Cartilage TE: from *in vitro* and *in vivo* models to the clinic

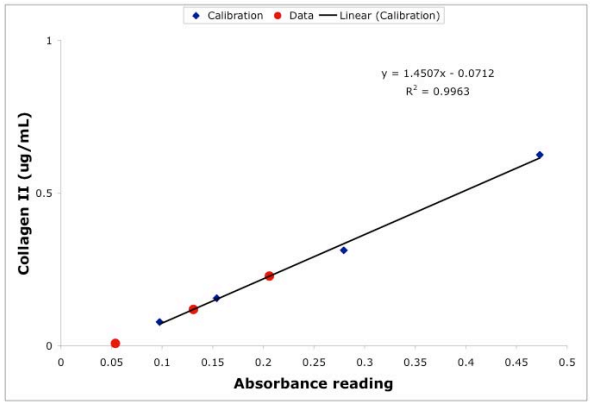
Module 3, Lecture 6

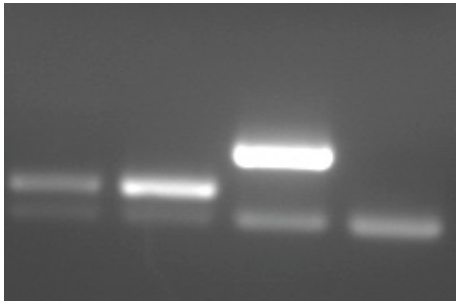
20.109 Spring 2010

Lecture 5 review

- What are some advantages of ELISA as a protein assay?

- What are some pros and cons of end-point RT-PCR as a transcript assay?



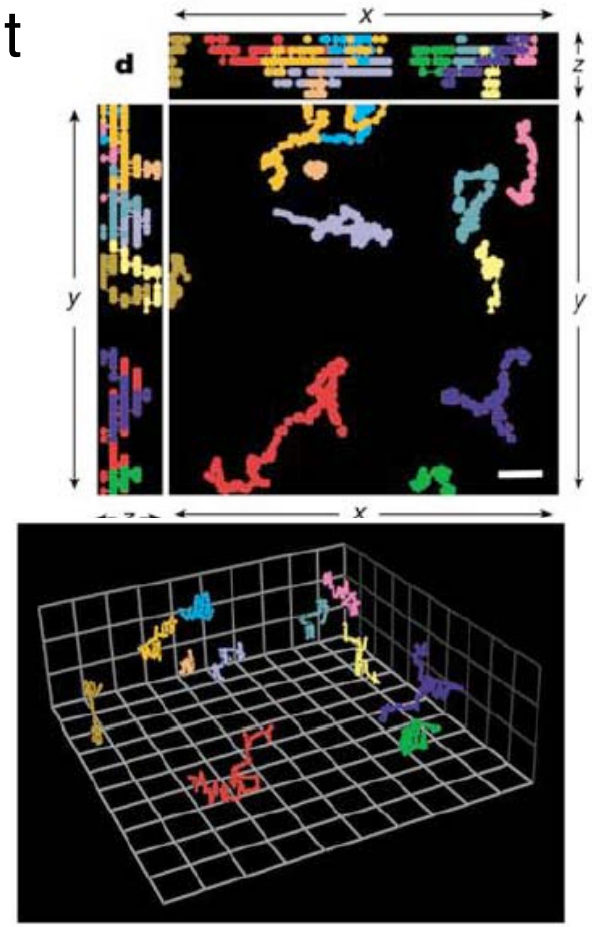


Topics for Lecture 6

- Imaging assays
- Cartilage TE *in vitro*
- Cartilage TE *in vivo*
- Cartilage TE in the clinic

Day 5-6: image analysis

- Imaging data is often high throughput
  - 4D: time, *x-y-z*
  - requires computation, *and*
  - human design/interpretation
- Many available analysis packages
  - some ~ $20-30K
  - NIH ImageJ = free
- Your analyses
  - cDNA band intensities
  - automated cell counts
  - optional: explore other features



Source: Mempel, T. R., et al. "T-cell Priming by Dendriticcells in Lymph Nodes Occurs in Three Distinct Phases." *Nature* 427 (8 January 2004): 154-159. doi:10.1038/nature02238.

