Introduction to Cell-Biomaterial Engineering

Module 3, Lecture 1

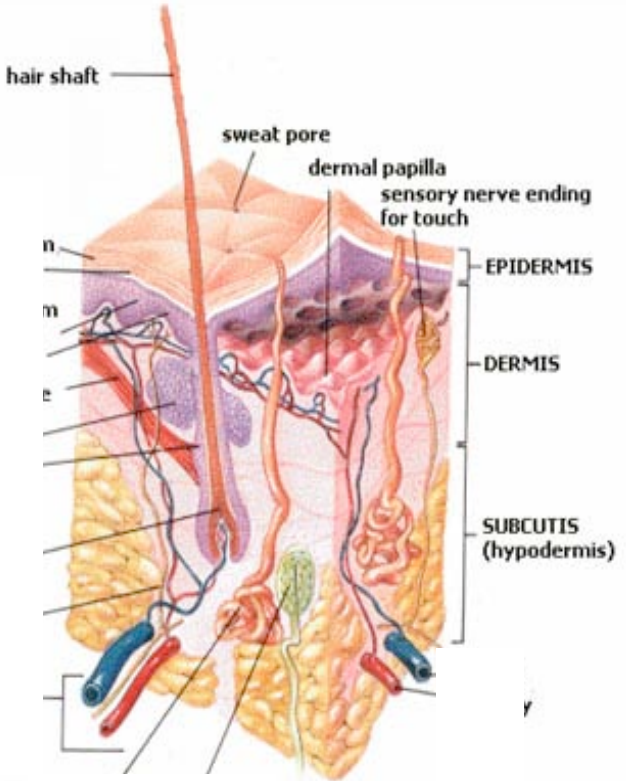
20.109 Spring 2010

Topics for Lecture 1

- Introduction to tissue engineering
  - motivation
  - basic principles + examples
- Introduction to Module 3
  - background: cells and materials
  - experiment: purpose and structure

Ability to repair tissue is limited

- Severe trauma (acute or disease-
  challenges tissue repair capacity
- Donor tissue
  - scarcity, immune response (graft o
- Autologous tissue
  - availability, donor site morbidity
- Permanent synthetic substitute
  - inflammation, mis-match, failure
- A new approach: promote regen
  of ~native tissue



[Public domain image,
Wikimedia Commons]

Tissue engineering: an emerging solution

"TE… applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function."

-R. Langer & J.P. Vacanti, *Science* **260**:920 (1993)

What is in a tissue engineer's toolkit?

How good are the outcomes?

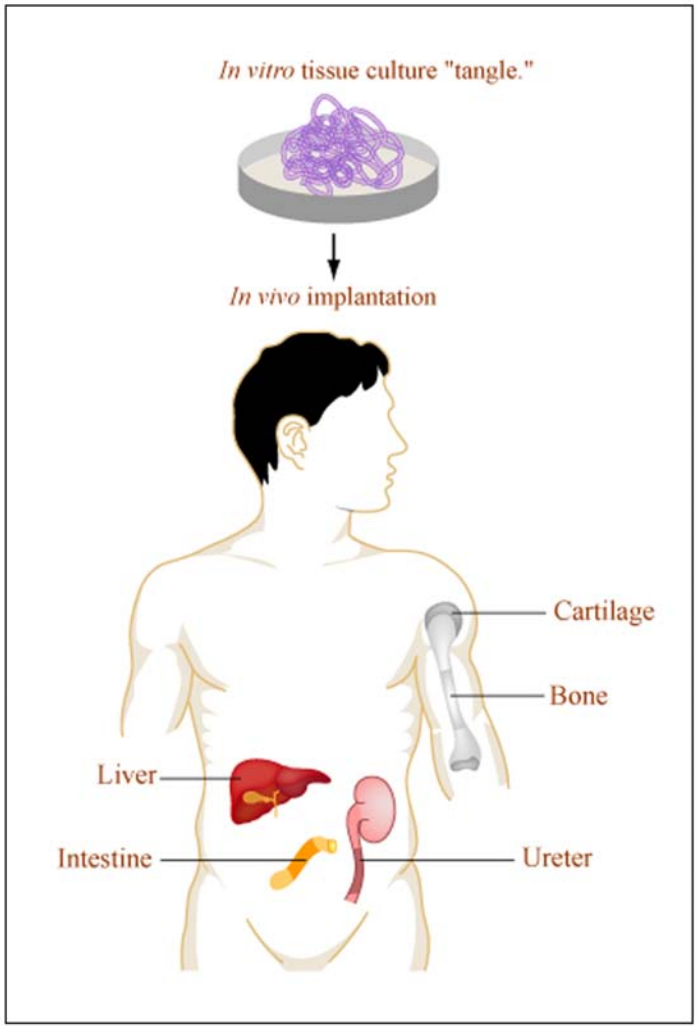
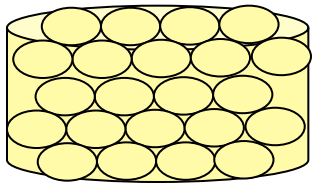


Image by MIT OpenCourseWare. After Langer and Vacanti (1993).

