# Overview of Module 3, Part II

Module 3, Lecture 7

20.109 Spring 2008

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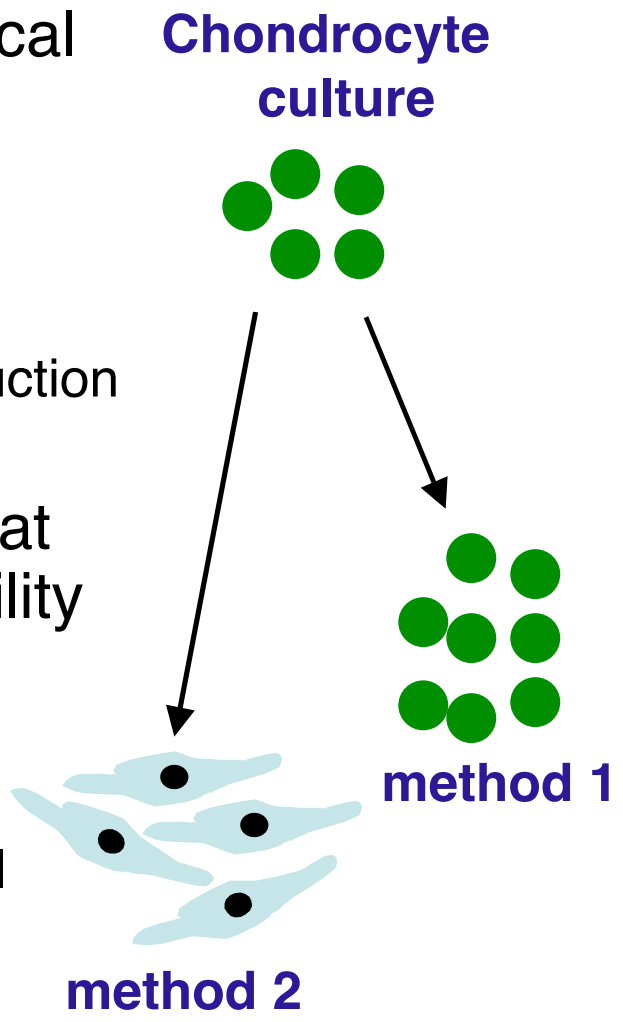
# Topics for Lecture 7

- Module 3 overview
  - what does each day contribute?
  - how could the findings be extended?
  - briefly back to cartilage TE big picture
- Intro. to drug delivery (chalkboard)
  - today if we have time, or as first part of lecture 8

# Overall goals of Module 3

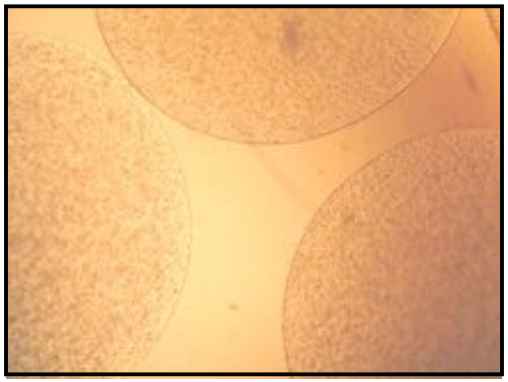
- Design experiment to study effects of local environment on cell de-differentiation
  - cell: primary chondrocytes, *in vitro* culture
  - local environment: material properties, cell density, culture medium composition
  - effects: viability, morphology, collagen production
- Grander purpose: discovering factors that maintain chondrocyte phenotype has utility for cartilage tissue engineering
  - determine properties of construct desired to regenerate cartilage
  - conditions for *ex vivo* cell expansion and cell transplantation



