

Introduction to Cell- Biomaterial Engineering

Module 3, Lecture 1

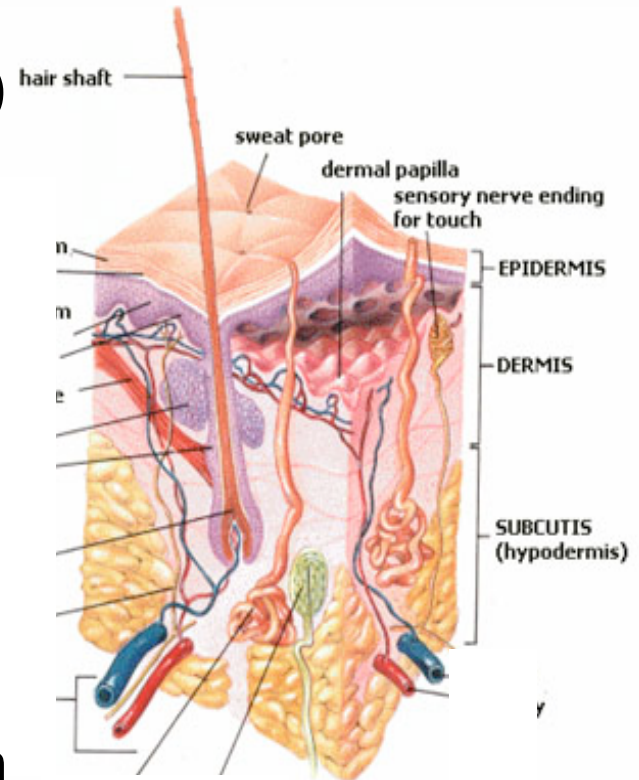
20.109 Spring 2010

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-state) challenges tissue repair capacity
- Donor tissue
 - scarcity, immune response (graft or host)
- Autologous tissue
 - availability, donor site morbidity
- Permanent synthetic substitute
 - inflammation, mis-match, failure
- A new approach: promote regeneration of ~native tissue



[Public domain image,
Wikimedia Commons]

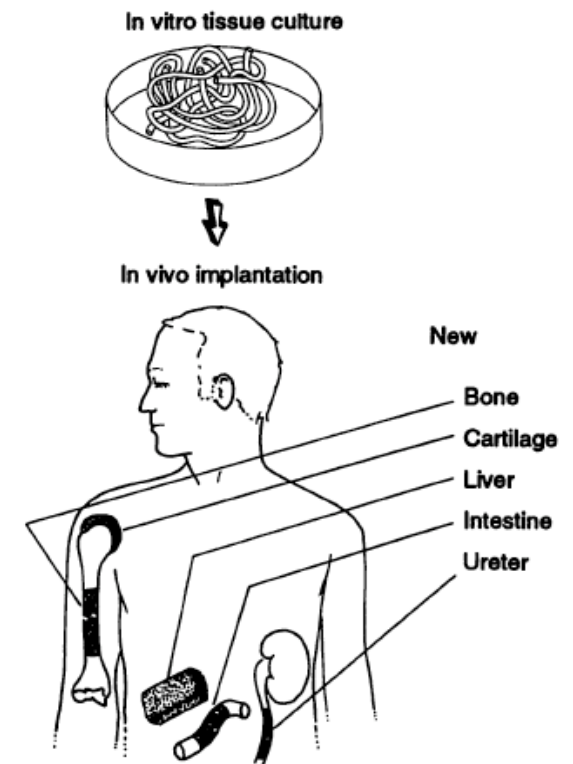
Tissue engineering: an emerging solution

“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?

