Cell viability in TE constructs; Cartilage structure/function

Module 3, Lecture 4

20.109 Spring 2008

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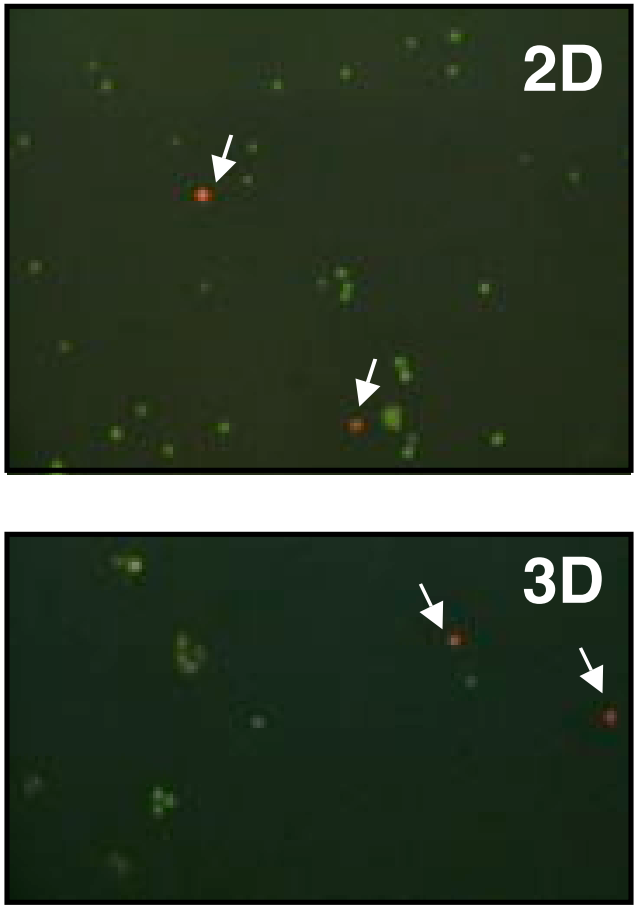
Topics for Lecture 4

- Review of Module 3 so far
- Cell viability: influence and measurement
- Overview of Module 3 week 3
- A closer look at cartilage
  - background on collagens and proteoglycans
  - how cartilage structure influences function

Module progress: week 2

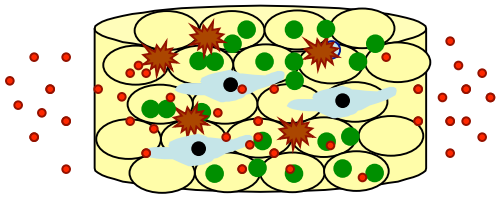
- Day 3: viability/cytotoxicity testing
- Practical matters
  - focusing takes practice
  - too low cell concentration
- Most groups found
  - low cell recovery, especially in 3D
  - mostly live cells in 2D samples
  - 3D samples more variable
- How can we explain these results?
- How can we improve the assay?
- Day 3: morphology observations
  - what did you see in brightfield?



W/F Green group

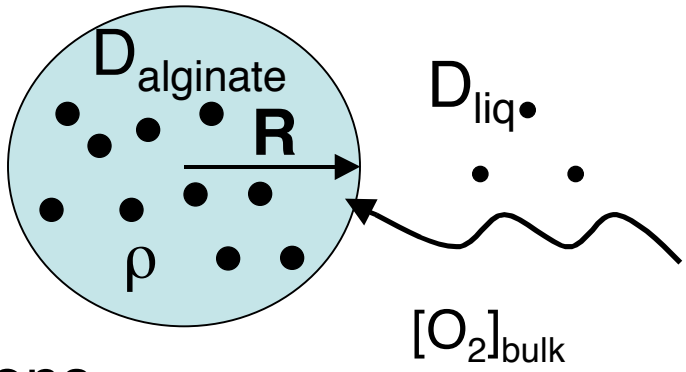
Factors affecting cell viability

- What factors affect viability in a TE construct?
- Cell level
  - density: competition for nutrients, O_2
  - interactions (+ or -) between different cell types
- Cytokine level
  - may promote viability and/or proliferation
  - may promote apoptosis
- Materials level
  - permeability of material (to nutrients, O_2)
  - pore size, percent porosity
  - toxicity of material or its degradation products



