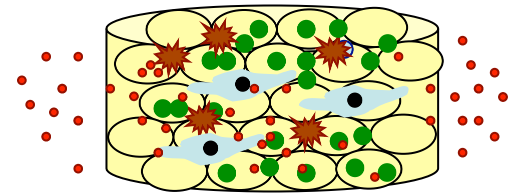
Biomaterials and Cell-Biomaterial Interactions

Module 3, Lecture 2

20.109 Spring 2013

Lecture 1 review

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



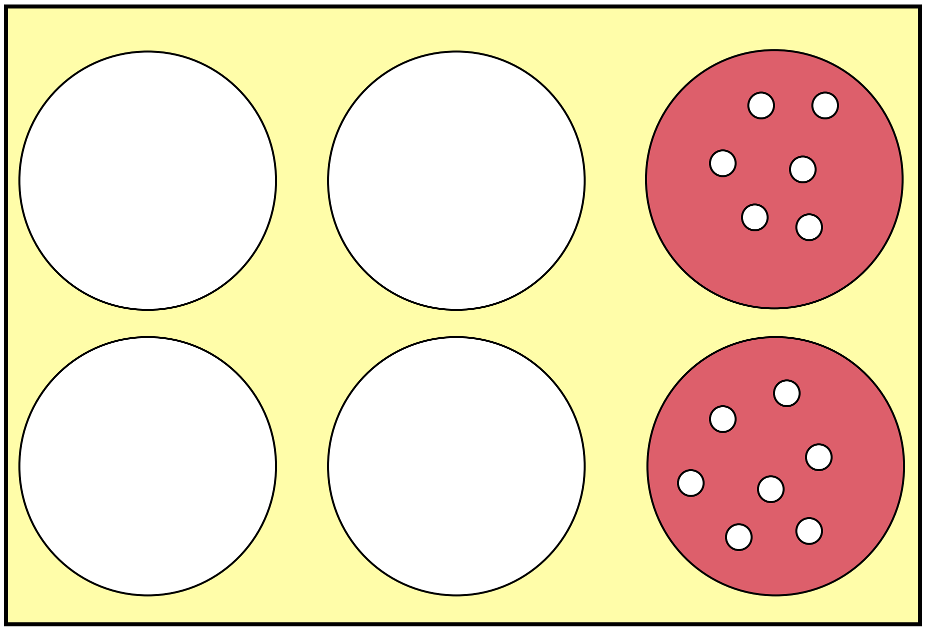
Topics for Lecture 2

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

Module 3 learning goals

- Lab concepts/techniques
  - mammalian cell culture and phenotypic assays
- Short informal report
  - accountability to 20.109 community
- Discussions in lecture
  - engage with meta-scientific issues, ethics, etc.
- Research idea presentation
  - investigate literature independently
  - exercise scientific creativity
  - design experiments to address a specific question

Today in Lab: M3D2



Condition 1 of 2

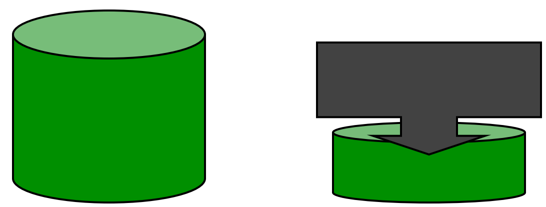
0.5 mL beads,
6 mL media

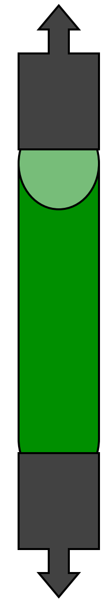
0.5 mL beads,
6 mL media

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.

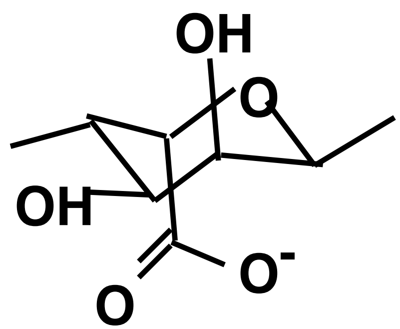
Properties of biomaterials

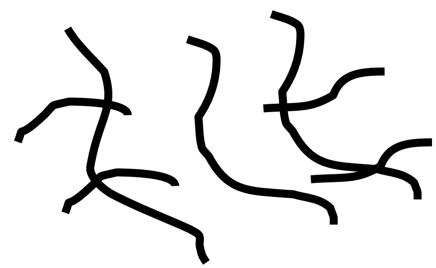
- Physical/mechanical
  - strength
  - elasticity
  - architecture (e.g., pore size)