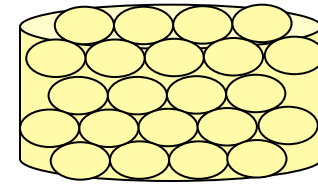


[Langer & Vacanti]

Scaffolds provide a framework

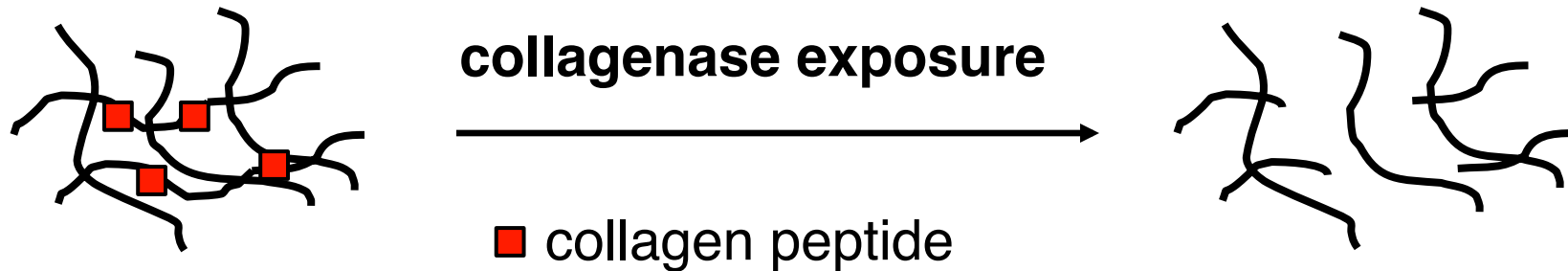
- Why a porous, degradable scaffold?

- mechanical support
- allow ingrowth, avoid inflammation
- promote nutrient+oxygen diffusion



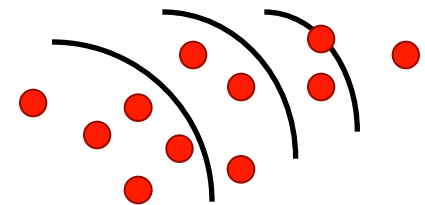
- How is the scaffold made degradable?

- cross-links susceptible to cleavage
- e.g., West JL & Hubbell JA, *Macromolecules* **32**:341 (1999)



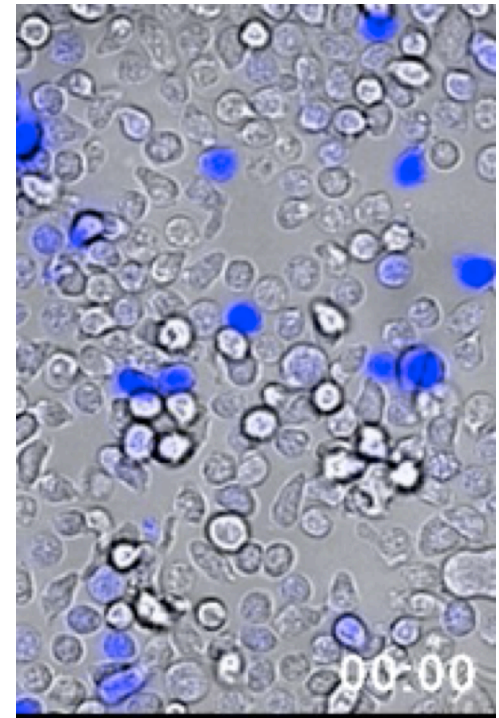
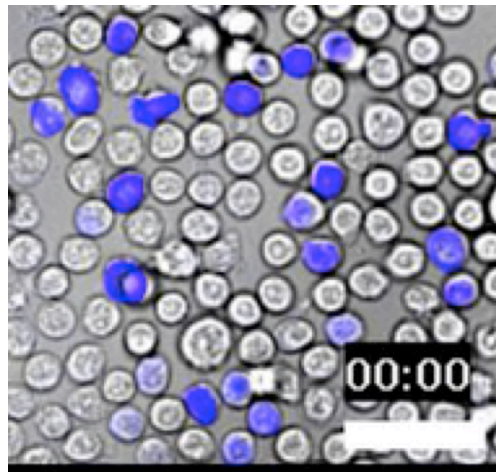
Cytokines promote cell functions

- 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).



