Introduction to Cell and Biomaterial Engineering

Module 3, Lecture 1

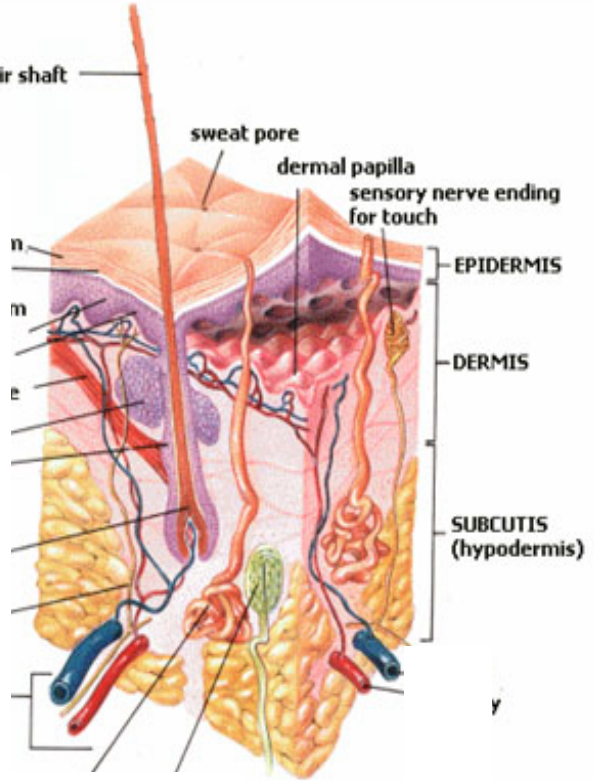
20.109 Spring 2014

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

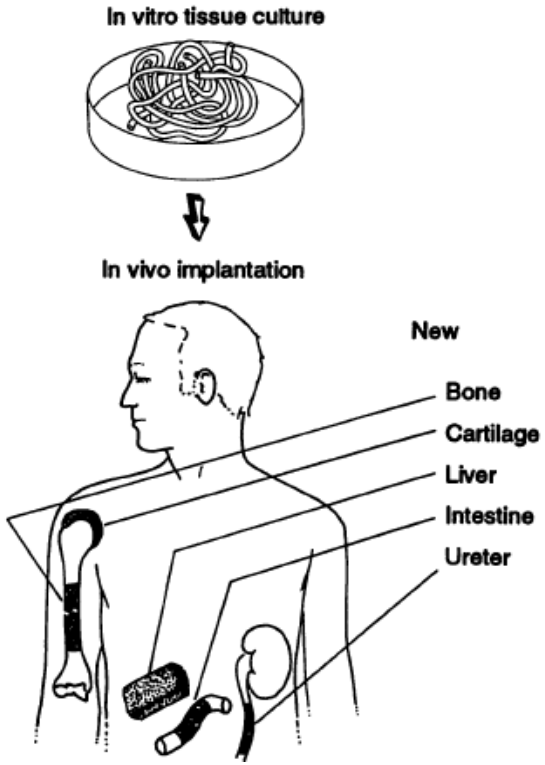


[Public domain image, Wikimedia Commons]

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)



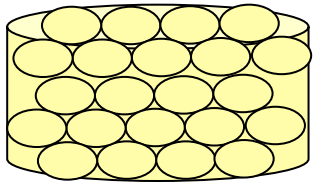
[Langer & Vacanti]

What is in a tissue engineer's toolkit?

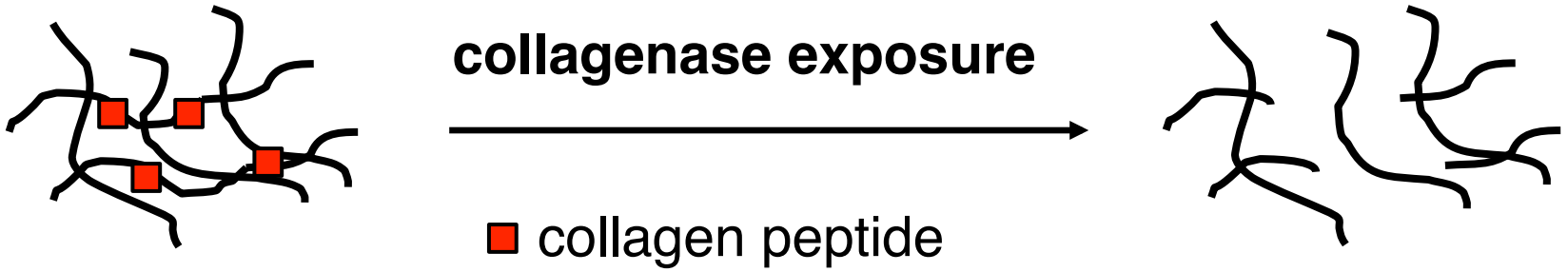
How good are the outcomes?

