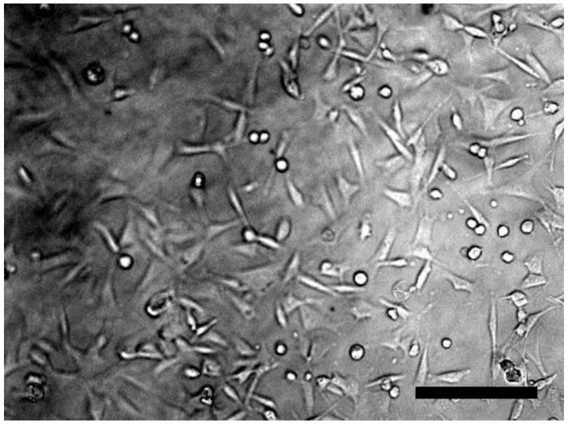
Cell Viability; Standards in Scientific Communities I

Module 3, Lecture 3

20.109 Spring 2013

Lecture 2 review

- What properties of hydrogels are advantageous for soft TE?
- What is meant by bioactivity and how can it be introduced?
- What are the two major matrix components of cartilage and how do they support tissue function?



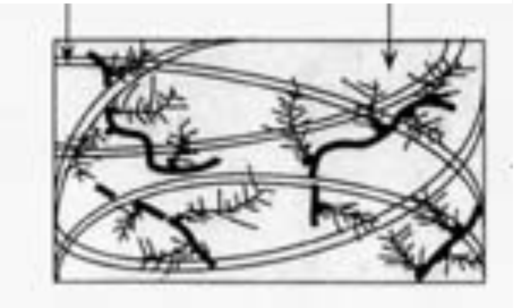


Image: VC Mow, A Ratcliffe, SLY Woo, eds *Biomechanics of Diarthrodial Joints* (Vol I). Springer-Verlage New York Inc., 1990.

Structure of healthy and OA cartilage

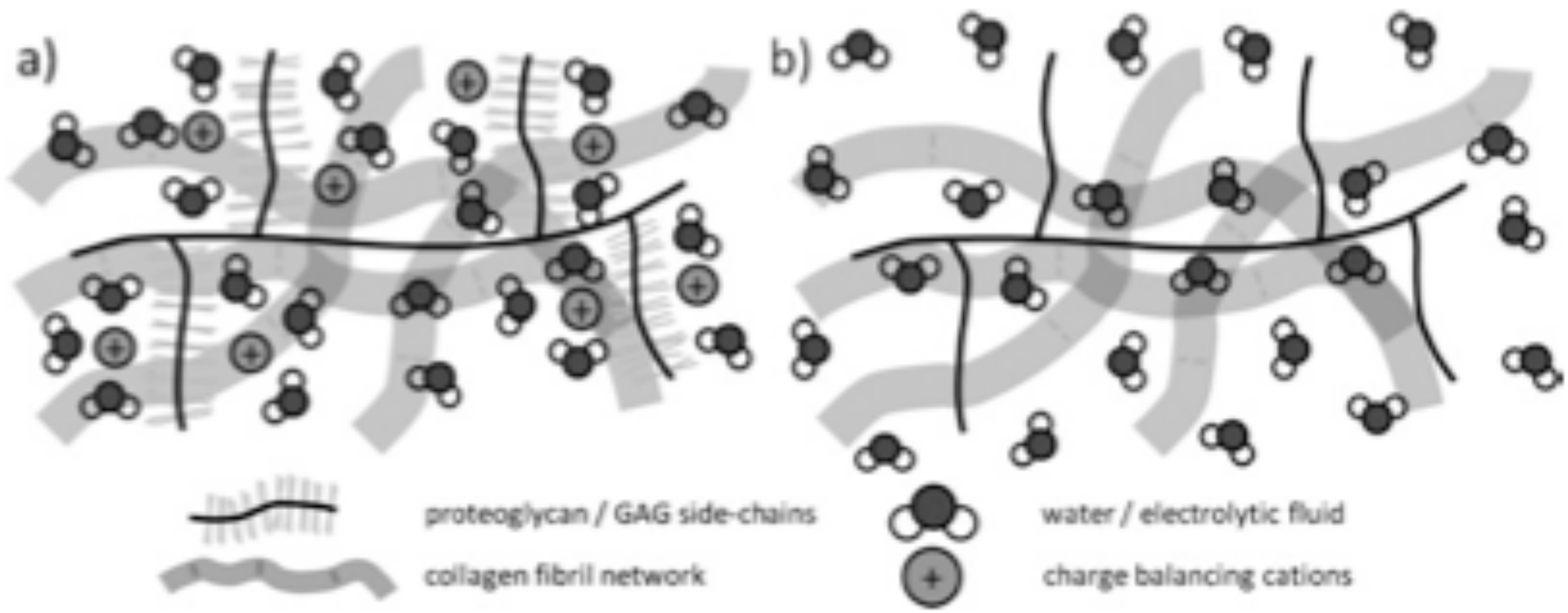


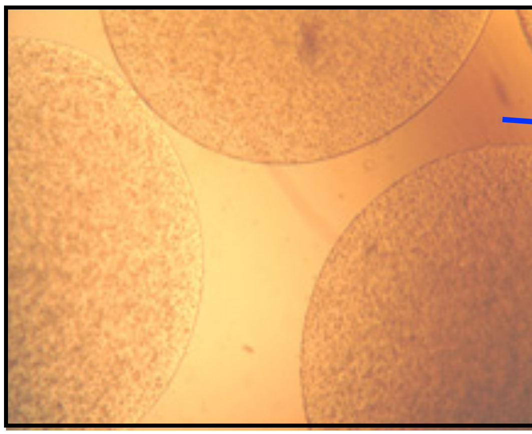
Image: DA Binks et al., *Br J Radiol* **86**:20120163 (2013).

