

# Introduction to Cell- Biomaterial Engineering

Module 3, Lecture 1

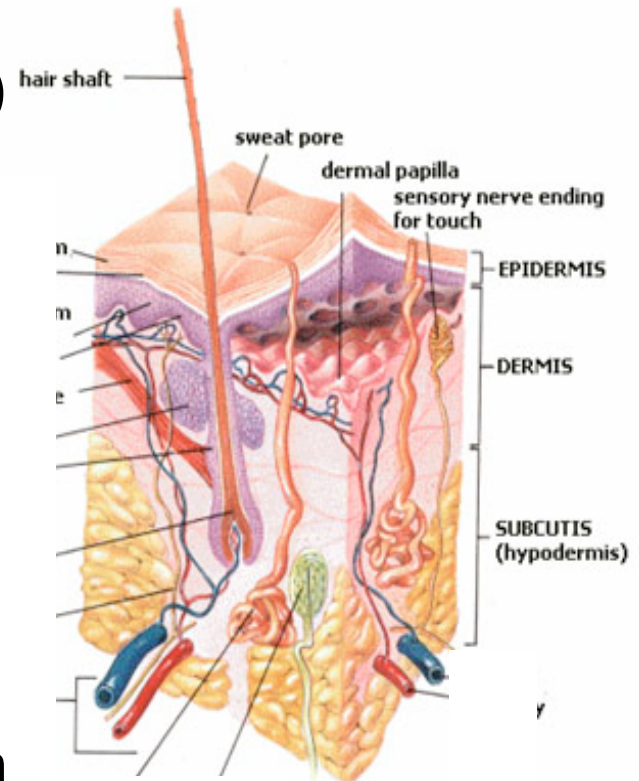
20.109 Spring 2009

# 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
  - failure, inflammation, mis-match
- A new approach: promote regeneration of ~native tissue



[Public domain image,  
Wikimedia Commons]

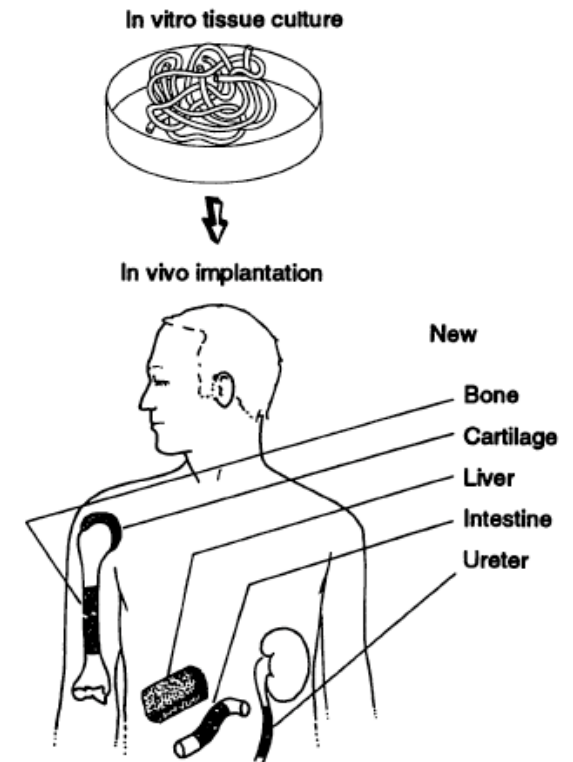
# Tissue engineering: an emerging solution

“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?**

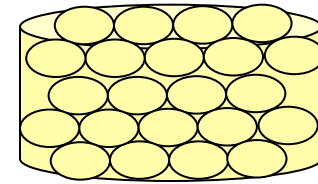


[Langer & Vacanti]

