Principles and Practice of Tissue Engineering

Module 3, Lecture 1

20.109 Spring 2008

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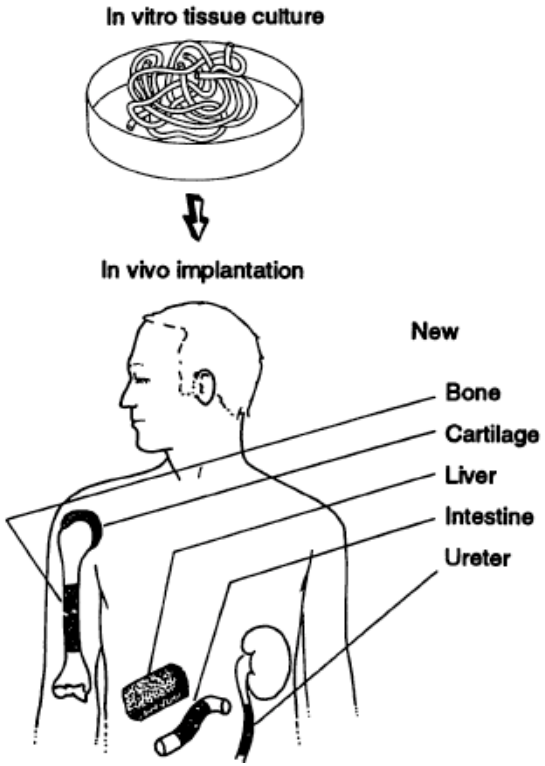
Topics for Lecture 1

- Introduction to tissue engineering
  - basic principles
  - examples
- Introduction to Module 3
  - background: cartilage
  - module structure
  - focus on week 1

What is tissue engineering?

"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)

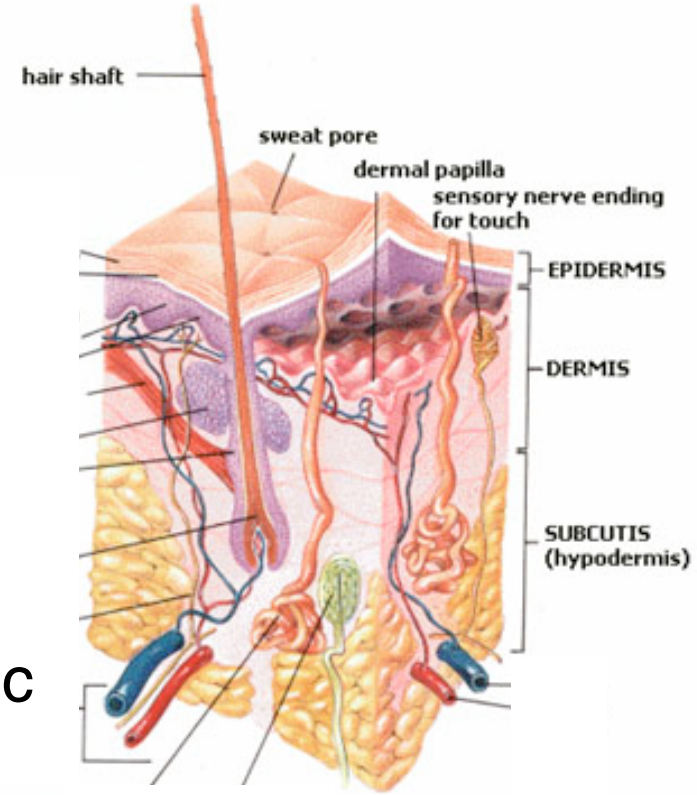


[Langer & Vacanti]

- By what means?
  - natural or synthetic materials and cells
- Which functions?
  - the replacement is not usually identical to native tissue

Why tissue engineering?

- Severe trauma (acute or disease-state) challenges natural repair
  - e.g., shallow vs. deep cuts or burns
  - scar tissue formation
- Donor tissue problematic
  - scarcity of available tissue
  - immune response (graft or host)
- Autologous tissue can be problematic
  - no available site with excess tissue
  - permanent damage at donor site

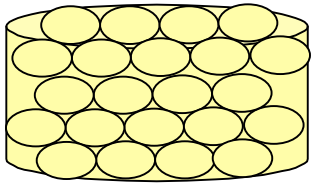


[Public domain image, Wikimedia Commons]