Nutrient use in 3D constructs

- Parameters affecting diffusion
  - size of construct (R)
  - cell density (ρ)
  - diffusivity (D)
  - bulk concentration $[O_2]_{bulk}$



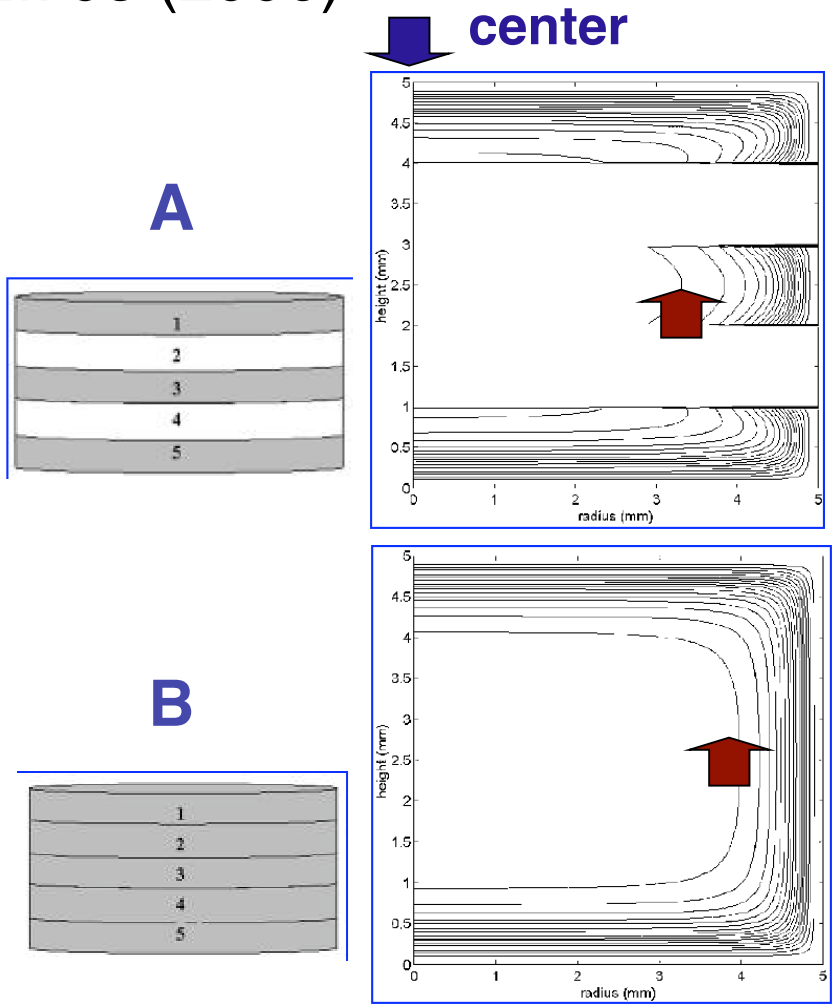
- In simple cases, boundary conditions can be used to get analytical solution

- Characteristic diffusion limit (nutrients, O_2): ~100 μm
  - diffusion profiles tend to correspond with viability profiles

- Solution *in vitro*: dynamic/perfusion culture
- Solution *in vivo*: promote angiogenesis quickly

Modeling cell viability in TE constructs

- J.C.Y. Dunn, et al. *Tissue Eng* **12**:705 (2006)
- Porous PLGA scaffolds
- Seeded cells in every other (A) or in each (B) layer
- Observed after 10 days
- Model
  - diffusion, O_2 use, and cell growth
  - quasi-steady state
  - no depletion in fluid
- Results
  - A has improved cell uniformity
  - cell growth matches O_2 tension
  - claim for predictive capability