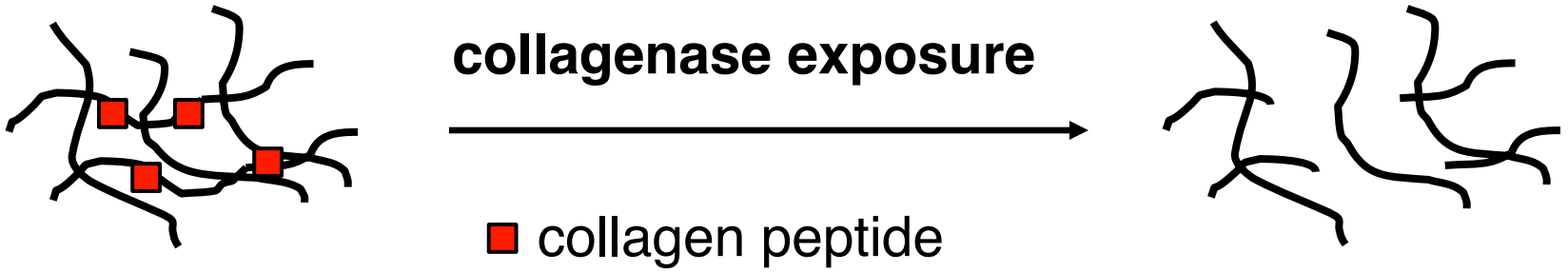
[Langer & Vacanti]

Scaffolds provide a framework

- Why a porous, degradable scaffold?
  - mechanical support
  - allow ingrowth, avoid inflammation
  - promote nutrient+oxygen diffusion

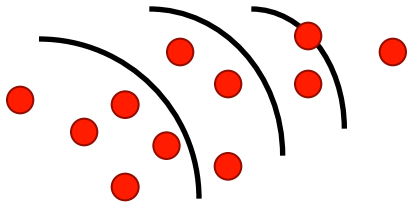


- How is the scaffold made degradable?
  - cross-links susceptible to cleavage
  - e.g., West JL & Hubbell JA, *Macromolecules* **32**:341 (1999)

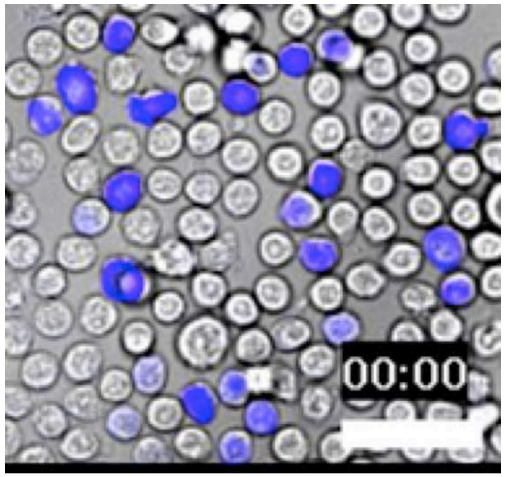


Cytokines promote cell functions

- Types of cytokines
  - growth factors (FGF, TGF, BMP)
  - angiogenic (VEGF)
  - chemokines (attract cells)
- Delivery of cytokines
  - release from scaffold or transplanted cells
- Example: CCL21 promotes T cell migration
  Stachowiak et al., *J Immunol* **177**:2340 (2006).

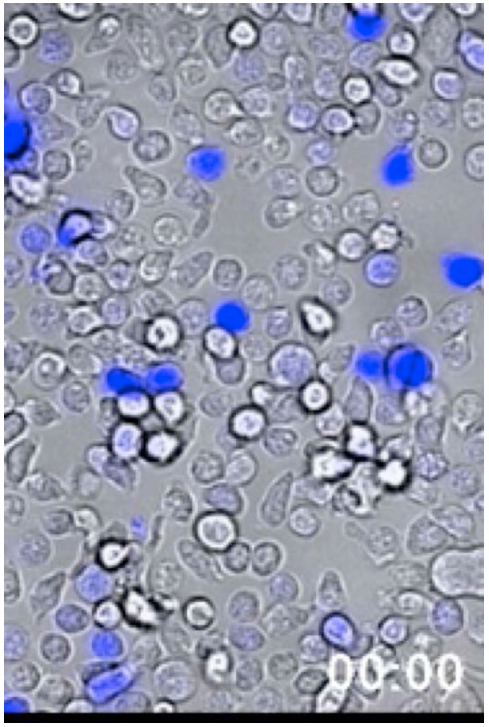


Control



See supporting video, "Chemokinesis control."