# Scaffolds provide a framework

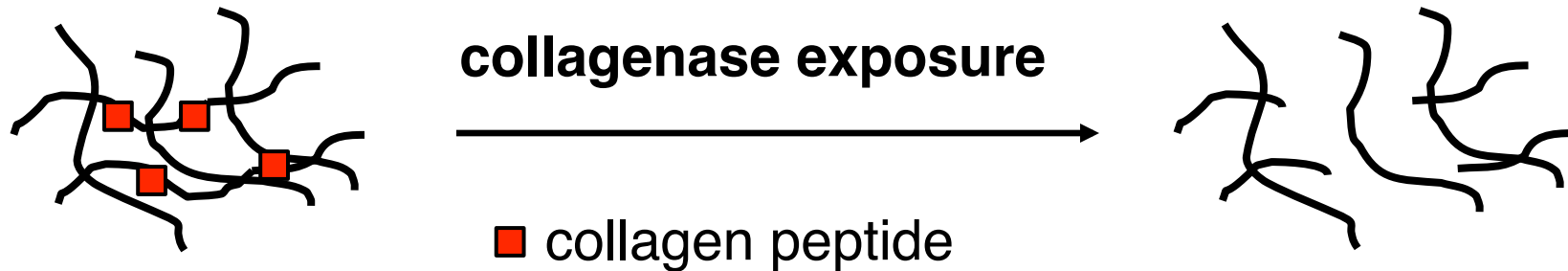
- Why a porous, degradable scaffold?

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



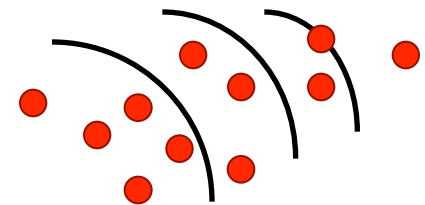
- How is the scaffold made degradable?

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



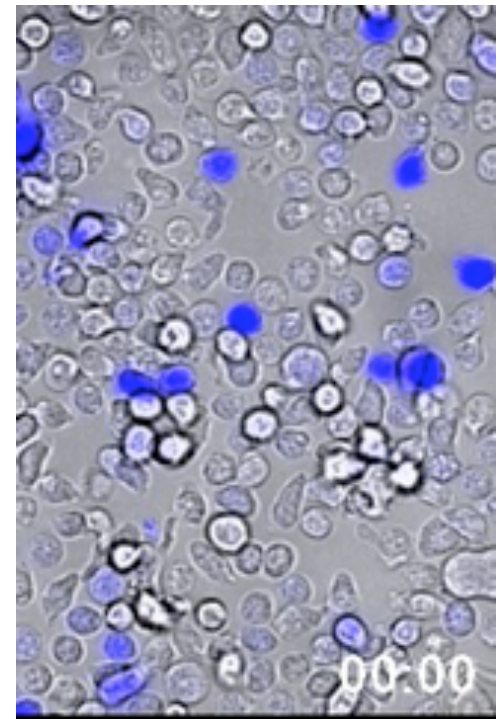
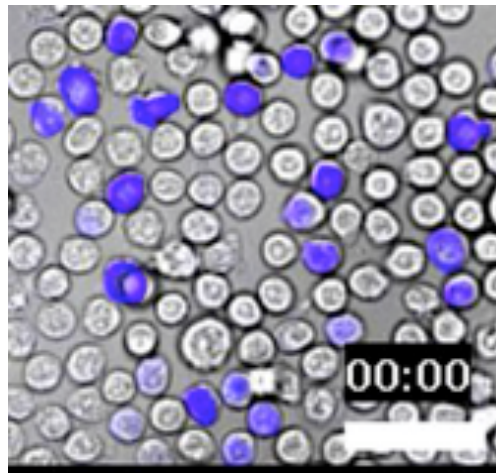
# Cytokines promote cell functions