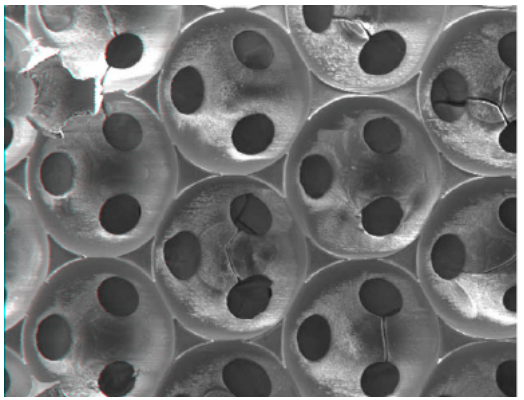


Modeling diffusion in a defined porosity

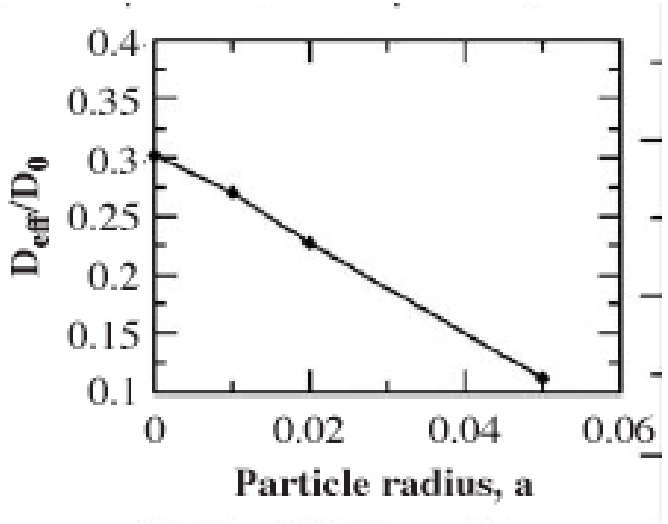
- S. Shanbhag et al. *Biomaterials* **26**:5581 (2006)

Kotov lab

- Diffusion in colloidal crystal templated scaffolds
  - geometrically defined model
  - Brownian dynamics (time evolution)
  - Monte Carlo simulations (particle moves, Boltzmann weighting)



- Results
  - $D_{eff} = 0.3\ D_o$ = upper-bound
  - decreases with size of inter-pores
  - with particle size (O_2 vs. protein)
  - with further confinement of particles by cells, or utilization by cells



Cell death: apoptosis and necrosis

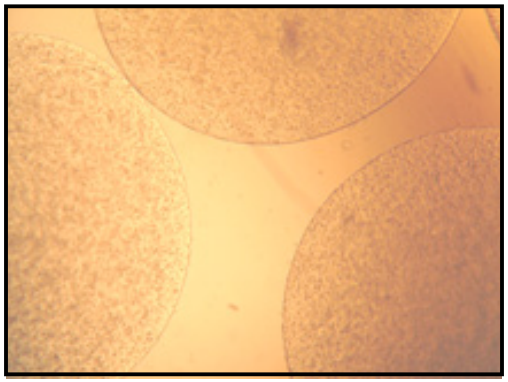
- Apoptosis
  - programmed cell death
  - role in development and immunity
  - process: cell condensation and fragmentation
  - misregulated apoptosis implicated in disease
- Necrosis
  - response to trauma
  - process: cells burst and release contents
  - necrotic cells promote inflammation
- Morphology or biochemical assays can distinguish apoptotic and necrotic cells