Components of a TE construct

scaffold/matrix

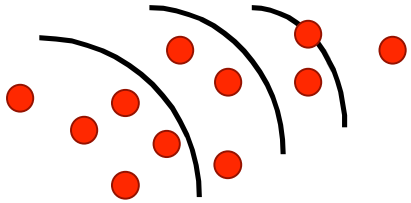
→ usually degradable, porous



soluble factors

→ made by cells or synthetic

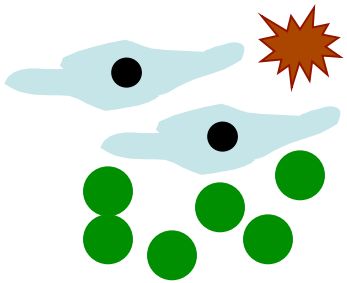
→ various release profiles



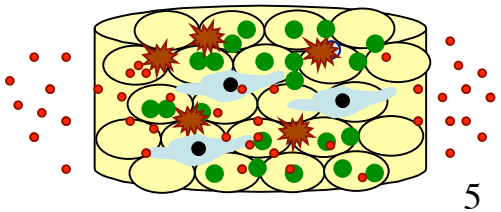
cells

→ precursors and/or differentiated

→ usually autologous

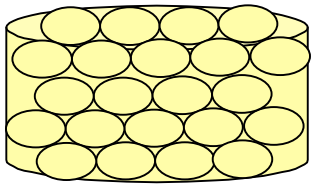


integrated implantable or injectable device



Principles of TE: scaffolds

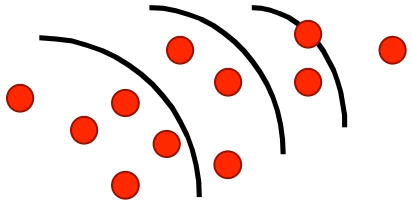
- Why a degradable, porous scaffold?
  - scaffold initially provides mechanical support for cells
  - degradability: scaffold may block new tissue growth, and/or overstimulate inflammation
  - porosity: promotes nutrient+oxygen diffusion



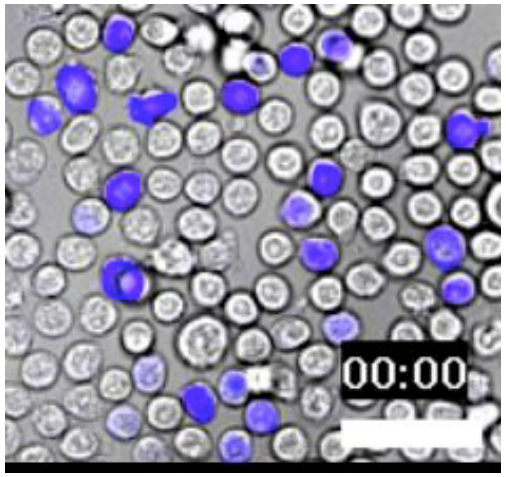
- How is the scaffold made degradable?
  - cross-links susceptible to chemical cleavage
  - cross-links susceptible to enzymatic cleavage
- Example: collagen or collagen-mimetic scaffolds
  - e.g., West JL & Hubbell JA, *Macromolecules* **32**:341 (1999)

Principles of TE: soluble factors

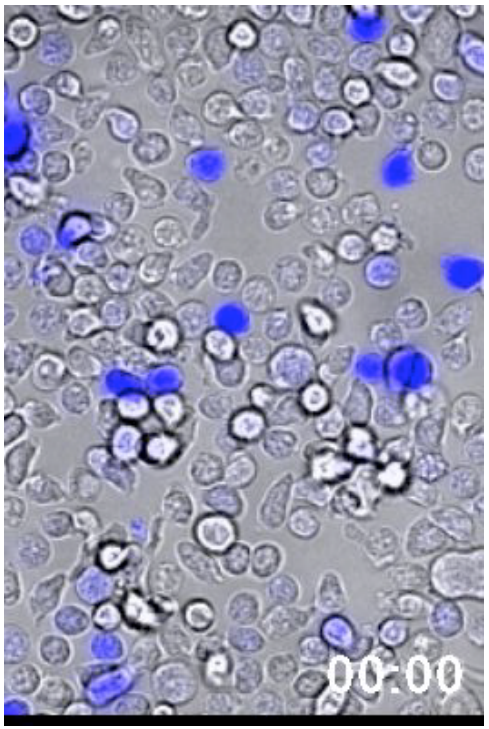
- Types of soluble factors (cytokines)
  - growth factors for proliferation or differentiation (TGF, BMP)
  - factors promoting angiogenesis (VEGF)
  - chemokines that attract the cell type(s) of interest
- Delivery of soluble factors:
  - release from transplanted cells or scaffold itself
- Example: CCL21 promotes T cell migration
  Stachowiak et al., *J Immunol* **177**:2340 (2006).



