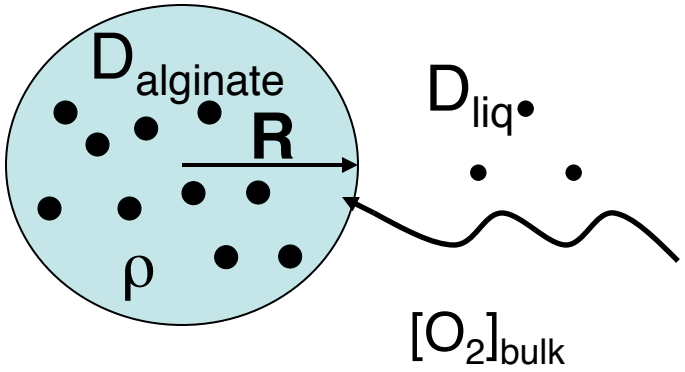
Cartilage TE: *in vitro* and *in vivo* models and assays

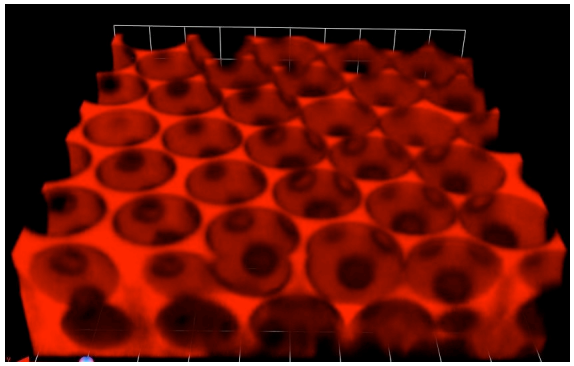
Module 3, Lecture 5

20.109 Spring 2009

Lecture 4 review

- What are the major distinguishing features of apoptosis vs. necrosis?
- How are cell viability and nutrient diffusion profiles related, studied, and potentially improved?
- What are some major features of a fluorescence microscope?

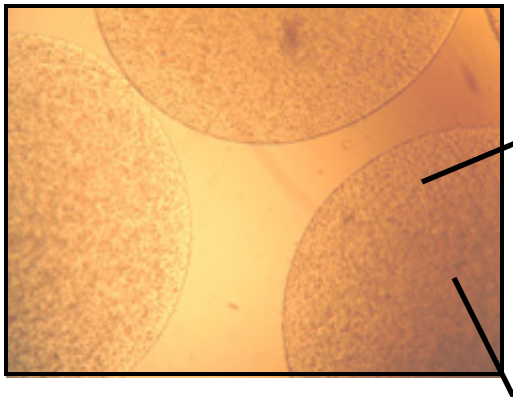




Topics for Lecture 5

- Gene and protein expression assays
- Cartilage TE *in vitro* and *in vivo*
- Clinical relevance

Module overview: 2nd half



1. Enzymatic digestion

Test for collagen proteins (by ELISA)