Scaffolds provide a framework

- Why a porous, degradable scaffold?
  - short-term: mechanical support, allow in-growth
  - long-term: avoid inflammation
  - promote nutrient + oxygen diffusion

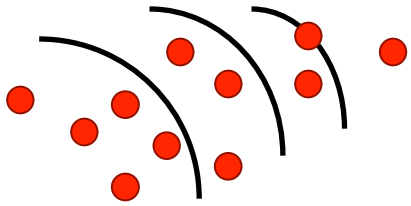


- How is the scaffold made degradable?
  - cross-links susceptible to cleavage
  - e.g., West JL & Hubbell JA, *Macromolecules* **32**:341 (1999)

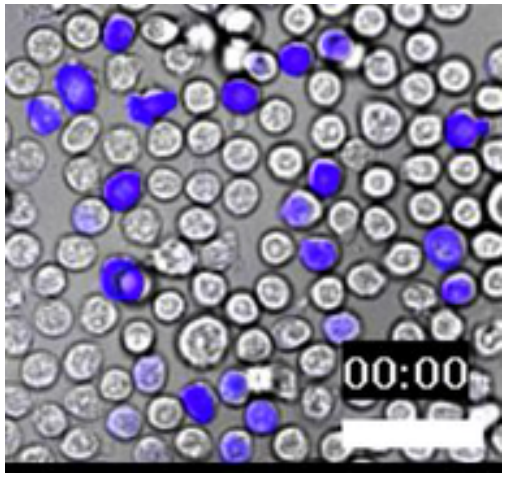


Cytokines promote cell functions

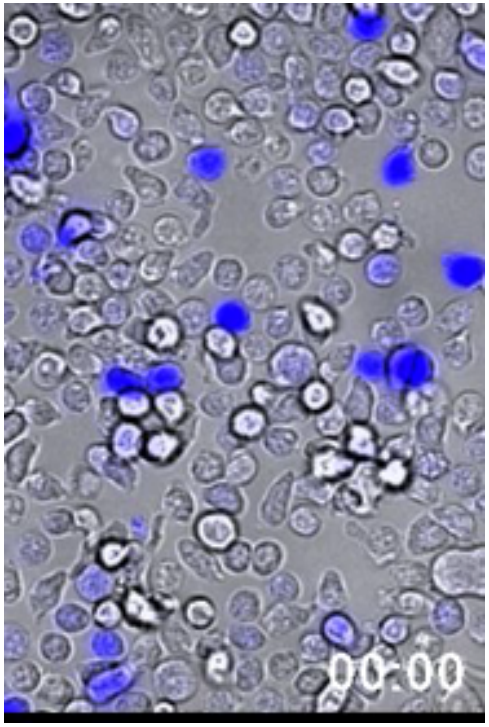
- Types of cytokines
  - growth factors (FGF, TGF, BMP)
  - angiogenic (VEGF)
  - chemokines (attract cells)
- Delivery of cytokines
  - release from scaffold or transplanted cells
- Example: CCL21 promotes T cell migration
  Stachowiak et al., *J Immunol* **177**:2340 (2006).



Control

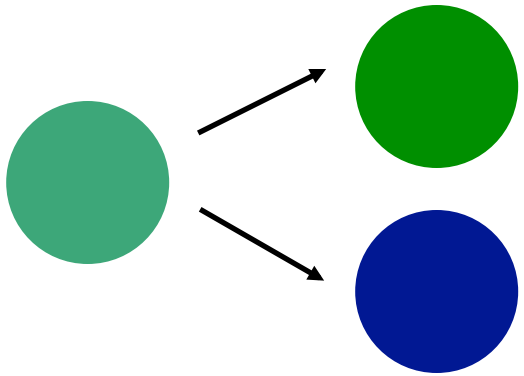


+CCL21



Cells make up tissues

- Progenitors vs. differentiated cells
  - stem cell (SC) sources: embryonic; adult; or induced
  - SC access issues: politically charged; rare; uncertain
  - ESC advantages: definitely form any cell type
  - ESC safety issues: genetic instability, tumors
  - differentiated cells difficult to stably maintain
- Transplanted vs. *in situ* cells
  - *in situ* rarity
  - *in situ* unique attraction difficult
  - transplantation safety risks
  - *ex vivo* expansion risks

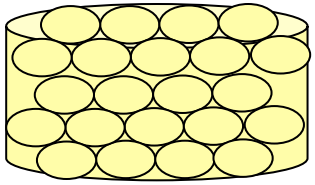


Stem cell short reviews: D.M. Choumerianou, et al. *Tissue Eng B* **14**:53 (2008).
S.M. Richardson, et al. *J Cell Physiol* **222**:23 (2010).