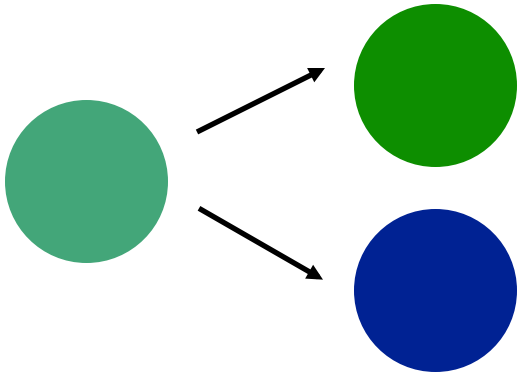
+CCL21



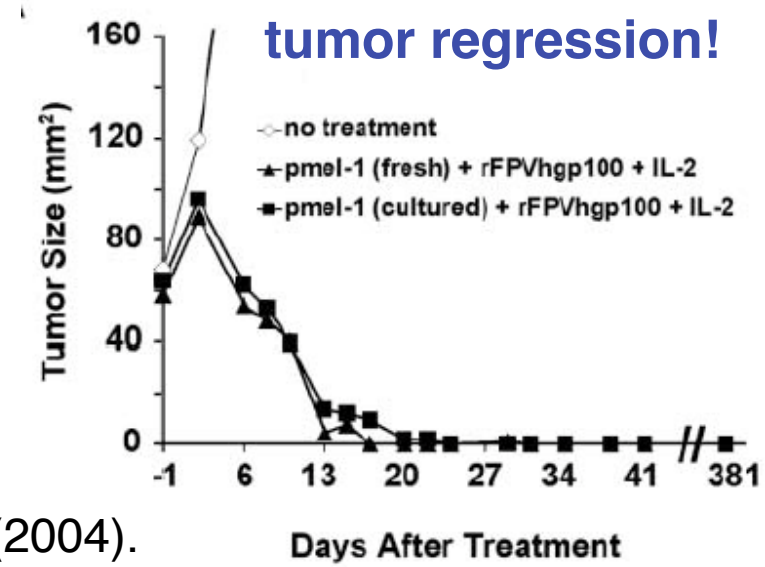
See supporting video, "Chemokinesis +CCL21."

Cells make up tissues

- Progenitors vs. differentiated cells
  - scarcity, function
- Transplanted vs. *in situ* cells
  - scarcity, safety



- Example: tumor-infiltrating lymphocytes (TIL)
  - T cells lose function in tumors
  - expand TIL *ex vivo,* treat with cytokines, and transplant
  - tested in mice



Review: Rosenberg, et al. *Nature Med* **10**:909 (2004).

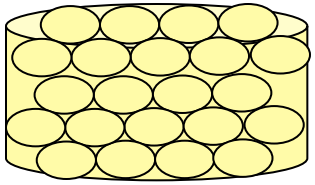
Data from: Overwijk, et al. J *Exp Med* **198**:569 (2003).

Source: Overwijk, W. W., et al. "Tumor Regression and Autoimmunity after Reversal of a Functionally Tolerant State of Self-reactive CD8+ T Cells."
J Exp Med 198, no. 4 (August 18, 2003): 569. doi: 10.1084/jem.20030590
Courtesy of Willem Overwijk, et al., and Rockefeller University Press.

