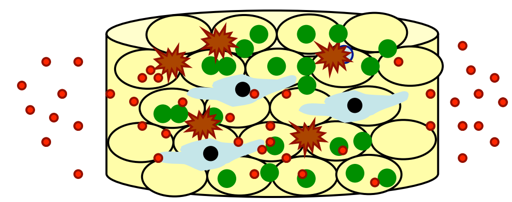
Biomaterials and Cell-Biomaterial Interactions

Module 3, Lecture 2

20.109 Spring 2012

Lecture 1 review

- What is tissue engineering?
- Why is tissue engineering?

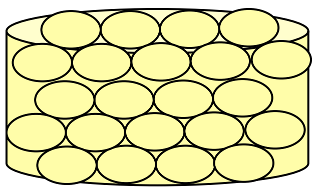


- Why care about cartilage?
- What are we asking in Module 3?

Lec1: components of a TE construct

scaffold/matrix

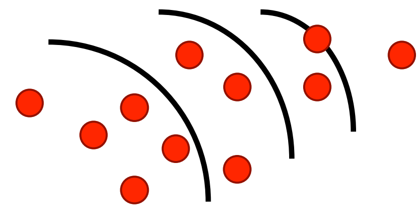
→ usually degradable, porous



soluble factors

→ made by cells or synthetic

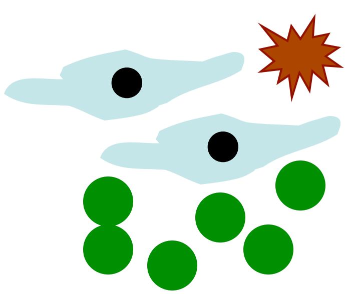
→ various release profiles

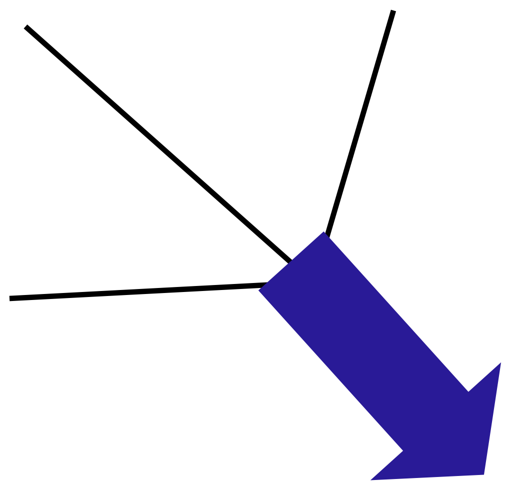


cells

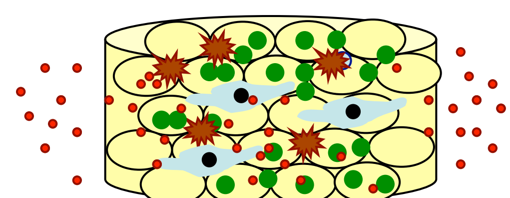
→ precursors and/or differentiated

→ often autologous



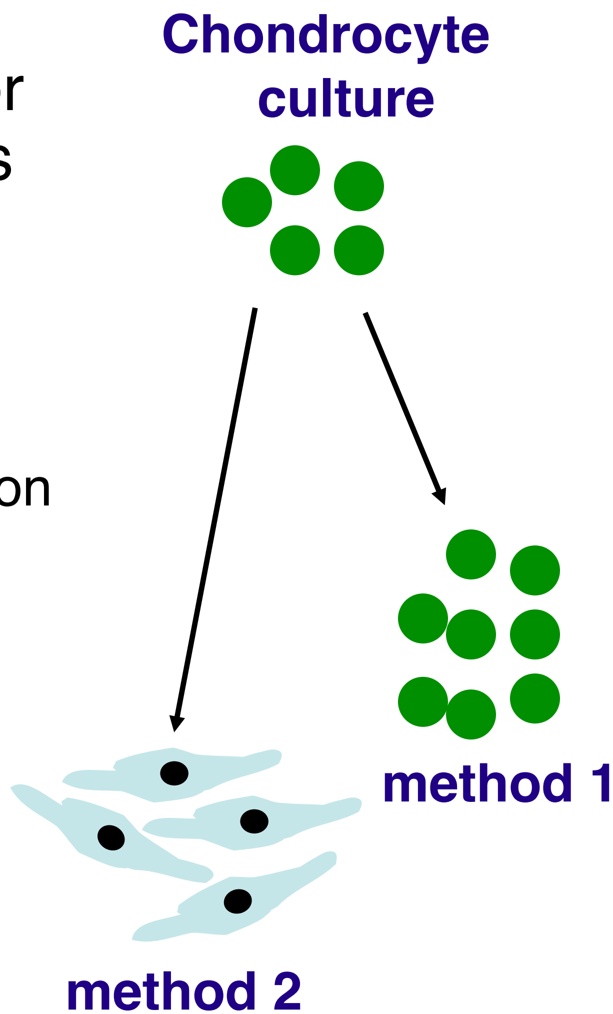


integrated implantable or injectable device



Lec1: specific goal and experiments