Fluorescence microscopy

- 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

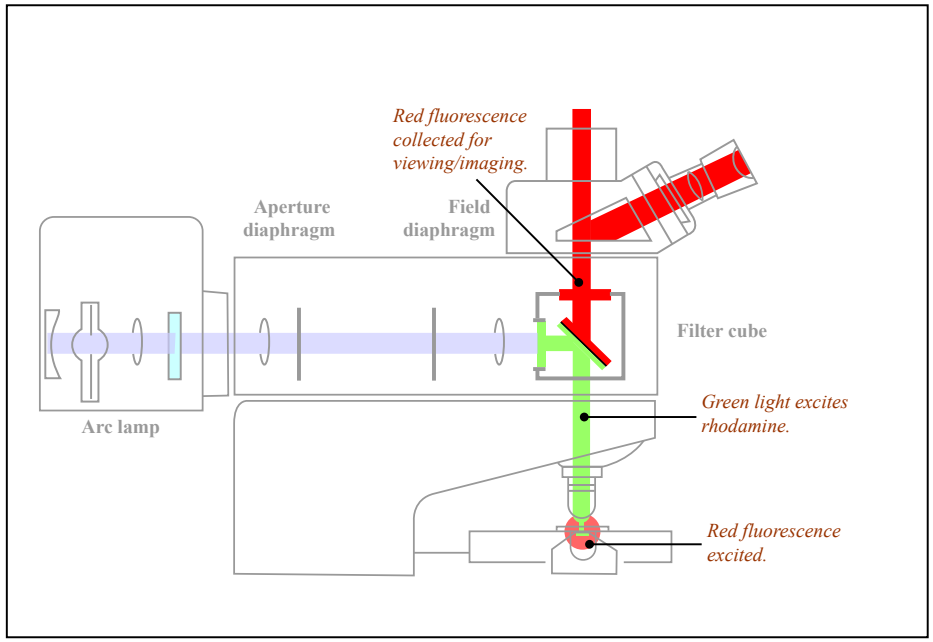


Image by MIT OpenCourseWare.