S. Elmore *Toxicol Pathol* **35**:495 (2007)

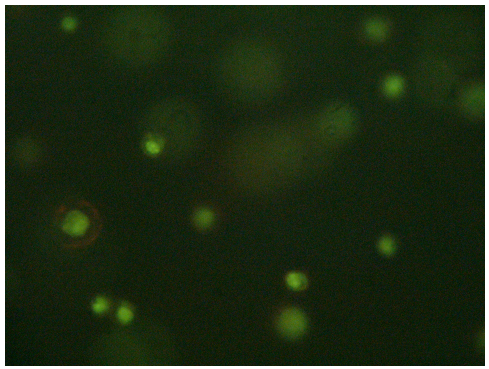
Module overview: lab

Day 1: design

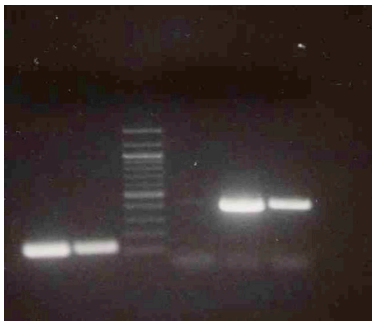
Day 2: seed cultures



Day 3: viability assay



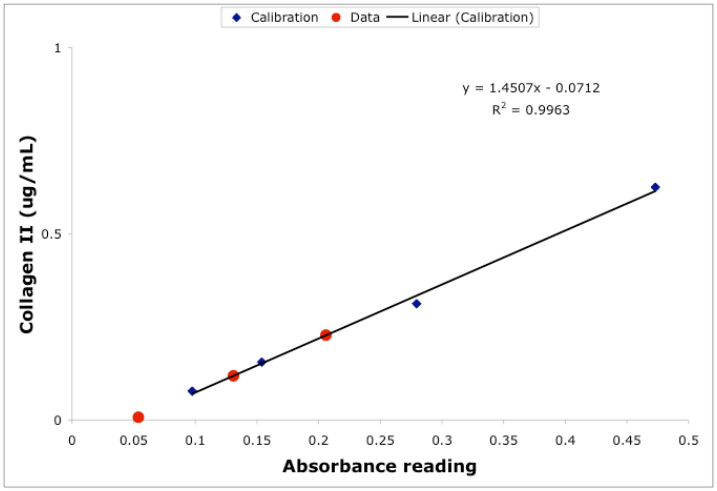
Day 4: prep RNA+cDNA



Day 5: transcript assay

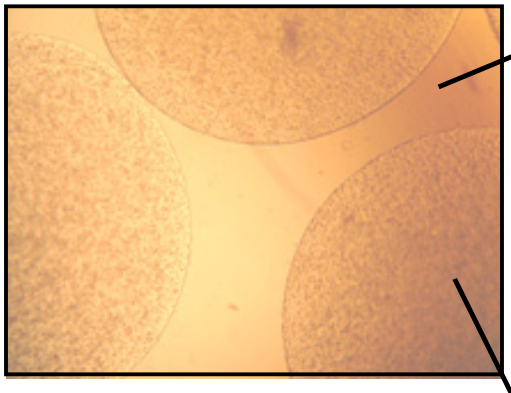
Day 6: protein assay

Day 7: remaining analysis



Day 8: your research ideas!

Module overview: week 4



1. Collect supernatant

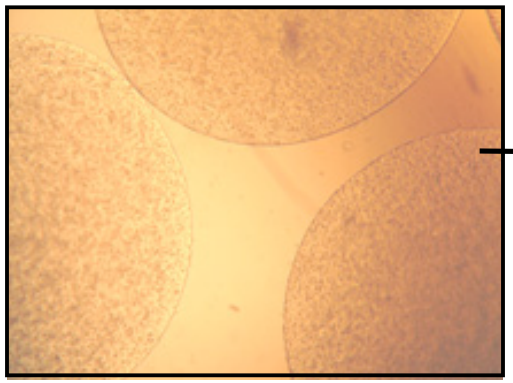
Test for collagen proteins (by ELISA)

2. Collect and lyse cells

Purify mRNA from cells → Amplify collagen cDNAs →

Compare collagen I and II transcript levels

Day 4: RNA isolation



1. Collect cells
   - lyse cells in buffer
   - homogenize over column

2. Isolate total RNA
   - on silica-gel columns that bind RNA > 200bp
   - using buffers, ethanol precipitation
   - enriched for mRNA due to size exclusion

