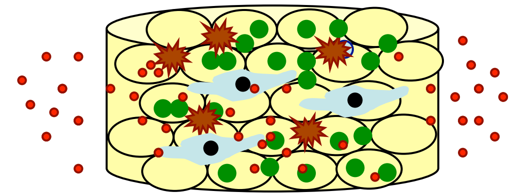
Biomaterials and Cell-Biomaterial Interactions

Module 3, Lecture 2

20.109 Spring 2014

Lecture 1 review

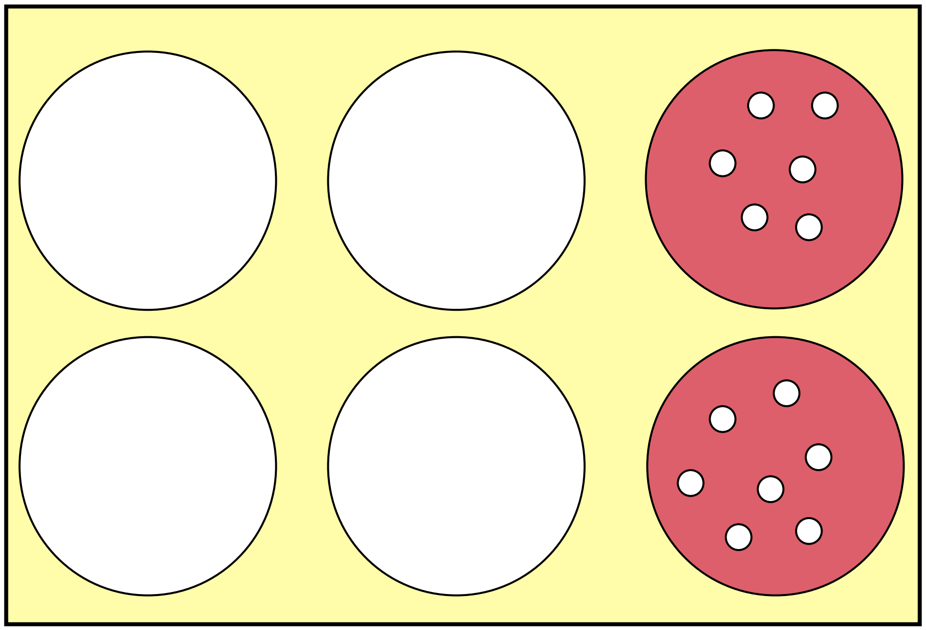
- What is tissue engineering?
- Why is tissue engineering?
- Why care about cartilage?
- What are we asking in Module 3?



Topics for Lecture 2

- Introduction to biomaterials in TE
  - properties
  - examples
- Cartilage composition
  - collagen
  - proteoglycans
  - structure → function

Today in Lab: M3D2



Condition 1 of 2

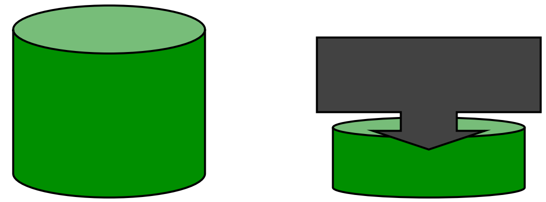
0.5 mL beads,
6 mL media

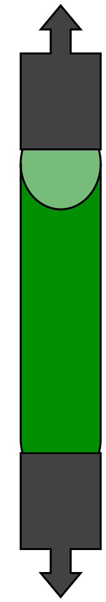
0.5 mL beads,
6 mL media

- 1 condition per plate (**2** plates total)
- 2 **wells** per plate (*split* 1 mL of beads)
- if contaminate 1 well on D3, still have 1 on D4

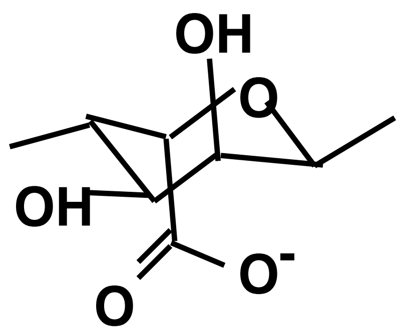
Properties of biomaterials

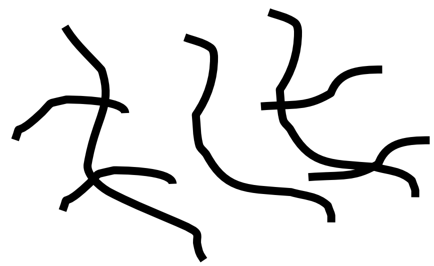
- Physical/mechanical
  - strength
  - elasticity
  - architecture (e.g., pore size)