Components of a TE construct

scaffold/matrix

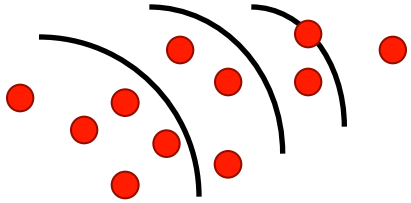
→ usually degradable, porous



soluble factors

→ made by cells or synthetic

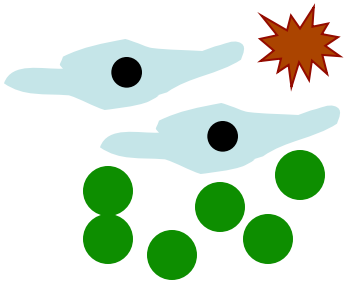
→ various release profiles



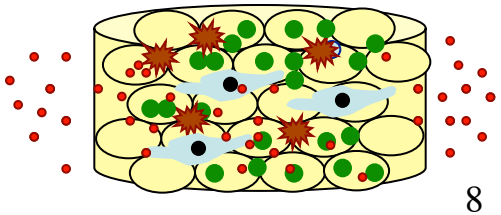
cells

→ precursors and/or differentiated

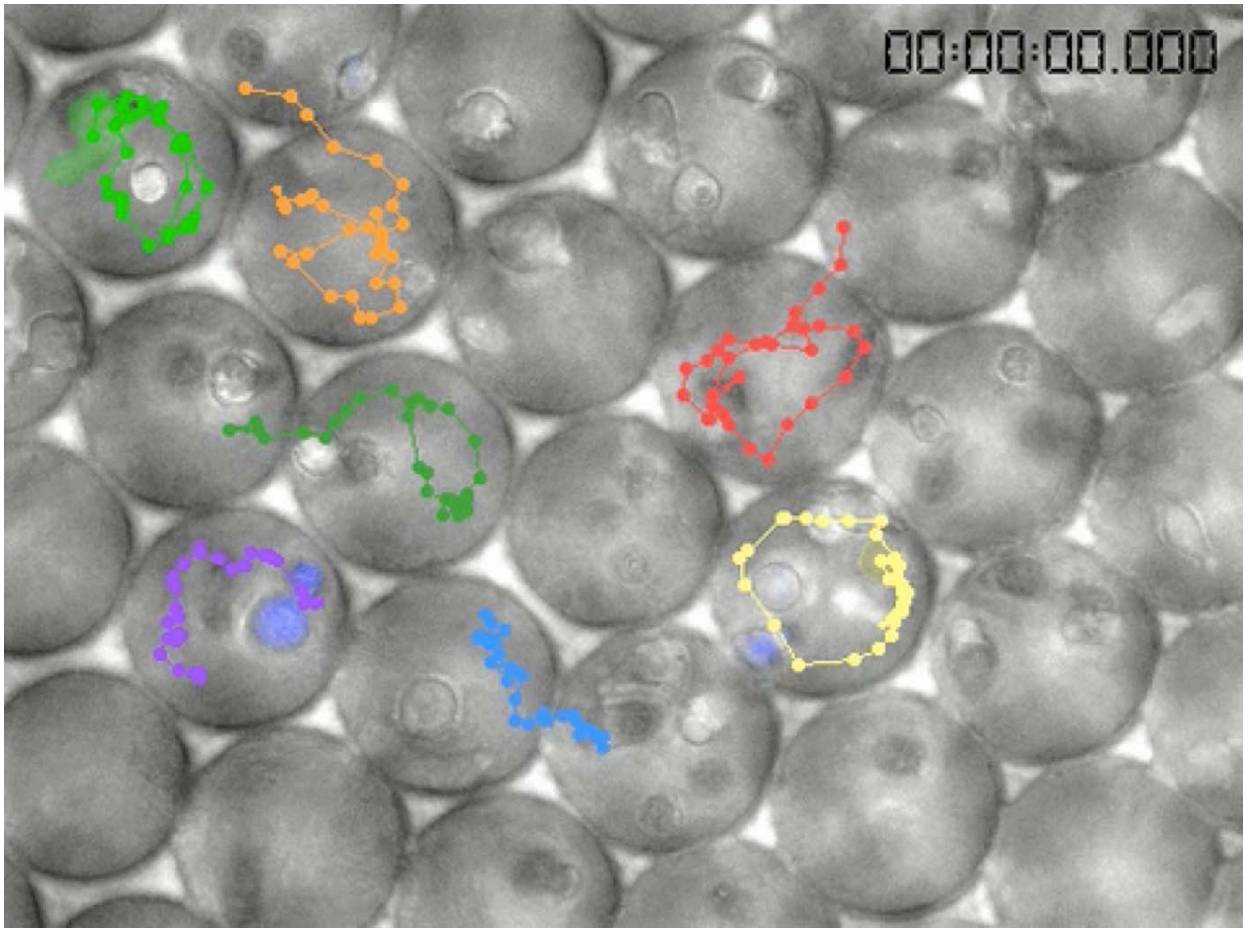
→ often autologous



integrated implantable or injectable device



Putting it all together: *in vitro* construct



See supporting video, "Cells in Scaffold."

Stachowiak et al. *J Biomed Mater Res*, **85A**: 815 (2008)

Supporting video for Stachowiak, A. N., and D. J. Irvine. "Inverse Opal Hydrogel-Collagen Composite Scaffolds as a Supportive Microenvironment for Immune Cell Migration." *J Biomed Mater Res* 85A, no.3 (2008): 815-828.