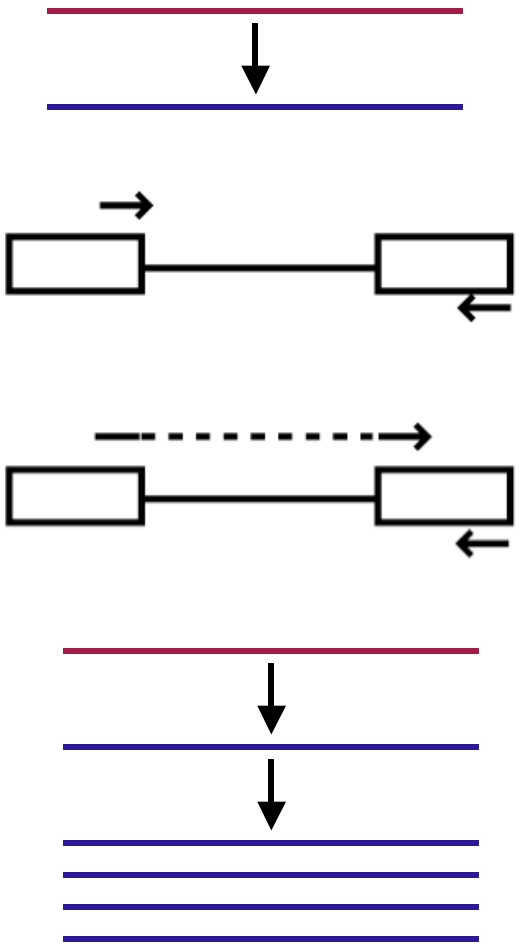
www.qiagen.com

Working with RNA requires extremely clean technique. Why?

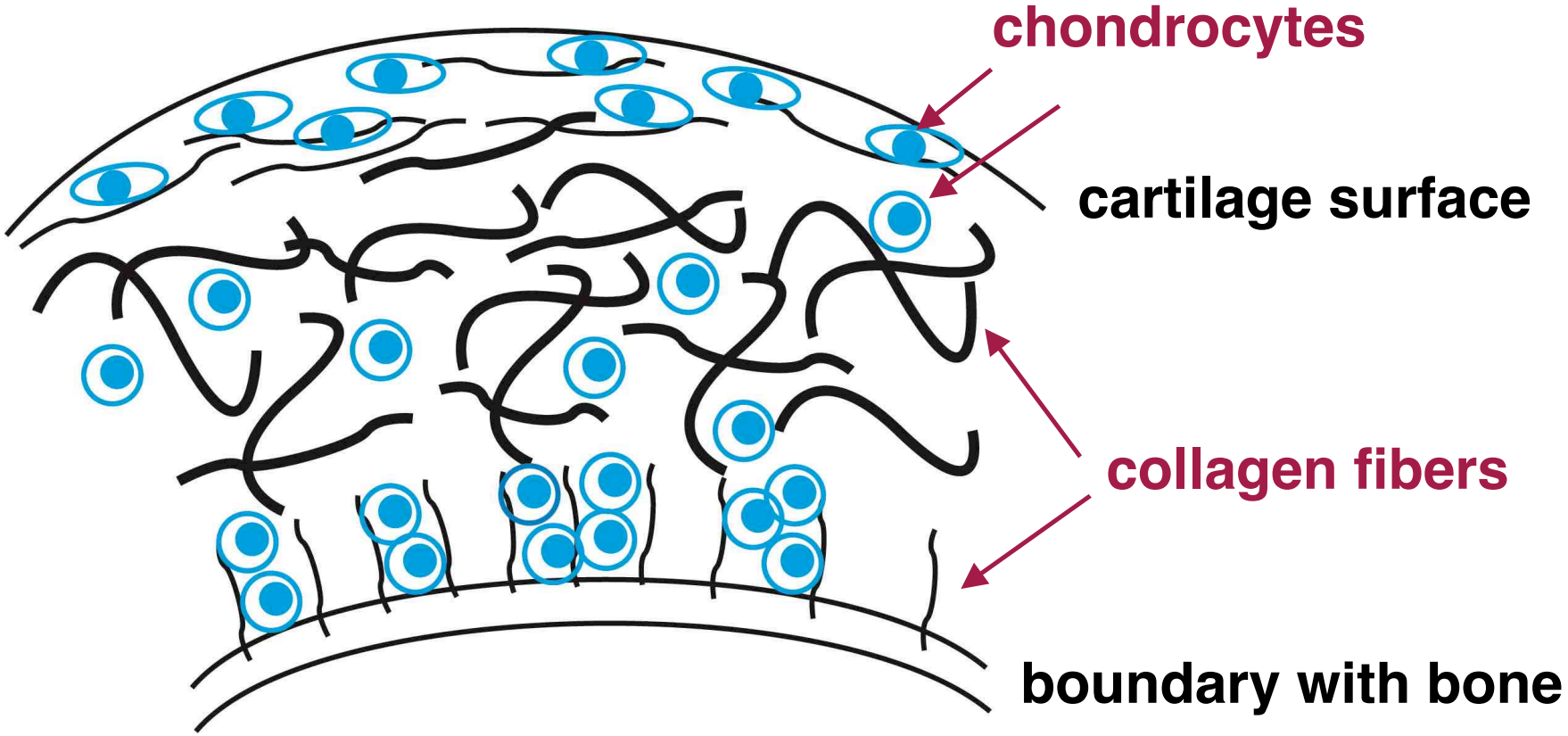
RNases are pervasive, e.g., on your hands