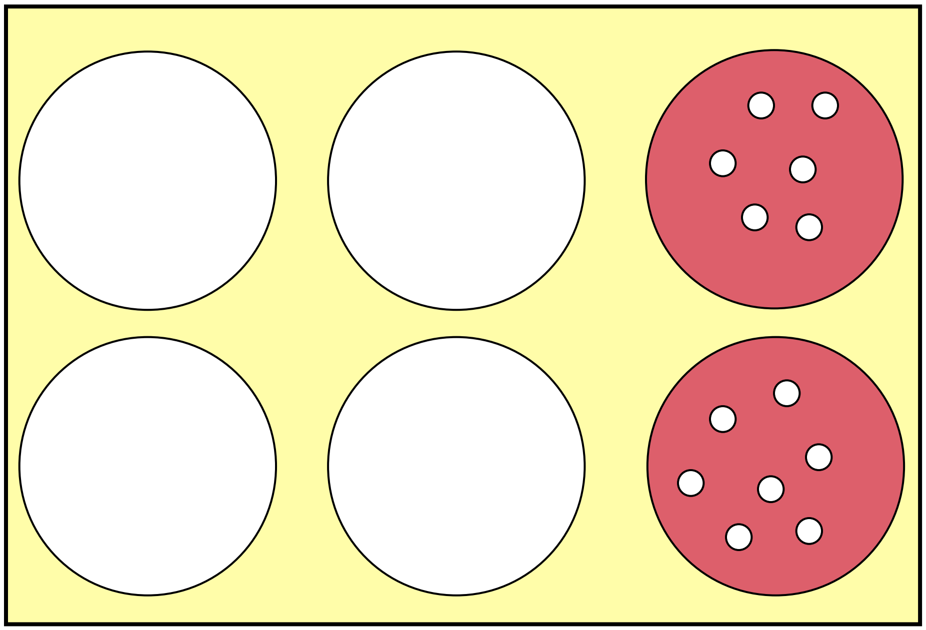
- **Goal:** examine effect of specific culture conditions on chondrocyte phenotype or stem cell differentiation to chondrocytes
- Observe cell morphology and viability
- Measure collagen content
  – Gene (qPCR) and protein (ELISA) expression
  – Collagen II:I ratio reflects cell state
- Measure proteoglycans
- Grander purpose: cartilage TE
  – conditions for *in vitro* cartilage production
  – conditions for *ex vivo* cell expansion
  – cures for joint diseases an unmet need



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



0.5 mL beads,
6 mL media

0.5 mL beads,
6 mL media

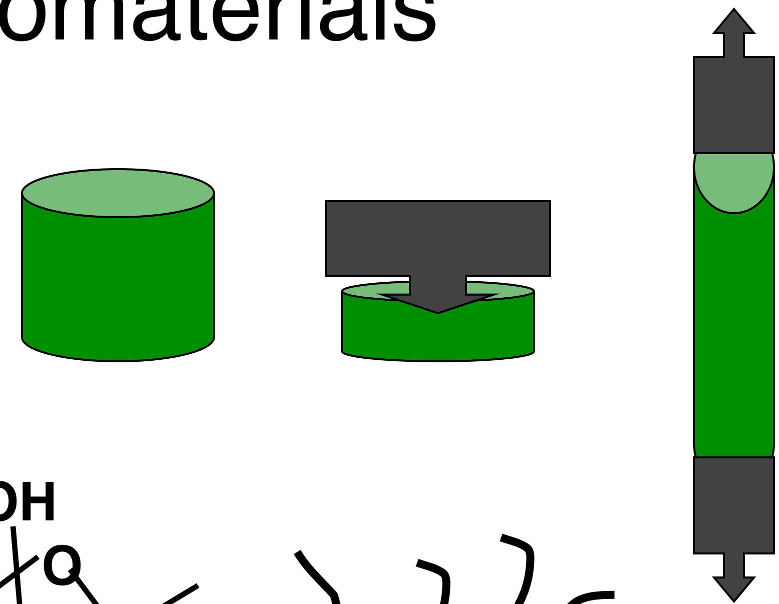
1 condition per plate (2 plates total).
2 wells per plate (split 1 mL of beads).
if contaminate 1 well on D3, still have 1 on D4.