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



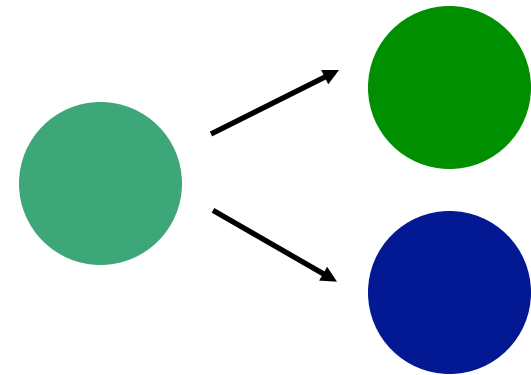
**+CCL21**

**Control**

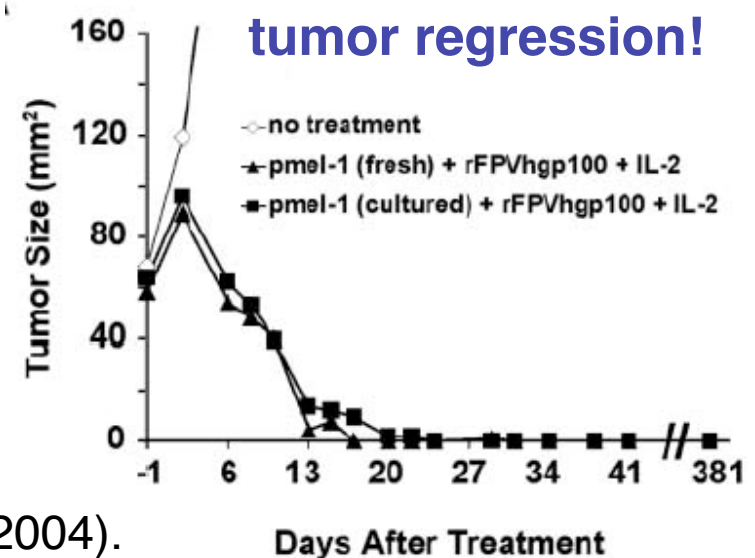


# Cells make up tissues

- 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



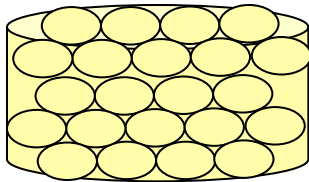
Review: Rosenberg, et al. *Nature Med* **10**:909 (2004).

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

# Components of a TE construct

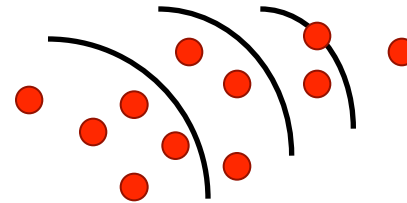
## scaffold/matrix

→ usually degradable, porous



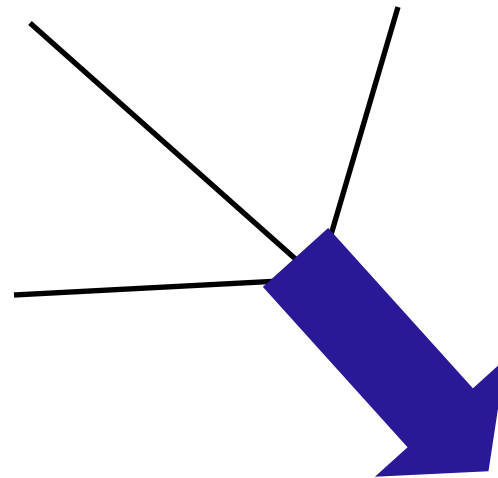
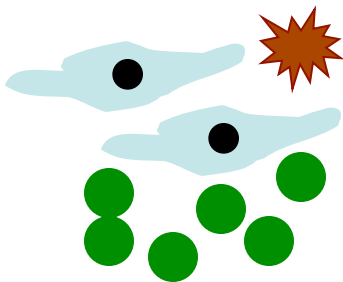
## soluble factors