Control

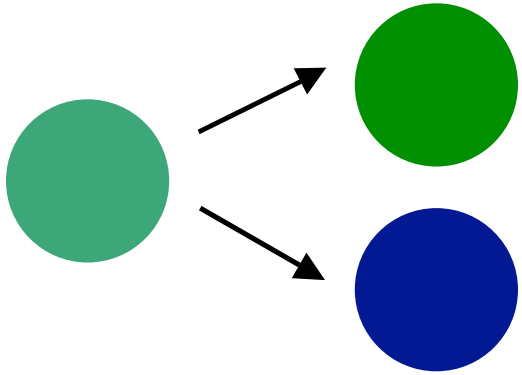


+CCL21

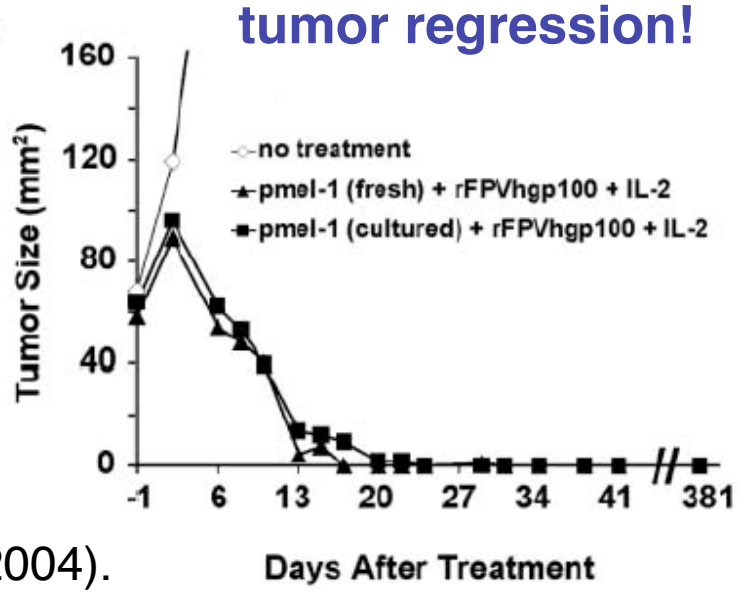


Principles of TE: cells

- Progenitors vs. differentiated cells
  - progenitors: hard to obtain large numbers
  - differentiated: may have lost functions
- Transplanted vs. *in situ* cells
  - cell expansion *in vitro*: can transplant large numbers