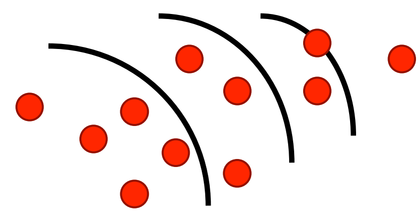


- Chemical
  - degradability
  - toxicity
  - water content





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



- Lifetime

The right material for the job

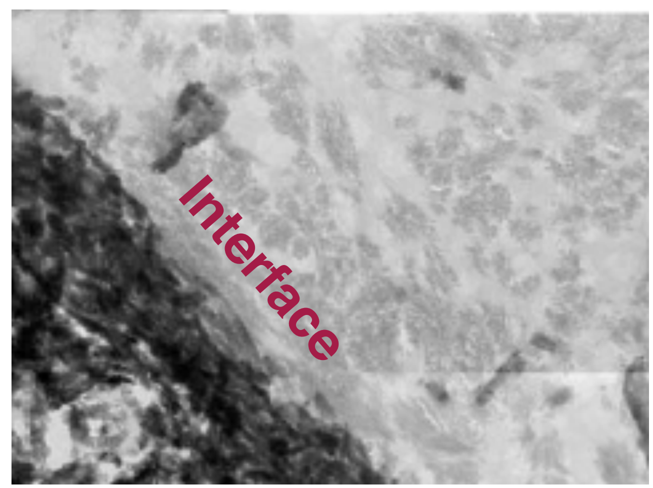
- Metals
  - Ti, Co, Mg alloys
  - pros: mechanically robust
  - applications: orthopedics, dentistry
- Ceramics
  - Al_2O_3, Ca-phosphates, sulfates
  - pros: strength, bonding to bone
  - applications: orthopedics, dentistry
- Polymers
  - diverse, tunable properties
  - applications: soft tissues

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

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

Metal hip implant

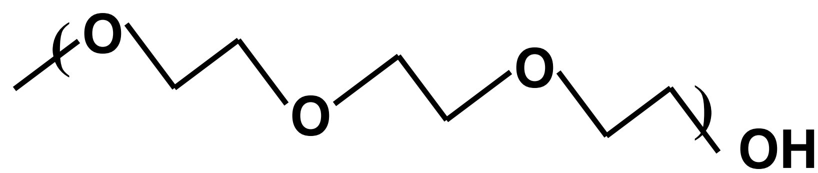
http://www.weisshospital.com/joint-university/hip/metal.html



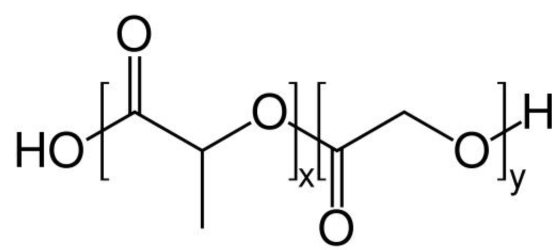
Si-HA Bone

Polymers are diverse and tunable

- Linear polymers
  - repeated chemical unit
- Co-polymers
  - heterogeneous repeats
- As MW increases
  - entanglements ↑
  - strength ↑
  - processability ↓
- Chemical group(s) affects
  - mechanical properties
  - stability/degradability
  - hydrophilicity
  - reactivity/modification ease
  - gas permeability



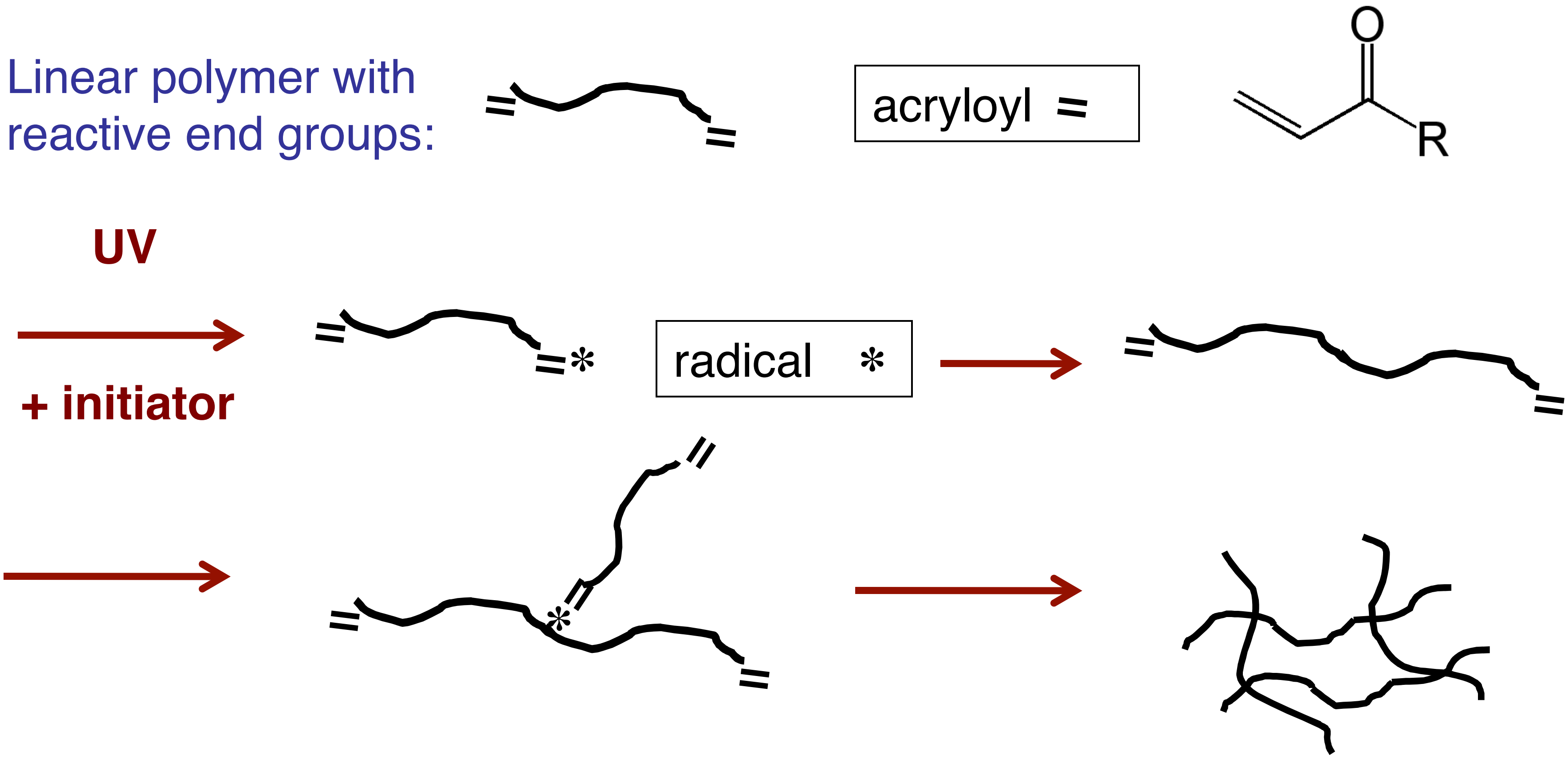
Poly(ethylene glycol)