Topics for Lecture 3

- Cell viability
  – measurement
  – contributing factors
- Standards in scientific communities
  – general engineering principles
  – standards in synthetic biology
  – standards in data sharing

Module progress: week 1

- Day 1: culture design
  - What did/will you test?



- Day 2: culture initiation
  - Cells receiving fresh media every day
  - Half-media exchange for groups with very soft beads

Fluorescence microscope parts

- Light source
  - Epifluorescence: lamp (Hg, Xe)
  - Confocal: laser (Ar, HeNe)
  - 2-photon: pulsed laser
- Filter cube
  - Excitation
  - Dichroic mirror
  - Emission
  - Band-pass vs. long-pass
- Detection
  - CCD camera: photons → voltages → pixel intensities

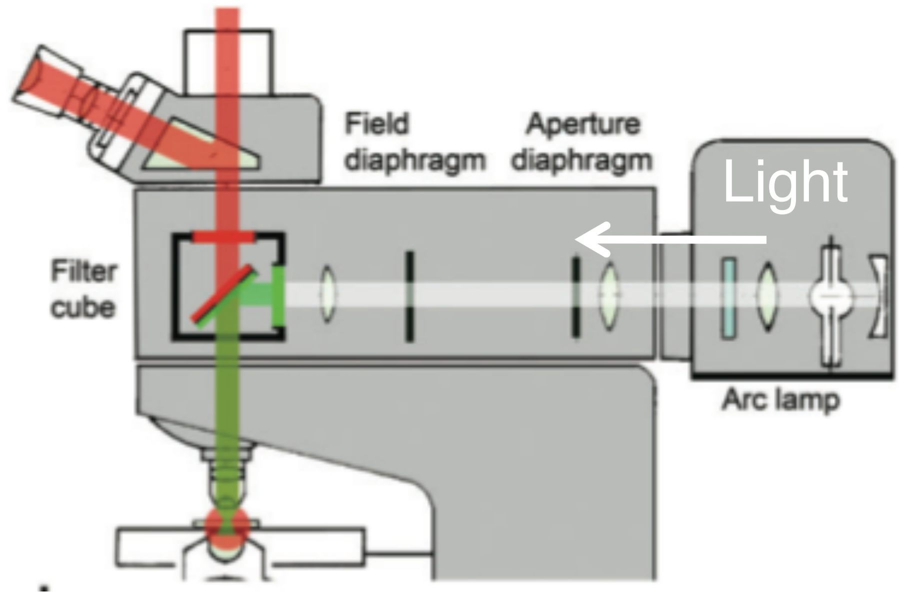
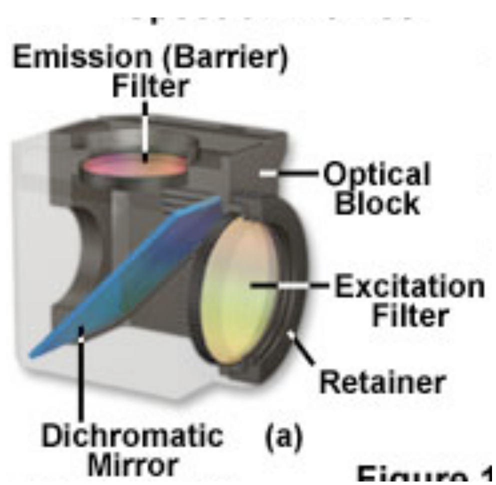
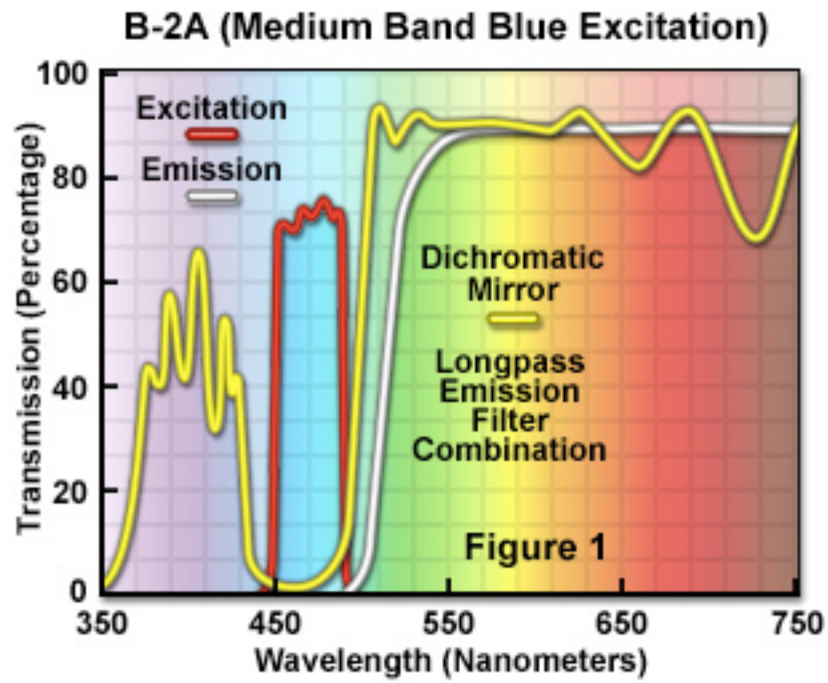


