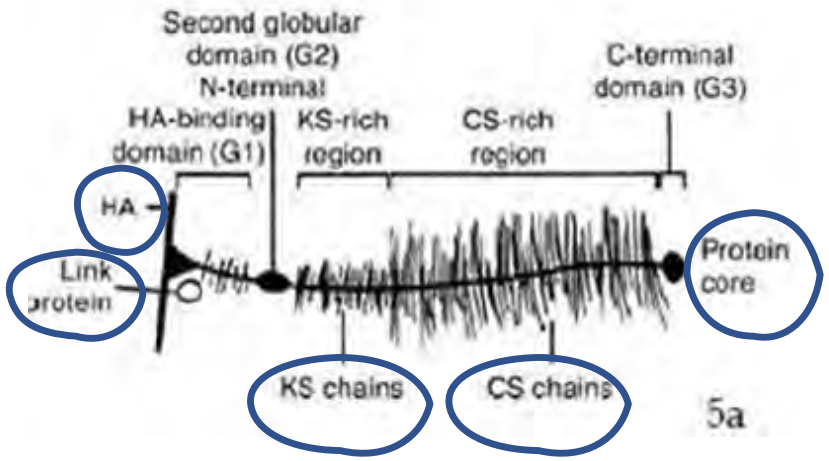
- Mutation of COMP
  - Psudoachondroplasia
  - Multiple epiphyseal dysplasia



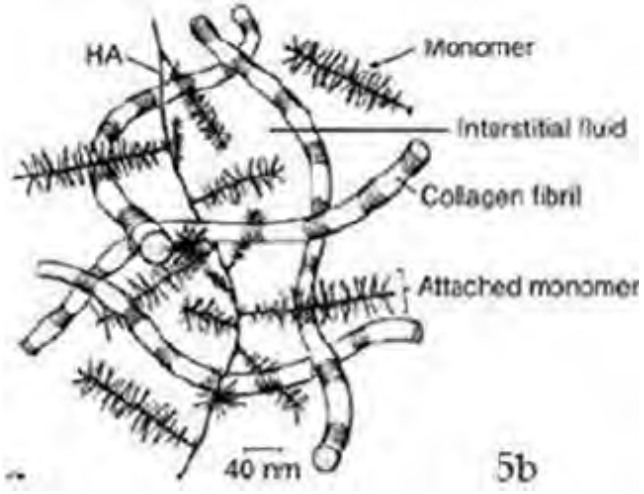
Cartilage tissue structure: aggrecan

Proteoglycan aggrecan molecule composed of:

- ✓ a protein core
- ✓ glycosaminoglycans chondroitin (CS) and keratan sulfate (KS)
- ✓ link protein attached to hyaluronic acid (HA) chain