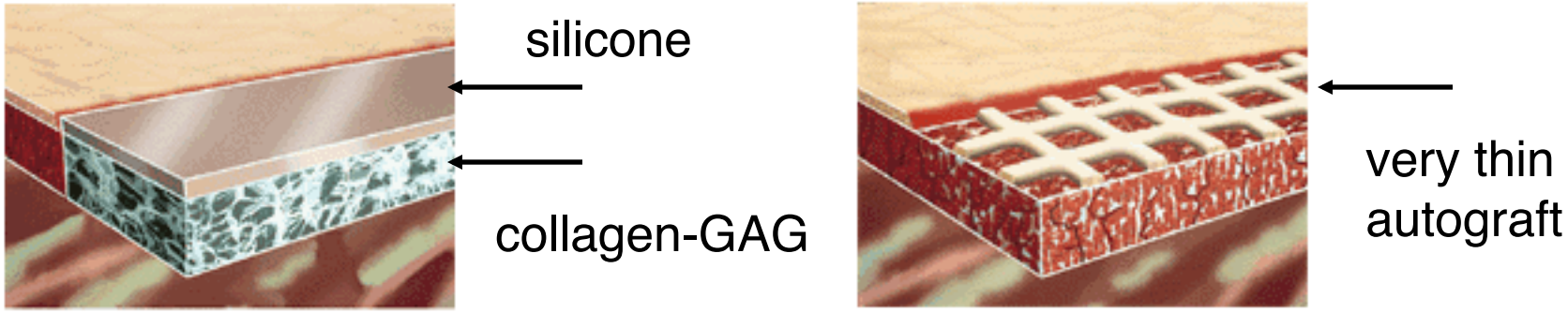
Interlude: Shmeat

http://www.colbertnation.com/the-colbert-report-videos/221975/march-17-2009/world-of-nahlej---shmeat

2:24 – 4:32

Commercial success in TE

- Regenerating severely burned skin
  - bilayer polymer [Yannas IV, et al. *Science* **215**:174 (1982)]
    - top: protects wound, retains fluid
    - bottom: provides scaffold for growth
  - forms neotissue comparable to native skin
  - sold as Integra Dermal Regeneration template