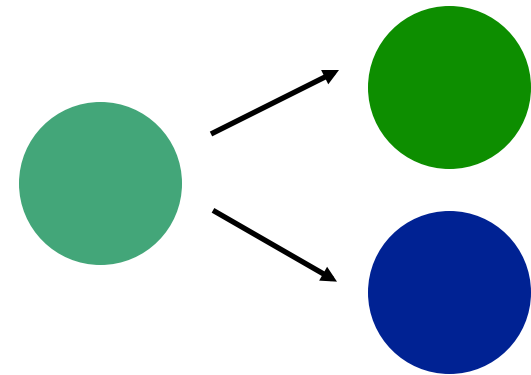
+CCL21

Control

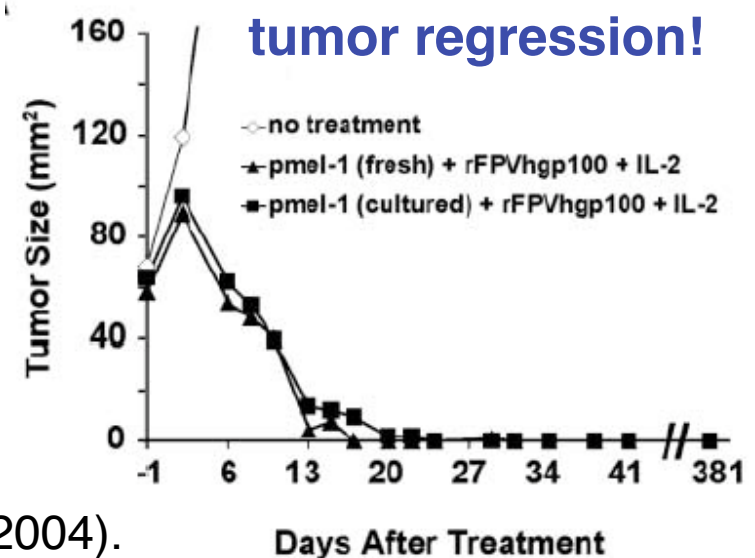


Cells make up tissues

- 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



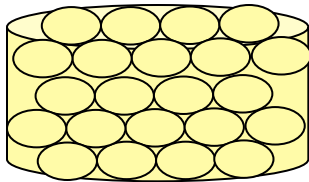
Review: Rosenberg, et al. *Nature Med* **10**:909 (2004).

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

Components of a TE construct

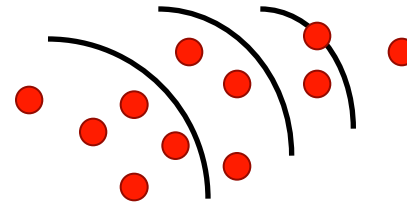
scaffold/matrix

→ usually degradable, porous



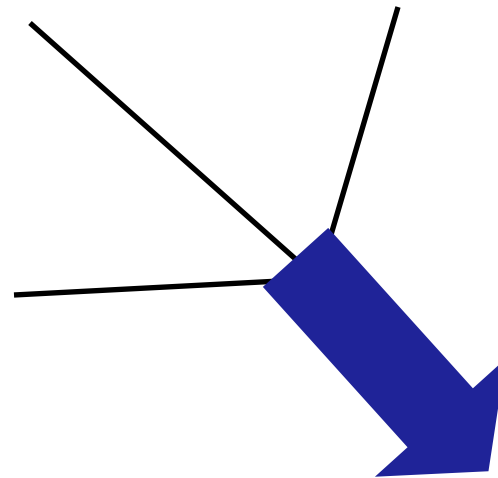
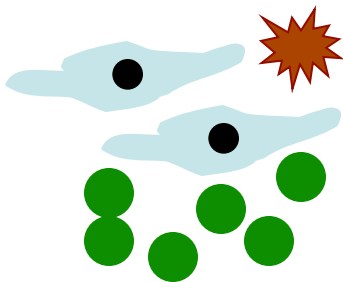
soluble factors