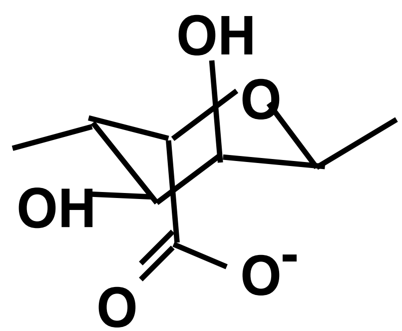
Topics for Lecture 2

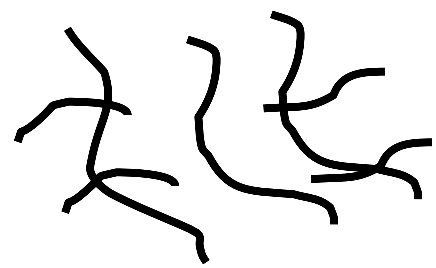
- Introduction to biomaterials
  - properties
  - examples
- Cartilage composition
  - collagen
  - proteoglycans
  - structure → function

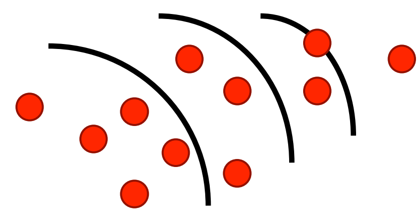
Properties of biomaterials

- 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

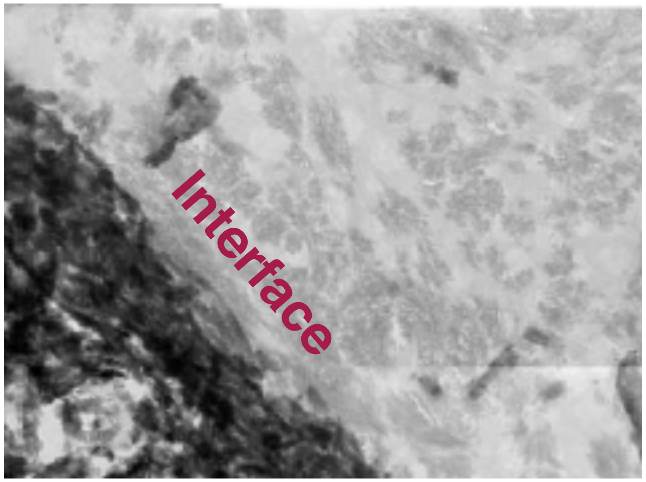
- Metals
  - Ti, Co, Mg alloys
  - pros: mechanically robust
  - applications: orthopedics, dentistry
- Ceramics
  - Al_2O_3, Ca-phosphates, sulfates
  - pros: strength, bonding to bone
  - applications: orthopedics, dentistry
- Polymers
  - diverse, tunable properties
  - applications: soft tissues

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

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

Metal hip implant

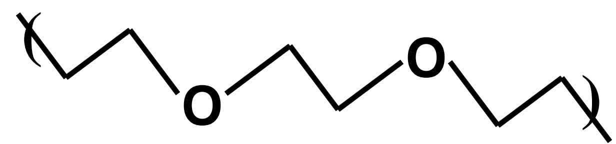
http://www.weisshospital.com/joint-university/hip/metal.html



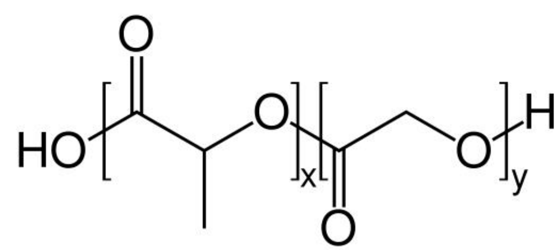
Si-HA **Bone**

Polymers are diverse and tunable

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



Poly(ethylene glycol)

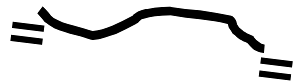


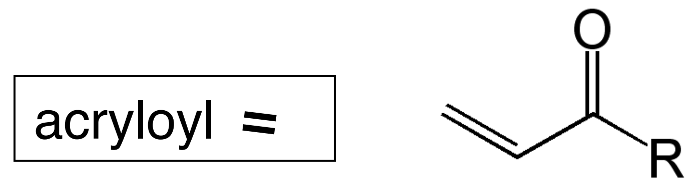
Poly(lactic-co-glycolic acid)