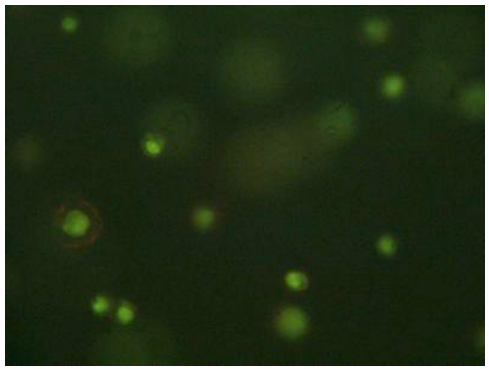
# Module overview: lab

Day 1: design

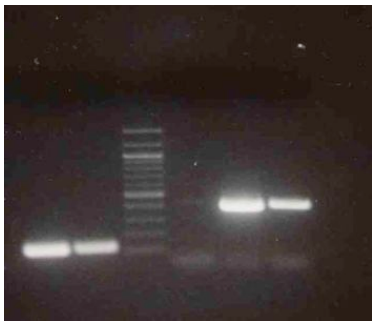
Day 2: seed cultures



Day 3: viability assay



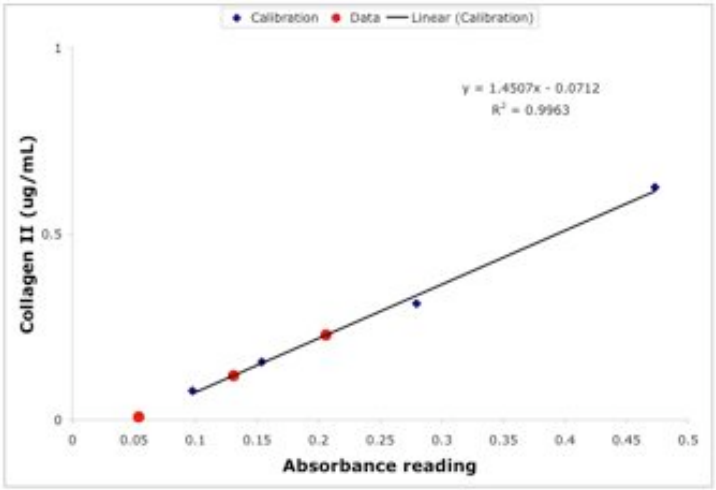
Day 4: prep RNA+cDNA



Day 5: transcript assay

Day 6: protein assay

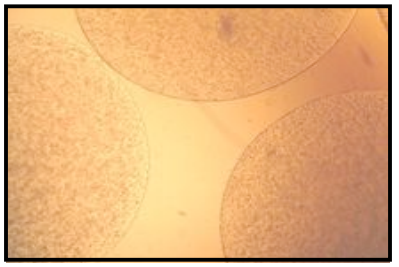
Day 7: remaining analysis



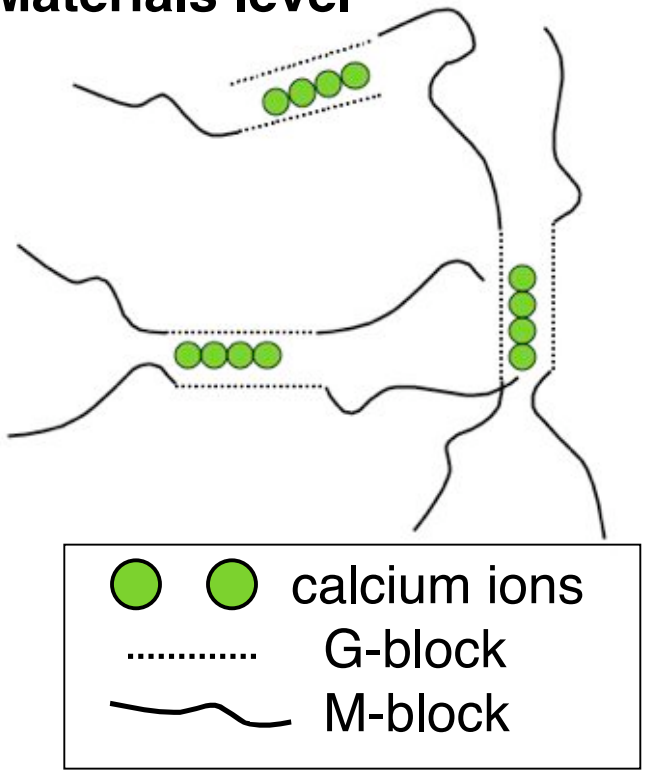

Day 8: your research ideas!

# Module progress: week 1

- Design parameters varied
  - change cell density in beads
  - architecture: 2D plastic vs. 3D alginate
  - type of alginate (viscosity, G/M ratio)
  - weight percent alginate
  - calcium cross-linker concentration
  - additives: collagen II, inhibitor of actin
  - application of compressive stress
- Some expectations
  - many conditions have tradeoff between viability and phenotype - examples?
  - optimal alginate rigidity not known a priori from literature (data comparison issue)