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

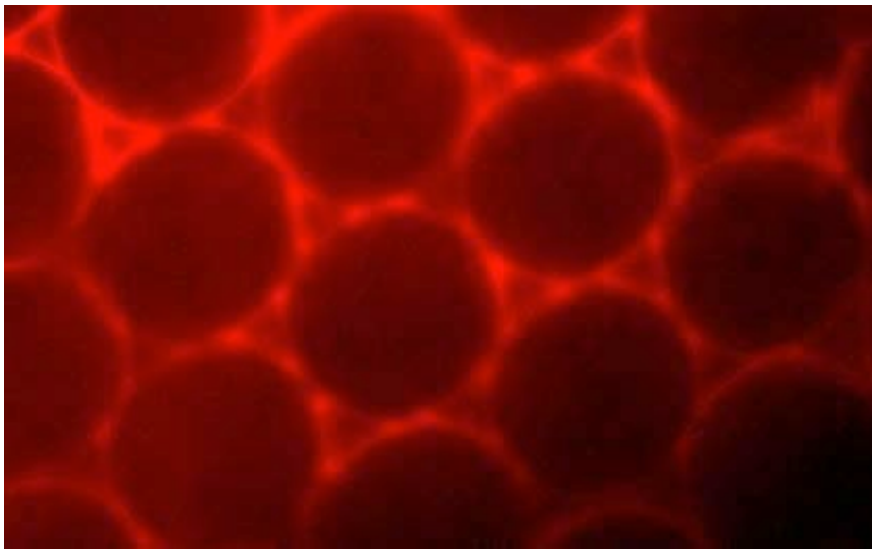
Specifications for Day 3 imaging

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

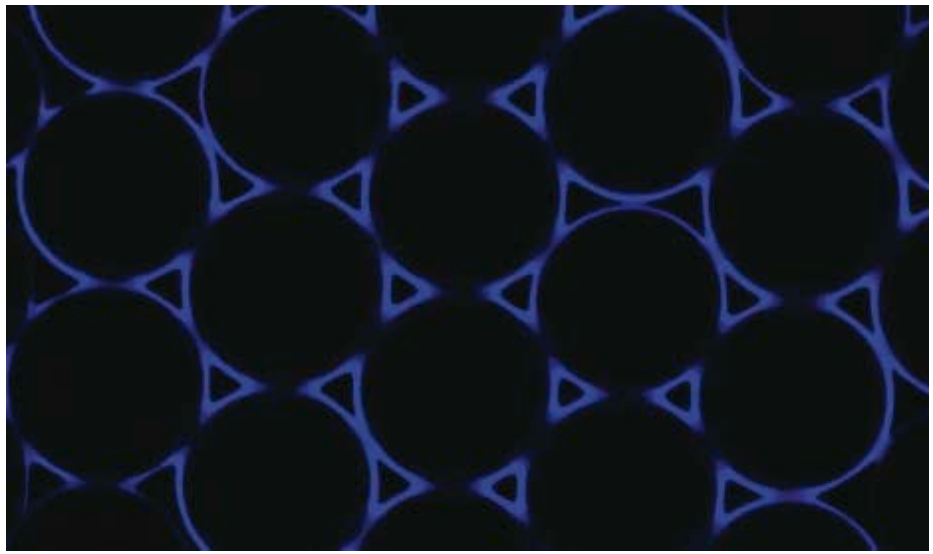
Images removed due to copyright restrictions.
1) Schematic of filter cube (optical block) - Figure 1a at
http://www.microscopyu.com/articles/fluorescence/filtercubes/filterindex.html
2) Spectrum profile graph for Nikon B-2A (Medium Band Blue Excitation) at
http://www.microscopyu.com/articles/fluorescence/filtercubes/blue/b2a/b2aindex.html

Types of microscopy

- Epifluorescence: noisy due to out-of-plane light
- Confocal: pinhole rids out-of-plane light
- 2-photon: femtoliter volume excited (in-plane)