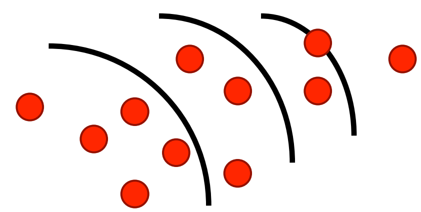


- Chemical
  - degradability
  - toxicity
  - water content





- Biological
  - motifs that cells recognize
  - release of soluble components



- Lifetime

The right material for the job

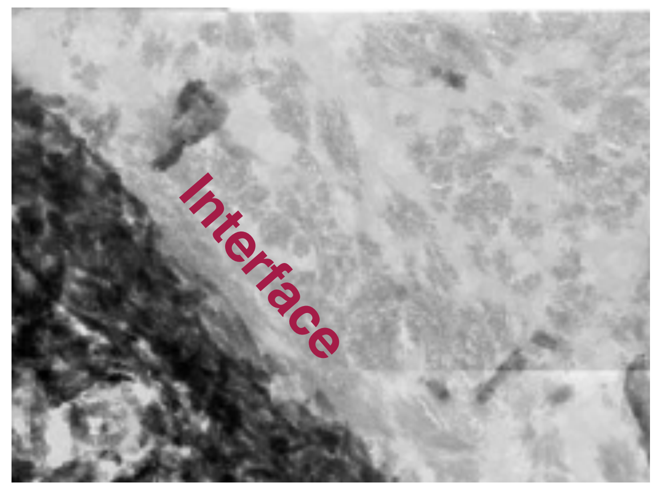
- Metals
  - Ti, Co, Mg alloys
  - pros: mechanically robust
  - applications: orthopedics, dentistry
- Ceramics
  - Al_2O_3, Ca-phosphates, sulfates
  - pros: strength, bonding to bone
  - applications: orthopedics, dentistry
- Polymers
  - diverse, tunable properties
  - applications: soft tissues

General: B. Ratner, ed. *Biomaterials Science*, 1996.

Image: Porter et al., *Biomaterials* **25**:3303 (2004).

Metal hip implant

http://www.weisshospital.com/joint-university/hip/metal.html

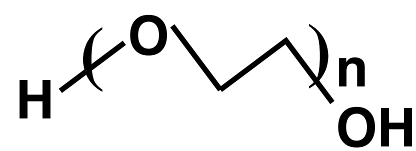


Si-HA **Bone**

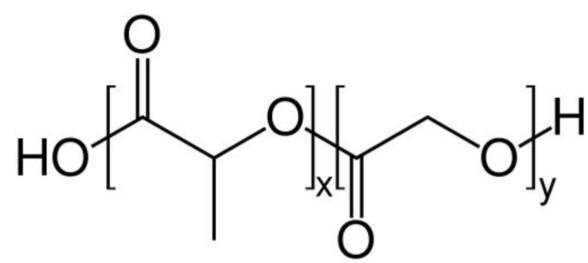
Basics of polymer structure

- Linear polymers
  - repeated chemical unit, or
  - heterogeneous repeats: co-polymer
- Homo- example: PEG
  - synthesis: epoxide ring-opening
  - adds one monomer at a time
- Co- example: PLGA
  - successive ester bonds formed
  - also by ring-opening!
- Many types of linear syntheses
- As MW increases
  - entanglements and strength ↑
  - processability ↓

Poly(ethylene glycol)



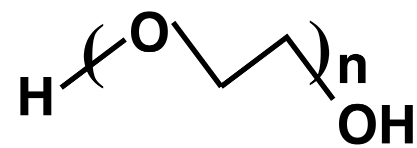
Poly(lactic-co-glycolic acid)



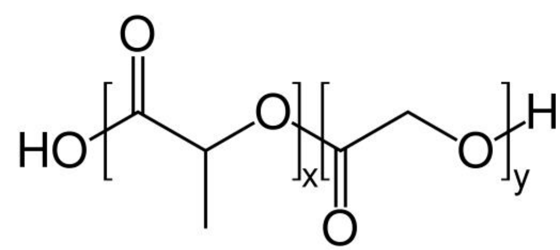
[public domain image]

Polymers are diverse and tunable

- Chemical groups affect
  - mechanical properties
  - stability/degradability
  - hydrophilicity
  - reactivity/modification ease
  - gas permeability
- PEG
  - unique relationship with water
  - resists protein adsorption
  - low MW easily excreted
- PLGA
  - ester bonds hydrolyzed
  - PLA more hydrophobic and degrades more slowly cf PGA



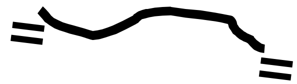
Poly(ethylene glycol)



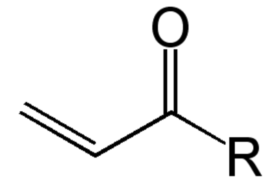
Poly(lactic-co-glycolic acid)