ECM structure of **collagen fibrils** intertwined in **aggrecan molecules**

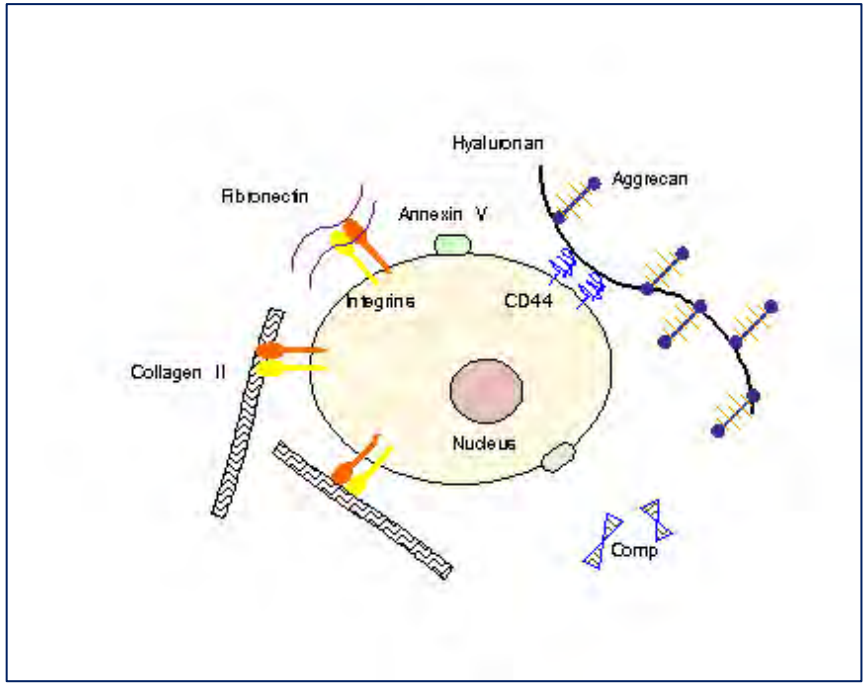


Cartilage homeostasis

- ✓ Aggrecan production: chondrocytes high capacity for aggrecan replacement
- ✓ Aggrecan degradation
  - Aggrecanases
    - ADAMTS-4 & -5
- ✓ Pathologies associated to aggrecan:
  - ✓ Aging cartilage: Short aggrecan molecules, lack of glycosaminoglycans , only HA binding site, $\downarrow water\ content$