→ made by cells or synthetic  
→ various release profiles

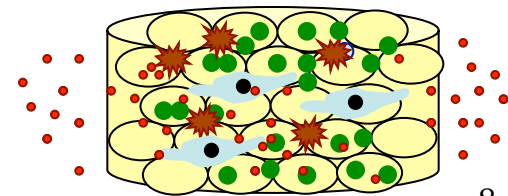


## cells

→ precursors and/or differentiated  
→ often autologous

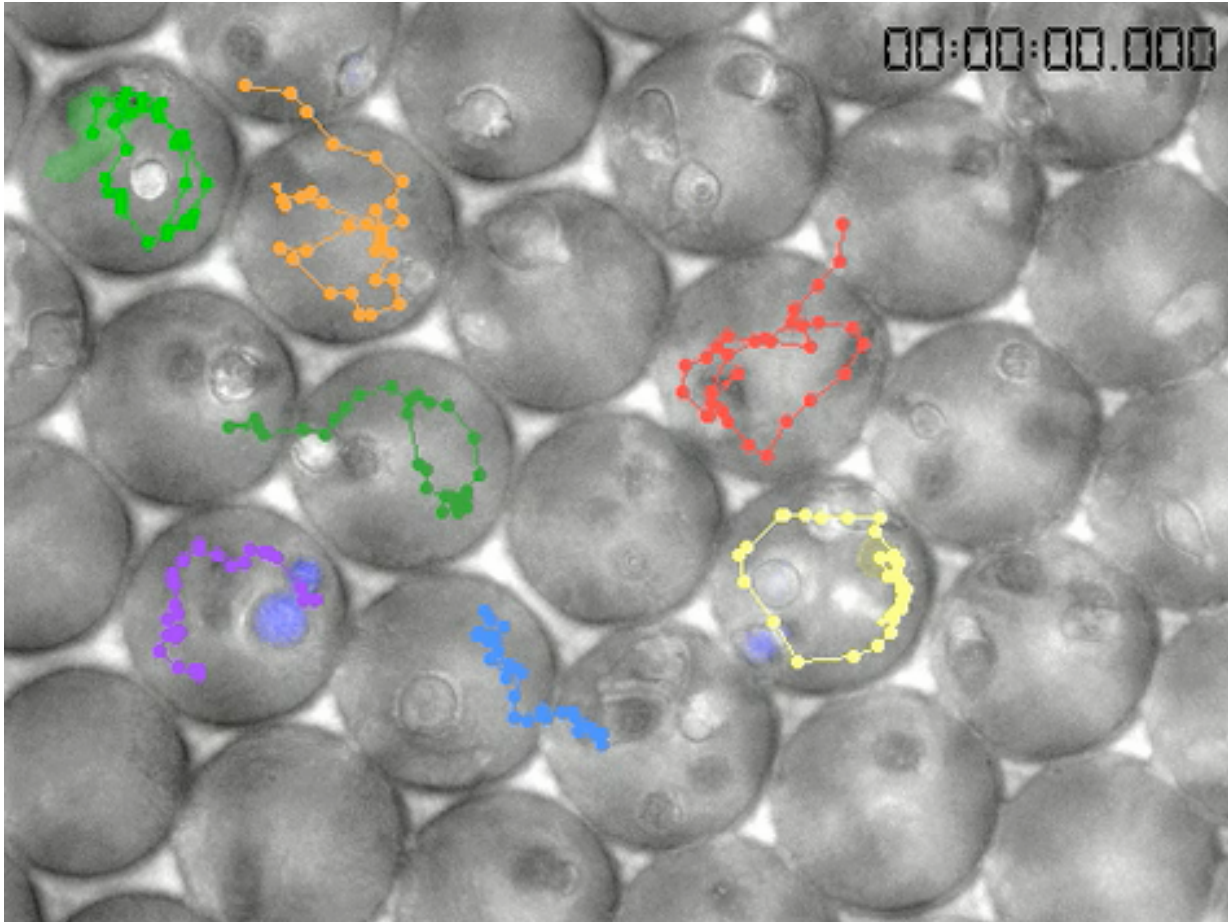


## integrated implantable or injectable device





# Putting it all together: *in vitro* construct



Stachowiak et al. *J Biomed Mater Res*, **85A**: 815 (2008) <sub>9</sub>

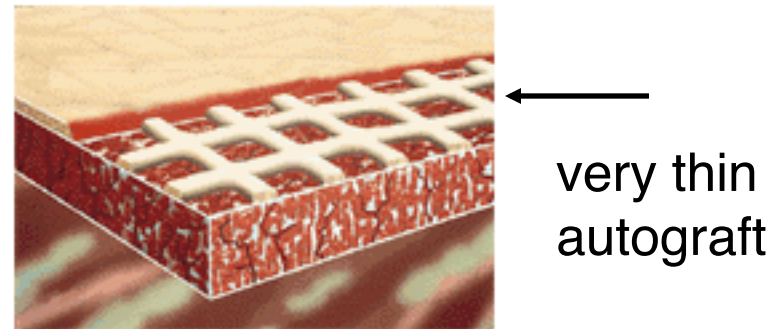
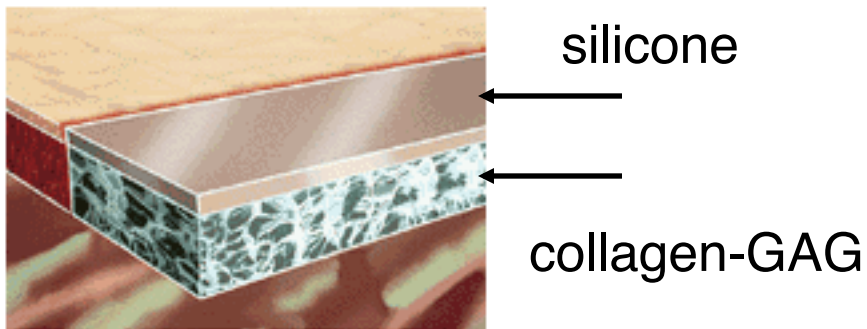
# Interlude: Shmeat