Components of a TE construct

scaffold/matrix

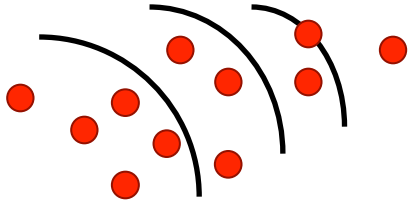
→ usually degradable, porous



soluble factors

→ made by cells or synthetic

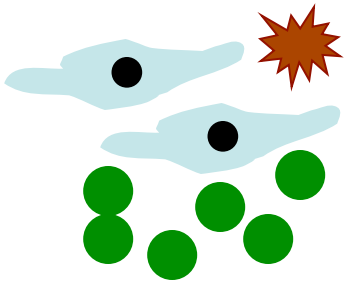
→ various release profiles



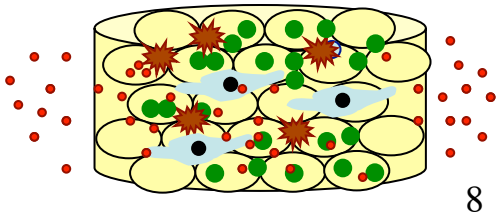
cells

→ precursors and/or differentiated

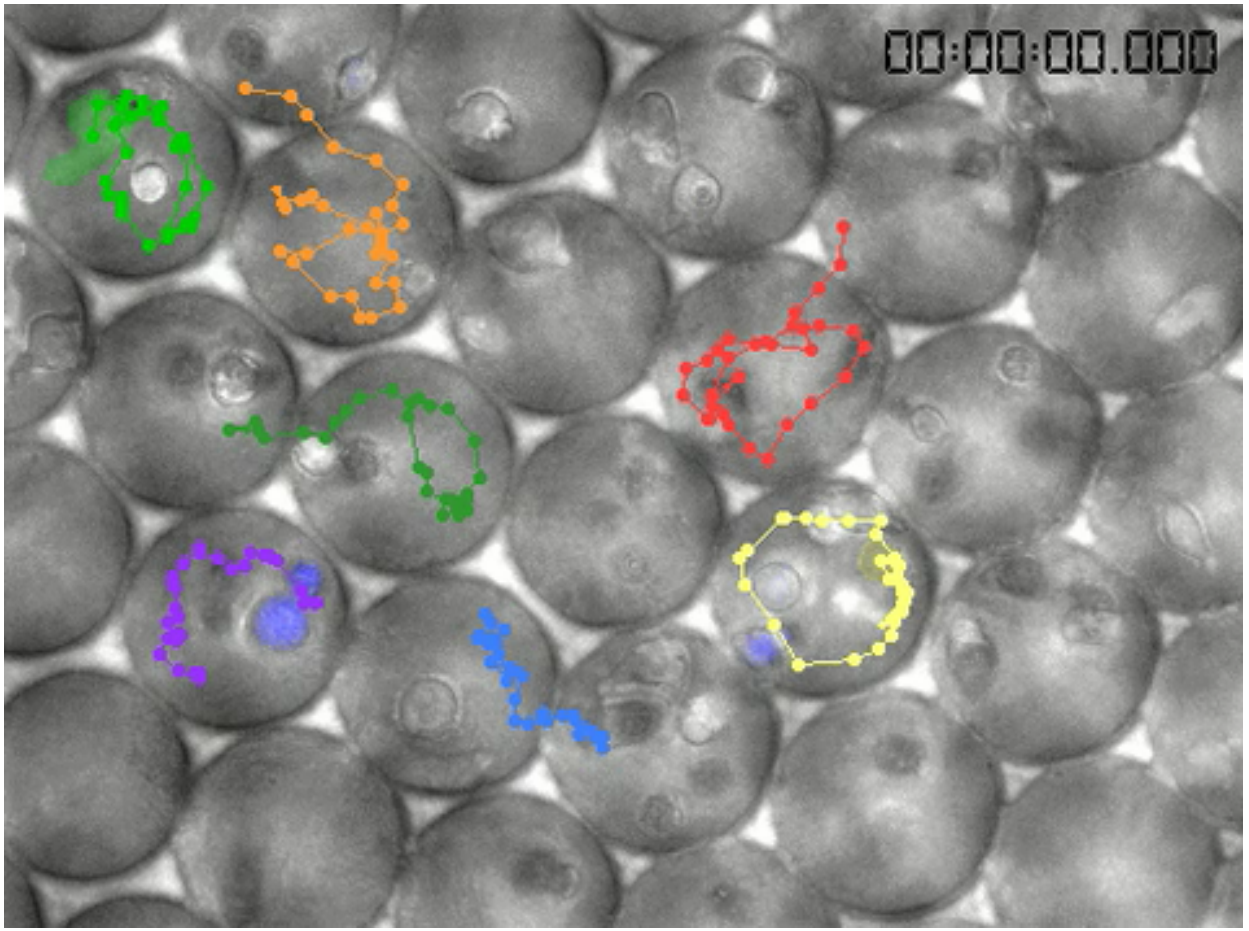
→ often autologous



integrated implantable or injectable device



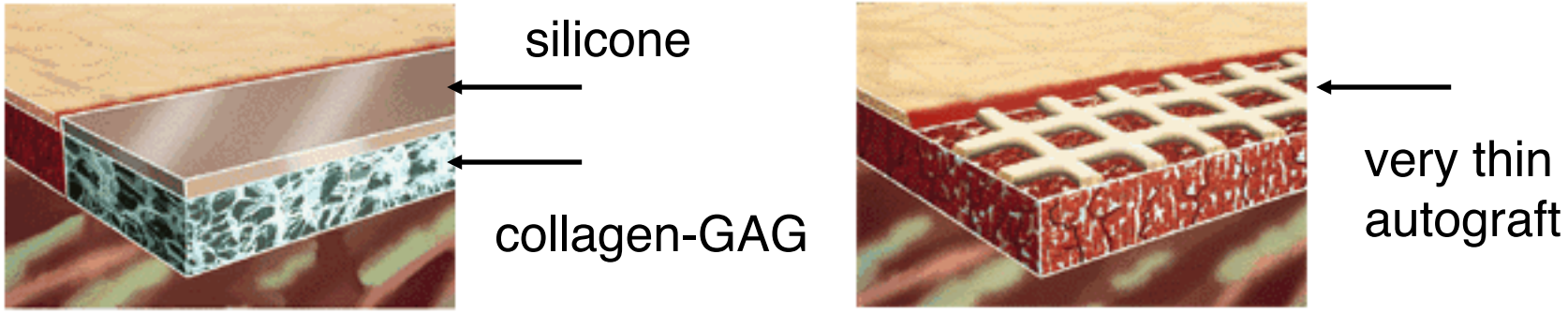
Putting it all together: *in vitro* construct



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

Early 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 (1996)



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