Image from: Lichtman & Conchello, *Nature Methods* 2:910 (2005)

Specifications for M3D3 imaging

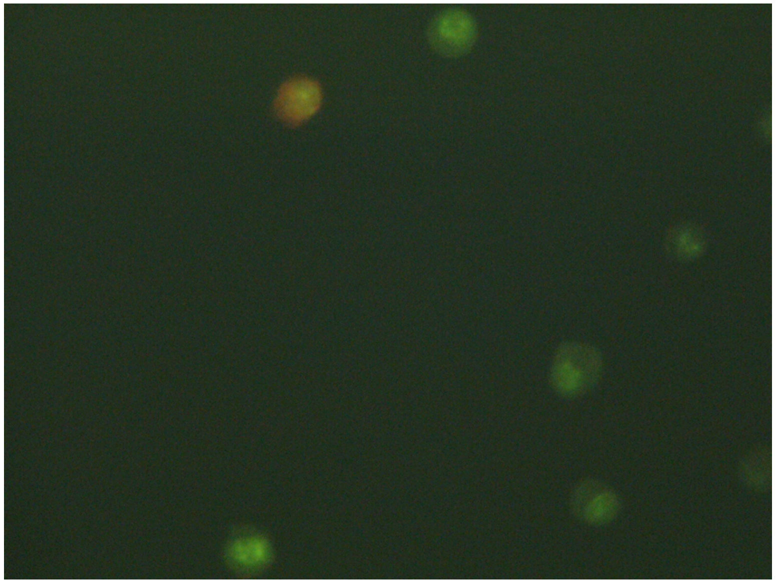
- Live/Dead Dyes
  - Green 490 ex, 520 em
  - Red 490 ex, 620 em
- Excitation 450-490 nm
- Dichroic 500 nm
- Emission 515^{+} nm

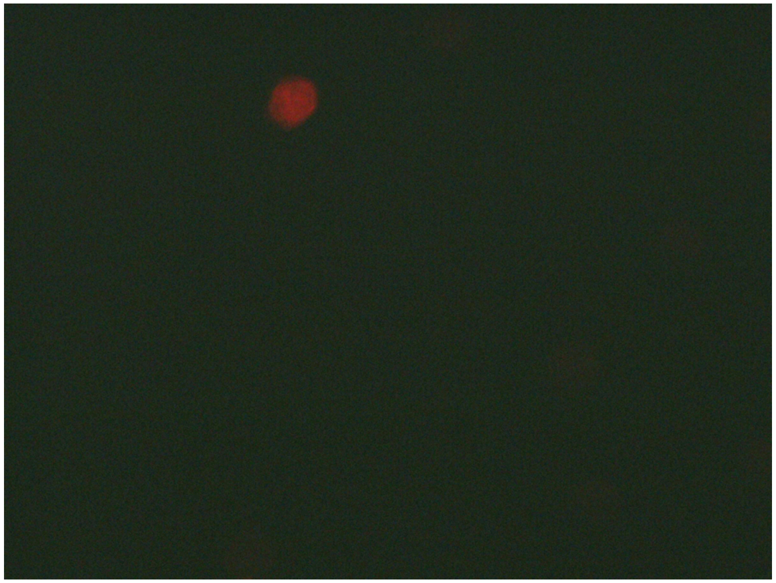




Images from: Nikon microscopy website: www.microscopyu.com

M3D3 viability assay





Green stain: SYTO10 = viability
Red stain: ethidium = cytotoxicity
} Assay readout: fluorescence

Working principle? **Relative cell-permeability**

Types of cell death

- Apoptosis
  - programmed cell death
  - role in development, immunity
  - cells condense, nuclei fragment
  - misregulation may cause disease
- Necrosis
  - response to trauma
  - cells burst and release contents
  - promotes inflammation
- Different morphology and biochemistry

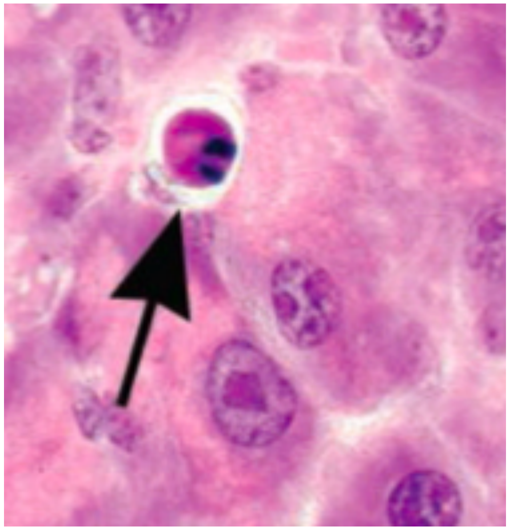
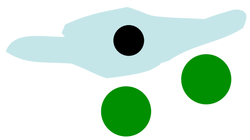