Poly(lactic-co-glycolic acid)

[public domain image]

Network polymer synthesis example



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

Network polymer

Properties of hydrogels

(Stachowiak & Irvine)

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

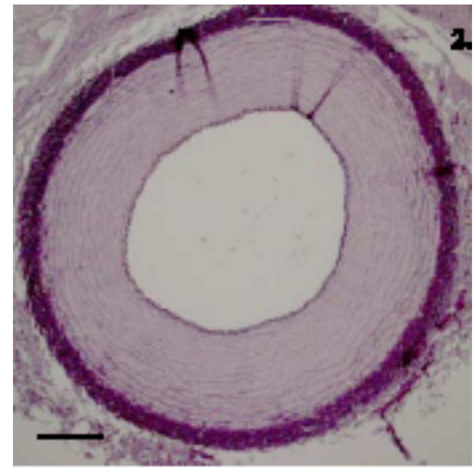
Review: Nguyen KT & West JL, *Biomaterials* **23**:4307 (2002)

Materials must be biocompatible

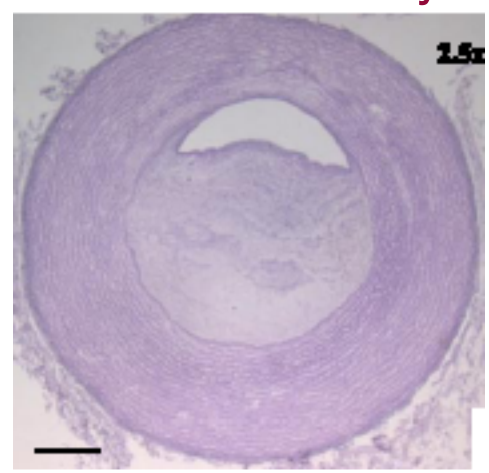
- Avoid bio-incompatibility
  - chemical toxicity: cells, genomes
  - immunogenicity
  - protein/cell adhesion → clotting
  - bacterial adhesion
- Material properties
  - material *and* its degradation products non-toxic
  - sterility
  - resistance to protein adhesion