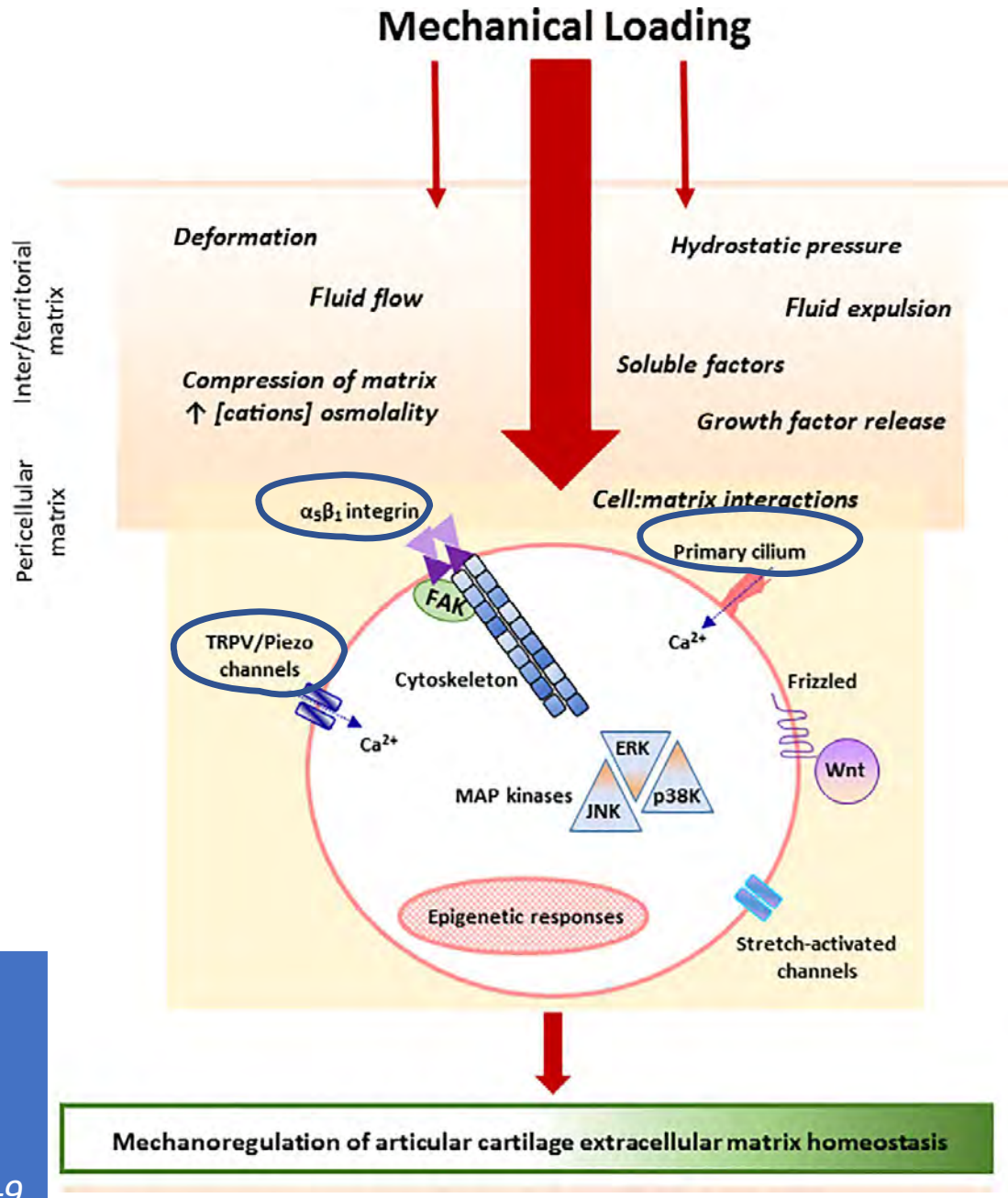
Chondrocytes

- ✓ Mature chondrocytes respond to biochemical, mechanical stimuli:
  - Producers of: matrix components, enzymes and enzyme inhibitors, GF, cytokines
  - High producers aggrecan
  - Low producers of collagen
- ✓ They maintain a **steady-state metabolism** secondary to equilibrium between anabolic and catabolic processes, resulting in the normal turnover of matrix molecules
- ✓ The mature articular chondrocyte embedded in its ECM is a resting cell **with no detectable mitotic activity** and a **low rate of synthetic activity**
- ✓ Energy metabolism depends **strongly on glucose** supply.
  - Facilitated glucose transport in chondrocytes is mediated by glucose transporter proteins (GLUTs)



Chondrocyte's Receptors

- ✓ **Integrins:**
  - Cell to extracellular Matrix (ECM) link
  - Mechanoreceptors
- ✓ **Ion channels**
  - Mechanical stimulation regulates the activity of ion channels localized on the plasma membrane due to **perturbations in membrane tension and lipid bilayer distortion**.
    - TRPV4 is a **Ca^{2+}** -permeable
- ✓ **The primary cilium**, a microtubule-based structure extending from the cell surface and protruding into the Pericellular Matrix



Example of interaction between ECM-receptors-chondrocyte metabolism:

Mechanical strain-induced α5β1-mediated membrane hyperpolarization: ↑ transcription of aggrecan and ↓ transcription of MMP-3

J Cell Biol 1999;145(1):183–9.

Matrix turnover, repair-degradation

- ✓Collagen turnover: slow
- ✓Proteoglycans: continuously produced
- ✓MMPs – MMPs inhibitors
- ✓Integrins: bind to collagen and:
  - Increase collagen synthesis OR increase degrading enzymes
- ✓Fibronectin
  - Fragmented fibronectin stimulates chondrocytes to produce degrading enzymes
  - Upregulated in OA

Joint Remodeling – MMPs – activators - inhibitors

- The most important of proteolytic enzymes since they can directly cleave collagen at a neutral pH are:
  - ✓ The collagenases MMP1 (interstitial collagenase), MMP3 (stromelysin-1), MMP8 (neutrophil collagenase), MMP13 (collagenase 3) and MMP18
- **MMPs activators / inhibitors**
  - ✓ **Serine and cysteine proteinases** are required to activate **pro-MMPs** after they are secreted.
  - ✓ Furthermore, **inhibitors of these proteinases** (such as tissue inhibitors of metalloproteinases (**TIMPs**) and inhibitors of serine proteinases (**SERPINs**)) are also present in the normal joint.

Nat Rev Rheumatol. 2017.

Joint Remodeling – Growth factors

- Growth and differentiation factors are considered positive regulators of homeostasis in mature articular cartilage
- They stimulate chondrocyte anabolic activity and, in some cases, inhibit catabolic activity.
- The best-characterized anabolic factors
  - insulin-like growth factor I (IGF-I)
  - members of the FGF and TGF-β/BMP families.
  - The PTHrP the Wnt/β-catenin pathways have been implicated in maintenance of cartilage homeostasis or OA disease processes.

Κύρια Σημεία

- Αρθρικός Υμένας
  - Παράγει αρθρικό υγρό, τρέφει χόνδρο, επιτηρεί ανοσολογικά την άρθρωση
  - Κύτταρα: Ινοβλάστες, μακροφάγα
  - Βασικά παράγωγα: HA, λουμπρικίνη, αγγρεκάνη, MMPs
- Αρθρικός Χόνδρος
  - Απορροφά φορτία
  - Matrix: Κολλαγόνο ΙΙ, αγγρεκάνη, συνδετικές πρωτεΐνες
  - Χονδροκύτταρα: απουσία πολλαπλασιασμού/ανναγέννησης, μεταβολίζουν γλυκόζη
- Αλληλεπίδραση υμένα, χόνδρου, τοπικών παραγόντων και συστηματικής κυκλοφορίας για τη διατήρηση της ομοιοστασίας της άρθρωσης