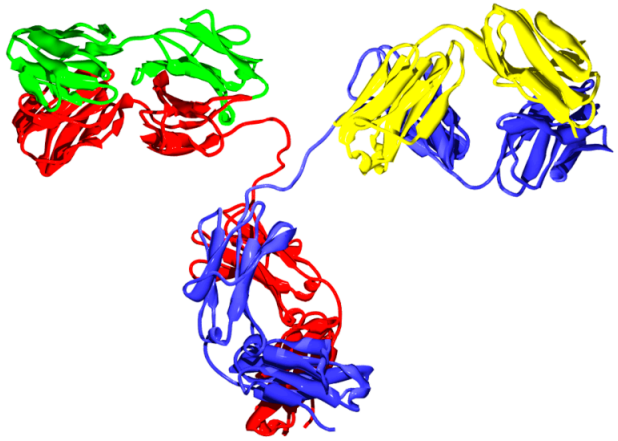
2. EDTA-citrate dissolution

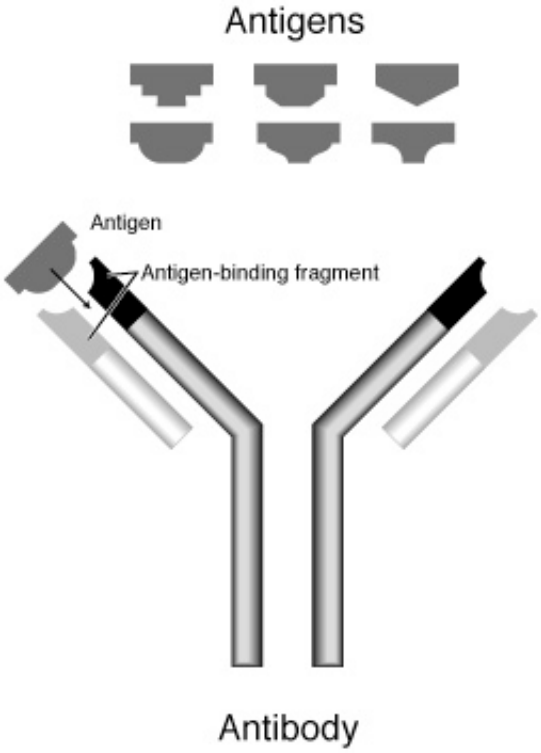
Purify mRNA from cells ⟶ Amplify collagen cDNAs ⟶

Compare collagen I and II transcript levels, normalized to GAPDH

Antibodies are specific and diverse

- Specificity
  - variable region binding, $K_D \sim$ nM
  - linear or conformational antigens
- Diversity
  - gene recombination
- Production
  - inject animal with antigen, collect blood
  - hybridomas (B cell + immortal cell)





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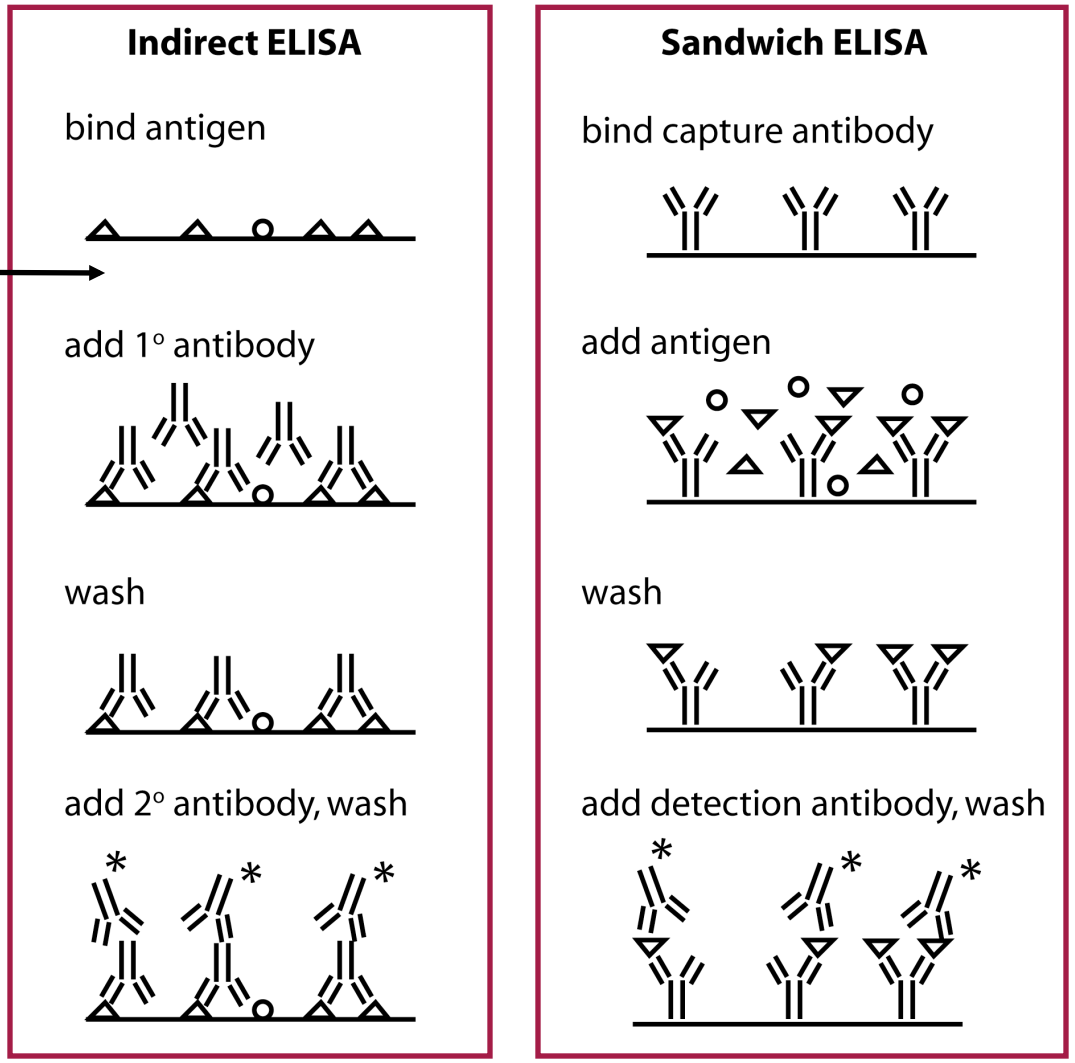
Day 5-7: protein analysis by ELISA