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

Normal artery



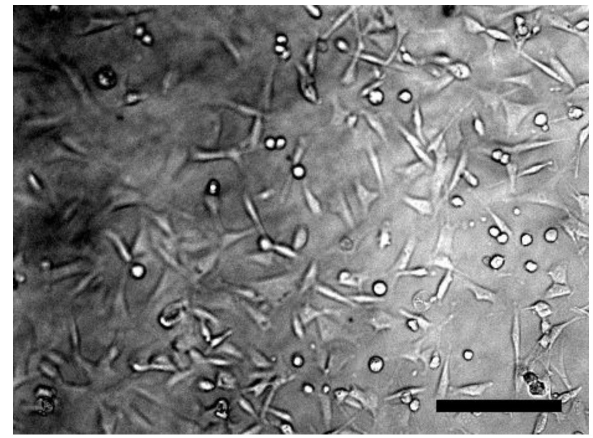
Occluded artery



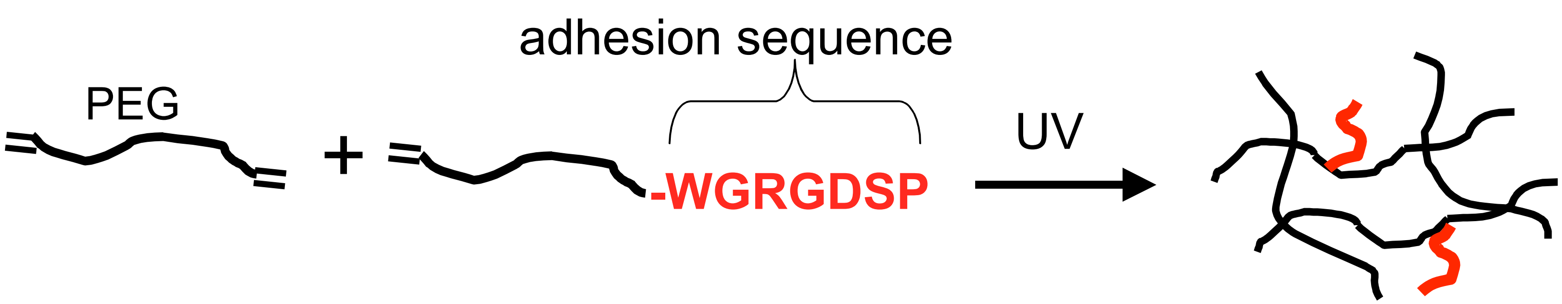
Beyond bioinert: bioactive materials

- 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: on reproducibility

Problem:

"In September, Bayer published a study describing how it had halted [a majority] of its early drug target projects because in-house experiments failed to match claims made in the literature."

http://online.wsj.com Dec 2nd, 2011

Solution?

"The initiative aims to help scientists validate their research findings by providing a mechanism for blind, independent replication by experts from Science Exchange's network of more than 1,000 providers at core facilities and contract research orgs."

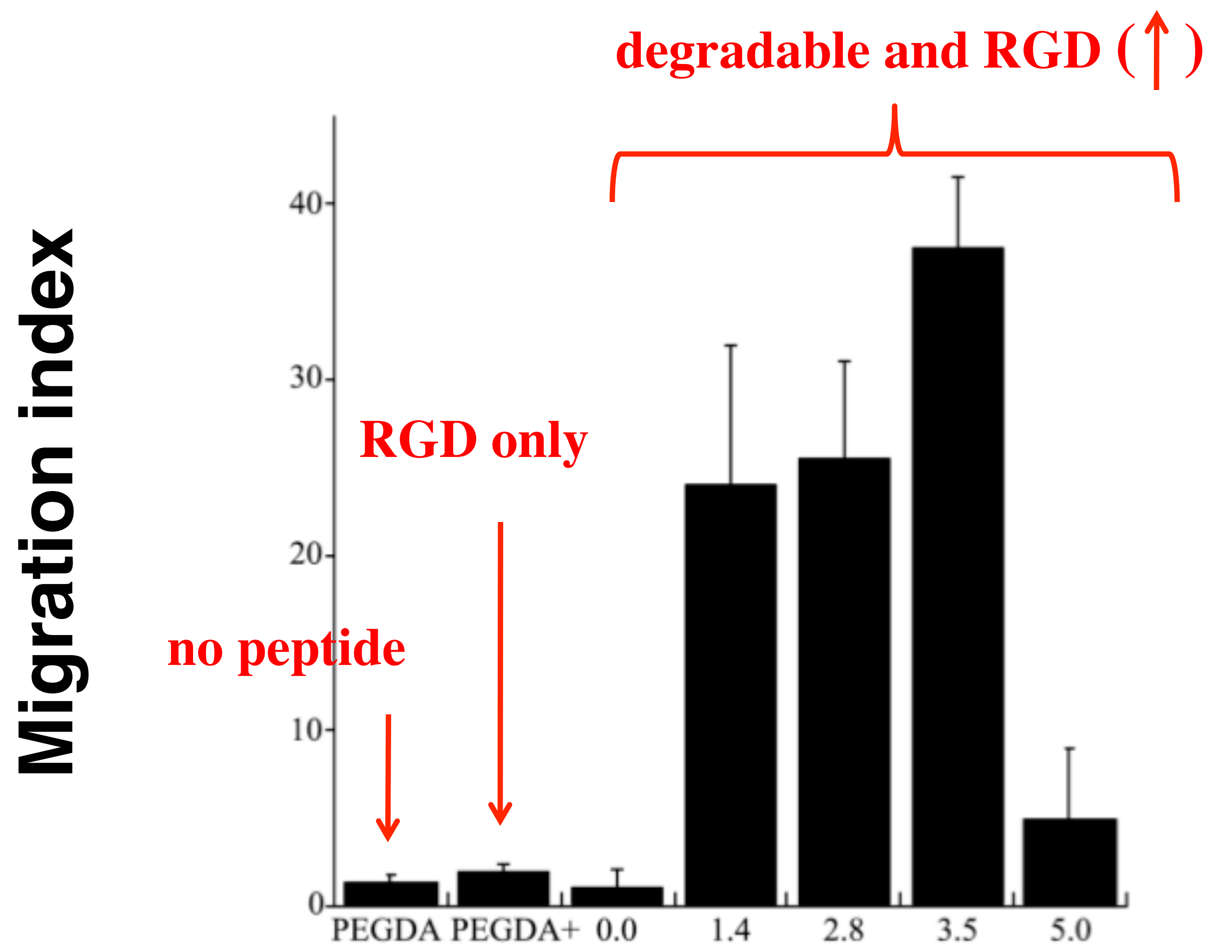
http://blogs.plos.org/everyone/2012/08/14/plos-one-launches-reproducibility-initiative/