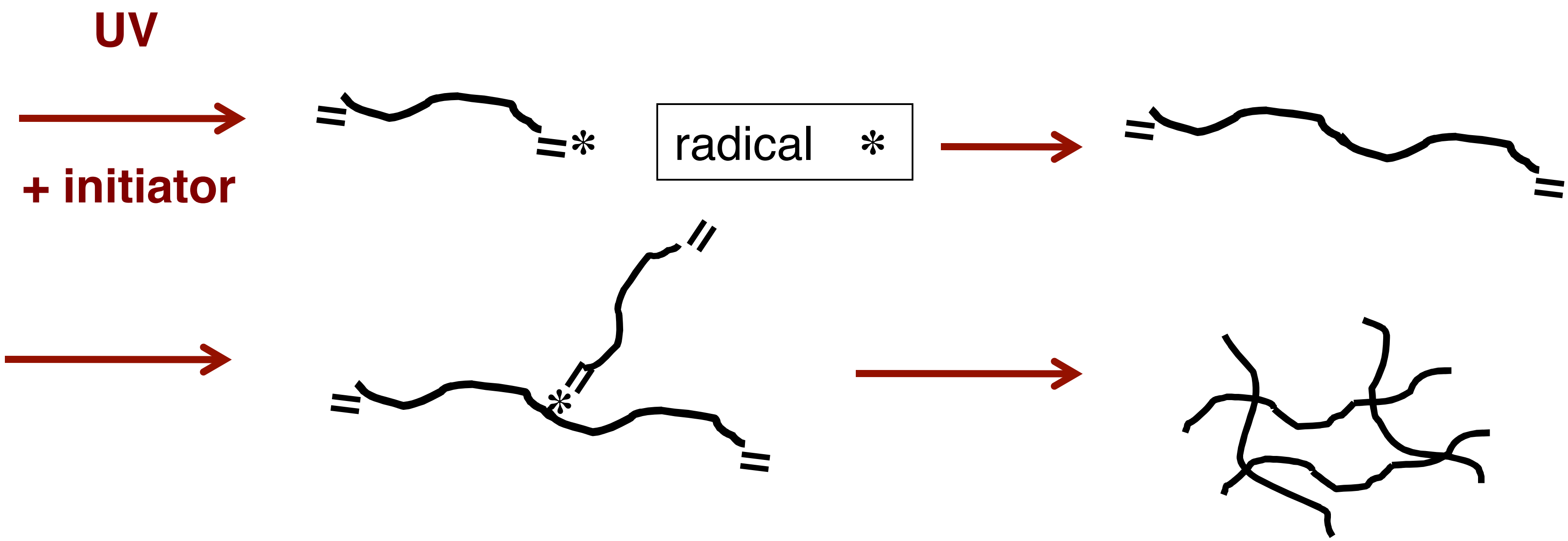
[public domain image]

Free radical polymerization

Linear polymer with
reactive end groups:







Network polymer

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

Properties of hydrogels

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

(Stachowiak & Irvine)

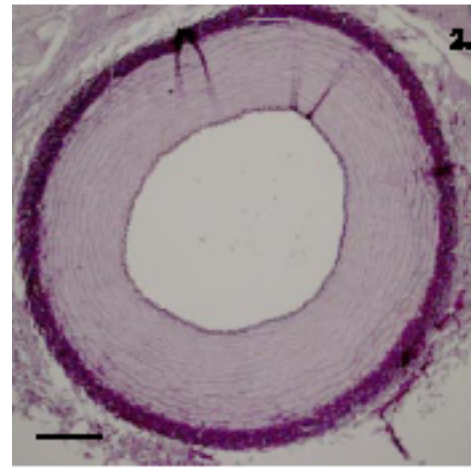
Review: Nguyen KT & West JL, *Biomaterials* **23**:4307 (2002)

Materials must be biocompatible

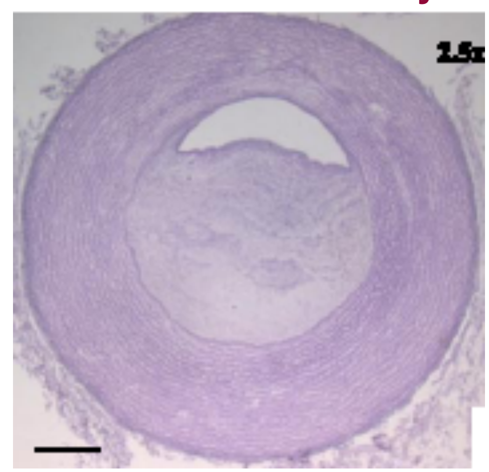
- Avoid bio-incompatibility
  - chemical toxicity
  - bacterial adhesion
  - immunogenicity
  - clot formation
- Material properties
  - material *and* its degradation products non-toxic
  - sterility
  - resistance to protein adhesion