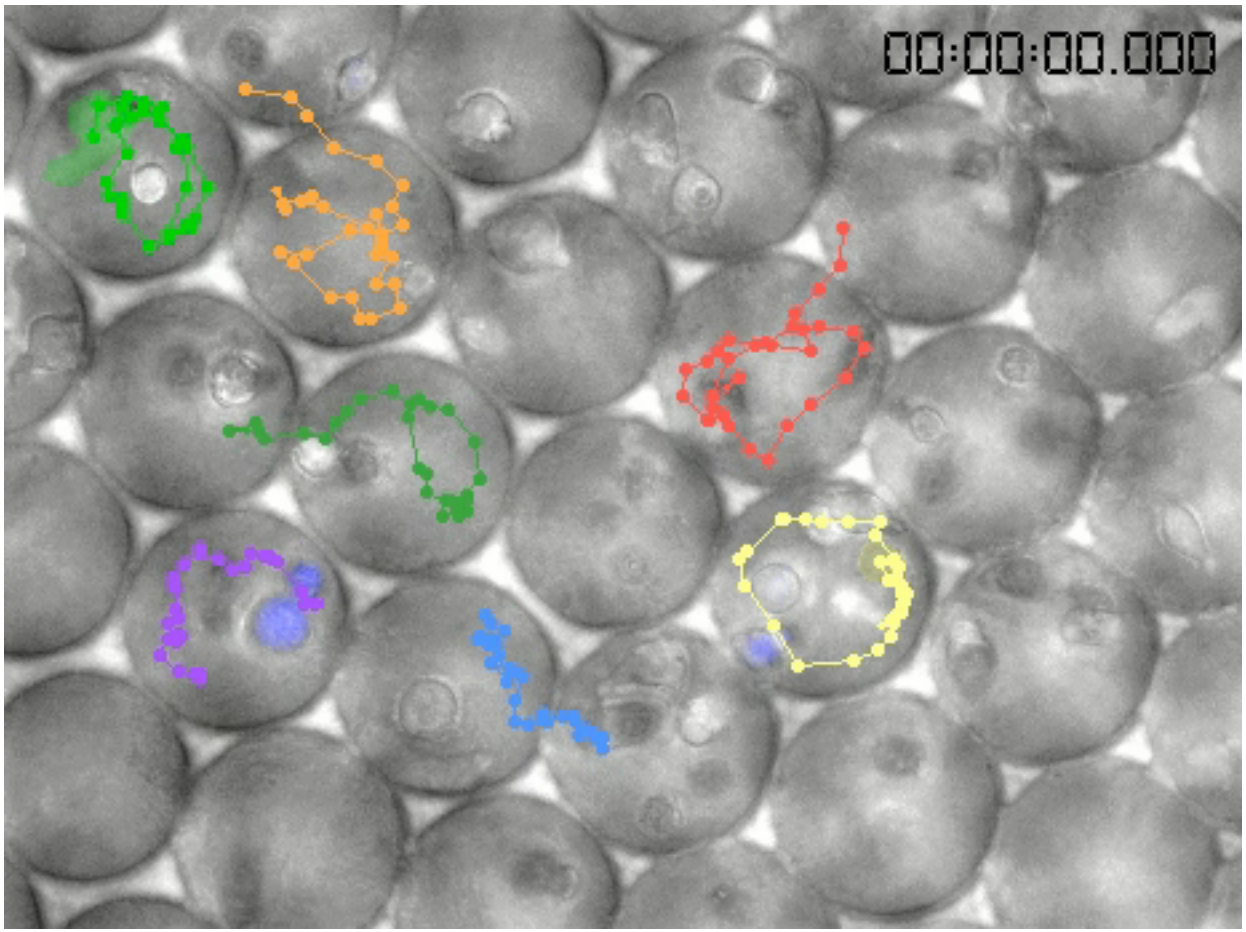
- Example: tumor-infiltrating lymphocytes (TIL)
  - T cells lose function in tumors
  - expand TIL *ex vivo,* treat with cytokines, and transplant: regression in some patients



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

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

Putting it all together: TE construct

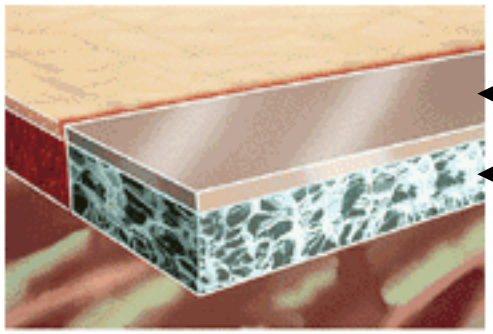


Stachowiak et al. *J Biomed Mater Res*, in press

Successful TE example

- 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
    - *not* contracted scar tissue
    - however, lacks sweat glands and follicles
  - sold as Integra Dermal Regeneration template

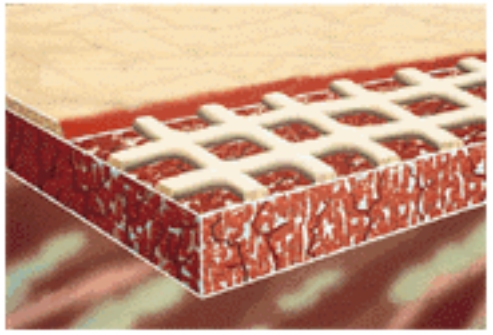
1.