→ made by cells or synthetic
→ various release profiles

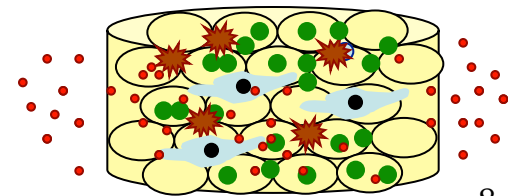


cells

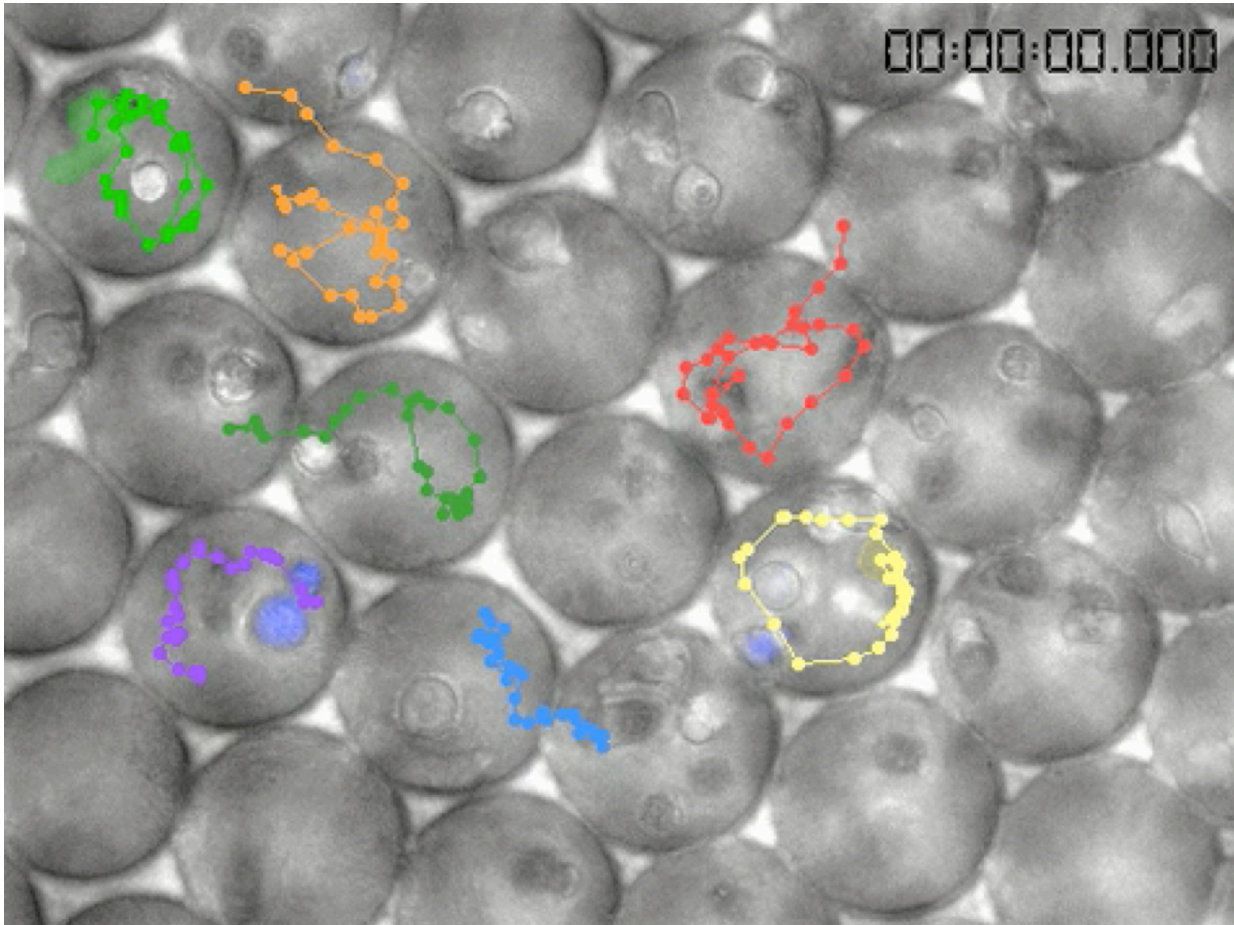
→ precursors and/or differentiated
→ often autologous