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

Normal artery



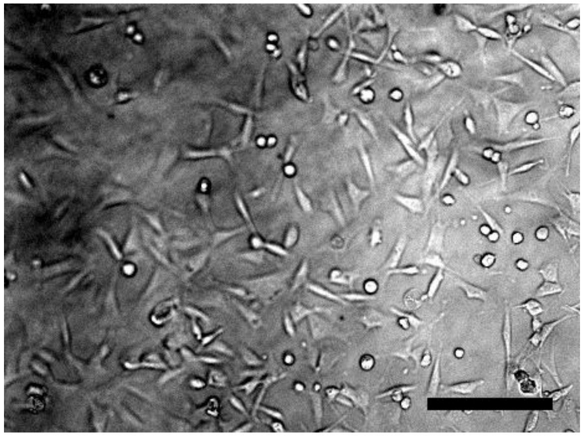
Occluded artery



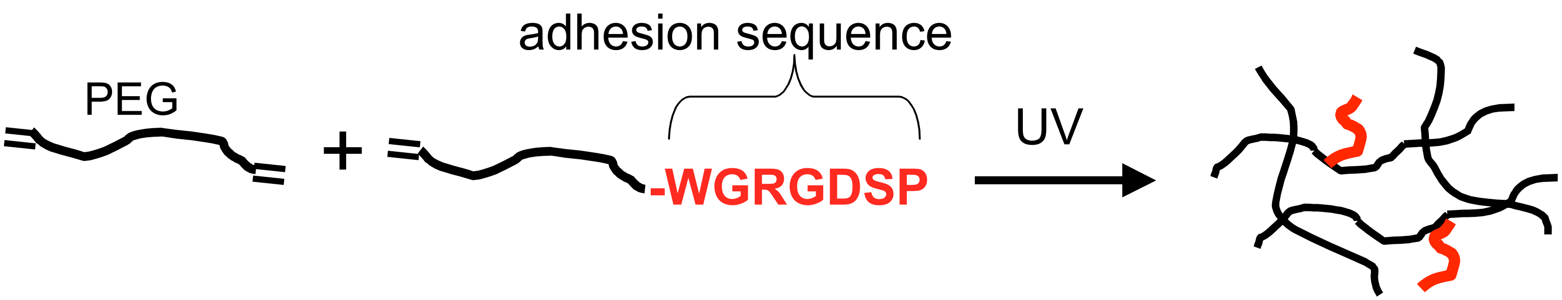
Beyond bioinert: bioactive materials

- 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

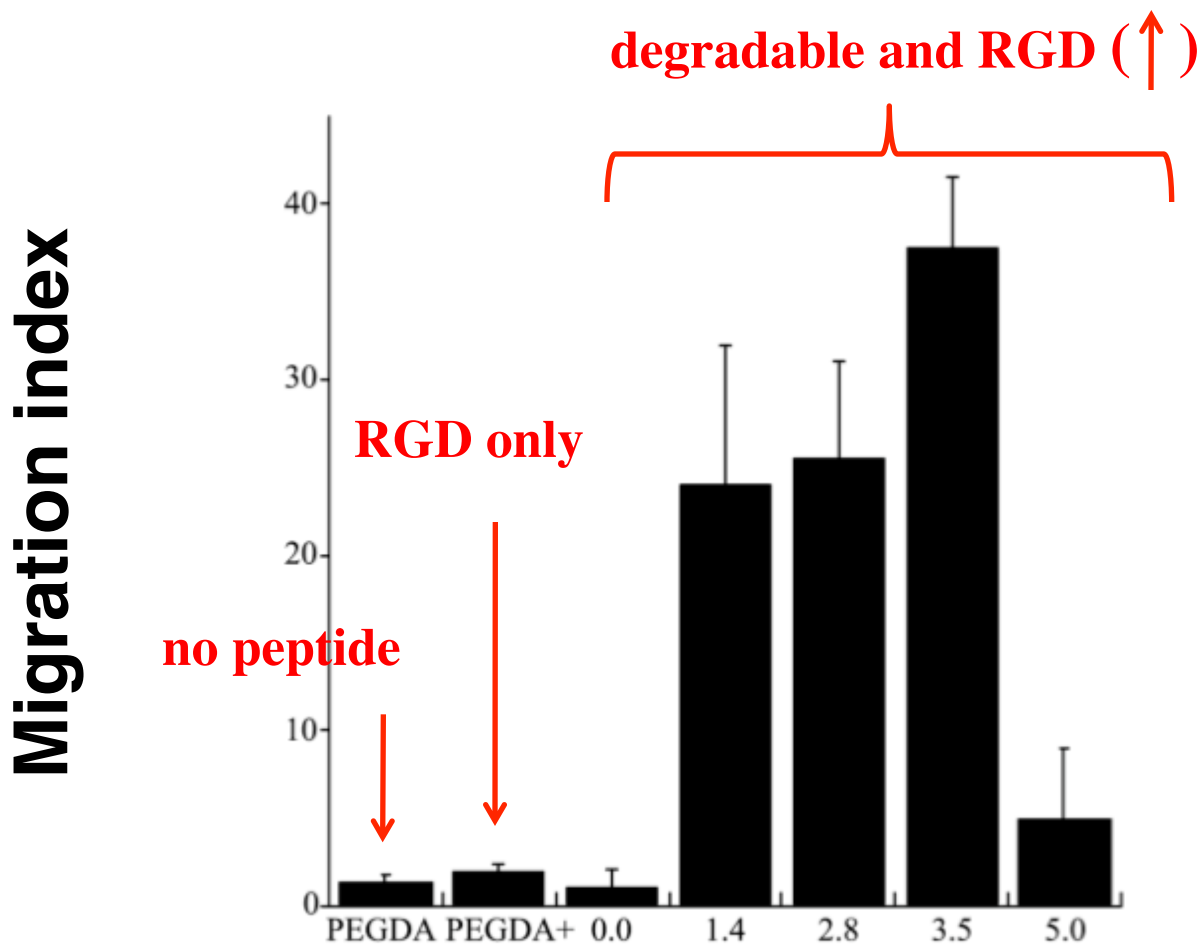
"In September, Bayer published a study describing how it had halted [a majority] of its early drug target projects because in-house experiments failed to match claims made in the literature."

Why might the problem of reproducibility (possibly) be getting worse, in biotech and some other fields?

Are there structural changes in the research community that could improve reproducibility?