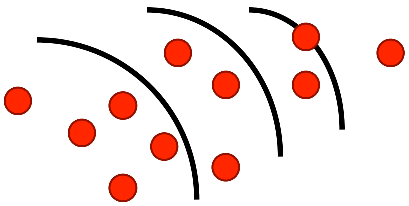
Image: S. Elmore *Toxicol Pathol* 35:495 (2007)

Factors affecting cell viability

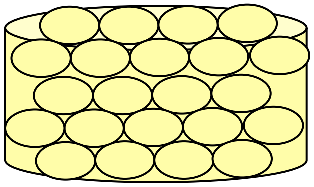
- Cell-related
  - density
  - contact



- Cytokine-related
  - proliferative
  - apoptotic

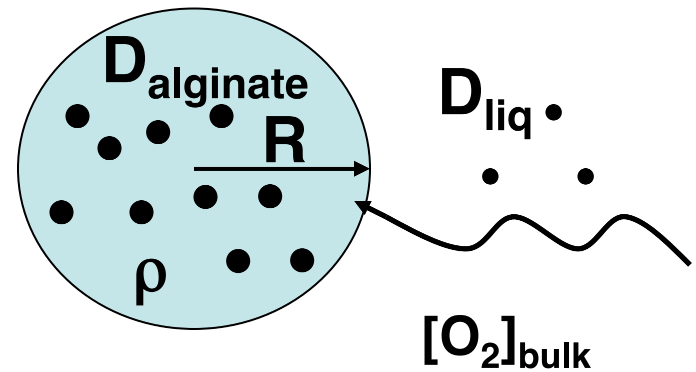


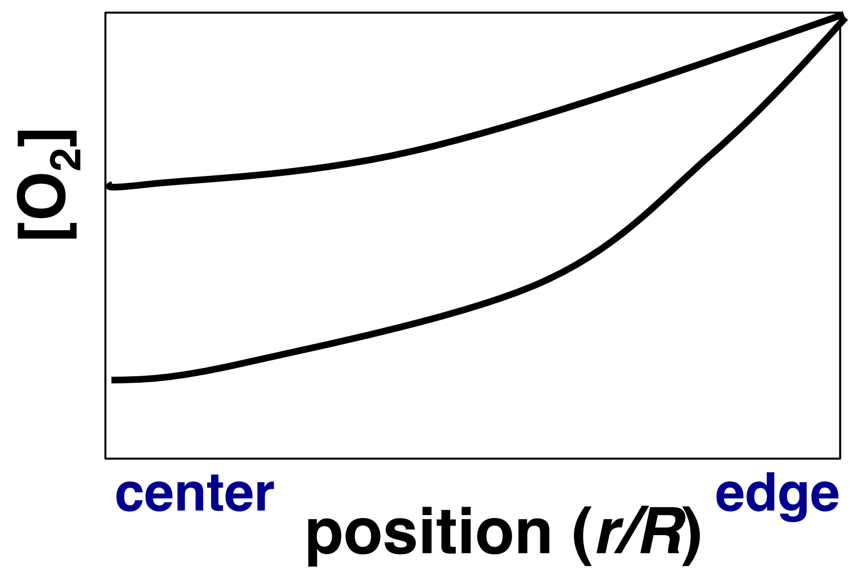
- Materials-related
  - bulk permeability
  - macro-porosity
  - toxicity



Diffusion in 3D constructs

- Nutrients and O_2
- Affected by
  - construct size R
  - cell density ρ
  - diffusivity D
  - conc. in medium $[O_2]_{bulk}$
- Concentration profile
  - can be solved Diff-Eq
  - [O2] ↓ toward center
  - steepness = f(D, ρ, ...)

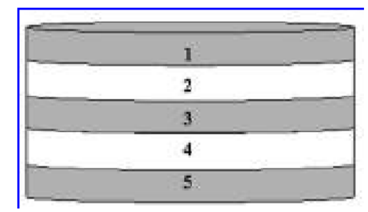




Modeling cell viability in TE constructs

- Porous PLGA scaffolds
- Seeded cells as in (A) or (B)
- Observed after 10 days
- Model includes
  - Diffusion
  - O_2 use
  - Cell growth
- Model assumes
  - $[O_2]_{bulk}$ is constant
  - Quasi-steady state