Day 4: RT-PCR

- RT = reverse transcriptase
  - what does this enzyme do?
- Unique primer design needs
  - how to isolate transcript but not genomic DNA?
- RT and PCR can be done in one reaction or two
  - enzyme de/activation by temperature
  - which enzymes when?
- What kinds of controls are desired?



Revisiting cartilage tissue



Avascular, highly water-swollen, heterogeneous tissue.

Collagen structure

- Collagen primary structure:
  - Gly-X-Y repeats
  - high proline, hydroxyproline content
- Collagen tertiary structure: triple helix
  - Gly contributes flexibility
  - Hyp contributes hydrogen-bonding
- Collagen quaternary structure: fibrils
  - true for many types, including I and II
  - cross-links via lysine and hydroxylysine
  - periodic banding structure observed

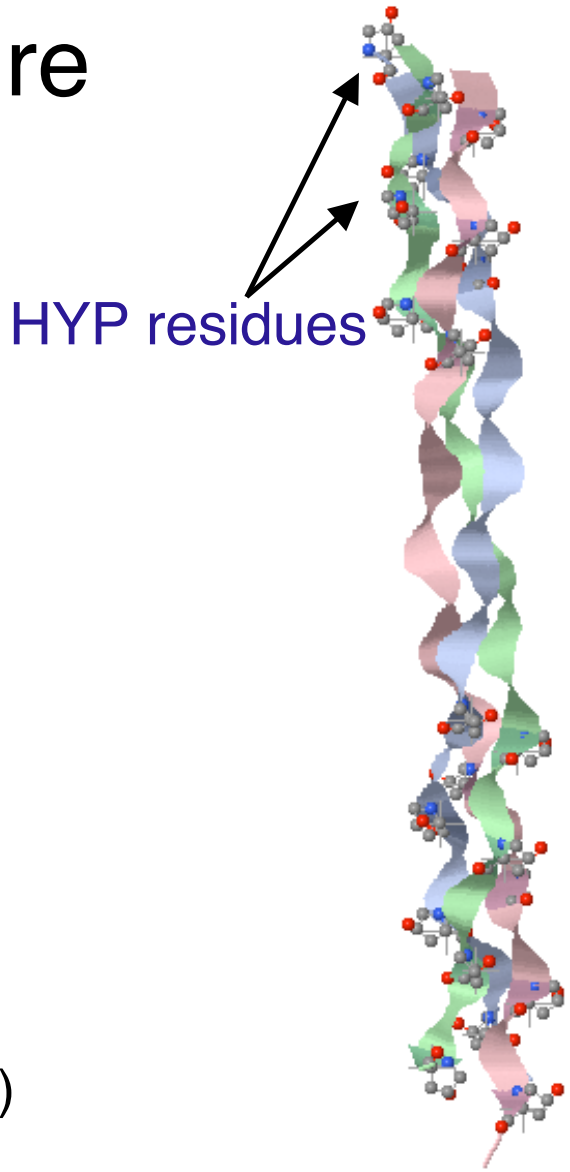
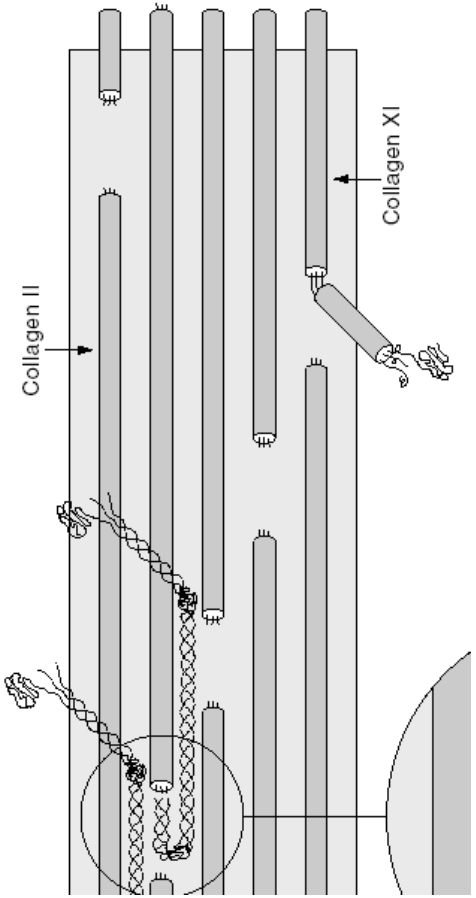