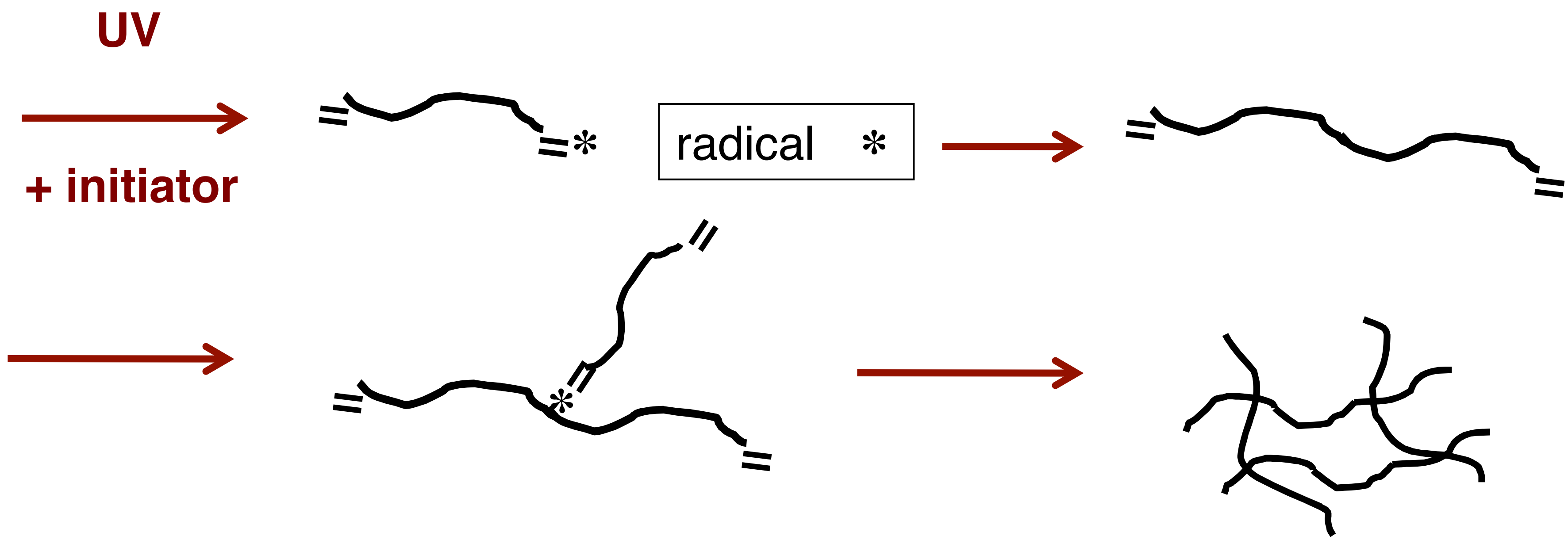
[public domain image]

Network polymer synthesis example

Linear polymer with reactive end groups: 

acryloyl =





Network polymer

- Network structure
  - covalently cross-linked chains
  - water-swollen (if hydrophilic)

Properties of hydrogels such as PEG

- Mimic soft tissues
  - water content
  - elasticity
  - diffusivity
- Synthesis at physiological conditions
  - temperature
  - pH
  - UV light: spatio-temporal control; safe; patterning potential
- Injectability
- Chemical modification

(Stachowiak & Irvine)

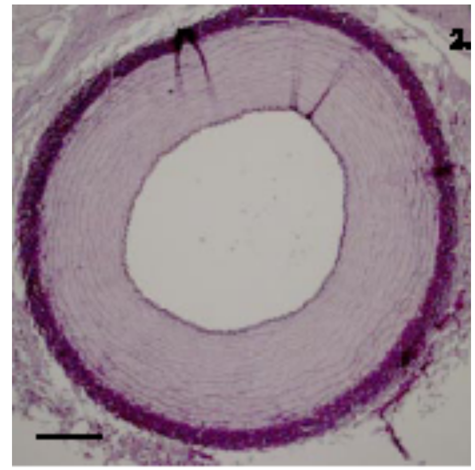
Review: Nguyen KT & West JL, *Biomaterials* **23**:4307 (2002)

Materials must be biocompatible

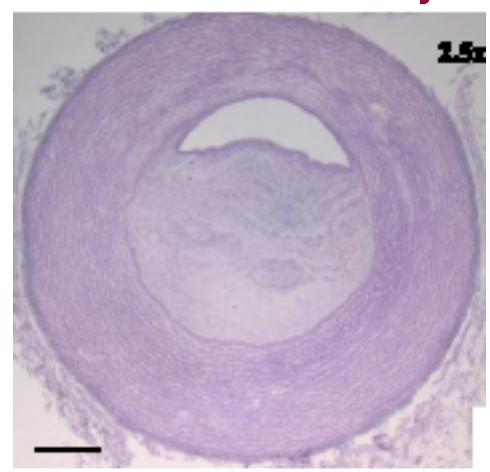
- Avoid bio-incompatibility
  - chemical toxicity: cells, genomes
  - immunogenicity
  - protein/cell adhesion → clotting
  - bacterial adhesion
- Material properties
  - material *and* its degradation products non-toxic and non-immunogenic
  - resistance to protein adhesion
  - Sterility
- PEG is a great bioinert material

Data from: Zavan B, et al., *FASEB J* **22**:2853 (2008).