silicone

collagen-GAG

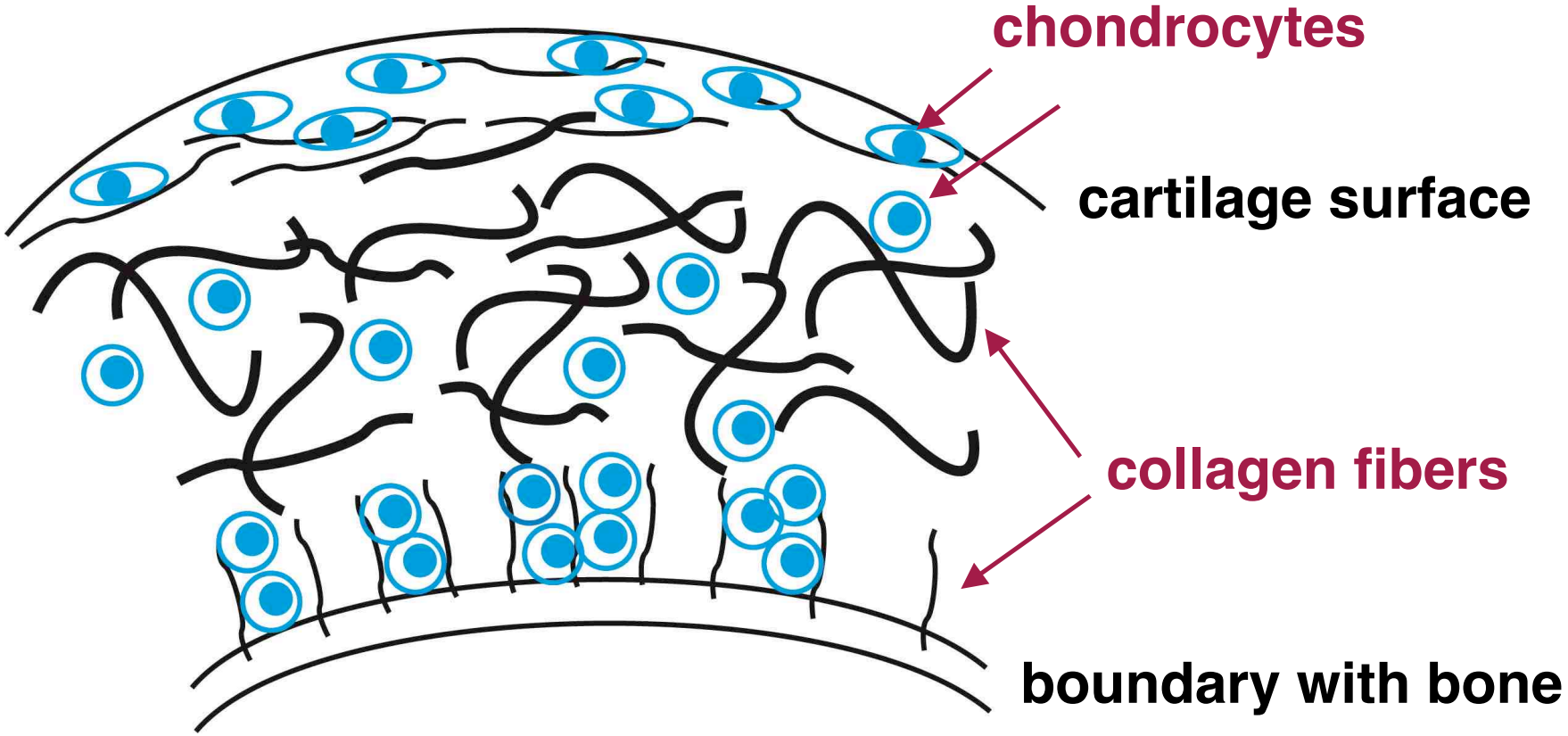
2.



very thin autograft

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

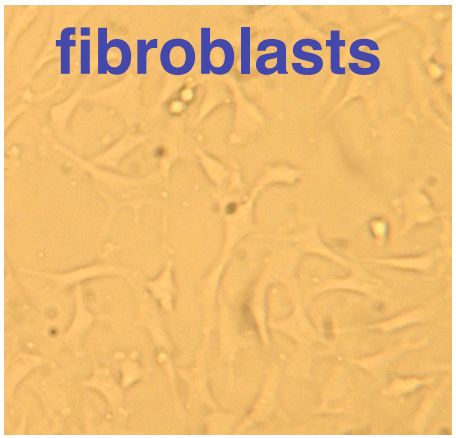
Our focus: cartilage tissue



Avascular, highly water-swollen, heterogeneous tissue.