Interlude: Shmeat

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

2:24 – 4:32

Current prospects for TE meat

- No one made PETA's chicken deadline!
  - even with 2 year extension to March 2014
- But: first "proof of concept" beef taste test by Dr. Mark Post in Aug. 2013
  - $375 K funding
- Research motivation
  - environment
  - animal ethics
  - technological feat
- Sales > 10 yrs off?

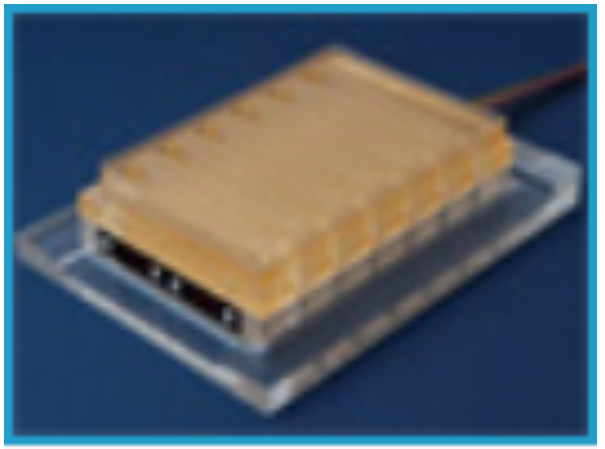
nytimes.com

A Lab-Grown Burger Gets a Taste Test

David Parry/Press Association, via European Pressphoto Agency

Beyond skin: commercial liver TE

- Developed at MIT!
  - Prof. Linda Griffith
  - Prof. Steve Tannenbaum
  - transfer to Zyoxel (CNBIO) 2012