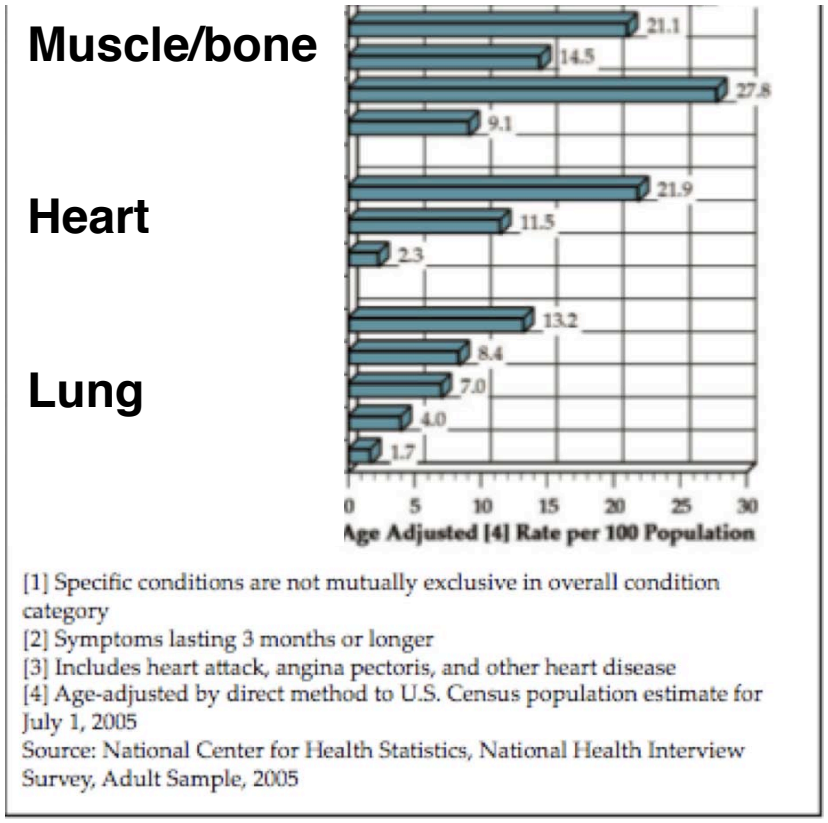


www.integra-ls.com/products/?product=46

Joint diseases: an unmet need

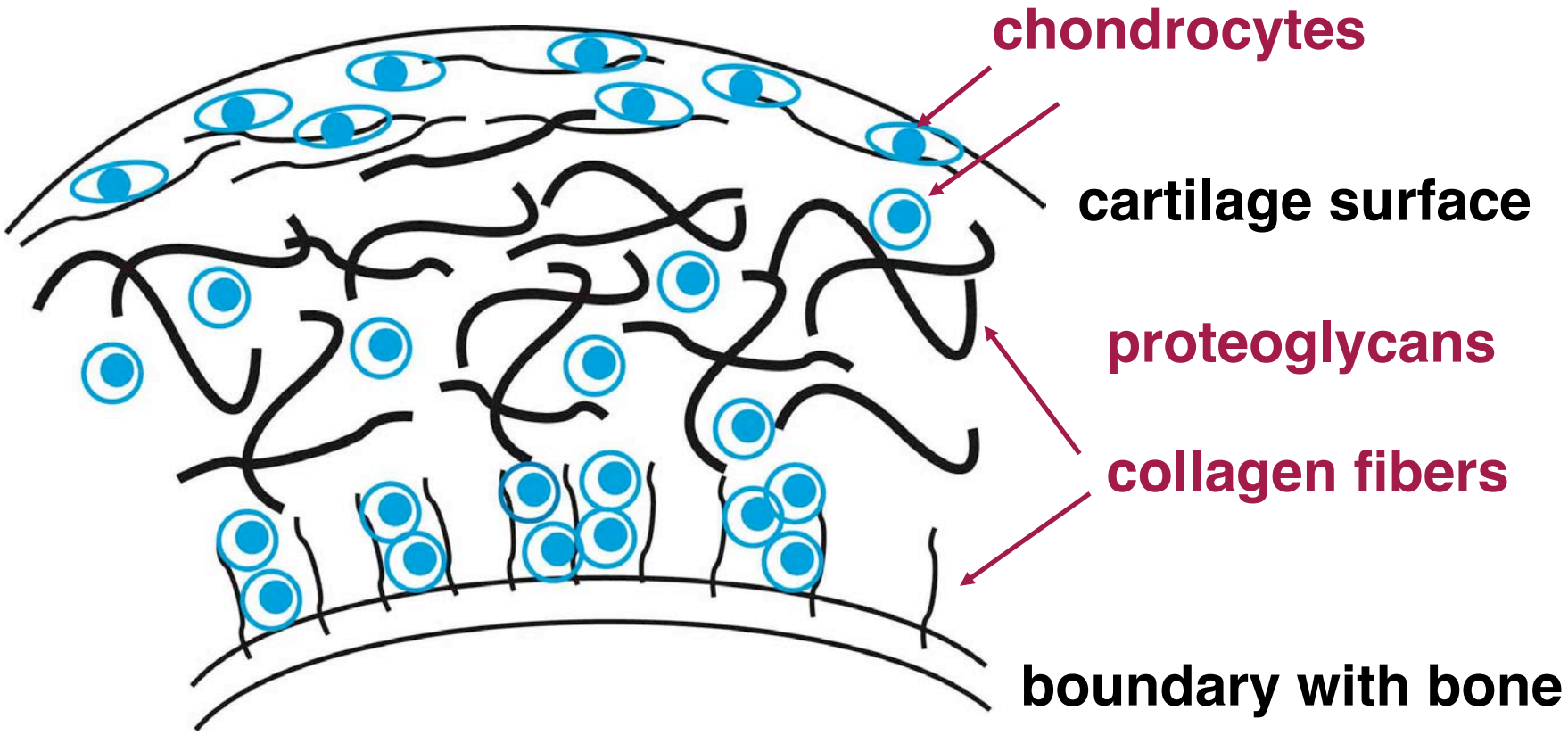
- Leading cause of physical disability in U.S.
- $100's billion in in/direct costs
- Osteoarthritis
  - common in elderly population
  - acute injury (athletes) → susceptibility to early disease
- http://www.youtube.com/watch?v=0dUSmaev5b0&feature=related
- Limited pharma solutions
  - pain management
  - targets unknown
  - cell therapies (Genzyme, Osiris)

Self-reported disease in U.S., 2005



[1] Specific conditions are not mutually exclusive in overall condition category
[2] Symptoms lasting 3 months or longer
[3] Includes heart attack, angina pectoris, and other heart disease
[4] Age-adjusted by direct method to U.S. Census population estimate for July 1, 2005
Source: National Center for Health Statistics, National Health Interview Survey, Adult Sample, 2005