Normal artery



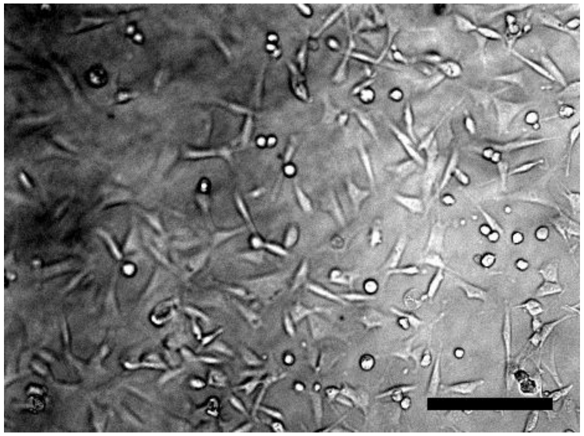
Occluded artery



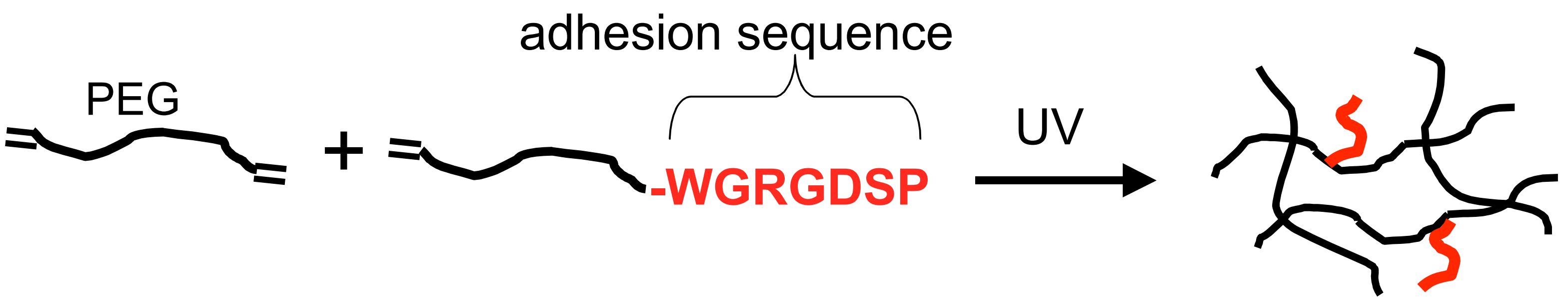
Beyond bioinert: bioactive materials

- Attach proteins/peptides for
  - *specific* cell adhesion
  - degradability
- Release cytokines for
  - proliferation
  - differentiation
  - attraction

Fibroblasts on polymer-peptide gels (Stachowiak).



- e.g., West JL and Hubbell JA *Macromolecules* **32**:241 (1999)



Interlude: scientific misconduct

CELL BIOLOGY

Stem-cell method faces fresh questions

Papers describing acid-bath technique under more scrutiny after institute's investigation finds errors in methodology.

Brigham and Women's stem cell study retracted over 'compromised' data

By Carolyn Y. Johnson / Globe Staff

A Brigham and Women's Hospital stem cell study, which raised the possibility that the human heart could repair itself, has been retracted after an internal investigation showed the researchers used compromised data.

Sources

Nature News

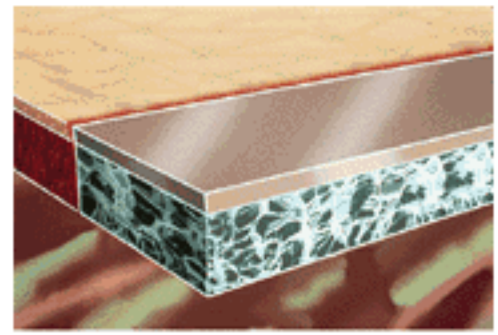
03/18/14

Boston Globe

04/09/14

Natural vs. synthetic polymers

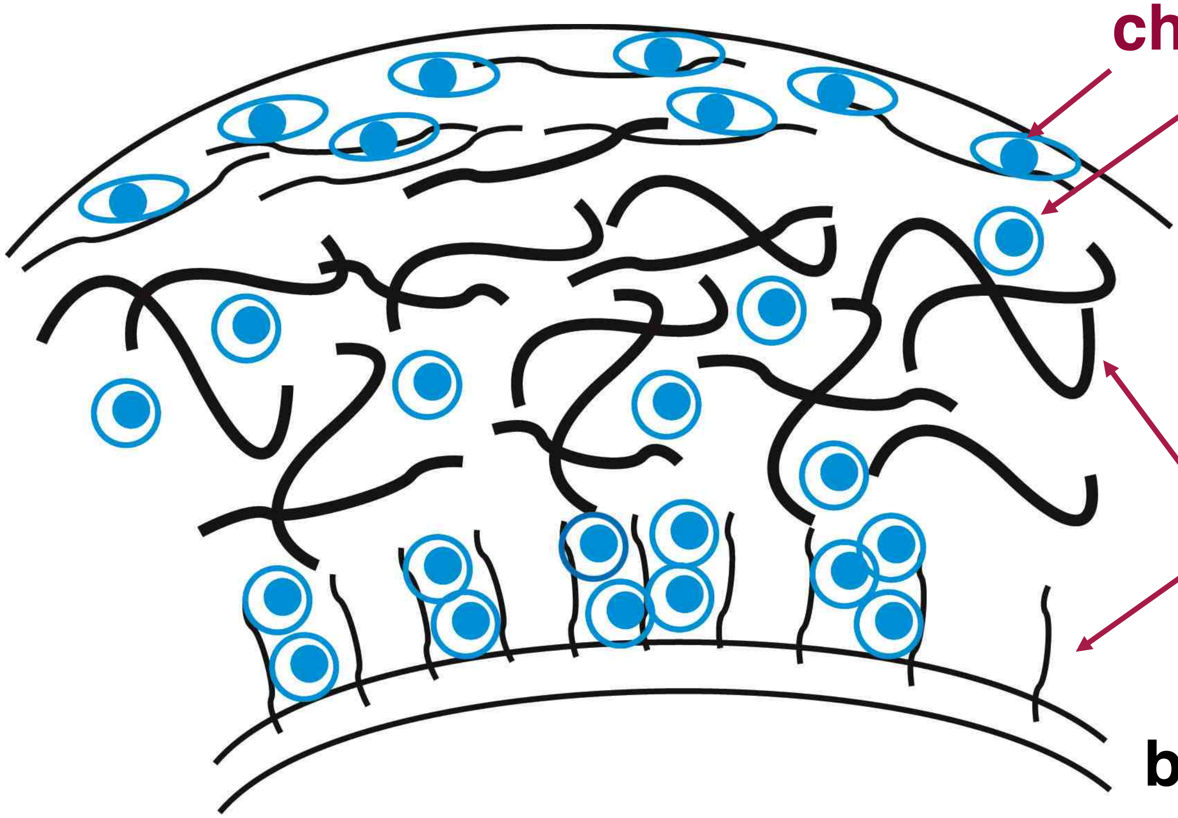
- Natural pros/cons
  - built-in bioactivity
  - lot-to-lot variation, unpredictable
  - poor mechanical strength
  - immunogenicity (xenologous sources)