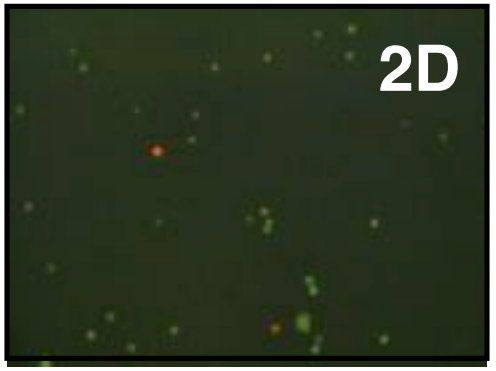


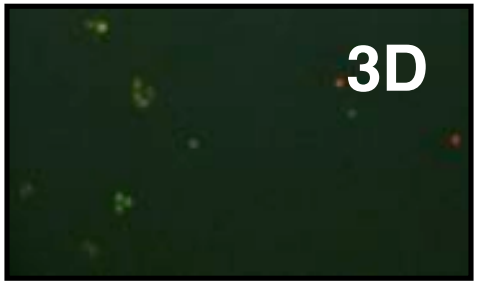
**Materials level**



# Module progress: week 2

- Morphology results
  - cells in 2D spread, cells in 3D round
  - results are consistent with our hypothesis, but morphology alone does not define cell type
- Viability/cytotoxicity results
  - mostly live cells in 2D samples
  - low cell recovery, variability in 3D samples
  - W/F Blue group: cell death in high calcium sample
  - T/R Purple+Red groups: possible cytotoxicity difference between Sigma and FMC alginate
  - potential explanations for results?



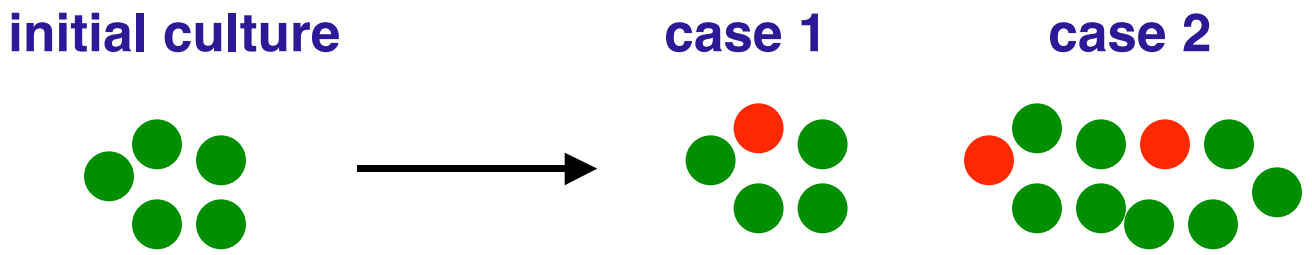




**W/F Green group**

| Alginate company | Alginate name | Viscosity | G/M Ratio |
|---|---|---|---|
| Sigma Aldrich | "low viscosity" | 250 cps at 2% | "high M" |
| FMC Biopolymer | Protanal LF 120M | 70-150 cps at 1% | ~40/60 |
| FMC Biopolymer | Protanal LF 10/60 | 20-70 cps at 1% | ~70/30 |

# Extending viability/cytotoxicity assay

- Why do we test for viability/cytotoxicity?
  - desire biocompatible TE construct
  - avoid materials toxic to cells
  - necrotic cells in turn can promote inflammation, vicious cycle
- How can we improve our assay?
  - improve recovery of cells from alginate with longer EDTA incubation and/or spin step
  - improve signal:noise with longer dye incubation
  - increase cell concentration, thus statistical reliability of data
- How can we learn more?
  - test for type of toxicity (apoptosis vs. necrosis), proliferation



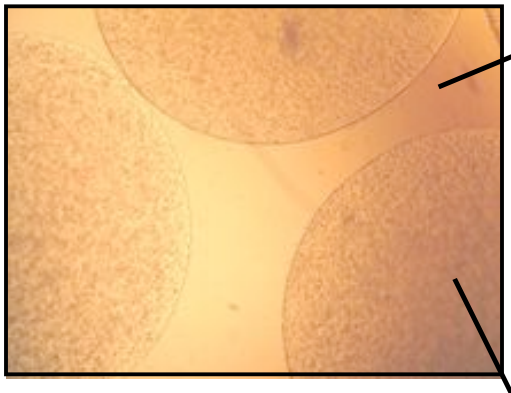
# Utility of modeling+simulations

- Model: mathematical description of physical phenomenon
  - e.g., relation between concentration of a diffusing species, its diffusivity, and distance traveled (macroscopic)
  - perform curve-fitting with real data to calculate parameters (Mod2)
- Simulations: apply models to compute "experiments"
  - e.g., follow the path of individual diffusing particles
  - often link a microscopic known and macroscopic unknown
- Example of TE relevance
  - diffusion of oxygen/nutrients tends to have ~100 μm limit
  - dependence on material permeability and pore structure: thus, modeling may fuel design of better TE constructs