Our focus: cartilage tissue

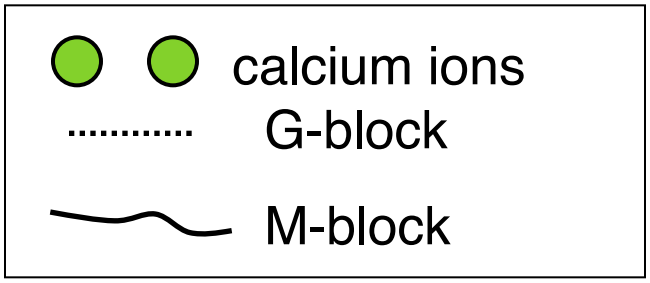


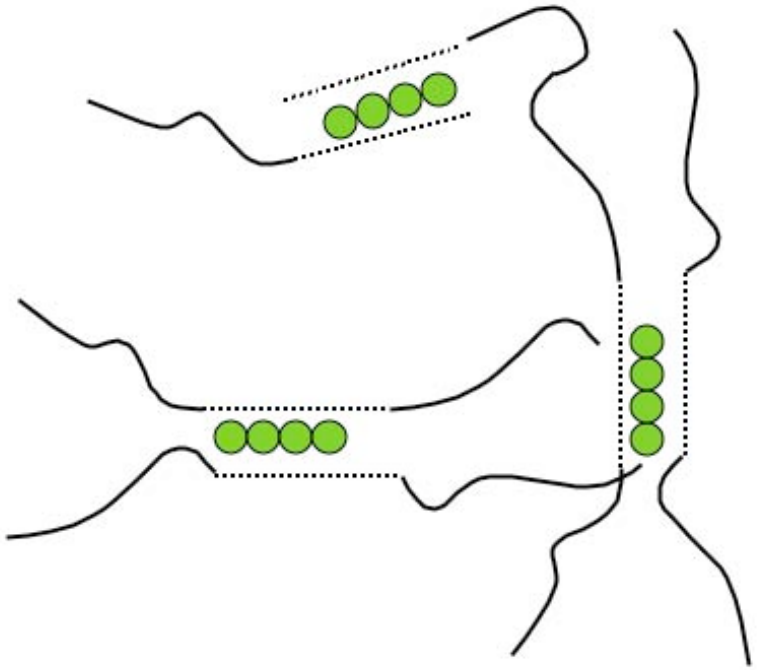
Water-swollen, heterogeneous, avascular tissue.

Alginate: material for 3D culture

- Seaweed-derived polysacharride
- Co-polymer of M and G acids
- G-block polymer chains cross-linked by cations (e.g., $Ca^{2+)}$
- Forms water-swollen gel

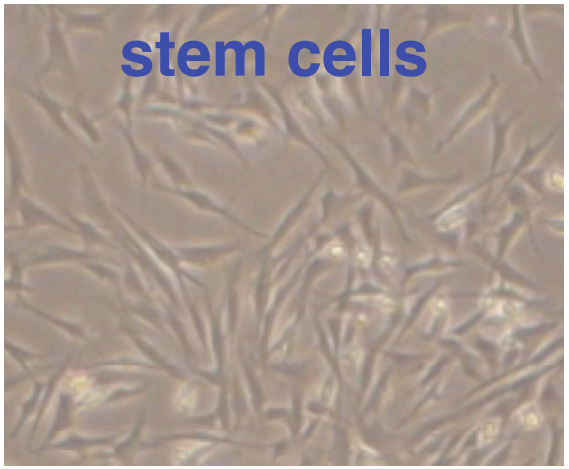
- G/M content and MW influence
  - mechanical properties
  - swelling
  - degradability
  - viscosity of solution

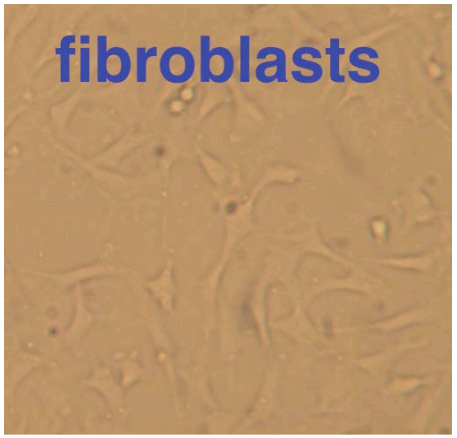




Cells for cartilage TE

	Stem cells	Chondrocytes
Obtained from…	Bone marrow	Digested cartilage
Recovery	Difficult, initially very few cells	Easy, many cells
Expansion	Many-fold	Minimal
Upkeep	FGF to expand, TGF-β1 to differentiate	Multiple factors to maintain phenotype