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

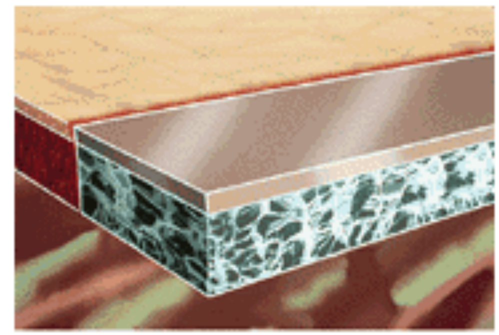
TE constructs to study cell migration



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

Natural vs. synthetic polymers

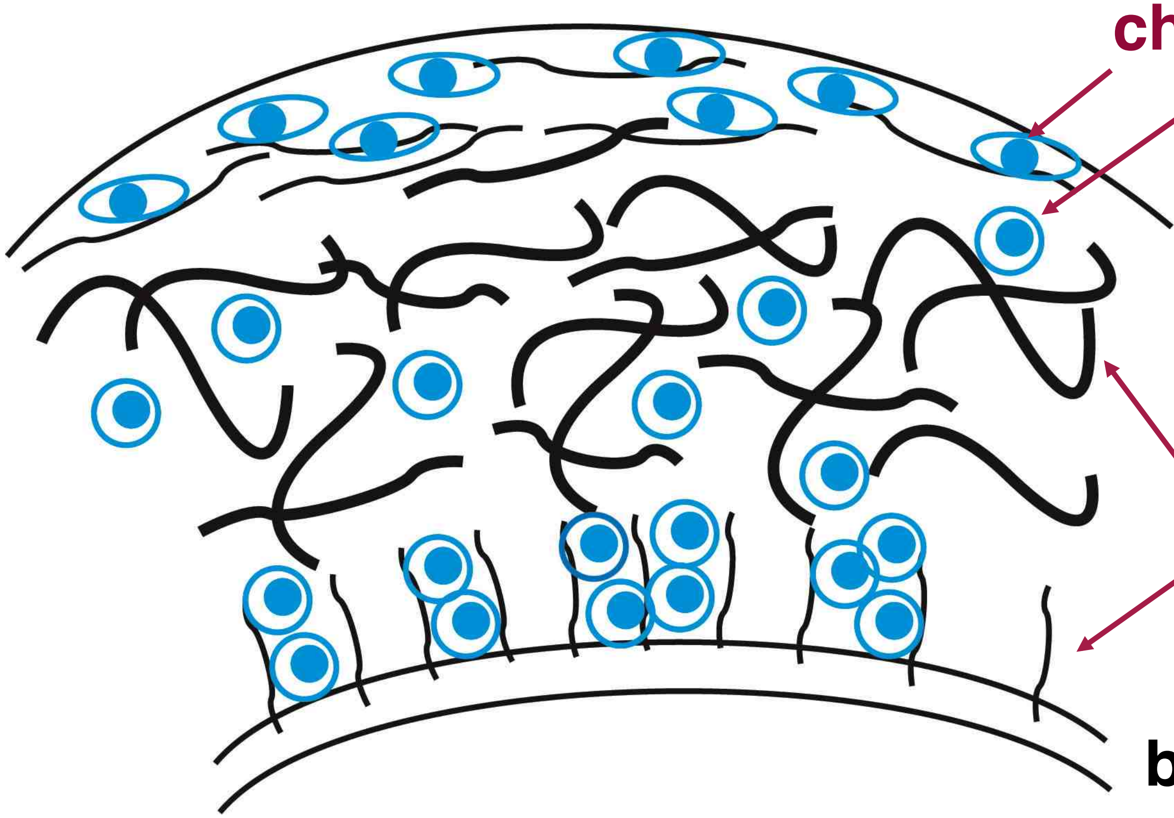
- Natural pros/cons
  - built-in bioactivity
  - poor mechanical strength
  - immunogenicity (xenologous sources)
  - lot-to-lot variation, unpredictable



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

- Synthetic advantages: tunable and reproducible

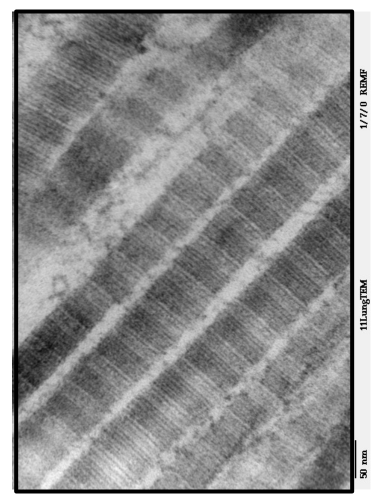
Revisiting cartilage structure



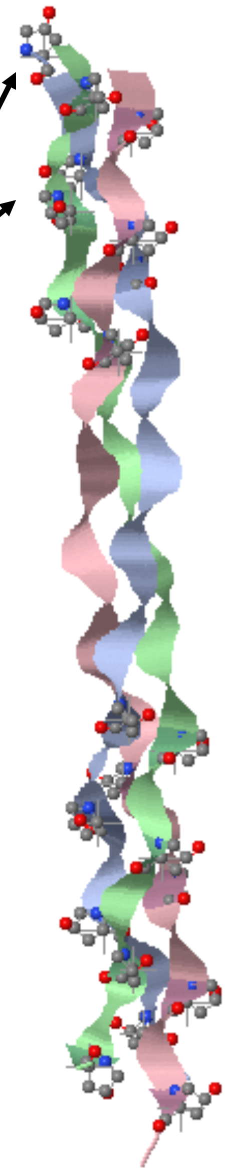
Water-swollen, heterogeneous, avascular and cell-poor tissue.