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

2:24 – 4:32

# Commercial success in TE

- Skin regeneration after severe burns
  - bilayer polymer [Yannas IV, et al. *Science* **215**:174 (1982)]
    - top layer protects wound, prevents fluid loss
    - bottom provides scaffold for growth
  - results in neotissue comparable to native skin
  - sold as Integra Dermal Regeneration template

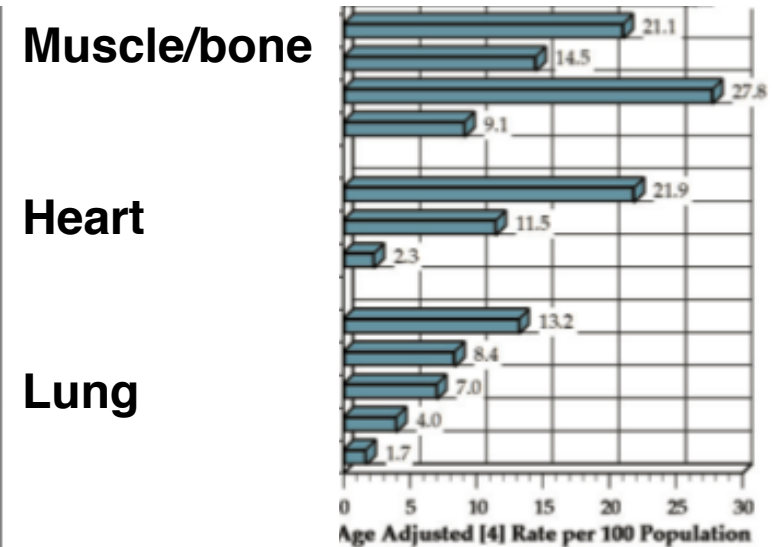


[www.integra-ls.com/products/?product=46](http://www.integra-ls.com/products/?product=46)