integrated implantable or injectable device



Putting it all together: *in vitro* construct



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

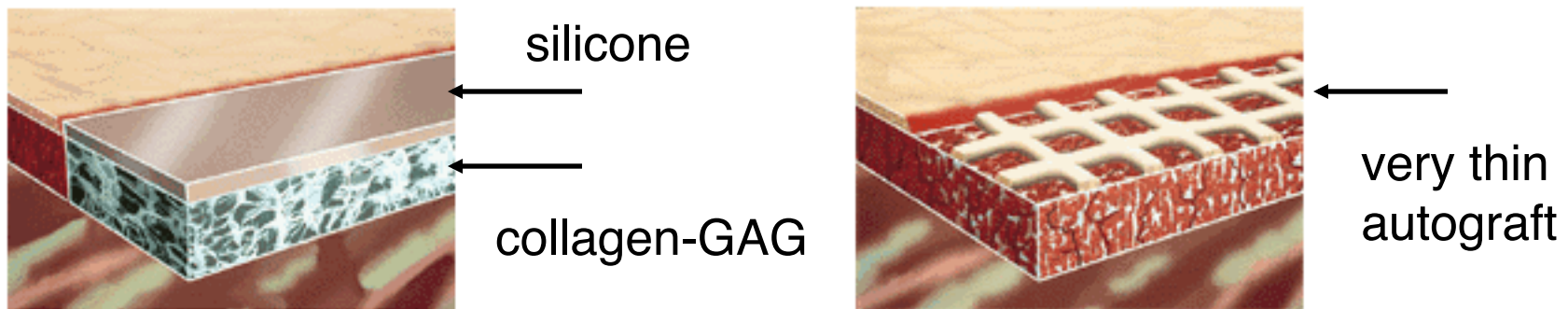
Interlude: Shmeat

<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

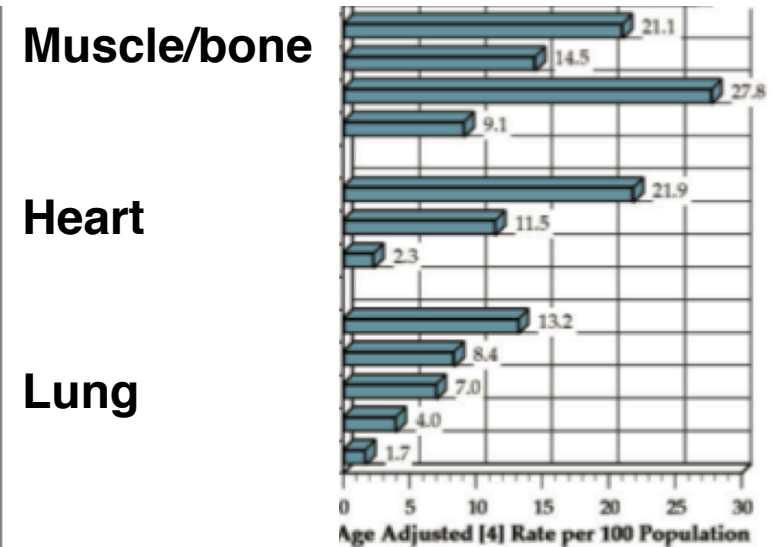


www.integra-ls.com/products/?product=46

Joint diseases: an unmet need

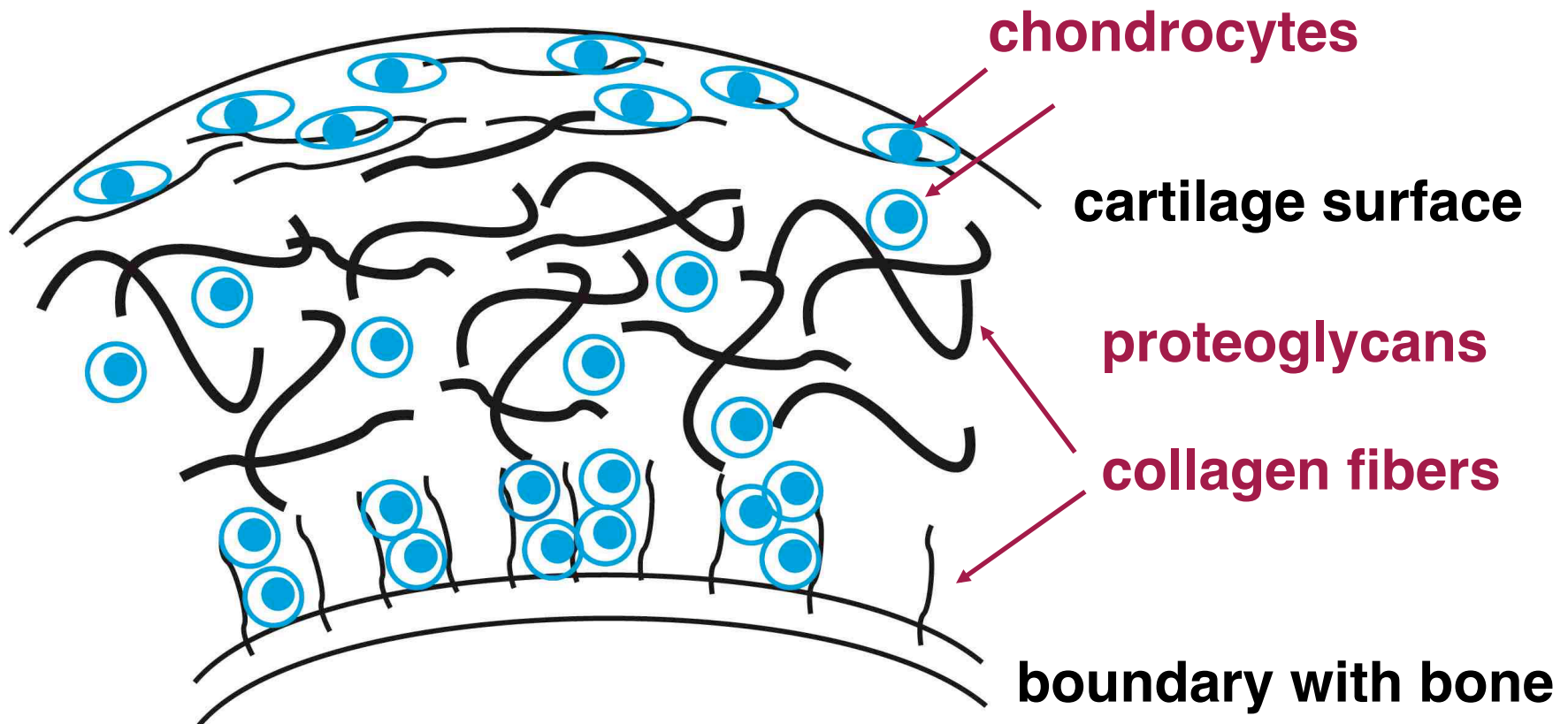