- Synthetic pros/cons
  - predicting biocompatibility is tough
  - mechanical and chemical properties readily altered
  - minimal lot-to-lot variation

- Synthetic advantages: tunable and reproducible

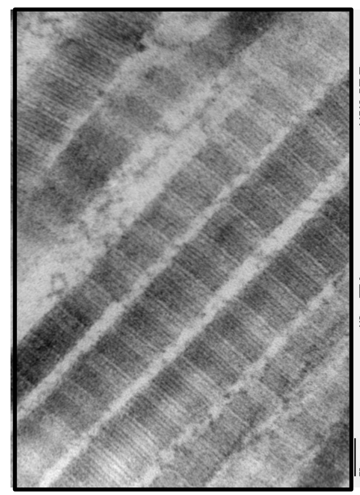
Revisiting cartilage structure

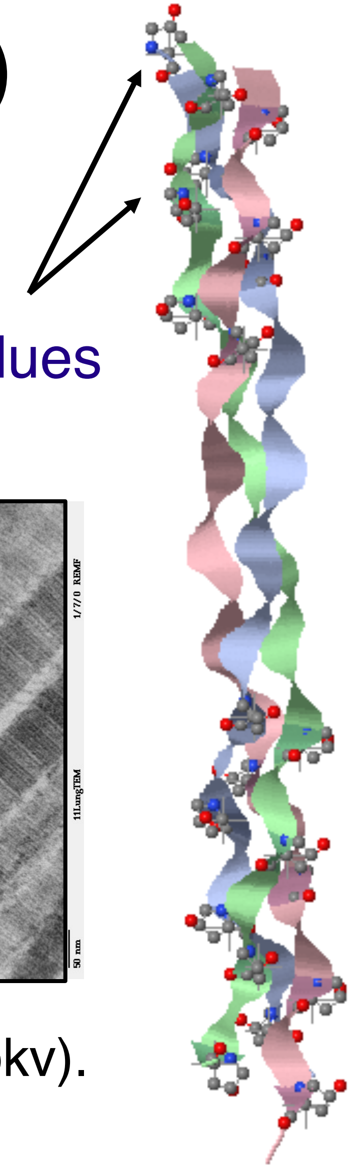


Water-swollen, heterogeneous, **avascular and cell-poor** tissue.

Structure of collagen(s)

- 1° structure:
  - Gly-X-Y repeats
  - proline, hydroxyproline
- 3° structure: triple helix
  - Gly: flexibility
  - Hyp: H-bonding
- 4° structure: fibrils
  - many but not all collagens
  - cross-links via lysine, hydroxylysine
  - periodic banding observable