A Cells in odd layers

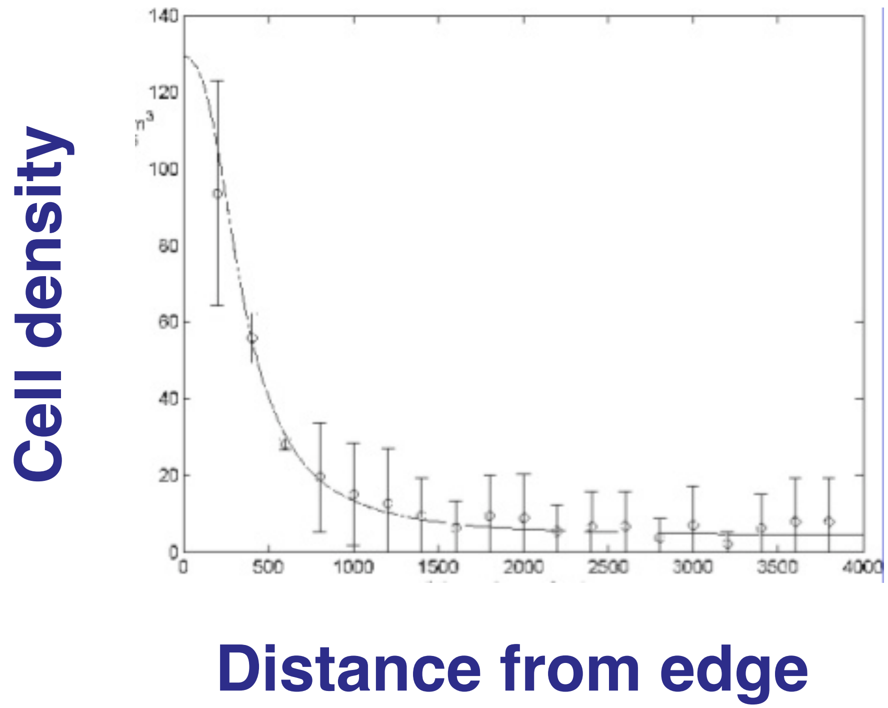


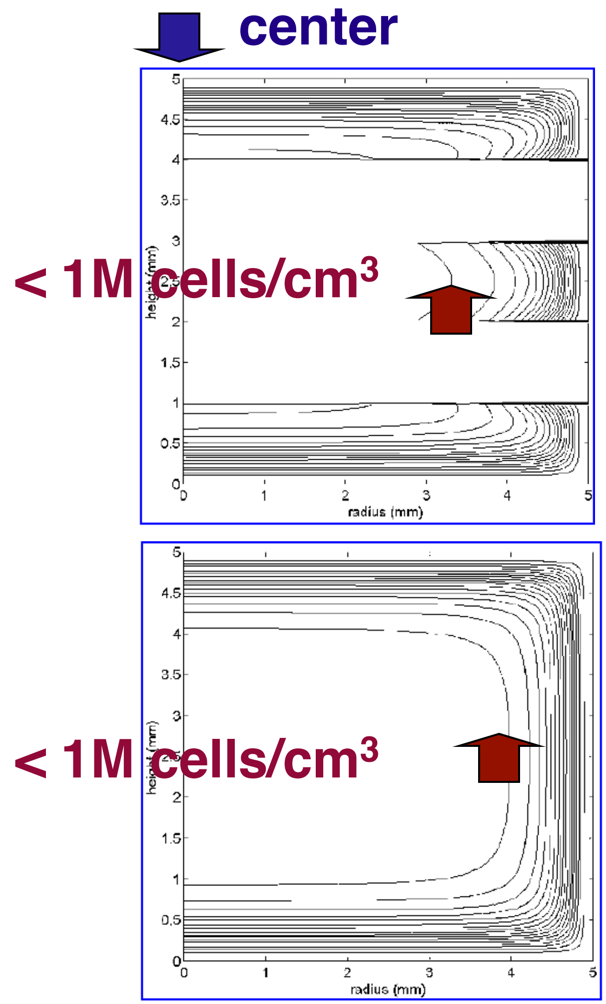
B Cells in all layers

J Dunn, et al. *Tissue Eng* **12**:705 (2006)

Viability model and experiment

- A more uniform than B
- Cell growth matches O_2 tension
- Claim of predictive capability





Dunn, et al.

Significance of diffusion in TE

- Characteristic limit ~100 μm
- Diffusion and viability profiles correlated
- How can we make thick tissues?
  - *in vitro*: dynamic/perfusion culture
  - *in vivo*: promote rapid angiogenesis

perfusion system
zeiss.com.sg

Interlude: perceptions of scientific progress

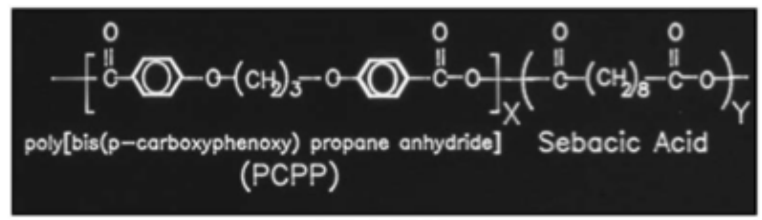
Read the highlighted excerpts from Chapter 7 of The Immortal Life of Henrietta Lacks

What scientific advances today bear a resemblance – in the hopes and/or fears they provoke – to tissue culture in the early 1900's? Does the TC historical perspective change your own thoughts or feelings about the promises and/or perils of current advances in science and technology? What role do scientists play in contributing to or correcting hype?

What moral responsibility do scientists have when they are speaking outside their domain but may be seen as experts?