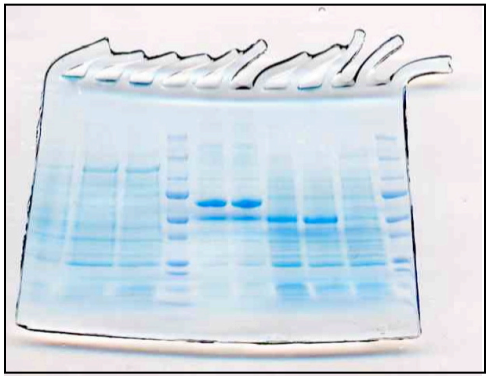
- ELISA: enzyme-linked immunosorbent assay
  - specific
  - sensitive
  - multiple kinds

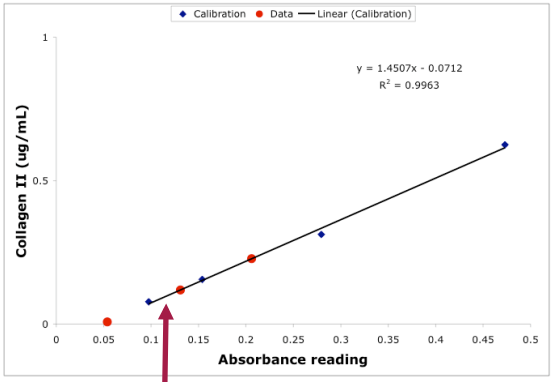
"blocking" step also needed



Common protein-level assays

- PAGE
  - simple and low cost
  - Coomassie detection limit ~ 0.3-1 ug/band (2-5 ng/band for silver staining)
  - cannot distinguish two proteins of same MW
- Western blot
  - can identify specific protein
  - detection limit ~1 pg (chemiluminescent)
  - only simple for denatured proteins
- ELISA
  - detects native state proteins
  - quantitative (standard curve)
  - high throughput