- Leading cause of physical disability in U.S.
- \$100's billion in in/direct costs
- Osteoarthritis
 - common in elderly population
 - acute injury (athletes) → susceptibility to early disease
- <http://www.youtube.com/watch?v=0dUSmaev5b0&feature=related>
- Limited pharma solutions
 - pain management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

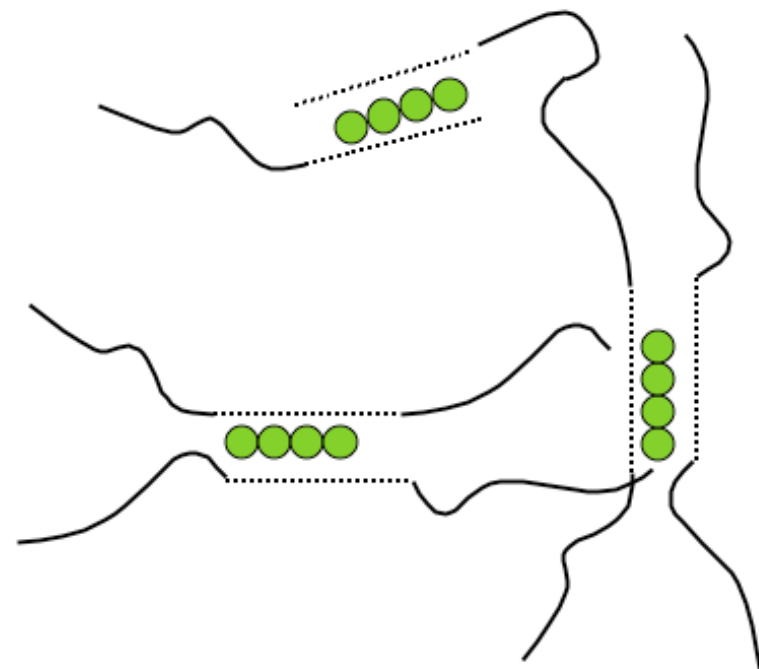
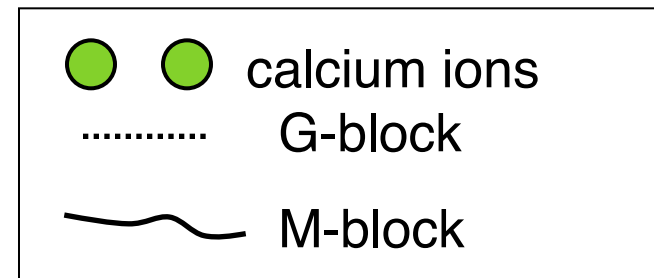
Our focus: cartilage tissue