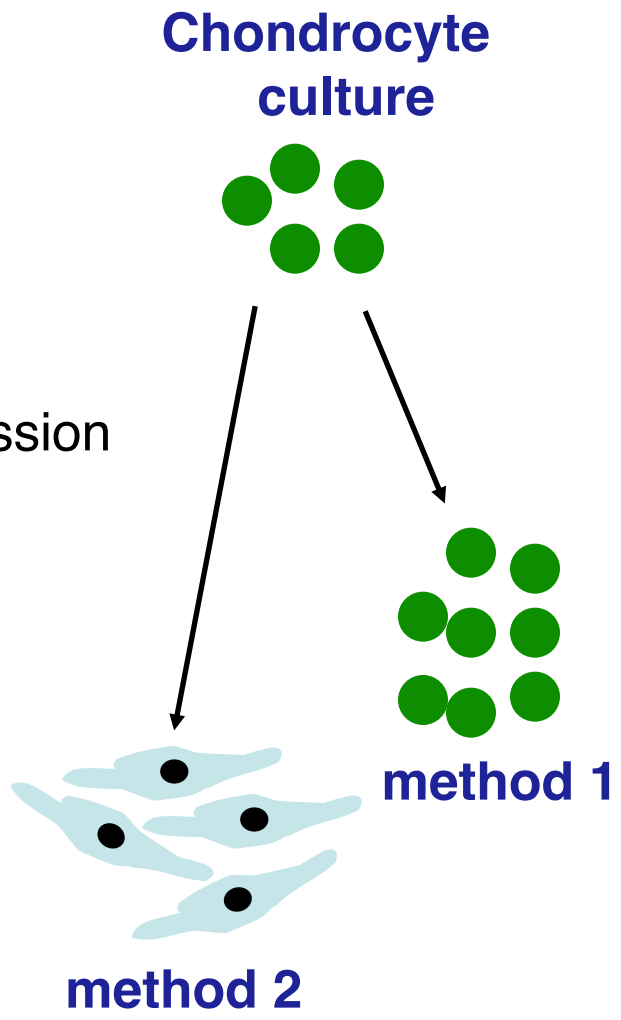


Specific goal and experiments

- **Goal:** examine effect of specific culture conditions on chondrocyte phenotype

- Observe cell morphology and viability
- Measure collagen content
  - Gene (RT-PCR) and protein (ELISA) expression
  - Collagen II:I ratio reflects cell state

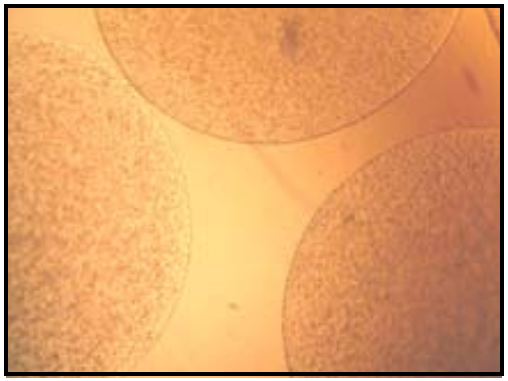
- Grander purpose: cartilage TE
  - conditions for *ex vivo* cell expansion
  - conditions for *in vitro* cartilage production



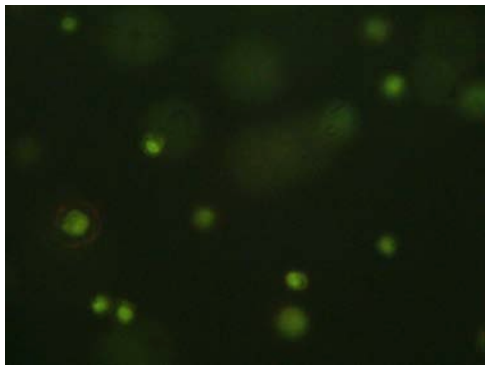
Module overview: lab

Day 1: design

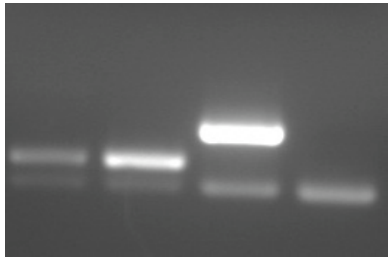
Day 2: seed cultures



Day 3: viability assay



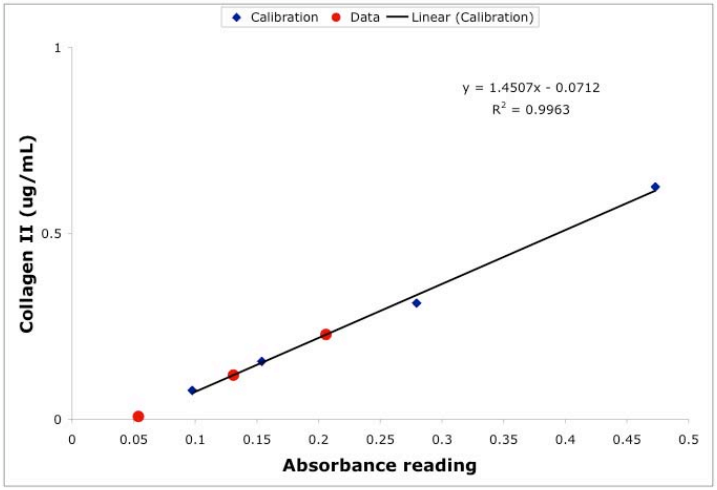
Day 4: prep RNA+cDNA



Day 5: transcript assay

Day 6: protein assay

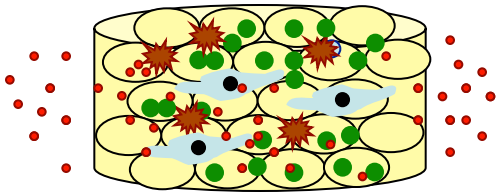
Day 7: remaining analysis



Day 8: your research ideas!

Lecture 1: conclusions

- Tissue engineering is an emerging interdisciplinary field
- Maintaining cell function is a key part of TE
- Alginate beads provide a culture system for researching soft tissues such as cartilage



Next time… more about engineered and natural biomaterials.

20.109 Laboratory Fundamentals in Biological Engineering

Spring 2010