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



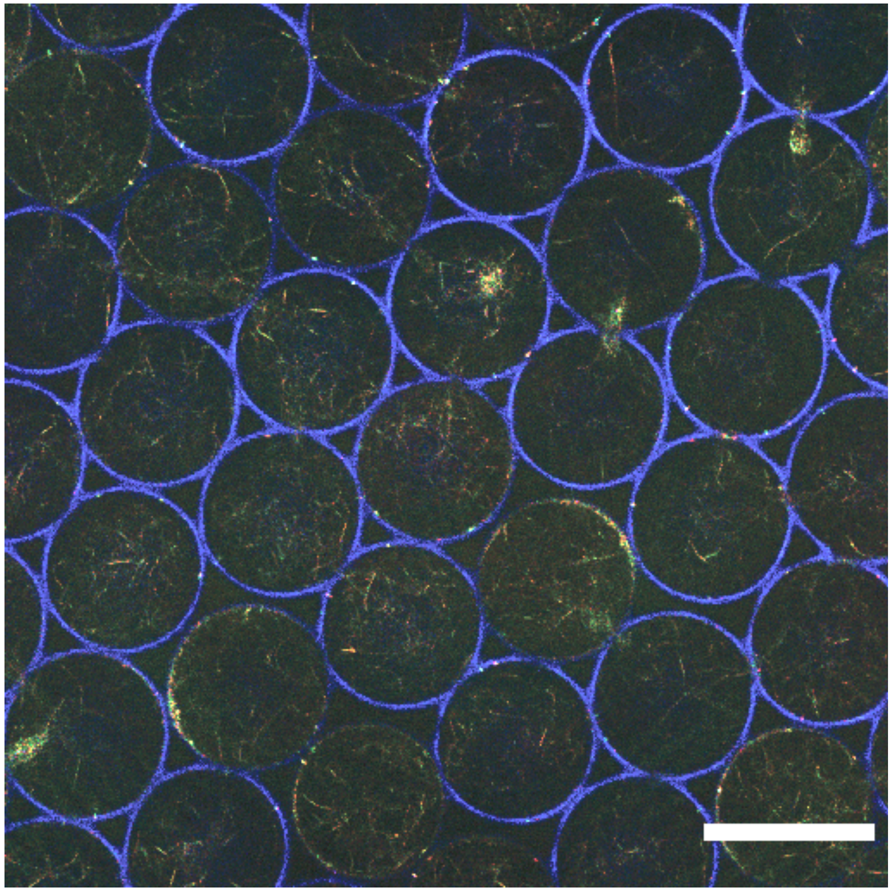
HYP residues



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)

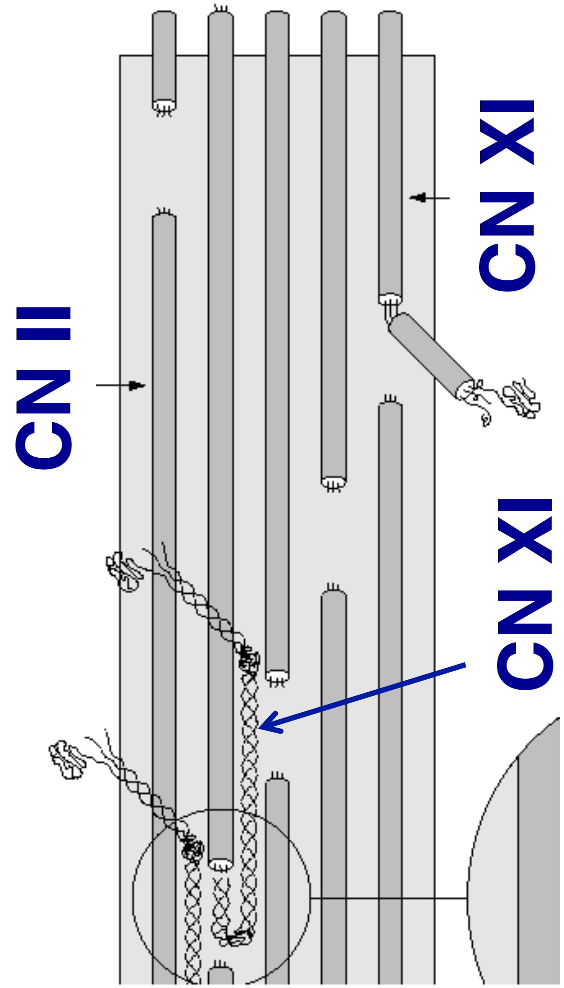
Macro structure of fibrillar collagen



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
  - others(III, VI, X, XII, XIV)
- Little collagen turnover in adult cartilage