Cartilage TE basics

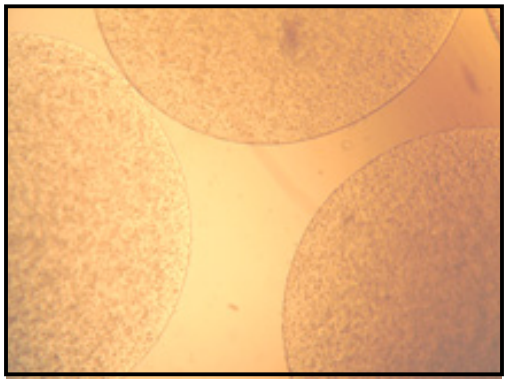
- Progenitor cells: mesenchymal stem cells
  - require growth factors for differentiation
  - may be difficult to obtain or work with
- Differentiated cells: chondrocytes
  - require special environment to maintain phenotype
  - otherwise, may de-differentiate to fibroblasts
- Our goal: *in vitro* culture of chondrocytes to preserve or destroy phenotype
  - observe collagen content, morphology, viability
  - collagen II:collagen I ratio reflects cell state
  - ultimately, knowledge of key environmental effects can help with design of cartilage TE constructs



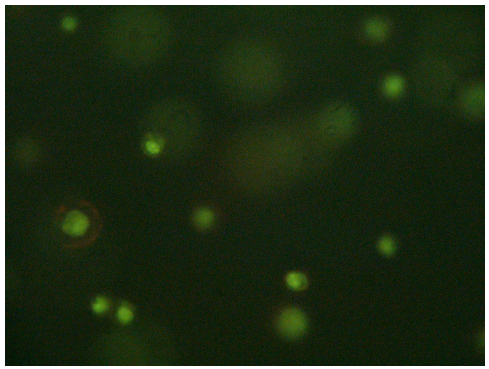
Module overview: lab

Day 1: design

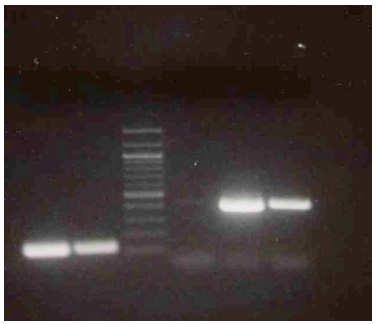
Day 2: seed cultures



Day 3: viability assay



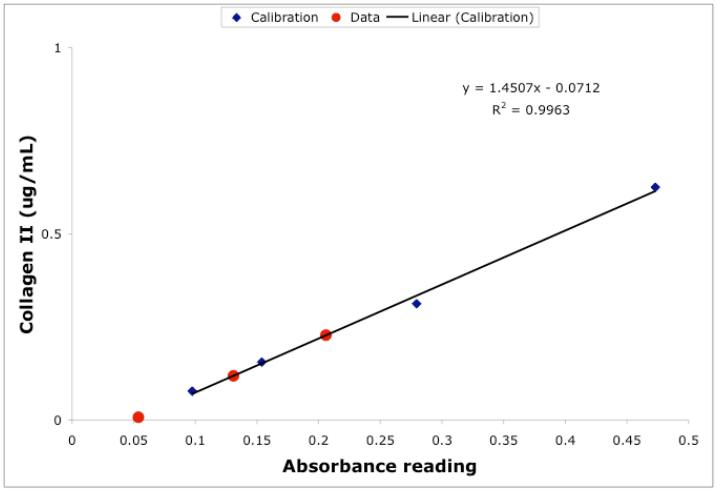
Day 4: prep RNA+cDNA



Day 5: transcript assay

Day 6: protein assay

Day 7: remaining analysis



Day 8: your research ideas!

Module overview: week 1

Days 1+2: design and seed cultures

- 2D culture: plastic surface
  - – prepare in duplicate
  - –design maintenance plan

flask 1 = flask 2

[bdbiosciences.com]

- 3D culture: alginate beads
  - – prepare in duplicate wells
  - – vary one parameter