# Joint diseases: an unmet need

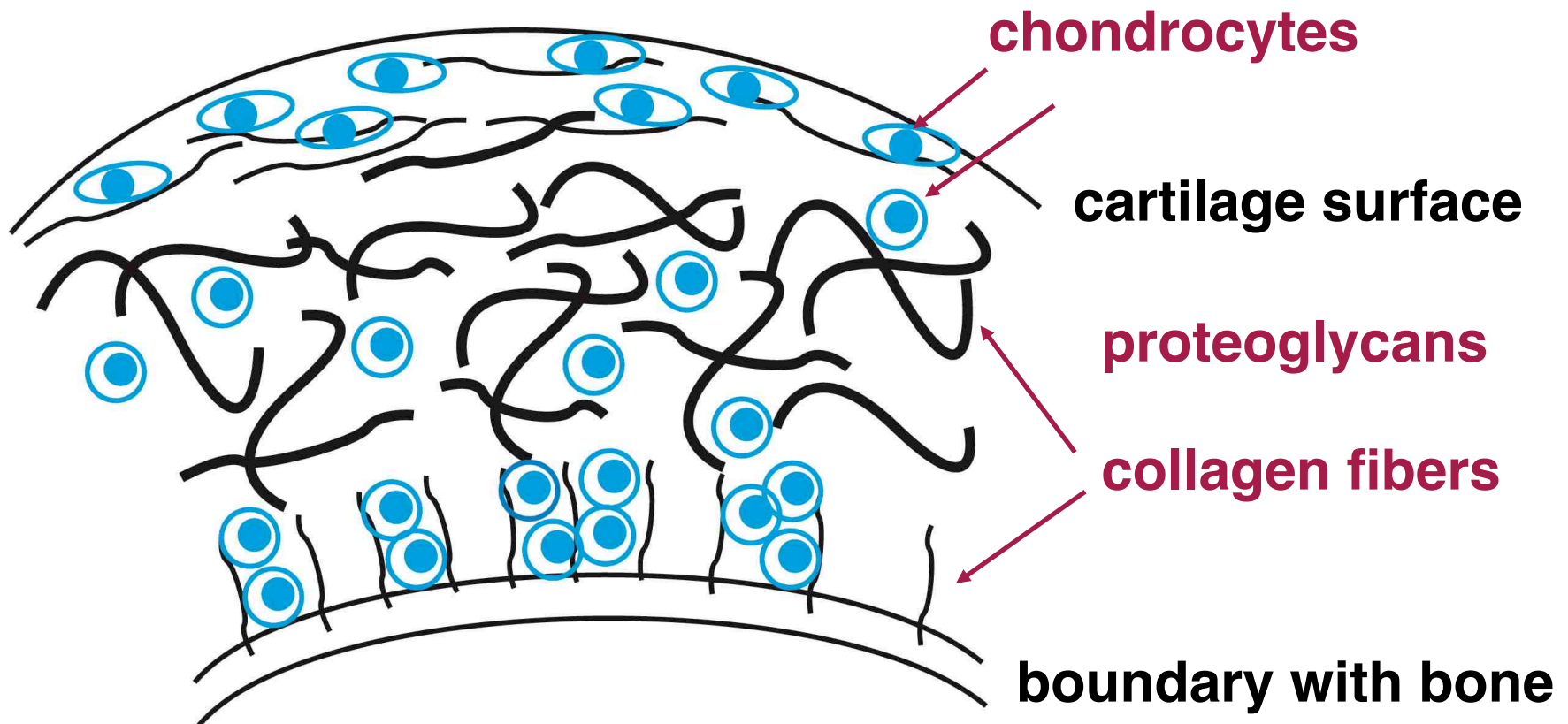
- Leading cause of physical disability in U.S.
- \$100's billion in in/direct costs
- Osteoarthritis
  - Common in elderly population
  - acute injury (athletes) → susceptibility to early disease
- <http://www.youtube.com/watch?v=0dUSmaev5b0&feature=related>
- Limited pharma solutions
  - pain management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

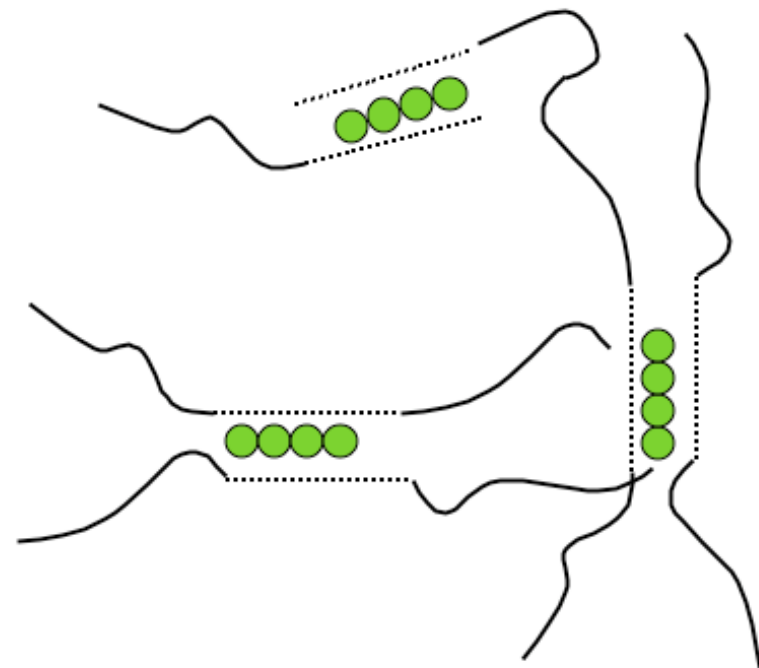
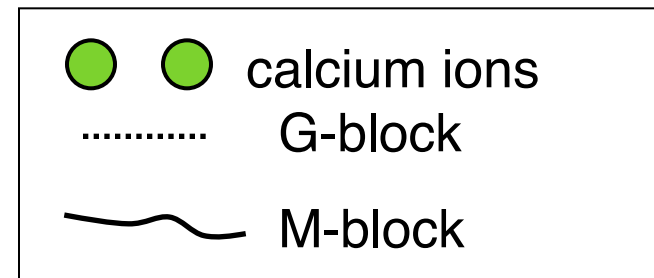
# Our focus: cartilage tissue



Avascular, highly water-swollen, heterogeneous tissue.