**1. design scaffolds → 2. model diffusion → 3. make best designs**

**→ 4. test scaffolds, compare to model → 5. redesign**

# Module overview: week 3



1. Collect supernatant

**Test for collagen proteins (by ELISA)**

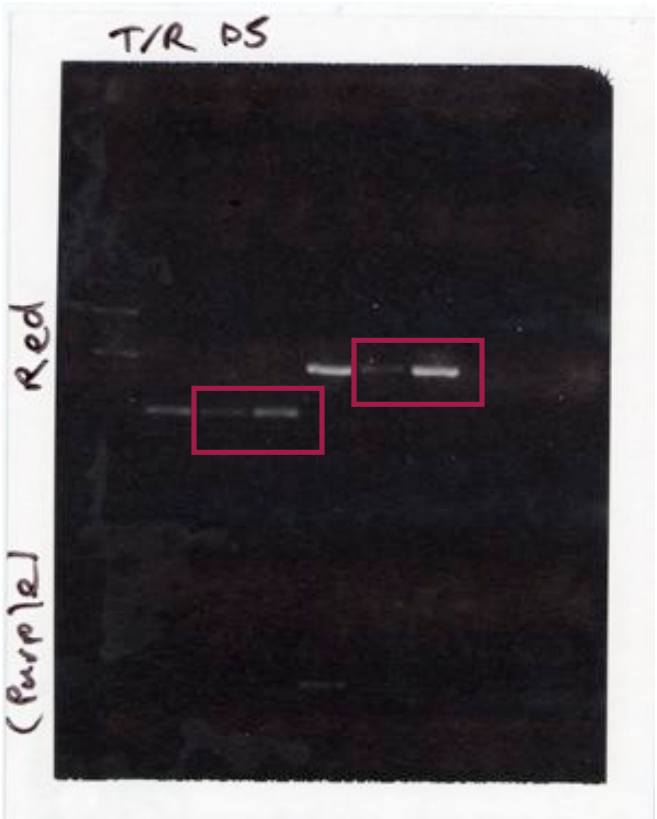
2. Collect and lyse cells

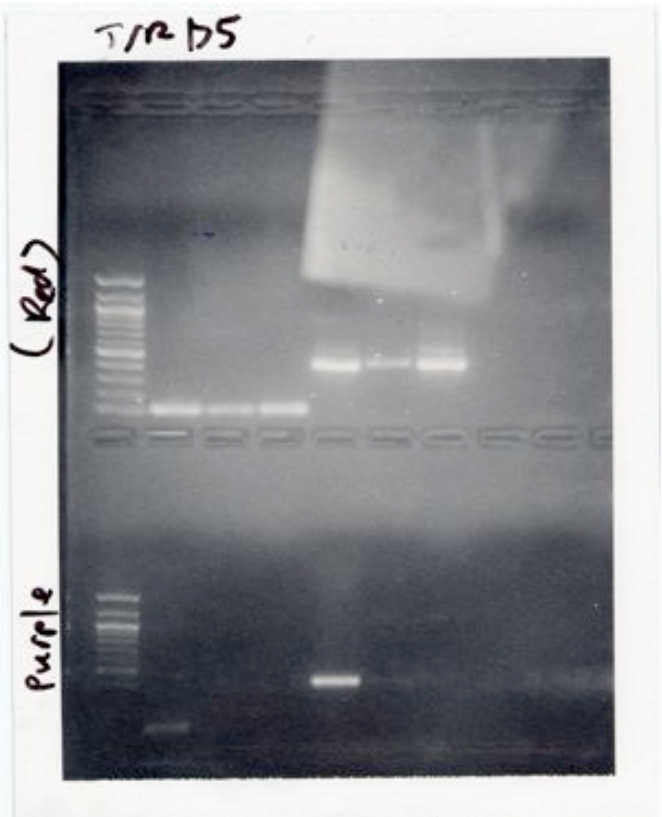
Purify mRNA from cells → Amplify collagen cDNAs →