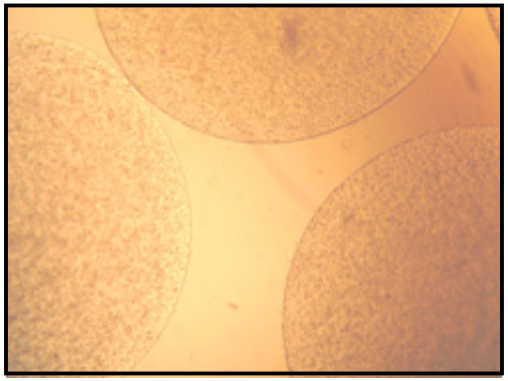
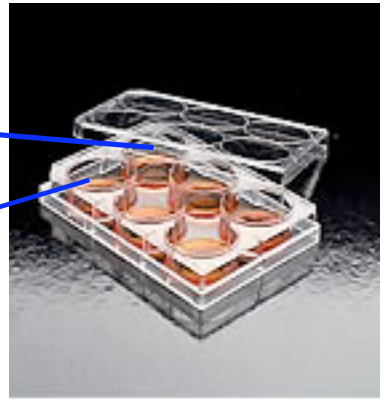
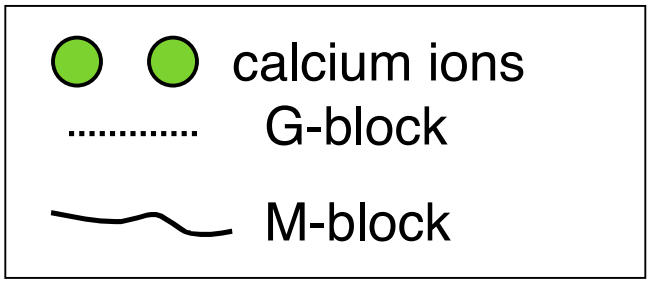


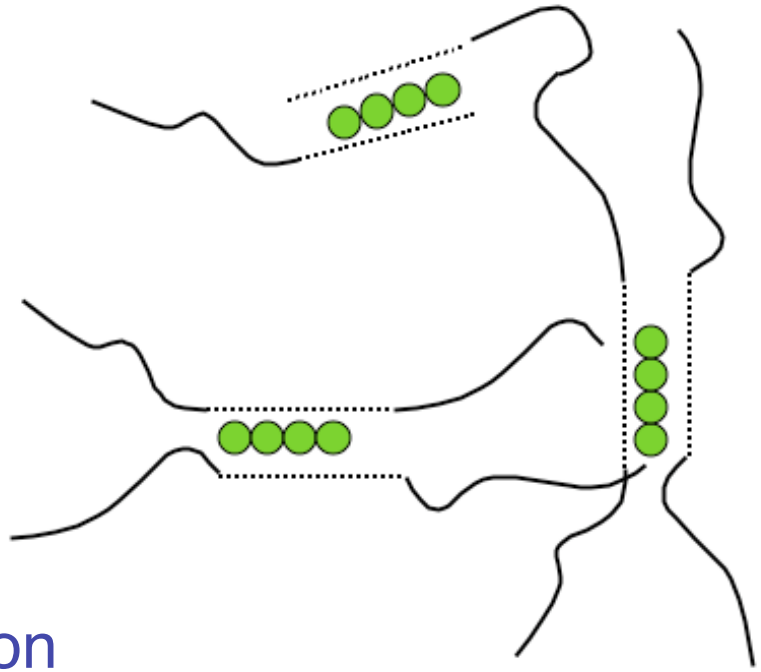
plate 1 ≠ plate 2



Alginate: material for 3D culture

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





Method preview:

liquid droplets → into Ca^{2+} solution → semi-solid gel beads

Lecture 1: conclusions

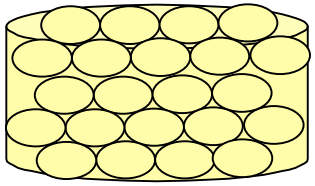
- Tissue engineering is an emerging field at the interface of engineering, science and medicine
- Maintaining cell function is a key part of TE
- Monolayer and alginate cultures provide a system for testing microenvironmental effects on chondrocytes

Next time… types of biomaterials, their properties, and cell-biomaterial interactions.

Ideas for varying culture conditions

scaffold/matrix

→ usually degradable, porous



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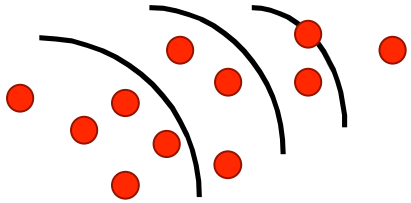

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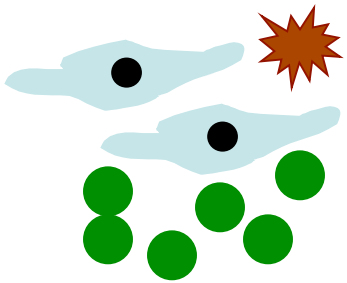

Other targets for change?
Most realistic options?