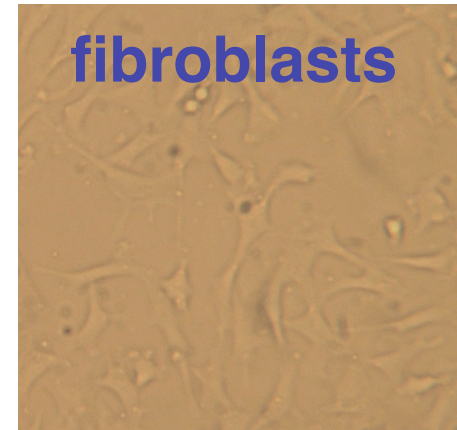
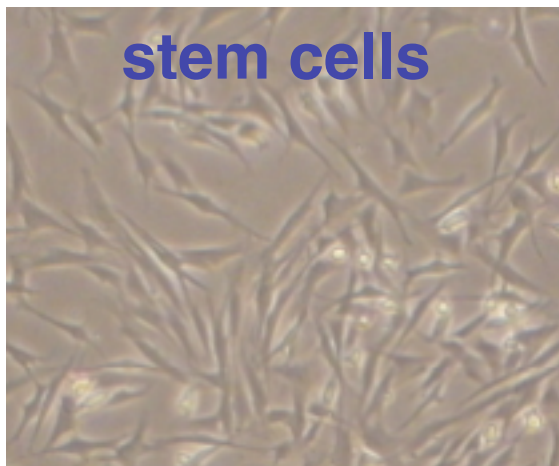
# Alginate: material for 3D culture

- Seaweed-derived polysacharride
- Co-polymer of M and G acids
- G-block polymer chains cross-linked by cations (e.g.,  $\text{Ca}^{2+}$ )
- Forms water-swollen gel
  
- G/M content and MW influence
  - degradability
  - swelling
  - mechanical properties
  - viscosity of solution



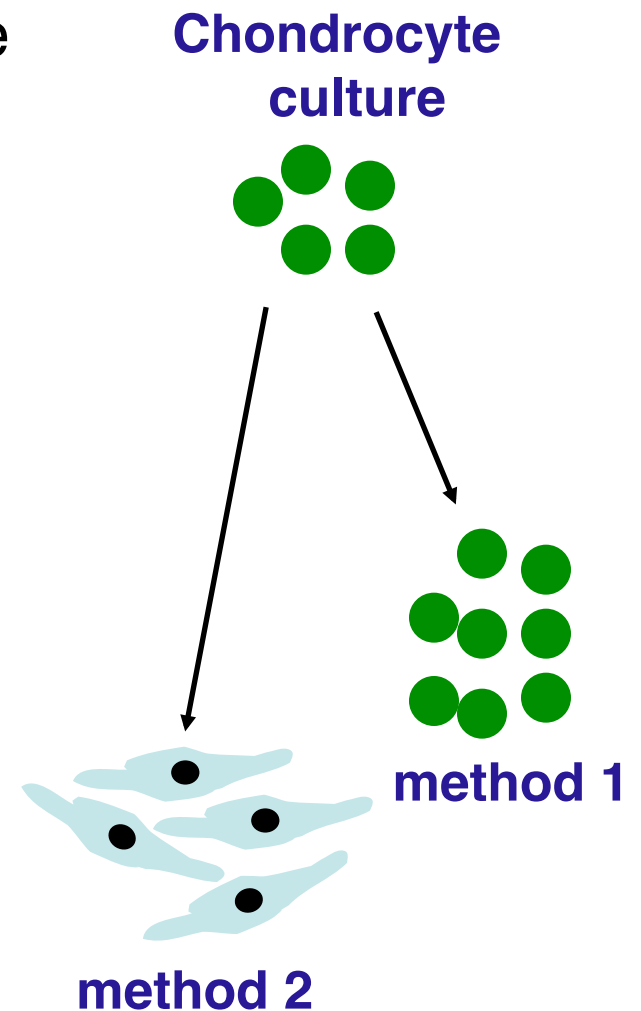
# Cells for cartilage TE

	<b>Stem cells</b>	<b>Chondrocytes</b>
Obtained from...	Bone marrow	Digested cartilage
Recovery	Difficult, initially very few cells	Easy, many cells
Expansion	Many-fold	Minimal
upkeep	Growth factors 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
  - RT-PCR and ELISA
  - Collagen II:I ratio reflects cell state
- Grander purpose: cartilage TE
  - conditions for *ex vivo* cell expansion
  - conditions for *in vitro* cartilage production