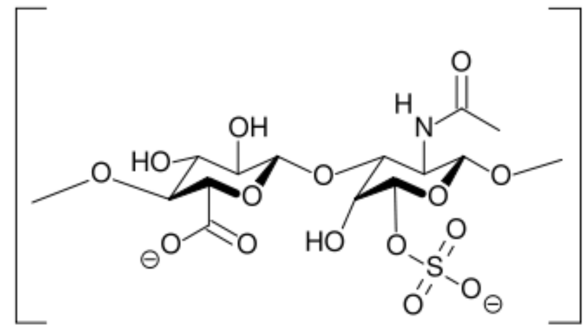


D.J. Prockop *Annu Rev Biochemritis Res* 64:403 (1995)
D. Eyre *Arthritis Res* 4:30 (2002)

D. Eyre (2002)

Proteoglycans are bulky and charged

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



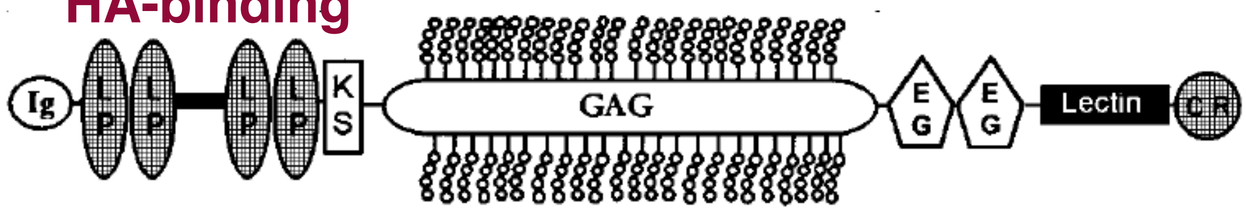
Chondroitin sulfate
(public domain image)

Aggrecan monomer

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

HA-binding

CS chains

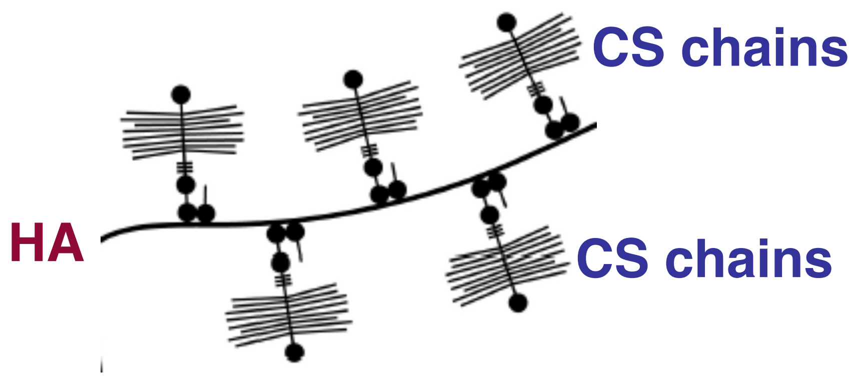


PG form heterogeneous aggregates

- 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

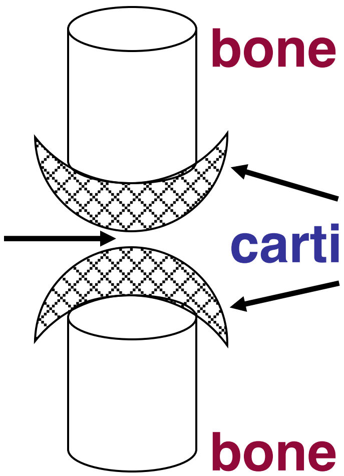
Aggrecan aggregate

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



Cartilage structure and function

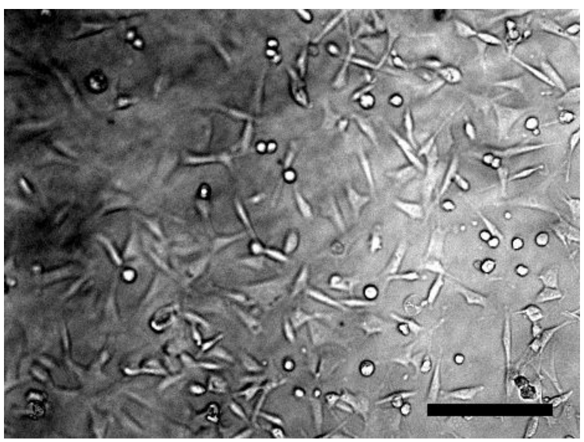
- Cartilage composition
  - dry weight: CN 50-75% ; PG 15-30%
  - water: 60-80%
  - cells: 5-10% (v/v)
- Requirements of a joint
  - load transfer (bone/bone, bone/muscle)
  - flexibility, lubrication
- Role of PG
  - high compressive strength (osmotic swelling)
  - low permeability reduces wear, H_2O 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: they mimic such tissue and are easy to modify.
- The composition of cartilage supports its functions.



Next time… intro to statistics, and to standards in scientific communities.