Image made using *Protein Explorer* (PDB ID: 1bkv)

E. Vuorio & B. de Crombrugghe *Annu Rev Biochem* **59**:837 (1990)

Collagen types in cartilage

- Collagen types vary with respect to
  - location: II in cartilage, vitreous humor; I in skin, bone, vitals, etc.
  - homo- (II) or hetero- (I) trimeric helices
  - supramolecular structure formation
  - glycoslyation
- Collagen composition in cartilage
  - Type II (fibrils) covalently linked to IX and XI
  - exact roles of IX and XI unknown
    - IX may form inter-fibrillar cross-links
    - XI may modulate collagen II fibril diameter
    - mutations to IX, XI, II cause disease
  - Types III, VI, X, XII, and XIV also present
- Little collagen turnover in adult cartilage

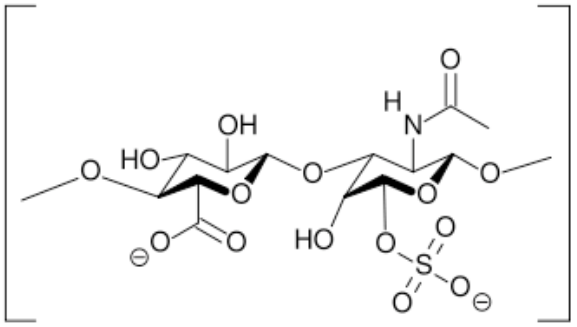


D. Eyre (2002)

D.J. Prockop *Annu Rev Biochemritis Res* **64**:403 (1995)
D. Eyre *Arthritis Res* **4**:30 (2002)

Proteoglycan structure

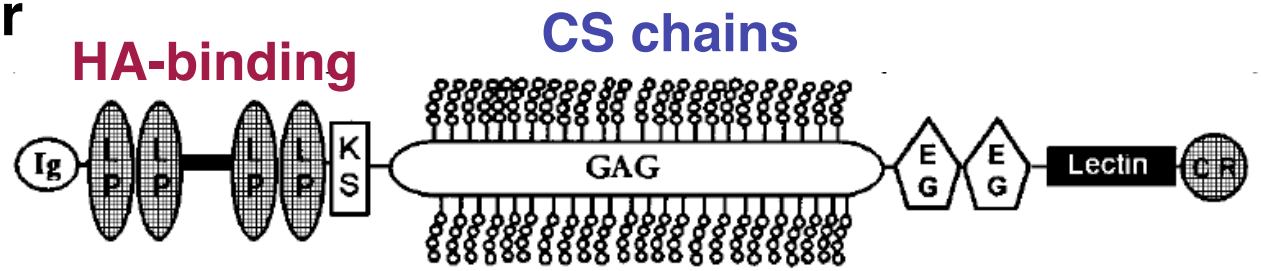
- Proteins with GAG side chains
  - many negatively charged groups COO^- SO_3^-
- Most common PG in cartilage is aggrecan
  - aggrecans polymerize via hyaluronin (HA)
  - GAG is primarily chondroitin sulfate (CS)
  - monomer > 1M, aggregates > 100M Da