Or just more problems?

http://scholarlykitchen.sspnet.org/2012/08/16/the-reproducibility-initiative-solving-a-problem-or-just-another-attempt-to-draw-on-research-funds/

http://www.xconomy.com/seattle/2012/10/02/the-reproducibility-initiative-a-good-idea-in-theory-that-wont-work-in-practice/

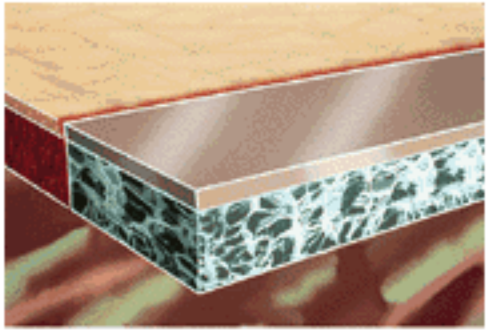
TE constructs to study cell migration



Gobin AS & West J, *FASEB J* **16**:751 (2002)

Natural vs. synthetic polymers

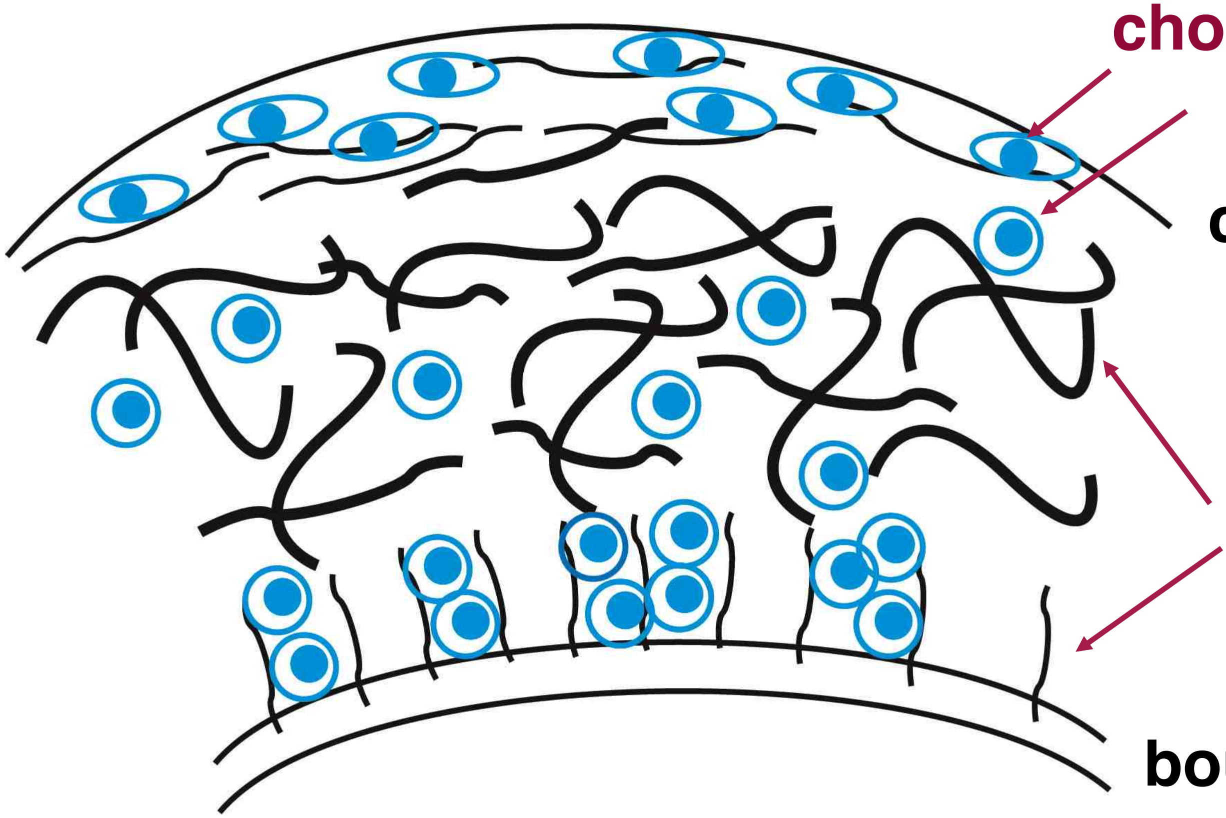
- Natural pros/cons
  - built-in bioactivity
  - poor mechanical strength
  - immunogenicity (xenologous sources)
  - lot-to-lot variation, unpredictable