Water-swollen, heterogeneous, avascular tissue.

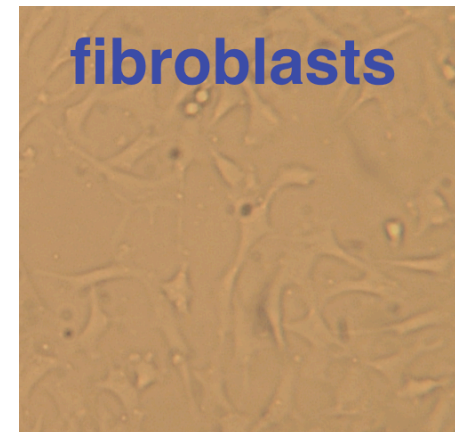
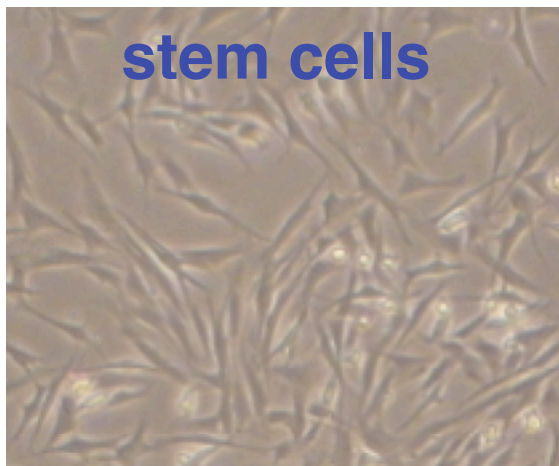
Alginate: material for 3D culture

- 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



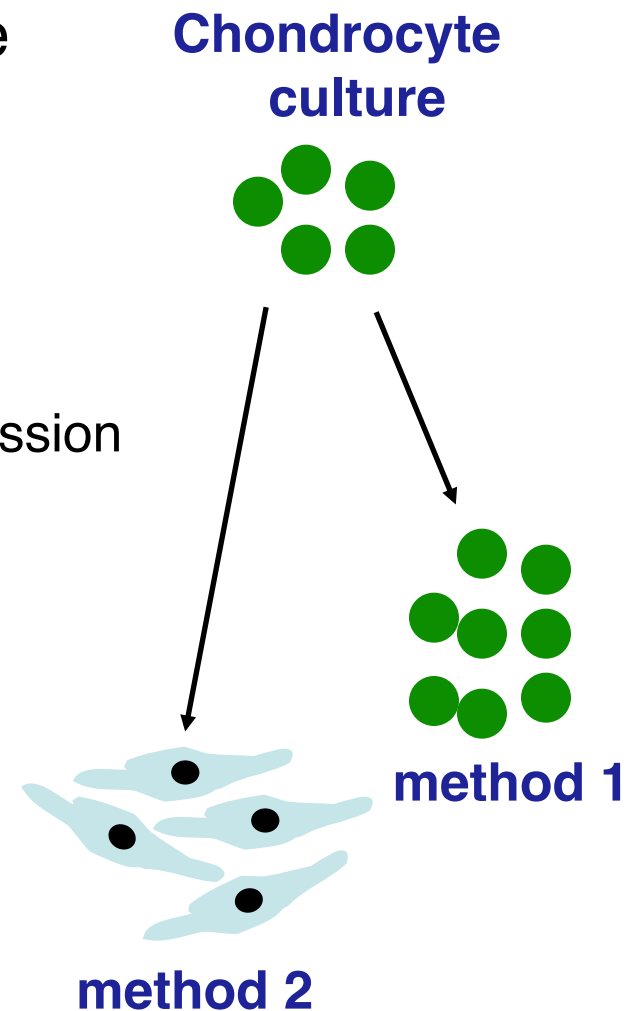
Cells for cartilage TE

	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- β 1 to differentiate	Multiple factors to maintain phenotype