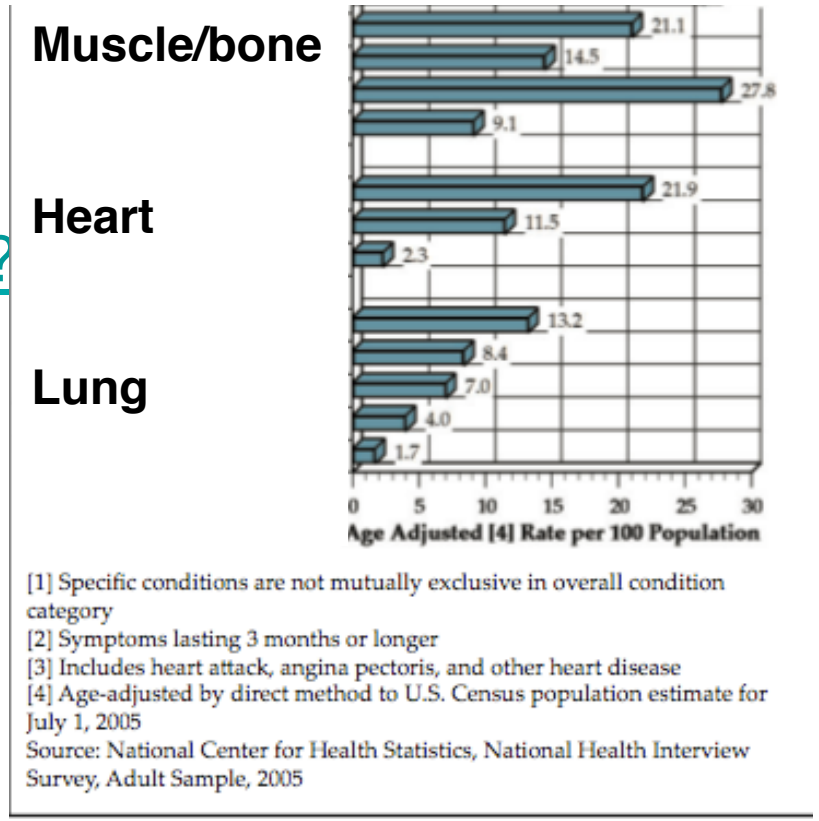
zyoxel.com

- Testing platform rather than therapy
  - true for some skin TE products also
  - liver especially interesting for drug development
- Continuous media perfusion
  - liver cells finicky
  - realistic for testing drug interactions

Joint diseases: an unmet need

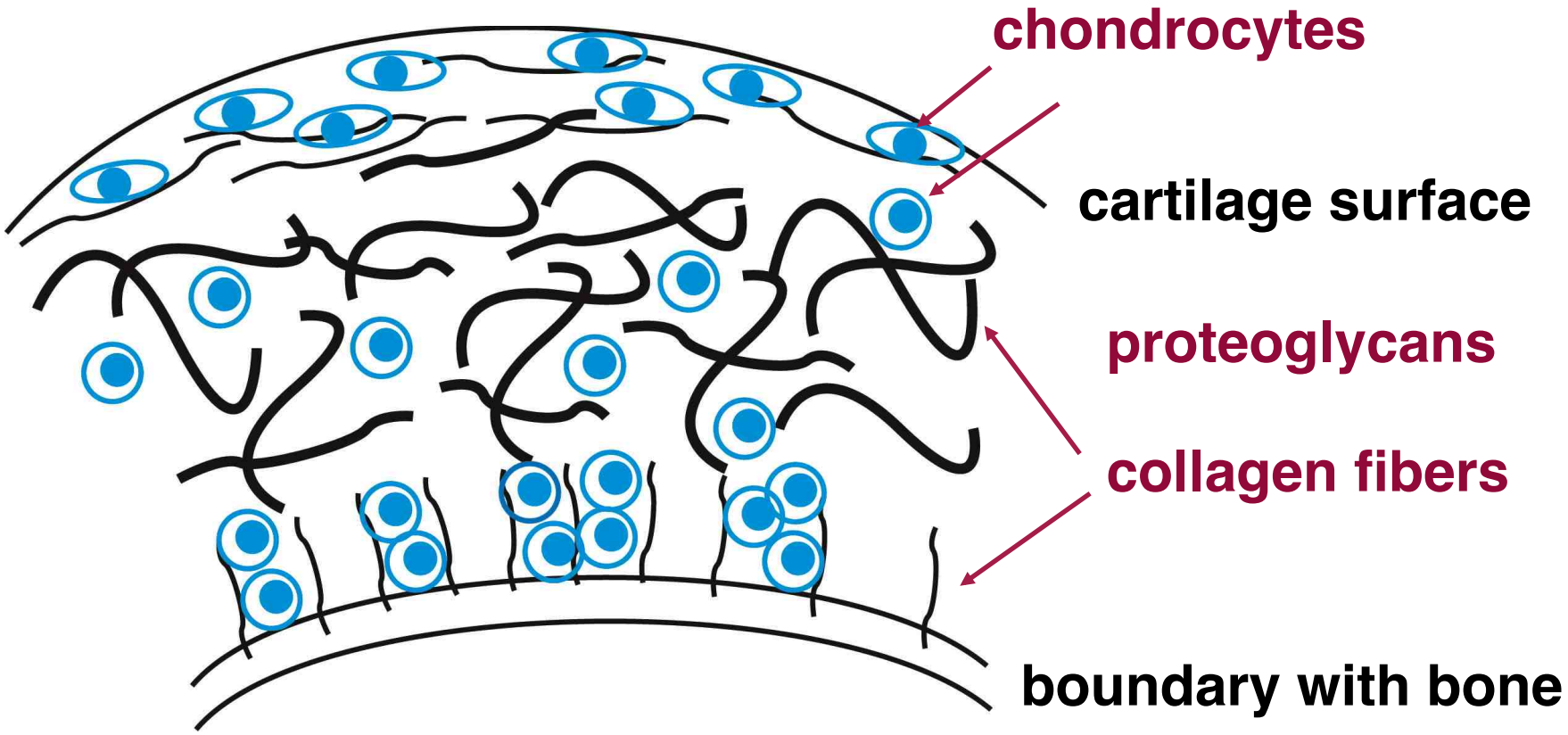
- Leading cause of physical disability in U.S.
- $100's billion in in/direct costs
- Osteoarthritis
  - common in elderly population
  - acute injury (athletes) → susceptibility to early disease
- http://orthoinfo.aaos.org/topic.cfm?topic=a00212
- Limited pharma solutions
  - pain management
  - targets unknown (aggrecenase?)
  - 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