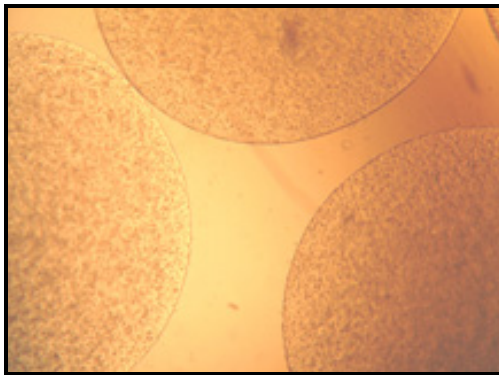




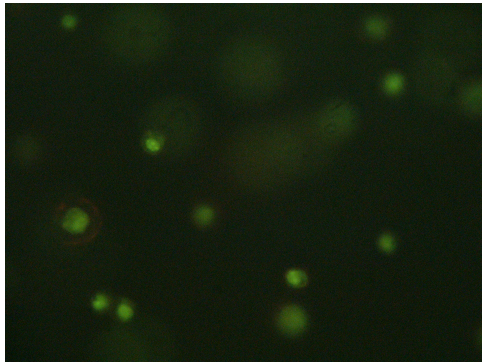
# Module overview: lab

Day 1: design

Day 2: seed cultures

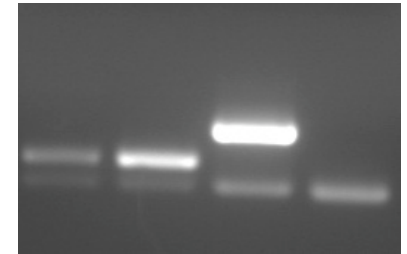


Day 3: viability assay



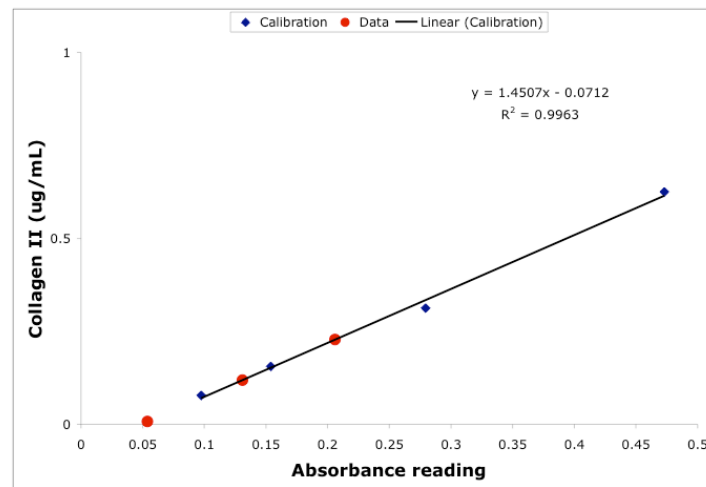
Day 4: prep RNA+cDNA

Day 5: transcript assay



Day 6: protein assay

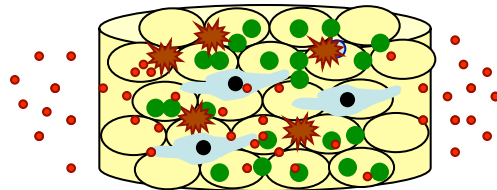
Day 7: remaining analysis



Day 8: your research ideas!

# Lecture 1: conclusions

- Tissue engineering is an emerging field at the interface of engineering, science and medicine
- Maintaining cell function is a key part of TE
- Alginate beads provide a culture system for investigating soft tissues such as cartilage



Next time... more about engineered and natural biomaterials.