- 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

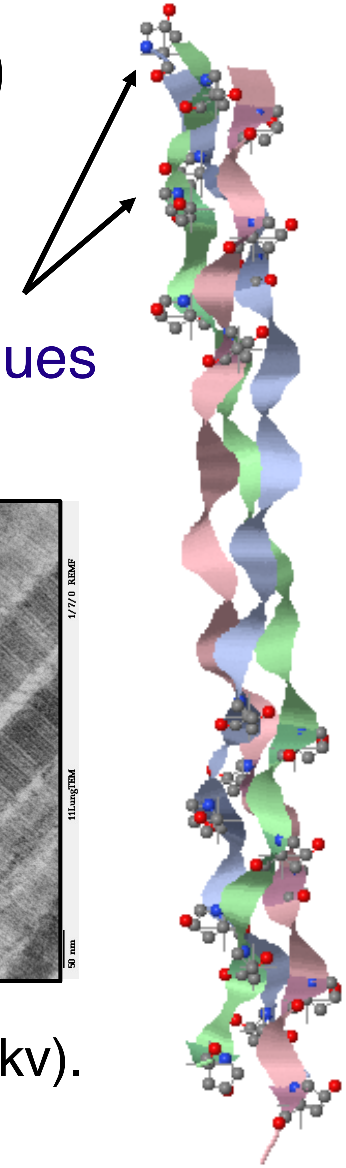


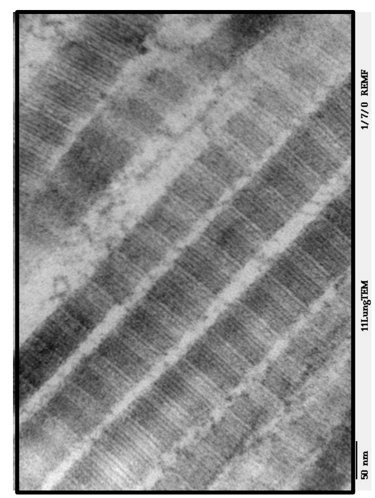
Water-swollen, heterogeneous, **avascular and cell-poor** tissue.

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

HYP residues

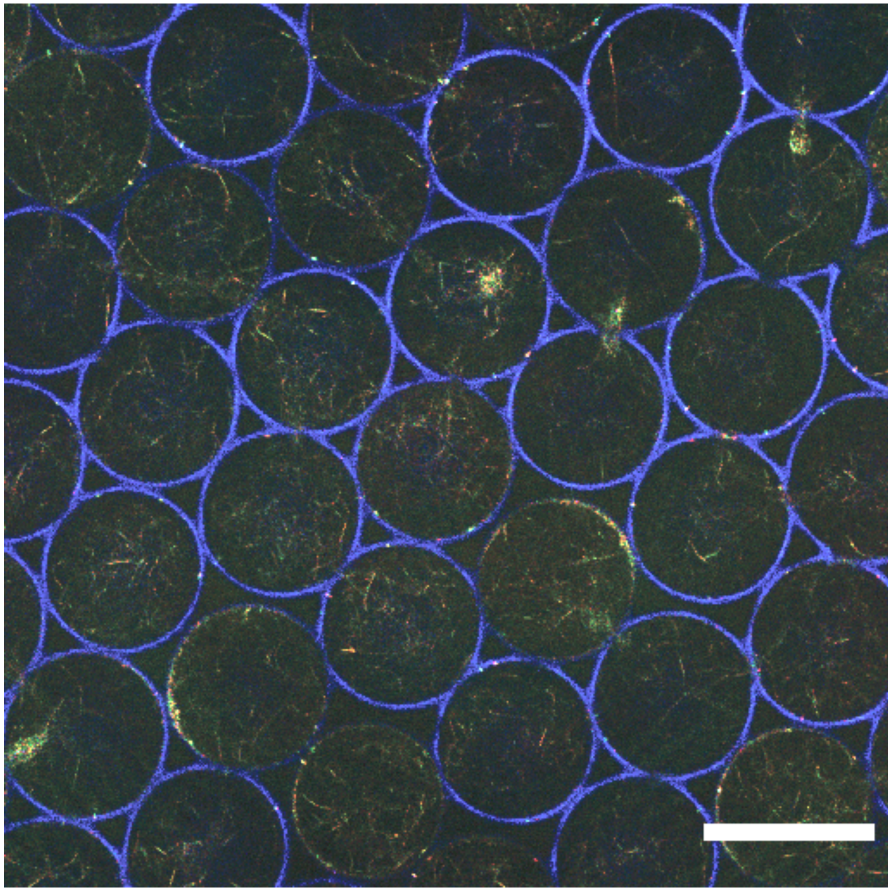




Molecular image made using *Protein Explorer* (PDB ID: 1bkv).
Fibril image from public domain.