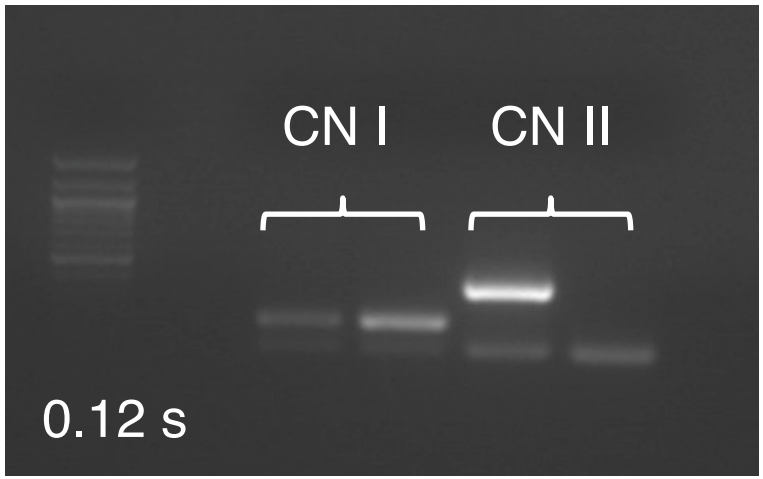


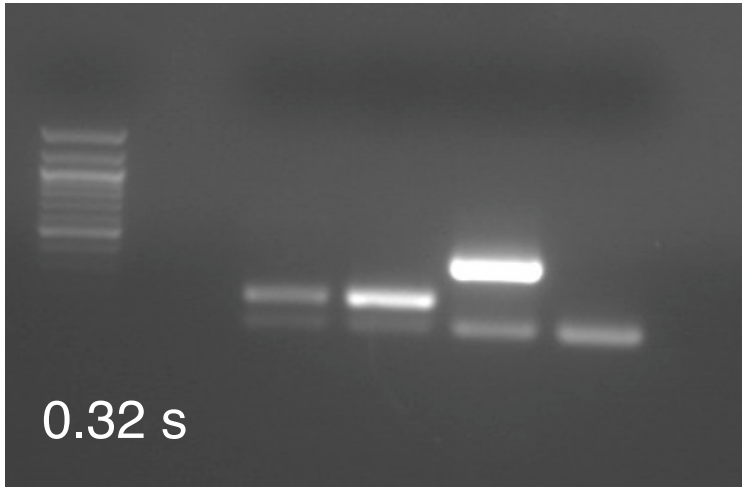
100 ng/mL protein

Current Protocols in Cell Biology, Molecular Biology

Day 4-5: transcript analysis

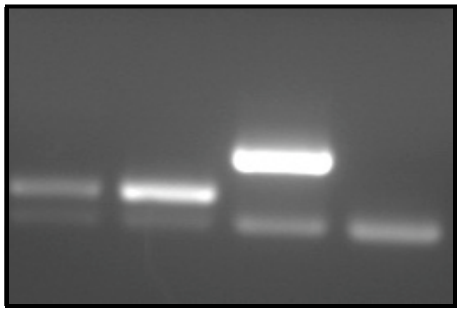
- Last time: RT-PCR
  - Collagen II + GAPDH
  - Collagen I + GAPDH
- Next: run out on a gel
- Measure band intensity/area
  - low dynamic range
  - exposure time
- Controls/references
  - GAPDH loading control
  - fresh stem cells
  - fresh chondrocytes

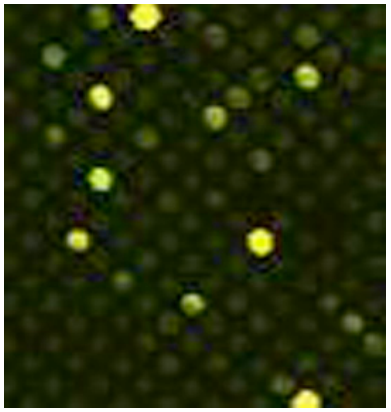




Common transcript-level assays

- RT-PCR (end-point)
  - simple, low cost
  - can be semi-quantitative
- Microarrays (end-point)
  - requires specialty equipment, high cost
  - complicated and fraught analysis
  - high throughput
- q-PCR (real-time)
  - some special equipment, medium cost
  - highly quantitative
  - potential for multiplexing
  - usually requires optimization (primers)



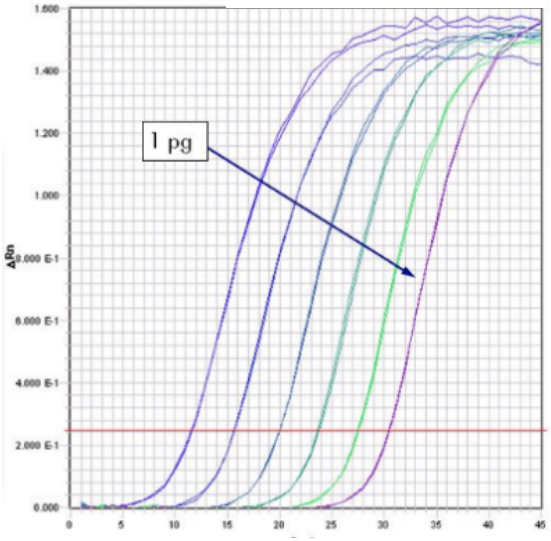


Current Protocols in Cell Biology, Molecular Biology

Introduction to qPCR

- Real-time tracking of DNA production
- Uses probes that fluoresce
  - when bind to any DNA
  - when bind to specific DNA (FRET)
- Why does PCR plateau?
- Several analysis methods
  - relative standard curve: fold-change of a transcript (normalized)
  - efficiency-correction: compare genes
  - absolute levels by radiolabeling