**Compare collagen I and II transcript levels**

# Module progress: week 3

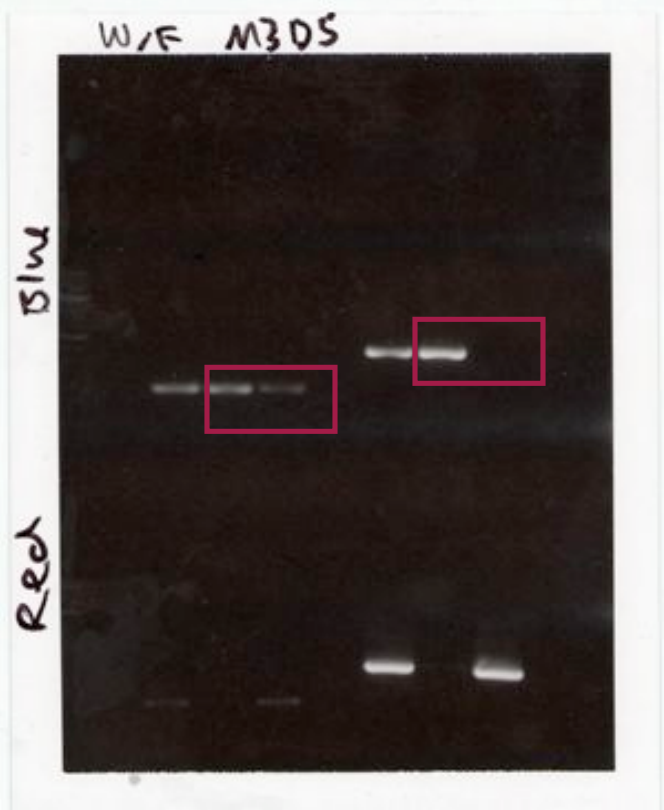
- Many collagen II:I ratios > 1, even for 2D samples
- Some bands too faint to get picture and quantify
- T/R Red group: high viscosity ratio > low viscosity II:I ratio



# Module progress: week 3

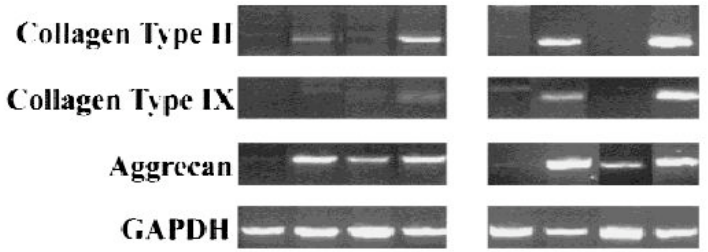
- W/F Blue group: no collagen II in high calcium sample?
- W/F Red group: 3D compression (II:I) > 3D control (II:I)
- Sources of error
  - low spec. readings
  - no re-blanking on spec.
  - protein contamination in RNA
  - no loading control to test above
- Other confounding issues
  - 2D cells not split often enough
  - cow-to-cow variation

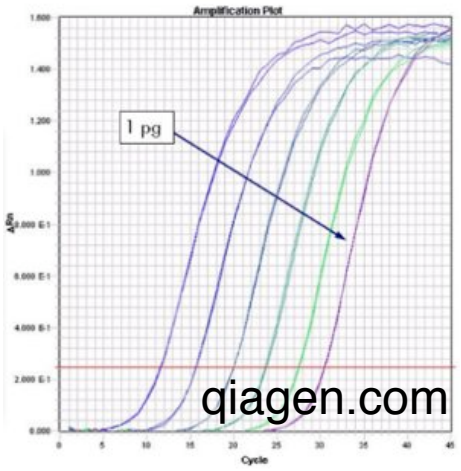


# How can we extend this assay?

- Improve RNA measurement
- Run different dilutions of DNA
  - could have time to do this on Day 6 or 7 *after* seeing first round results
  - extends dynamic range of assay
- Co-amplification of internal control
  - housekeeping gene should not change expression based on culture method
  - thus serves as loading control
- Test whether primers for I and II are equally efficient at amplification
  - G/C content and location (ends)
  - potential for primer hairpins, dimers
- Quantitive (real-time) PCR

Image from: Caterson et al., *J Biomed Mater Res* **57**:394 (2001)