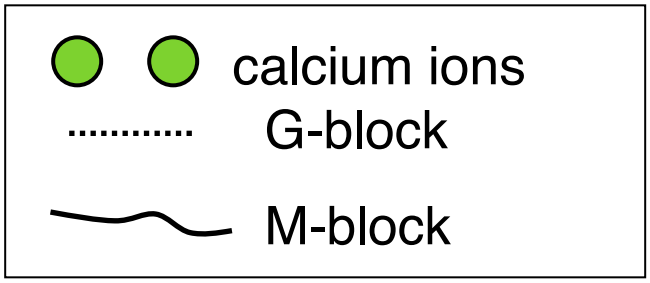
Water-swollen, heterogeneous, **avascular and cell-poor** tissue.

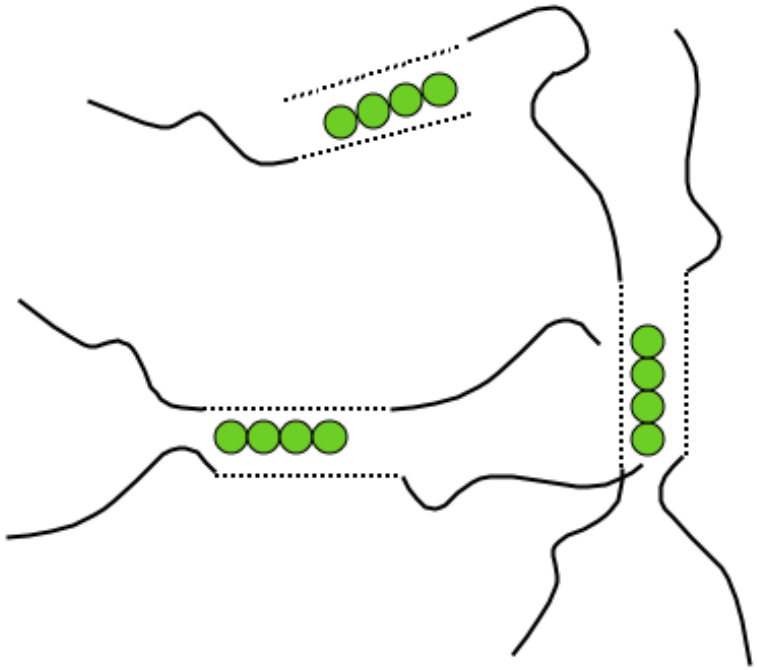
Alginate: material for 3D culture

- 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
  - swelling
  - mechanical properties
  - degradability (τ)