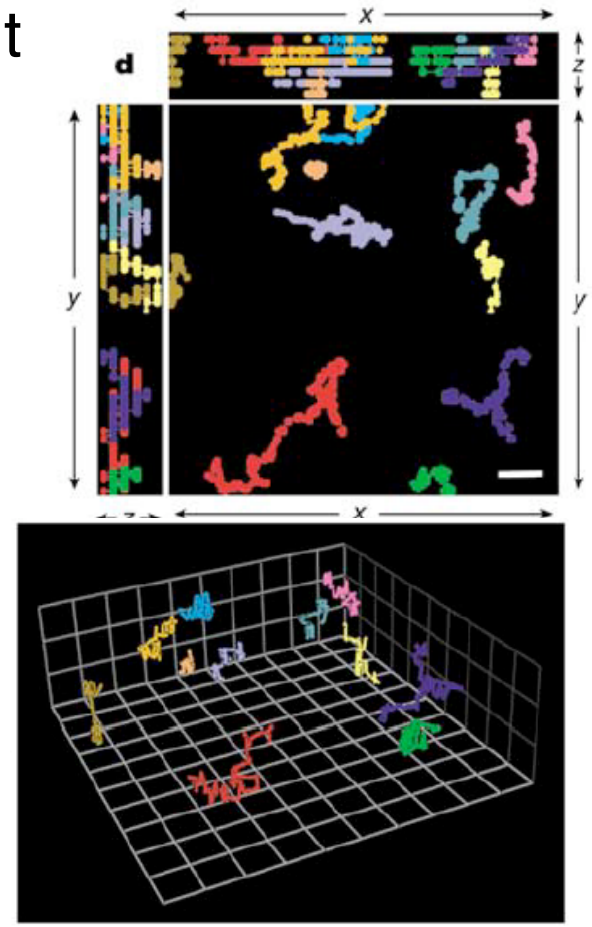
qiagen.com



Current Protocols in Cell Biology, Molecular Biology

Day 5-6: image analysis

- Imaging data is often high throughput
  - potentially 4D: time-lapse, *xyz*
  - require computation, as well as
  - human design and interpretation
- Many available analysis packages
  - specialty packages may run $20-30K
  - NIH ImageJ freely available
- Your analyses
  - relative intensities of cDNA bands
  - automated cell counting
  - optionally explore other features



Images from: T.R. Mempel, et al. *Nature* **427**:154 (2004)

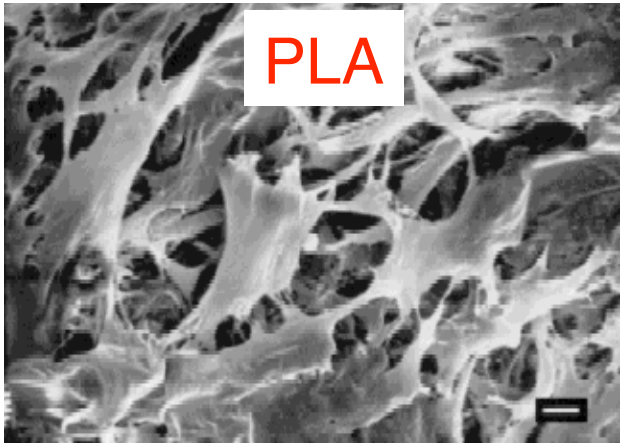
Interlude:

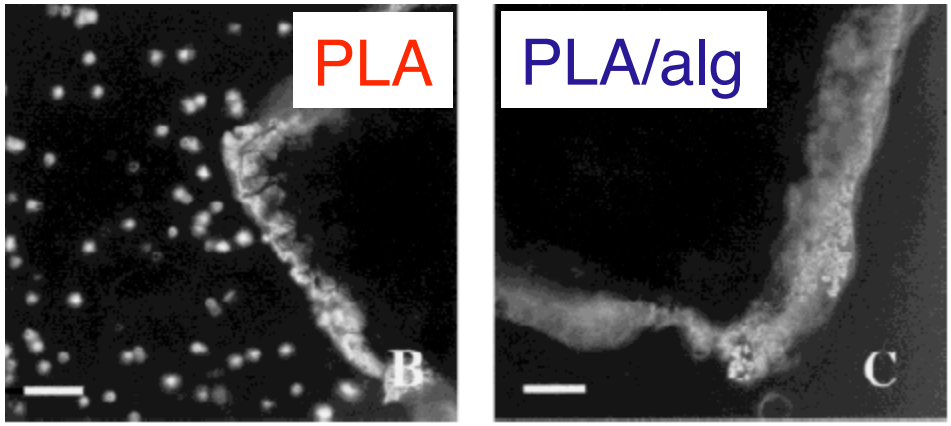
1. Stand/stretch a minute

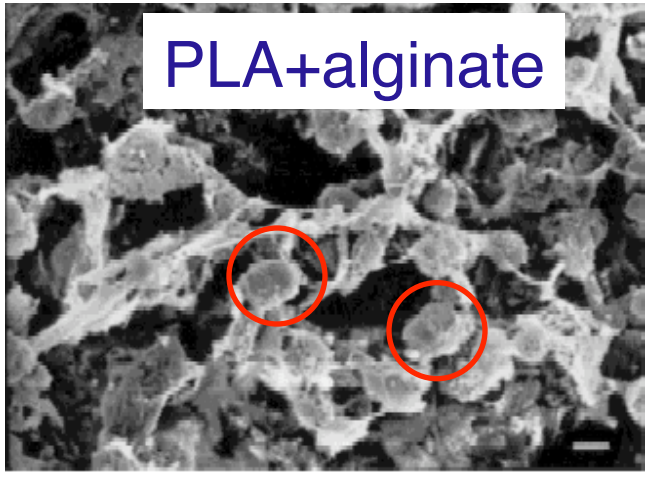
2. What TE topics would you like to hear more about (list on board)?

Polymer composite for cartilage TE

- Porous PLA scaffold + stem cells
- Cells loaded in medium
  - elongated morphology
- Cells loaded in alginate
  - round morphology
  - improved cell retention