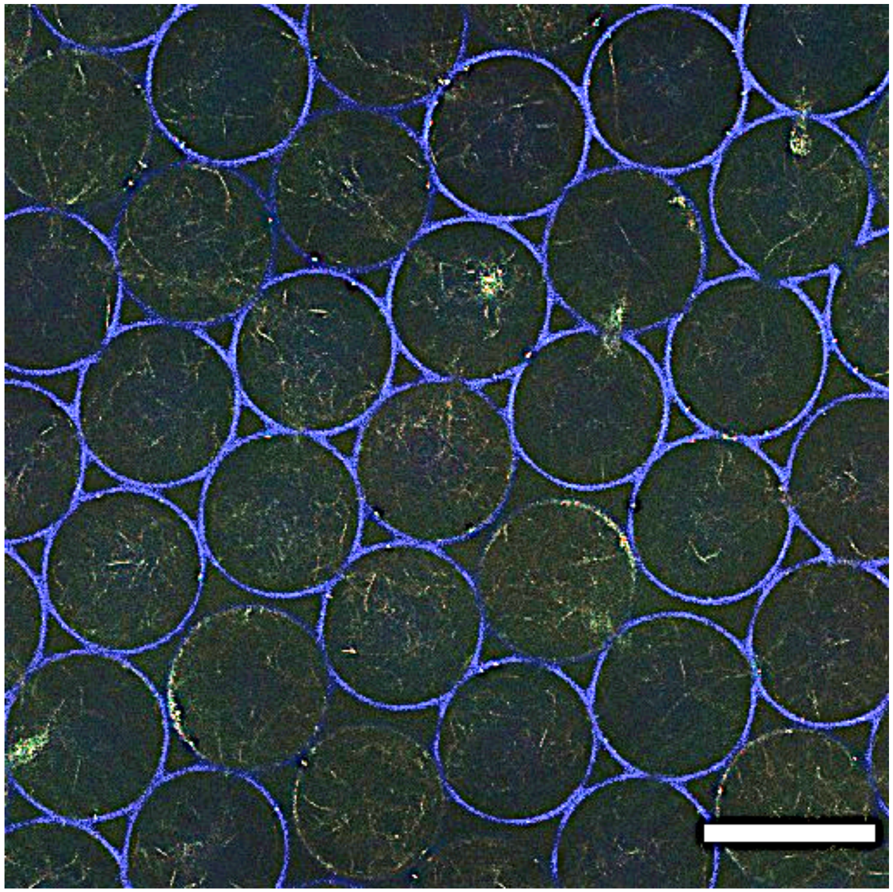




Molecular image made using *Protein Explorer* (PDB ID: 1bkv).
Fibril image from public domain.

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

Macro structure of fibrillar collagen



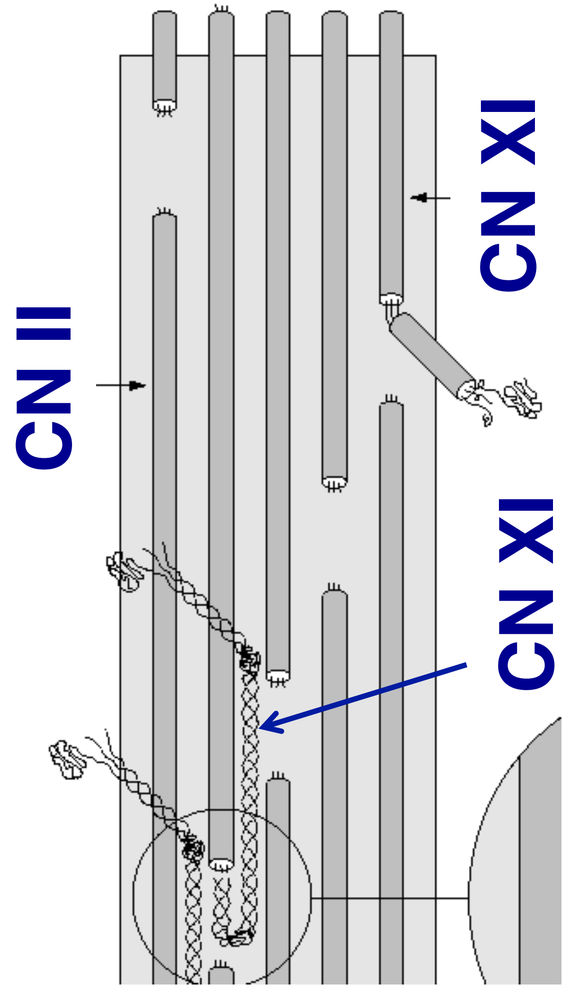
PEG scaffold:
- microporous (bead template)
- strict order

CN filler:
- nanoporous
- apparent disorder

A. Stachowiak and D.J. Irvine, confocal reflection microscopy of collagen-filled synthetic scaffold.

Collagen composition in cartilage

- Collagen types vary in
  - location
  - glycoslyation
  - higher-order structure
  - homo- (II) or hetero- (I) trimers
- Cartilage collagens
  - Type II with IX and XI
  - exact roles of IX and XI unknown
    - inter-fibrillar cross-links
    - modulate fibril diameter
    - integration with rest of ECM
  - others(III, VI, X, XII, XIV)
- Little collagen turnover in adult cartilage

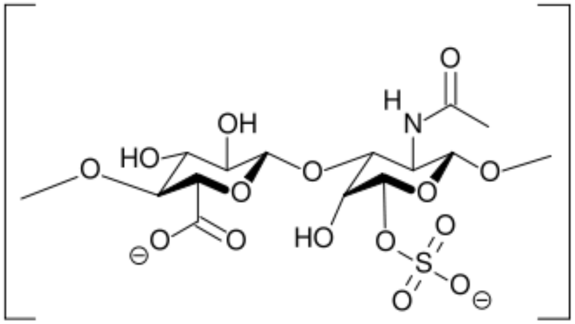


D. Eyre (2002)

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

Proteoglycans are bulky and charged

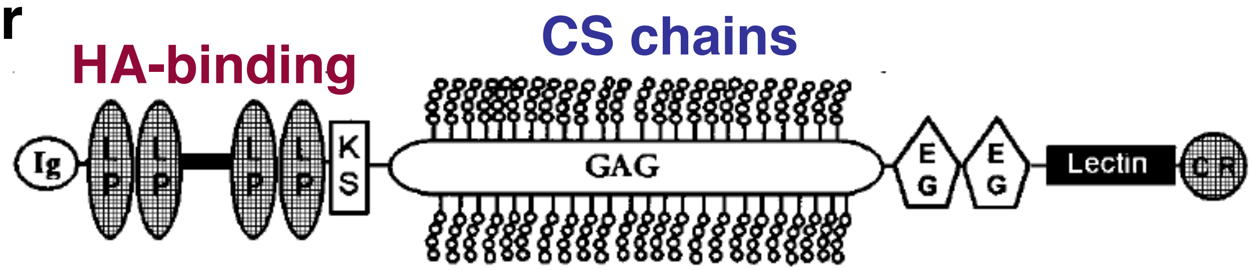
- PG: proteins with GAG side chains
  - GAG is glycosaminoglycan
  - many charged groups: COO^- , SO_3^-
  - electrostatic repulsion
- Main cartilage PG is aggrecan
  - GAG is primarily chondroitin sulfate (CS)
  - aggrecans polymerize via hyaluronin (HA)