Thinking critically about module goals

- Local: compare 2 culture conditions → cell phenotype?
- Global: toward cartilage tissue engineering
- All well and good, but…
- Can we move beyond empiricism – tissue *engineering*
- Broadly useful biomaterials example
  - goal: wide degradation range
  - result: times from weeks to years
  - process: models and experience

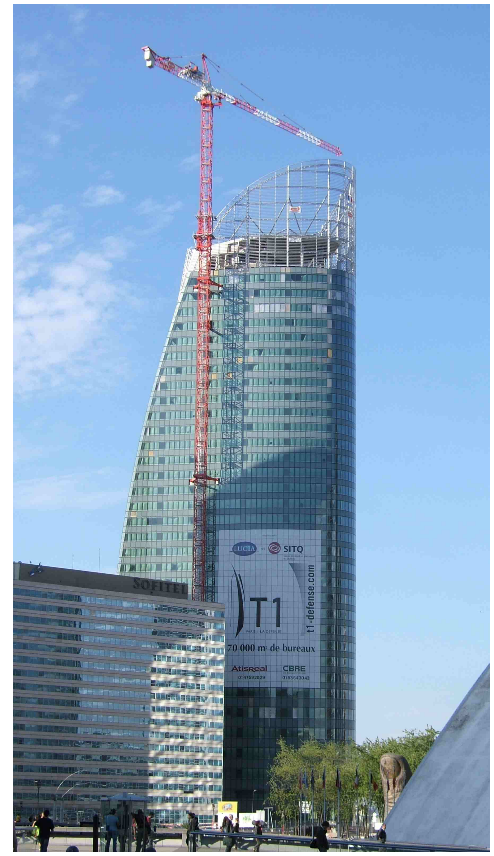


"a lot of chemical calculations later, we estimated that the anhydride bond would be the right one"

Image and quote: Robert Langer, *MRS Bulletin* **31** (2006).

Biology: too complex to engineer?

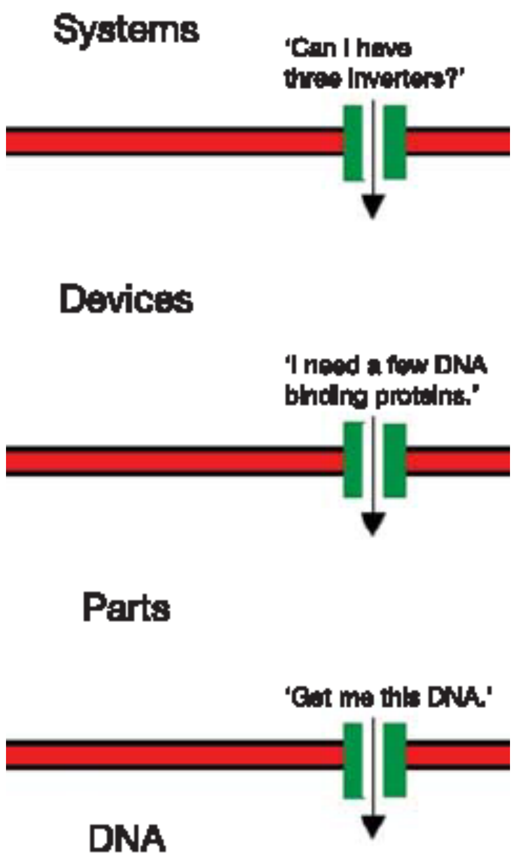
- Systematic vs. *ad hoc* approach
- D. Endy, *Nature* **438**:449 (2005)
- Need for “foundational technologies”
- Decoupling
  - e.g., architecture vs. construction
- Abstraction
  - e.g., software function libraries
- Standardization
  - screw threads, train tracks, internet protocols
- What can and/or should we make standard to engineer biology?



Public domain image (Wikimedia Commons)