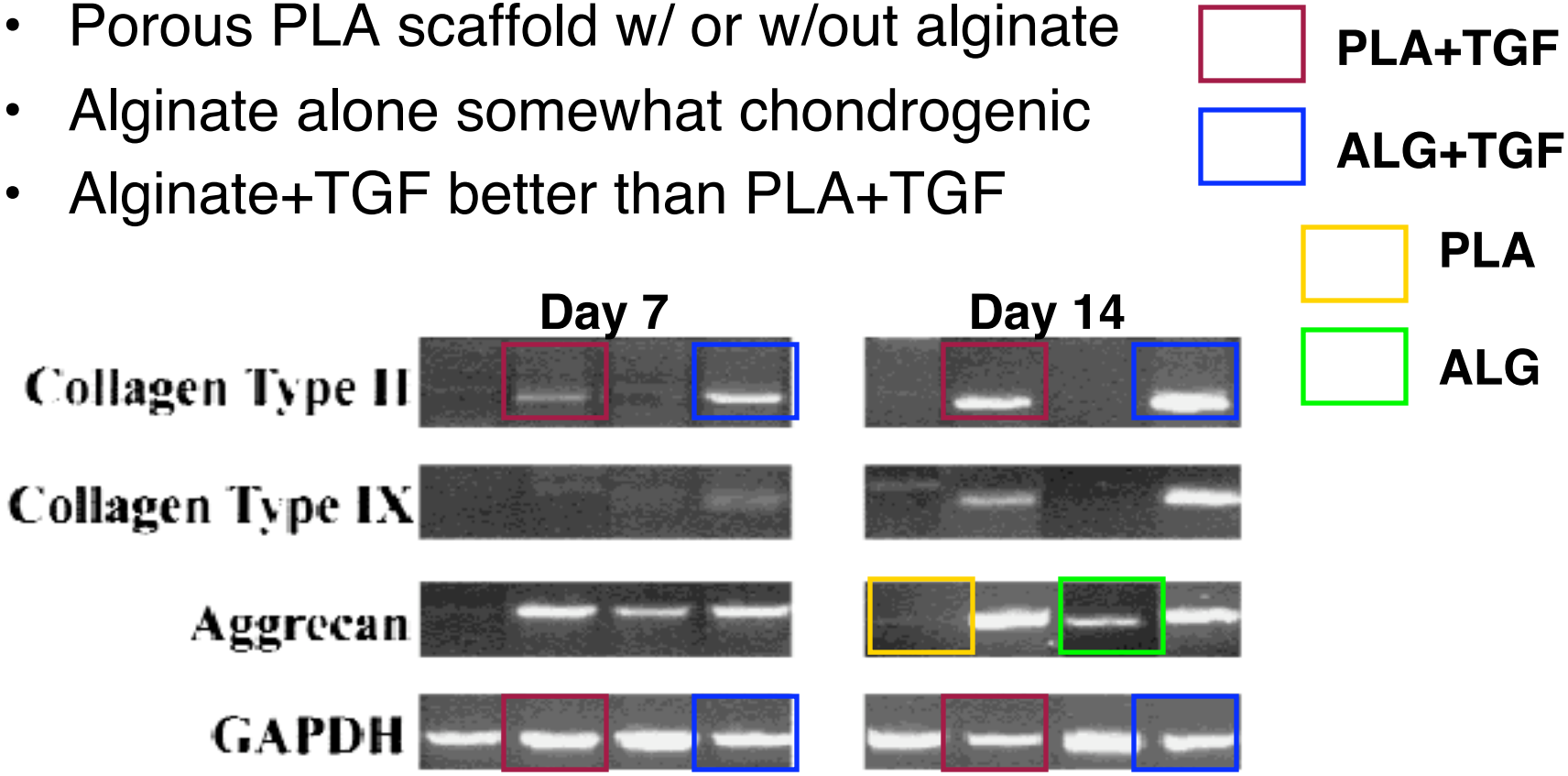




Caterson et al., *J Biomed Mater Res* **57**:394 (2001)

Chondrogenesis *in vitro*

- Porous PLA scaffold w/ or w/out alginate
- Alginate alone somewhat chondrogenic
- Alginate+TGF better than PLA+TGF



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

Scaffold-free *in vitro* cartilage TE

- Method: rotational culture of rabbit chondrocytes with no cytokines
- Results
  - Mostly dynamic culture optimal: less apoptosis, more rigid disc
  - Newly synthesized extracellular matrix: primarily CN II and PG
  - Organized architecture, similar to *in vivo*
- A scaffold-free method is inherently biocompatible
  - Any disadvantages?
  - Pros/cons of *cell*-free methods?

T. Nagai et al., *Tissue Eng* **14** (2008)

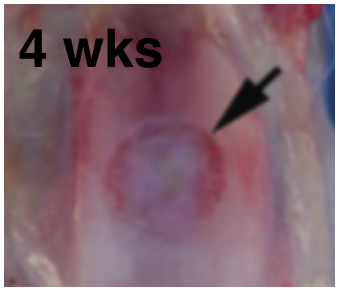
Static

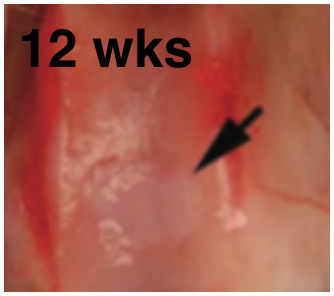
Dynamic, 3 d

Dynamic, 3 w

Cells and scaffolds *in vivo*

- Y. Liu et al. *Tissue Eng* **12**:3405 (2006)
- Stem cells and/or injectable natural matrix (gelatin/HA) in rabbit knee defects
- Matrix and cells both contributed; synergy