Chondroitin sulfate
(public domain image)

Aggrecan monomer

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

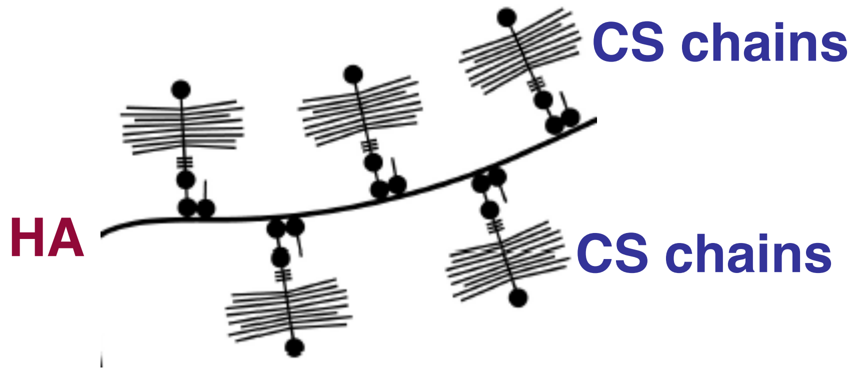


PG form aggregates of varying sizes

- Monomer > 1M, aggregates > 100M Da
- Average size decreases
  - with age
  - with osteoarthritis (OA)
- Aggrecenase inhibitors may be an OA target
- High negative charge density leads to osmotic swelling

Aggrecan aggregate

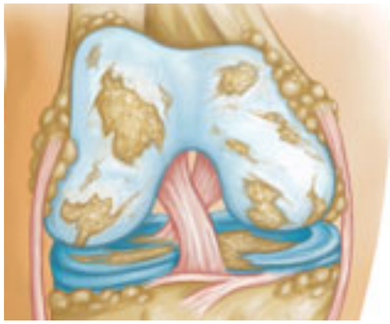
C.B & W. Knudson
Cell & Dev Bio
12:69 (2001)

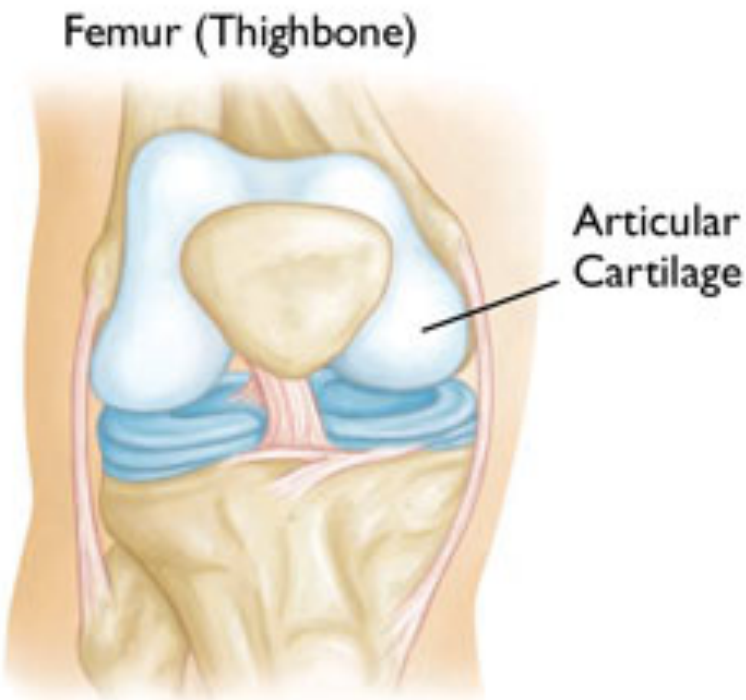


Macro structure of the knee

- See also movie: http://orthoinfo.aaos.org/topic.cfm?topic=a00212
- Requirements of a joint
  - load transfer
    - bone/bone
    - bone/muscle
  - flexibility
    - synovial fluid lubricates

OA knee



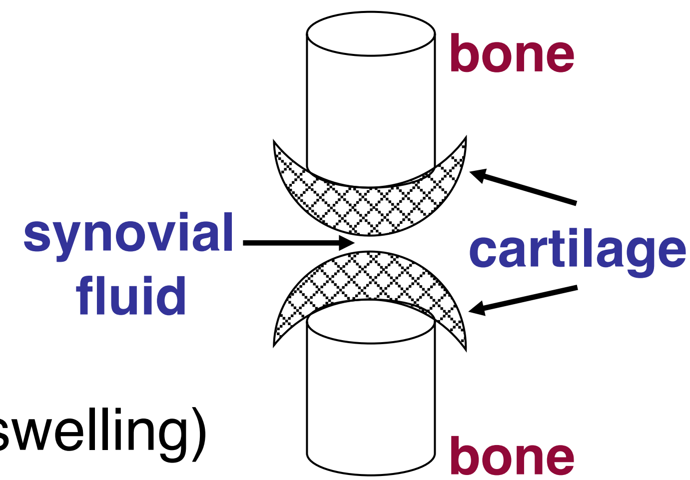


Healthy knee

http://orthoinfo.aaos.org/topic.cfm?topic=a00212

Cartilage structure and function

- Cartilage composition
  - dry weight: CN 50-75% ; PG 15-30%
  - water: 60-80%
  - cells: 5-10% (v/v)
- Role of PG
  - high compressive strength (osmotic swelling)
  - low permeability and high drag coefficient reduce wear on joint → H_2O bears some load
- 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