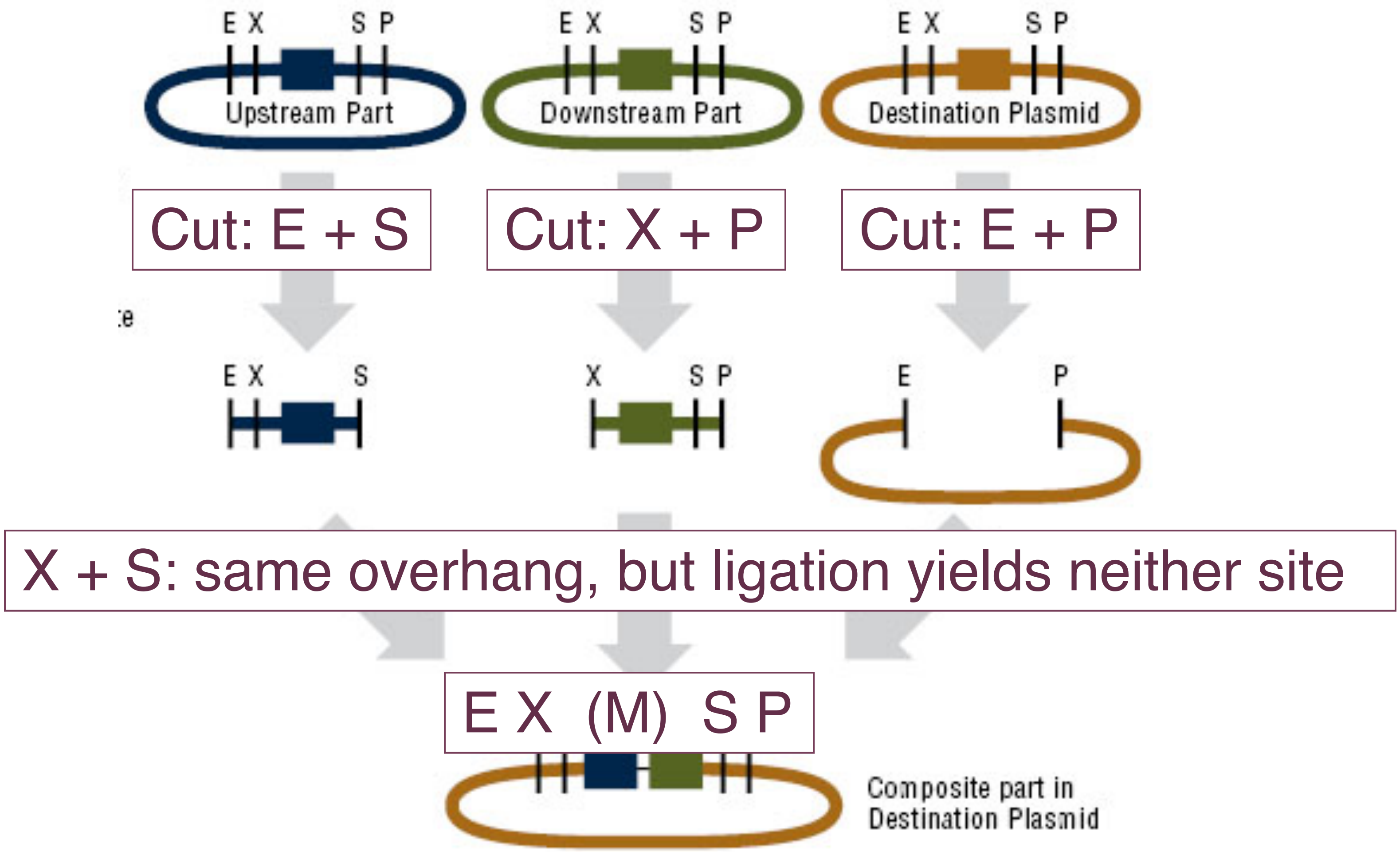
Apply principles to synthetic biology

- Synthetic biology, in brief: "programming" cells/DNA to perform desired tasks
  - artemisinin synthesis
  - genetic circuit
- Decoupling
  - DNA design vs. fabrication (rapid, large-scale)
- Abstraction
  - DNA → parts → devices → systems
  - materials processing to avoid unruly structures
- Standardization
  - standard junctions to combine parts
  - functional (e.g., RBS strength)
  - system conditions
  - assays



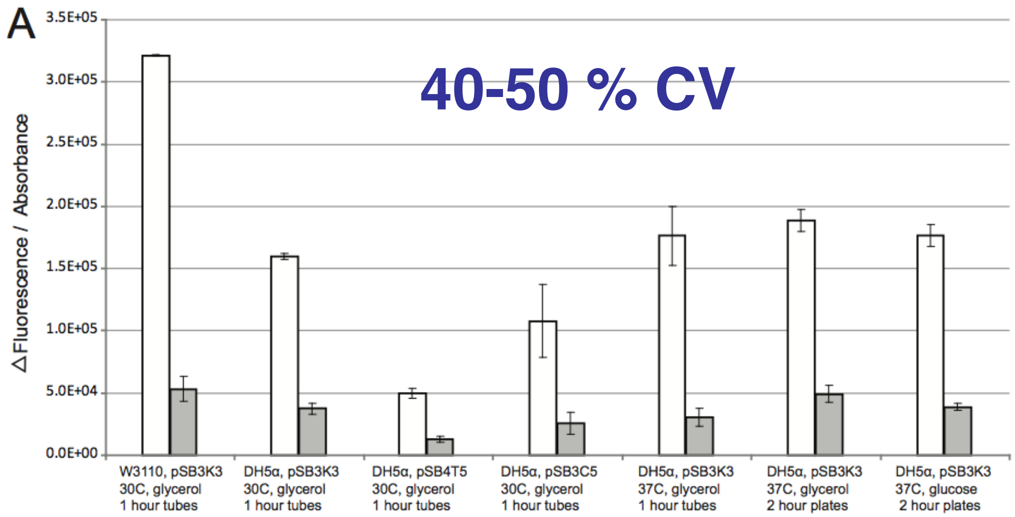
D. Endy, *Nature* **438**:449 (2005).