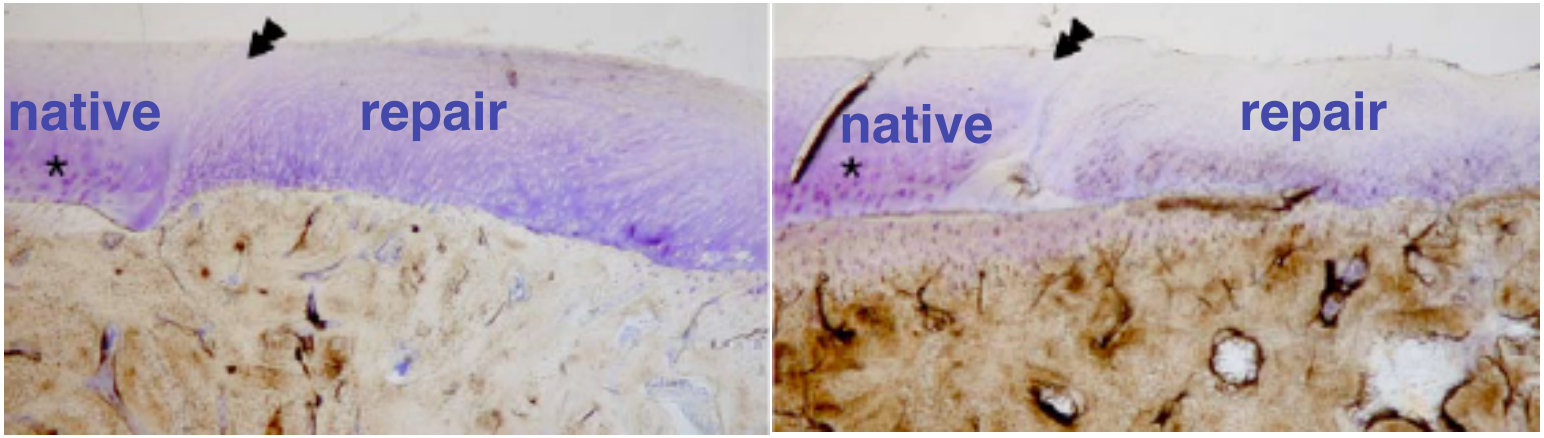




		Grade (Points)				
Group	*Interval Until Animals Were Sacrificed (Wks)*	*Restoration of Osteochondral Architecture*	*Repair Tissue Integration*	*Cellular Morphology*	*Matrix Staining*	*Total Score*
Untreated	4	0.13	0.25	0.00	0.00	1.88
	8	0.63	0.50	0.38	0.13	4.59
	12	1.00	1.13	0.13	0.25	5.63
MSCs only	4	0.63	0.25	0.38	0.00	3.39
	8	1.50	1.50	0.38	0.25	8.01
	12	2.13	1.25	1.25	2.13	11.64
sECM only	4	3.00	0.50	1.13	0.88	10.89
	8	3.25	0.50	1.25	2.13	12.76
	12	3.75	2.75	1.38	2.75	17.13
MSCs + sECM	4	3.25	1.50	2.00	2.38	15.38
	8	3.50	2.25	3.63	2.63	18.64
	12	4.00	3.00	4.38	3.00	21.38

Large animal *in vivo* model

- D. Barnewitz et al. *Biomaterials* **27**:2882 (2006)
- Biodegradable scaffold with autologous cells
- Examined horses and dissected joints after 6-12 months
- Matrix synthesis, implant integration with native tissue
- Why use a large animal model (vs. small)?



Advantages of working *in vivo*

- Ability to mimic human disease-state
- Ability to mimic therapy/surgery applied to humans
  - especially true for large animal models
- Can compare results to “gold standard” treatment
- The construct interfaces with an actual wound, the immune system, etc. - more realistic environment
- Toxicity studies more meaningful

Cartilage pathology

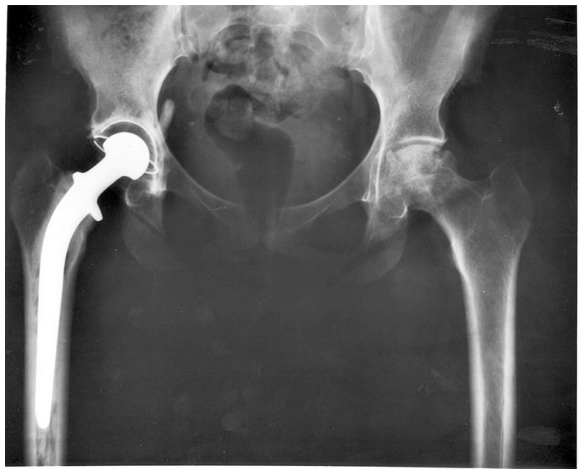
- Cartilage has little regeneration capacity
- Early damage can promote later disease
- Osteoarthritis pathology
  - PG and collagen loss, PG size ↓
  - ↑ water content, ↓ strength
  - chondrocyte death
- Symptoms
  - loss of mobility
  - pain

Image from OPML at MIT: *http://web.mit.edu/cortiz/www/AFMGallery/AFMGallery.html*.

V.C. Mow, A. Ratcliffe, and S.LY. Woo, eds. *Biomechanics of Diarthrodial Joints* (Vol. I) Springer-Verlag New York Inc. 1990

Clinical treatments

- Strategy 1: enhance/provoke healing
  - biologics: hyaluronic acid, TGF-B, etc.
  - damage bone to stimulate stem cells
- Strategy 2: replace tissue
  - cell and/or scaffold implantation
    - immature therapy
  - joint replacement
    - synthetic or donated tissue
    - invasive or fiber-optic (partial)
- Other or supplemental
  - mechanical or electrical stimulation
  - debridement (rid debris)



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S.W. O'Driscoll. *J Bone Joint Surg* **80**:1795 (1998)
S. Poitras, et al. *Arth Res Ther* **9**:R126 (2007)

Lecture 5: conclusions

- Antibodies to diverse targets (e.g., proteins) can be made and used for detection/measurement.
- Trade-offs exist (e.g., between simplicity and accuracy) for different transcript-level assays.
- Various *in vitro* and *in vivo* models have been developed for cartilage tissue engineering.

Next time: Atissa on presenting with a partner, and come ready to discuss your projects.

Lectures 7/8: special topics, review, loose ends