Principles of osmotic pressure

- Water must have equal chemical potential in both compartments: $\mu_{H2O,1} = \mu_{H2O,2}$
- Solutes decrease μ, pressure increases μ
- Infinite water would equalize [solute], but influx limited
- Charges must also be balanced (Donnan equilibrium)

Simplified cartilage model

	H_2O + high $[Na^+]/PG^-$	**1**
Membrane: PG can't cross →		
	H_2O + low $[Na^+]$	**2**

Poor water retention in OA cartilage reduces load sharing, increases wear

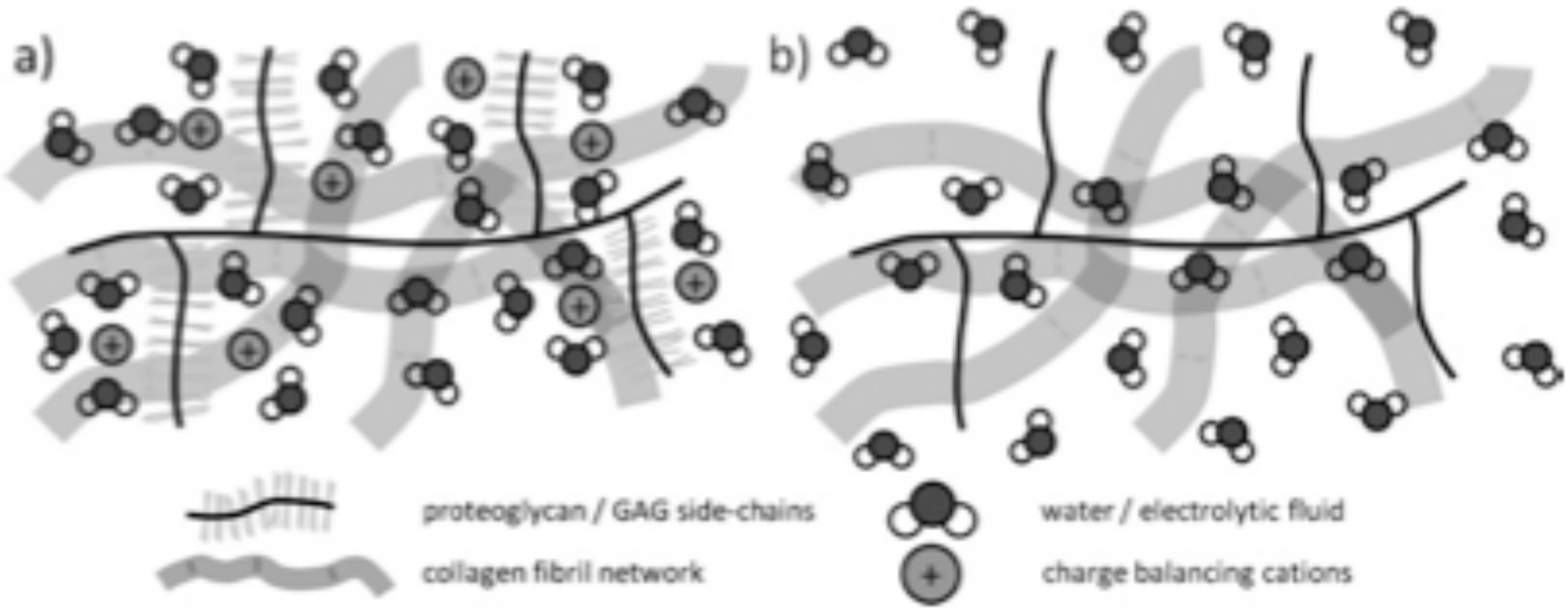
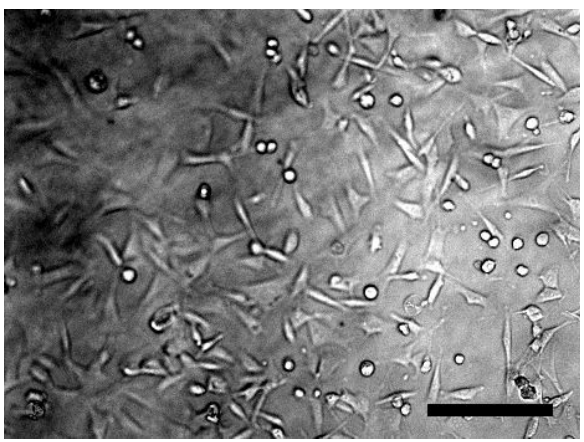


Image: DA Binks et al., *Br J Radiol* **86**:20120163 (2013).

Lecture 2: engineered and native biomaterials

- Diverse biomaterials are used in TE.
- Cell-material interactions can be (+), (-), or neutral.
- Hydrogels are useful for soft tissue engineering: similar properties and easily tunable.
- The composition of cartilage supports its functions.



Next time… cell viability and imaging; intro to standards in scientific communities.