Assembly standard for plasmids



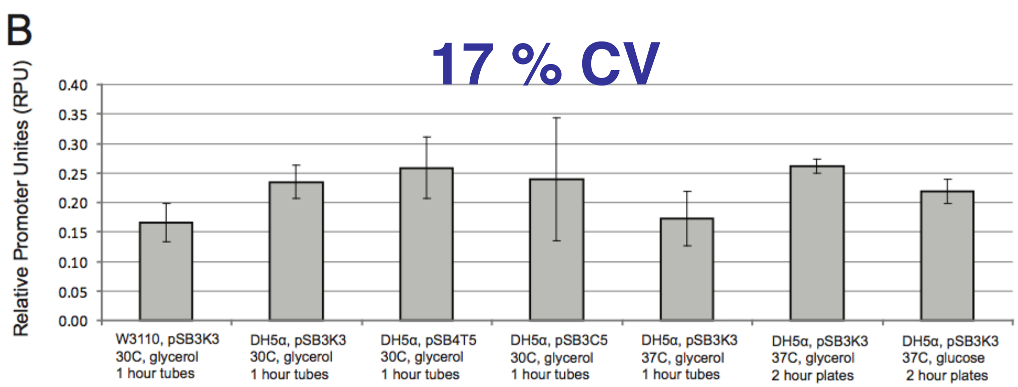
Development: T.F. Knight, R.P. Shetty, D. Endy; Image: neb.com

Functional standard for promoters



Absolute promoter strength

Variation due to cell strain, equipment, media, lab, etc.



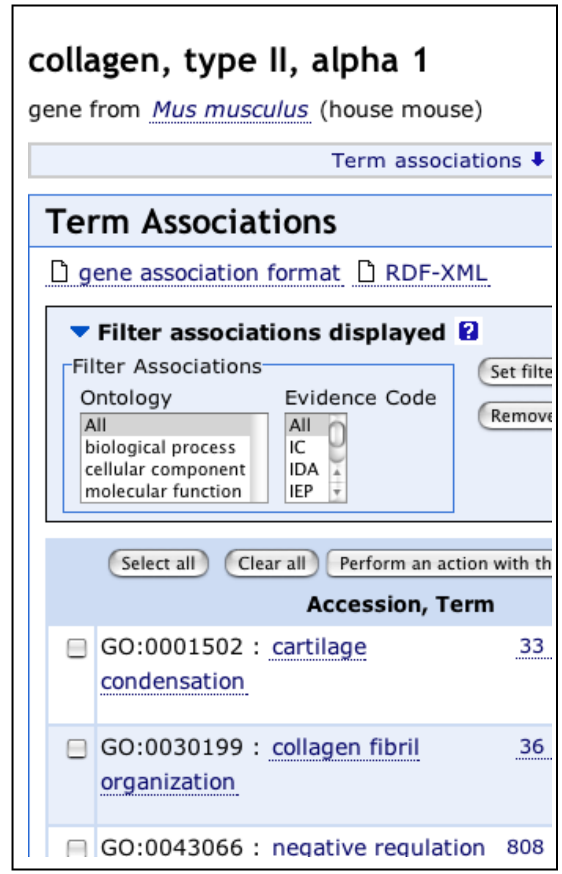
Relative promoter strength

Variation reduced 2-fold.

J.R Kelly et al., *J Biol Eng* **3**:4 (2009)

Data standards: what and why?

- Brooksbank & Quackenbush, *OMICS*, **10**:94 (2006)
- High-throughput methods are data-rich
- Standards for **collection** and/or **sharing**
- To be continued…

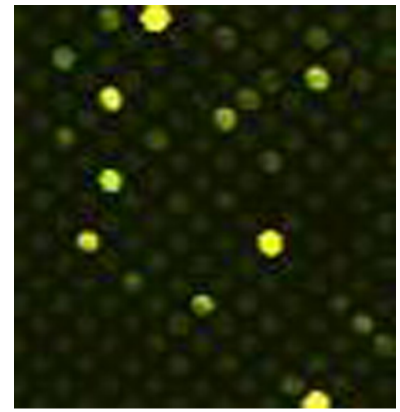


www.geneontology.org

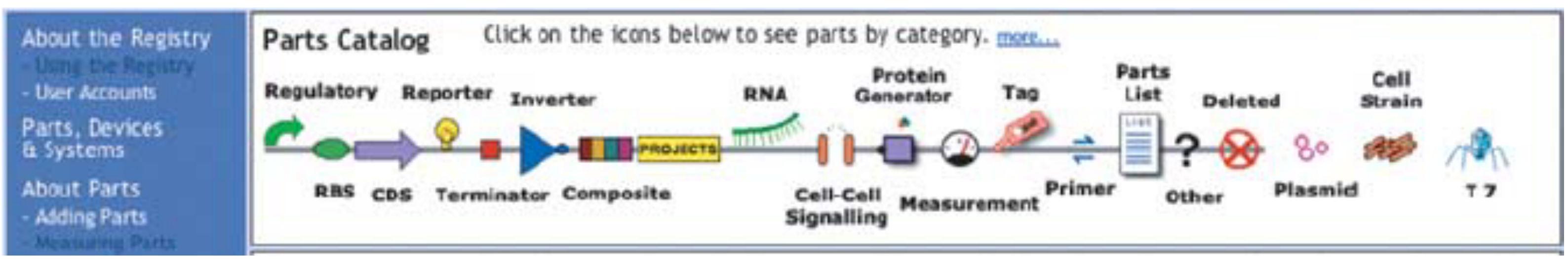
Lecture 3: conclusions

- Cell viability in TE constructs is affected by cell, material, and soluble factors.
- Standardizing data sharing and collection is of interest in several BE disciplines.

Microarray data



From D. Endy, *Nature* **438**:449 (standardized biological "parts")



Next time: TE-specific lecture and *discussion* of standards.