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

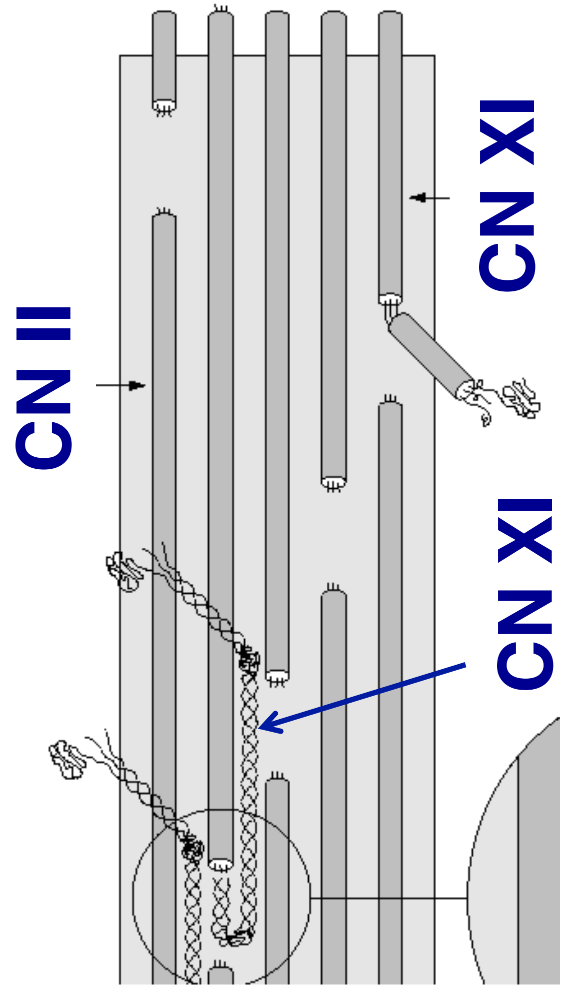
Macro structure of fibrillar collagen



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

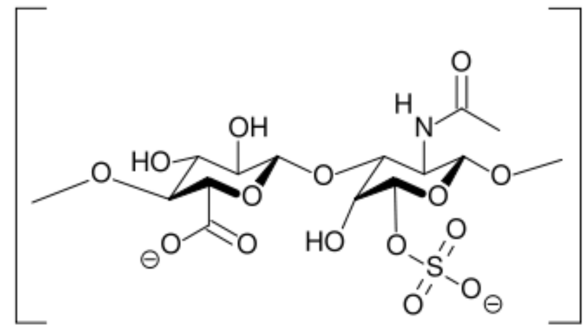


D. Eyre (2002)

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

Proteoglycans are bulky and charged

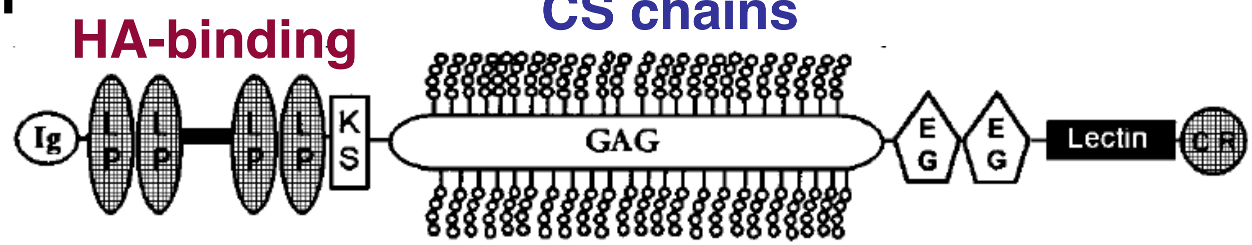
- PG: proteins with GAG side chains
  - GAG is glycosaminoglycan
  - many charged groups: COO^- , SO_3^-
  - electrostatic repulsion
- Main cartilage PG is aggrecan
  - GAG is primarily chondroitin sulfate (CS)
  - aggrecans polymerize via hyaluronin (HA)



Chondroitin sulfate
(public domain image)