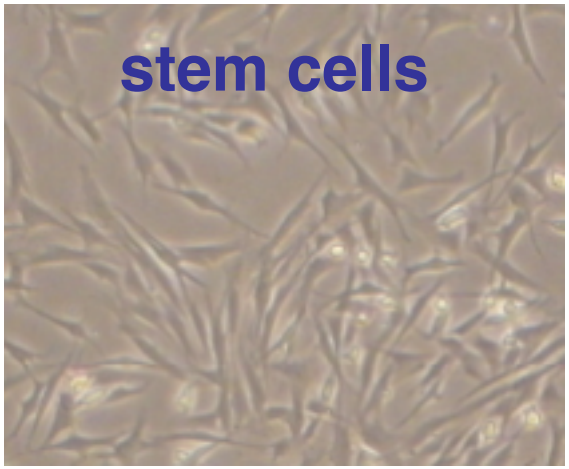
  - viscosity of solution

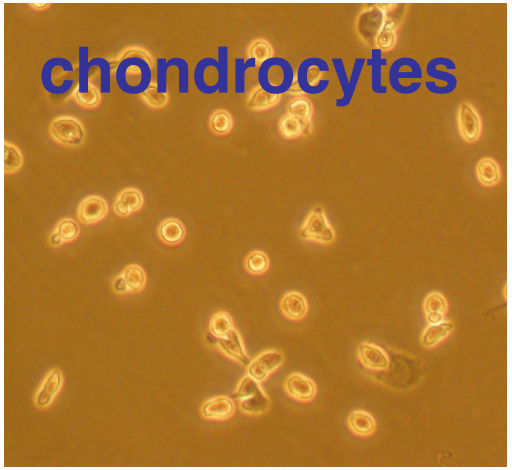


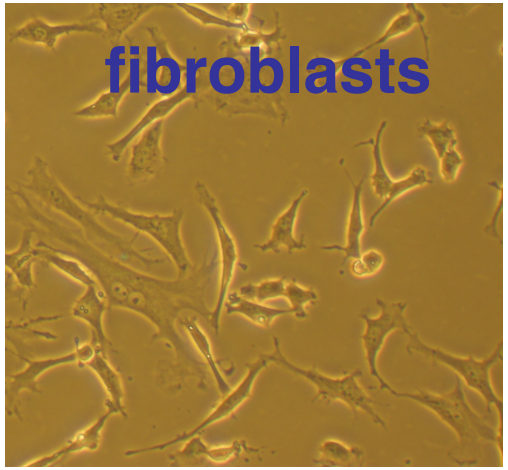


Cells for cartilage TE

	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





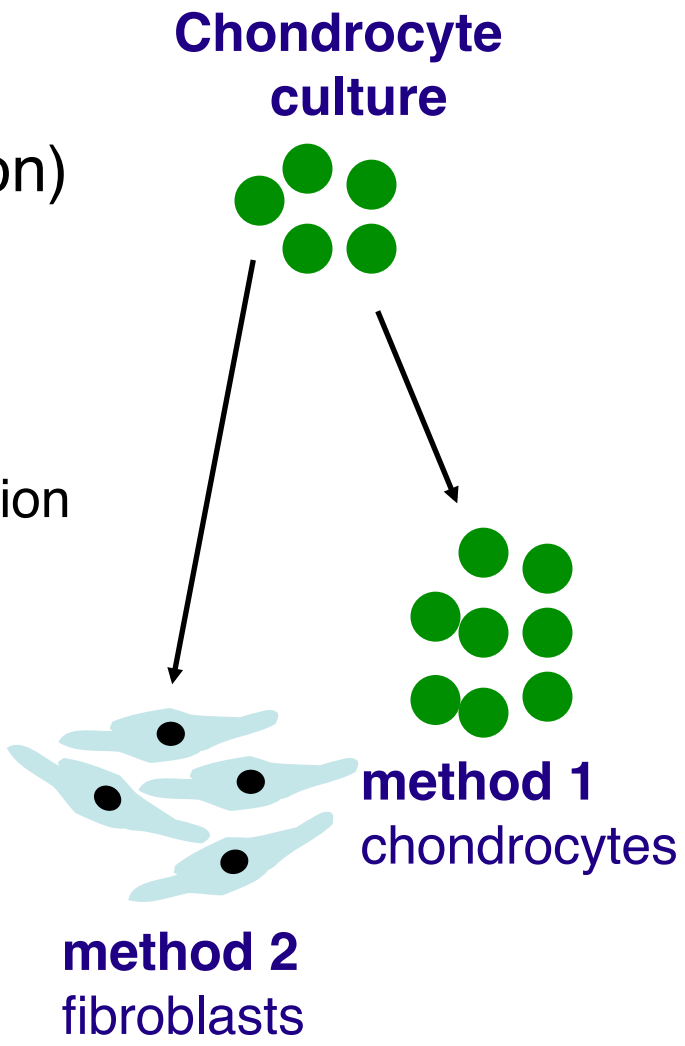


Specific goal and experiments

- **Goal:** compare effects of two culture conditions on chondrocyte phenotype (CDR maintenance or SC differentiation)