Specific goal and experiments

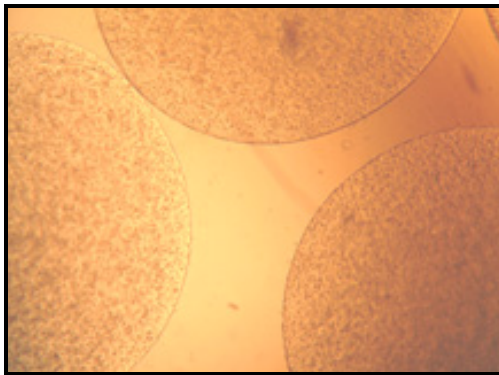
- **Goal:** examine effect of specific culture conditions on chondrocyte phenotype
- Observe cell morphology and viability
- Measure collagen content
 - Gene (RT-PCR) and protein (ELISA) expression
 - Collagen II:I ratio reflects cell state
- Grander purpose: cartilage TE
 - conditions for *ex vivo* cell expansion
 - conditions for *in vitro* cartilage production



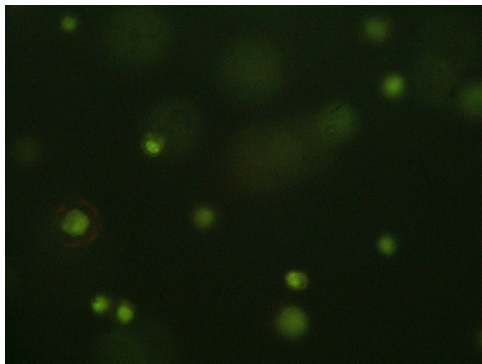
Module overview: lab

Day 1: design

Day 2: seed cultures



Day 3: viability assay

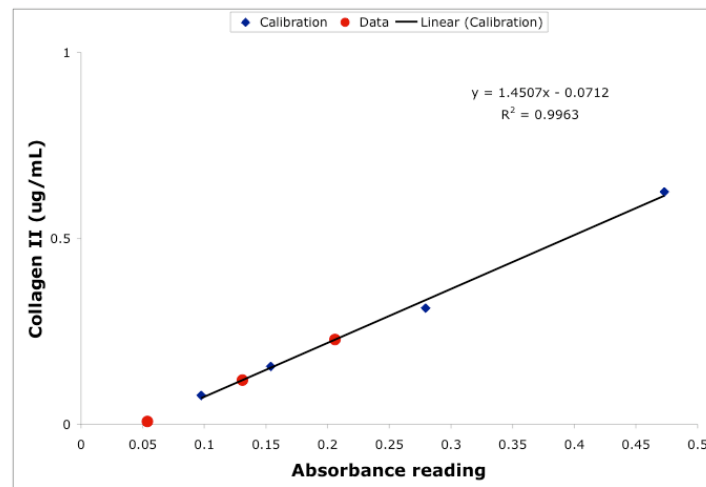
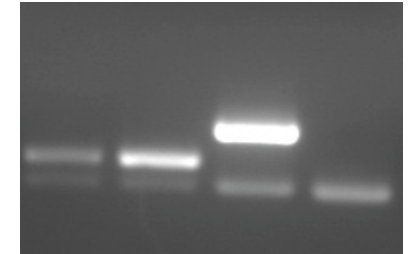


Day 4: prep RNA+cDNA

Day 5: transcript assay

Day 6: protein assay

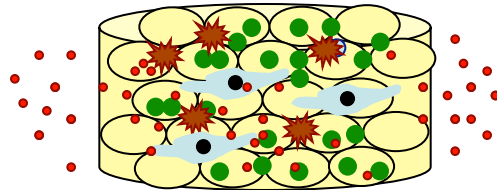
Day 7: remaining analysis



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.