Chondroitin sulfate
(public domain image)

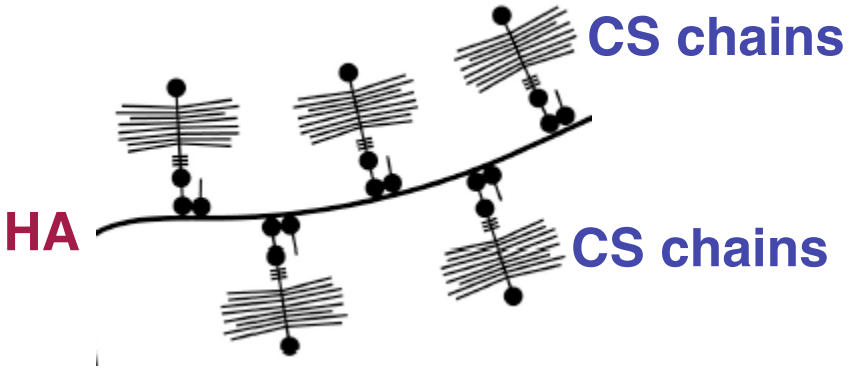
Aggrecan monomer

R.V. Iozzo *Annu Rev Biochem* **67**:609 (1998)



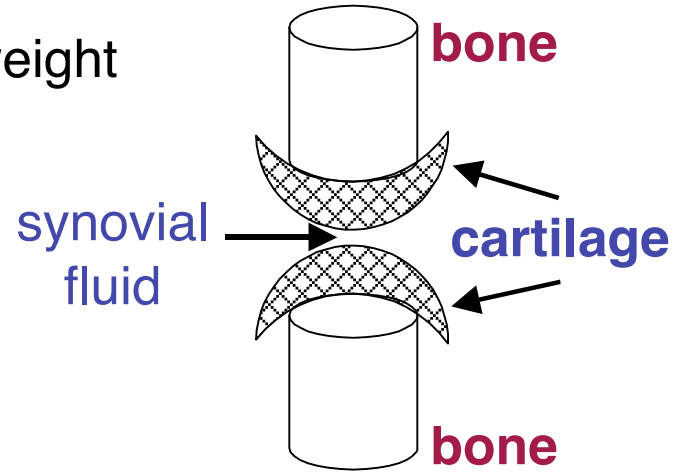
Aggrecan aggregate

C.B & W. Knudson *Cell & Dev Bio* **12**:69 (2001)



Cartilage structure and function

- Composition of cartilage
  - CN is 50-75% and PG is 15-30% of dry weight
  - water: 60-80%
  - cells: 5-10% by volume
- Requirements of a joint
  - load transfer (bone/bone, bone/muscle)
  - flexibility, lubrication
- Role of PG
  - high compressive strength due to osmotic swelling: water is pumped out during compression
  - low permeability, friction coefficient reduces wear and tear
- Role of CN
  - high tensile strength (~GPa)
  - contain swelling forces of PG



V.C. Mow, A. Ratcliffe, and S.LY. Woo, eds. *Biomechanics of Diarthrodial Joints* (Vol. I) Springer-Verlag New York Inc. 1990

Lecture 4: conclusions

- Cell viability in TE constructs is affected by factors at the cell, materials, and cytokine level.
- Modeling is one useful tool to study the effects of nutrient diffusion on cell viability.
- RT-PCR is a technique for studying gene expression, with special considerations beyond PCR.
- The structure of the cartilage extracellular matrix promotes its function in joints.

Next time: gene and protein assays, *in vitro* and *in vivo* models for cartilage TE