- Observe cell morphology and viability
- Measure collagen content
  - gene (qPCR) and protein (ELISA) expression
  - collagen II:I ratio reflects cell state
- Measure proteoglycans

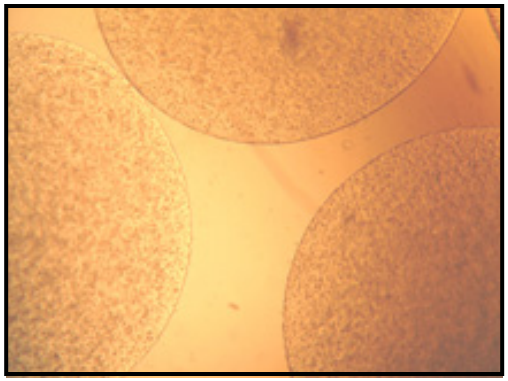
- Big picture impact: cartilage TE
  - conditions for *in vitro* cartilage production
  - conditions for *ex vivo* cell expansion



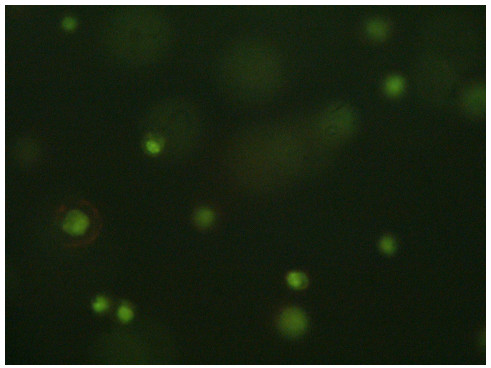
Module overview: lab days

Day 1: design

Day 2: seed cultures

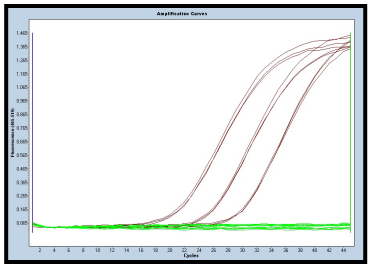


Day 3: viability assay



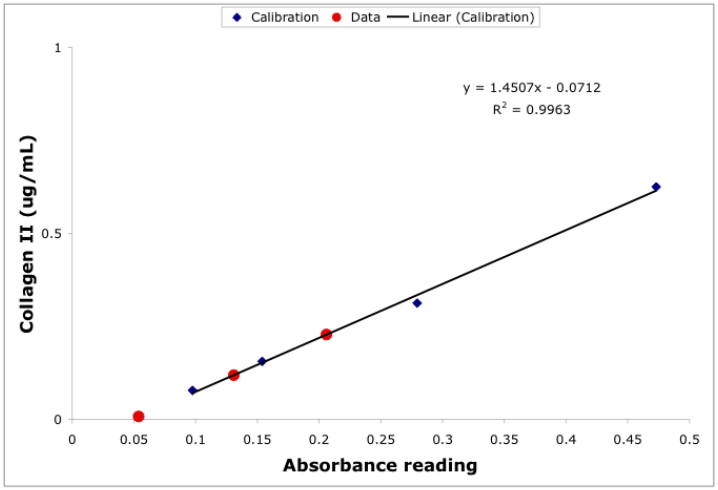
Day 4: prep RNA + cDNA

Day 5: transcript assay



Day 6: protein assay

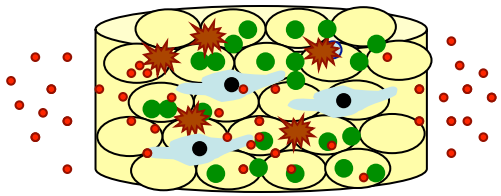
Day 7: remaining analysis



Day 8: your research ideas!

Lecture 1: intro to TE and M3

- Tissue engineering is an emerging interdisciplinary field
  - showing signs of becoming a maturing field
- Maintaining cell function is a key part of TE
- Alginate beads provide a culture system for soft tissue (e.g., cartilage) research



Next time… more about both engineered and natural biomaterials.