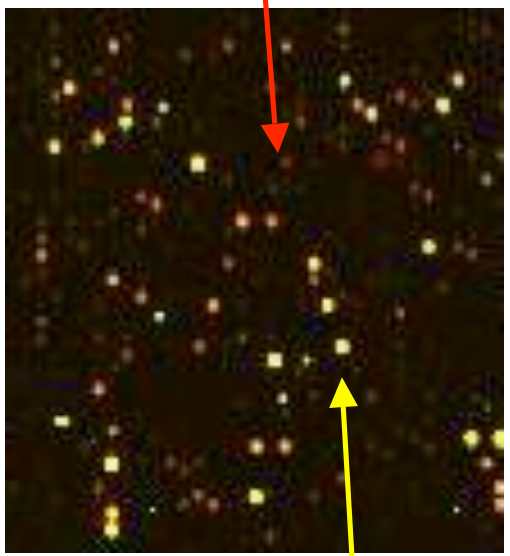


# High throughput gene expression assay: microarrays

- Isolate total RNA from cells, as we did
- Make cDNA *pool* using -TTTT- primers
  - selects all mRNAs, mixed amplification product
  - contrast PCR: specific primers to amplify one cDNA
- Distinguish two experimental conditions
  - one RNA pool gets oligo dT with to red fluorophore
  - other gets oligo dT conjugated to green fluorophore
- Hybridize cDNA pool to microarray
  - microarray contains complementary cDNA pool
  - one DNA per tiny spot
  - potentially reflects entire genome
- Red vs. green fluorescence shows *relative* gene expression between two samples

Red: Gene A expression in 1 > 2



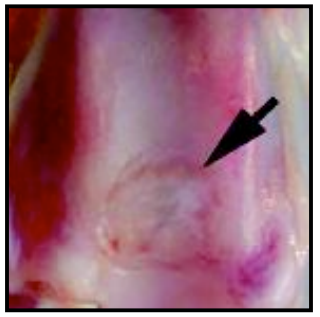
Yellow: Gene B expression equal in conditions 1 and 2

# Module progress: week 4

- ELISA results overall
  - a few folks *clearly* saw CN II presence in their 3D sample, but not in 2D sample or any supernatants
  - many folks saw *possible* CN II signal (above blank, but below lowest standard) in their samples
  - CN I assay not fully optimized: slow development, but some signal
- Main advantage of ELISA in our experiment?
  - recognizes proteins in native state (not so in typical Western)
- How could we improve results next time around?
  - optimize antibody amounts, incubation times, etc.
  - optimize sample collection: take supernatant *after* trypsinization, lyse cells for internal (vs. surface) proteins
  - perform sandwich ELISA: improved sensitivity - why?
- Overall, what might you test/do differently next time?
  - hard to change just one parameter for natural materials

# Cartilage TE: from models to therapy

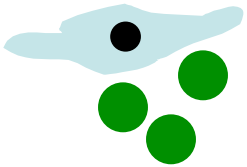
- Experiments *in vitro*
  - cell lines or primary cells in different cultures
  - assay gene expression, protein production, cytotoxicity, mechanical properties
- Experiments *in vivo*
  - small and large animal models
  - animal can undergo new therapy (e.g., scaffold-cytokine combo) and compare to standard surgery
  - more realistic toxicity, tissue growth results
  - must develop implantation, retention strategies
- Clinical treatment
  - autologous chondrocyte implantation (ACI) used clinically
  - cell culture technique and treatment marketed as *Carticel* by Genzyme [www.carticel.com]
  - meant for small defects from injury, not pervasive disease



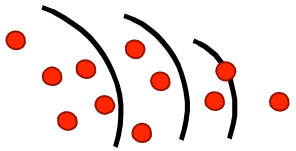
Y. Liu et al. *Tissue Eng* **12**:3405 (2006)

# Comparing TE strategies

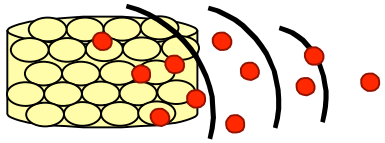
- Cell therapy alone
  - pro: no* introduction of foreign material
  - requires biopsy and time for expansion
  - requires some retention mechanism, may leak



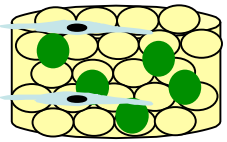
- Cytokine therapy alone
  - pro: simplicity (injection at site)
  - repeated injections, cytokine production costly, limited efficacy



- Scaffold+cytokine therapy
  - pro: slow release of cytokine
  - difficulty developing appropriate scaffold: non-toxic, degrades at correct rate, cytokine stays functional



- Scaffold+cells therapy
  - pro: cells themselves could make cytokine indefinitely



- Scaffold+cytokine+cells
  - combine advantages to address early- and late-stage needs

# Drug delivery topics to cover

- Goals of drug delivery/controlled release field
- Methods of engineering delivery timecourse
- Human practice issues raised (pros/cons)