Aggrecan monomer

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

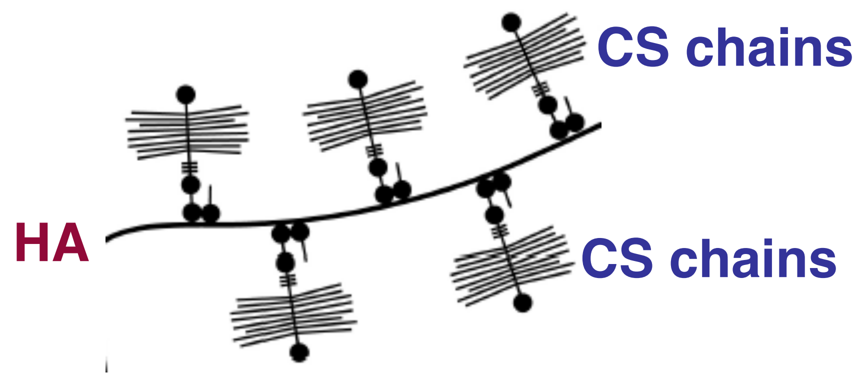


PG form aggregates of varying sizes

- 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

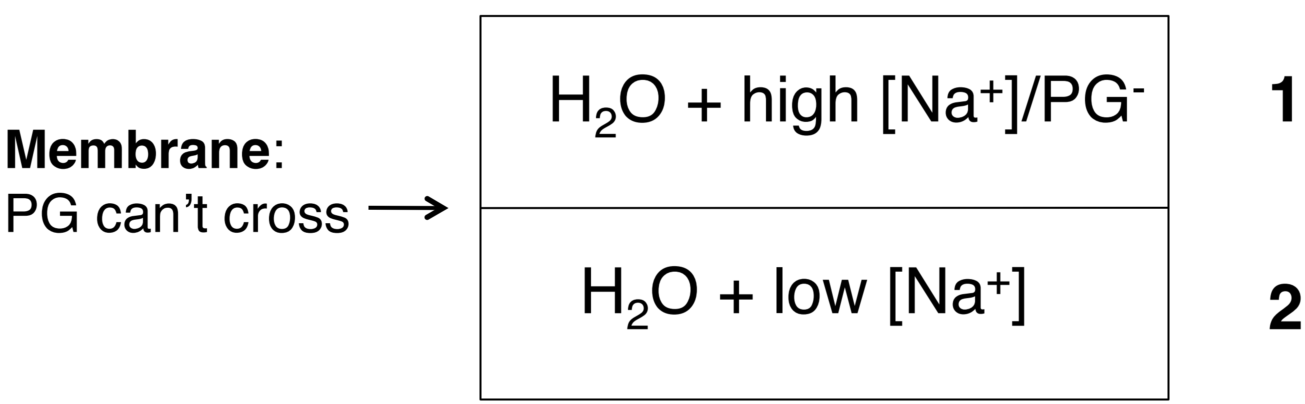
C.B & W. Knudson
Cell & Dev Bio
12:69 (2001)



Principles of osmotic pressure

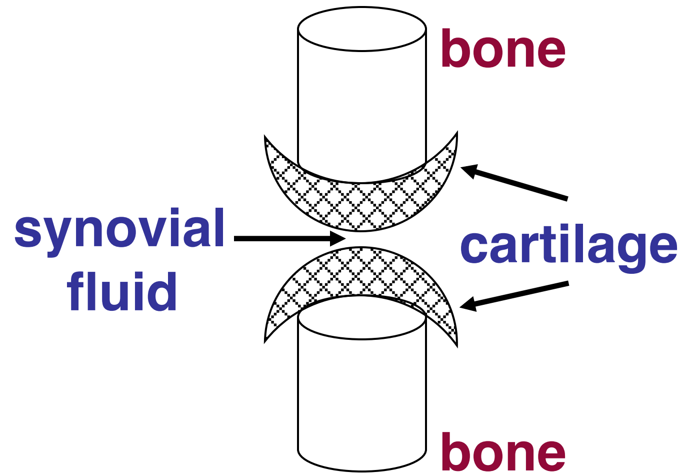
- Water must have equal chemical potential in both compartments: $\mu_{H2O,1} = \mu_{H2O,2}$
- Solutes decrease μ, pressure increases μ
- Infinite water would equalize [solute], but influx limited
- Charges must also be balanced (Donnan equilibrium)

Simplified cartilage model



Cartilage structure and function

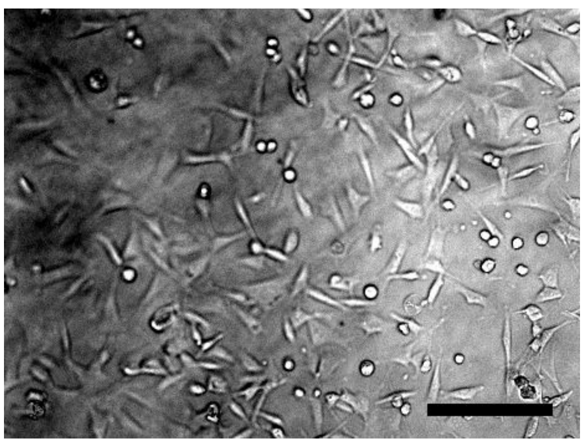
- Cartilage composition
  - dry weight: CN 50-75% ; PG 15-30%
  - water: 60-80%
  - cells: 5-10% (v/v)
- Requirements of a joint
  - load transfer (bone/bone, bone/muscle)
  - flexibility, lubrication
- Role of PG
  - high compressive strength (osmotic swelling)
  - low permeability reduces wear, H_2O 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

Lecture 2: conclusions

- 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.