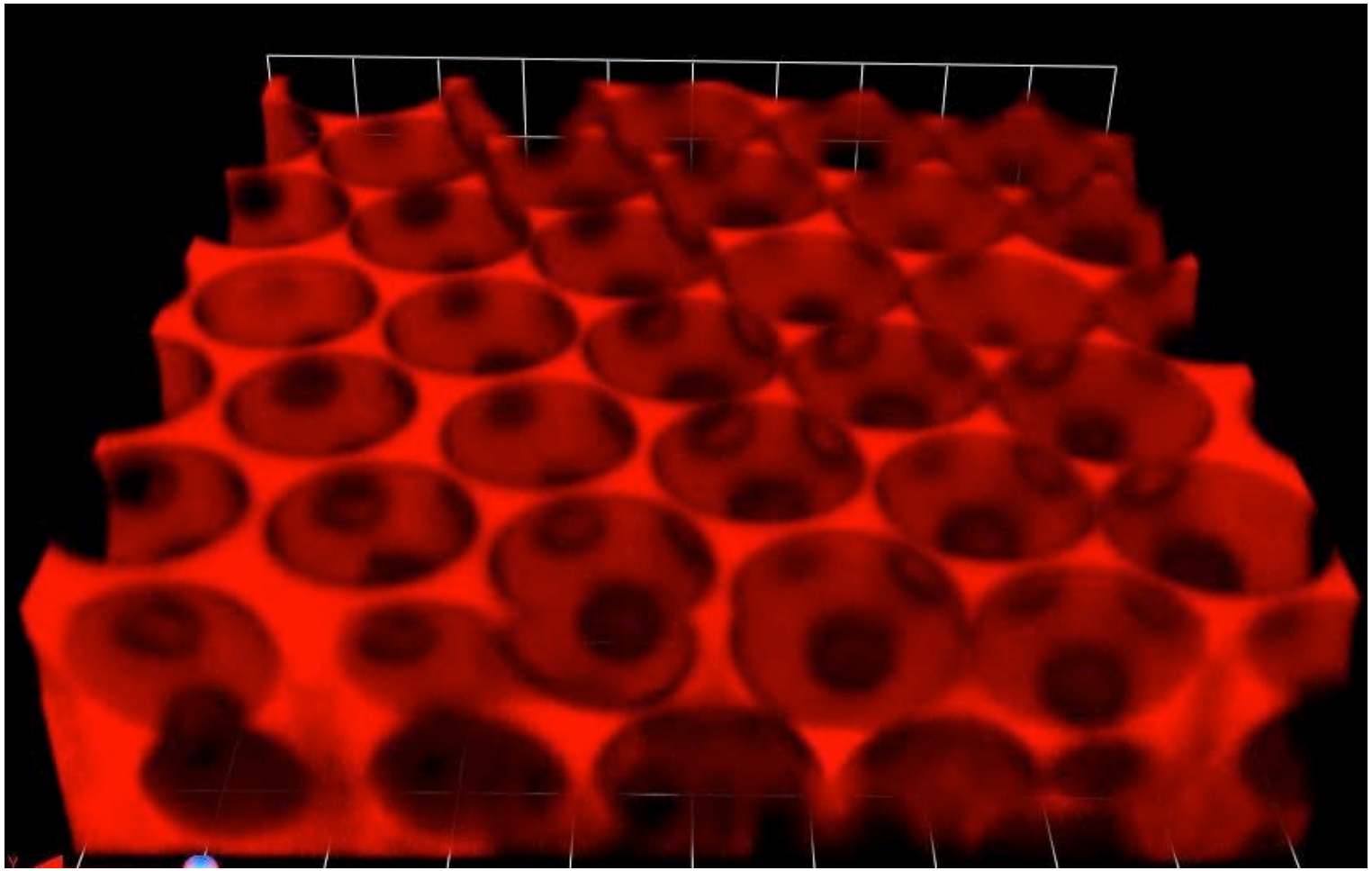


Epifluorescence



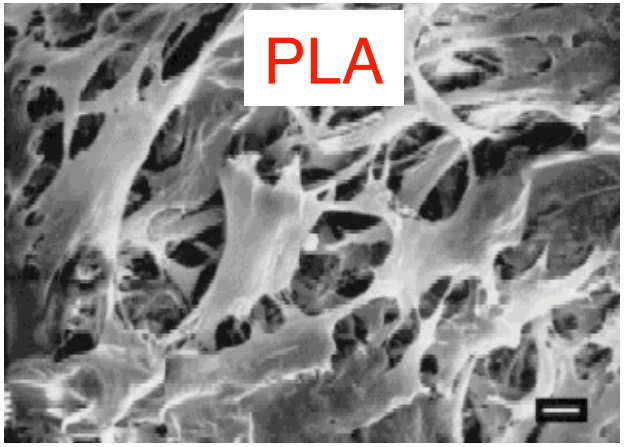
Confocal

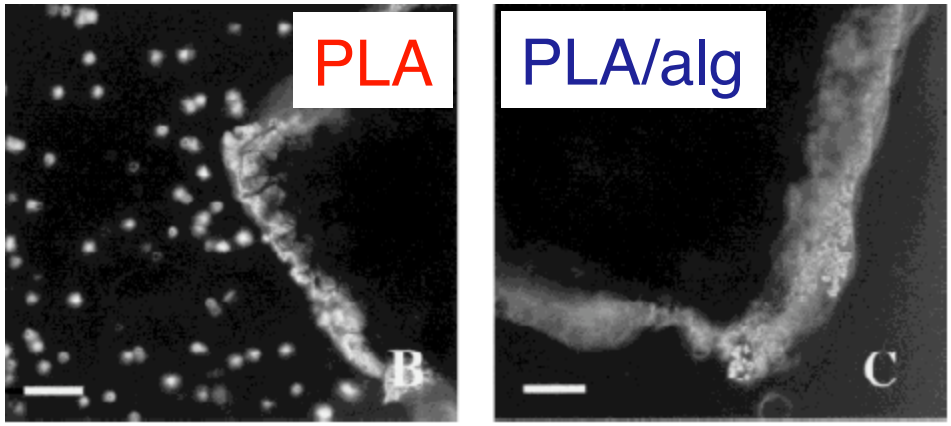
Confocal uscopy permits 3D reconstruction

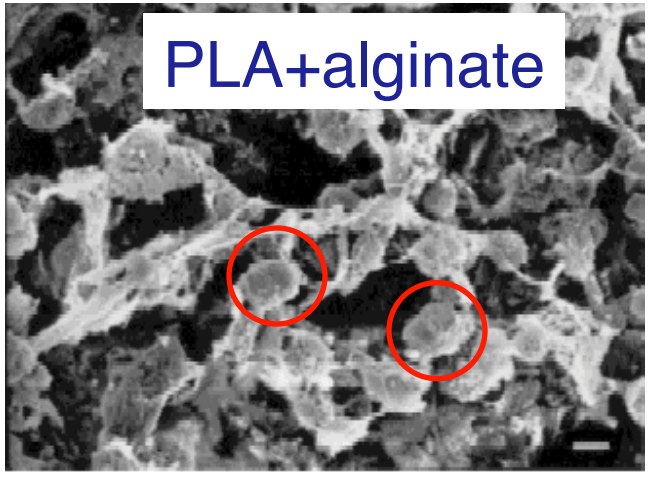


Polymer composite for cartilage TE

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



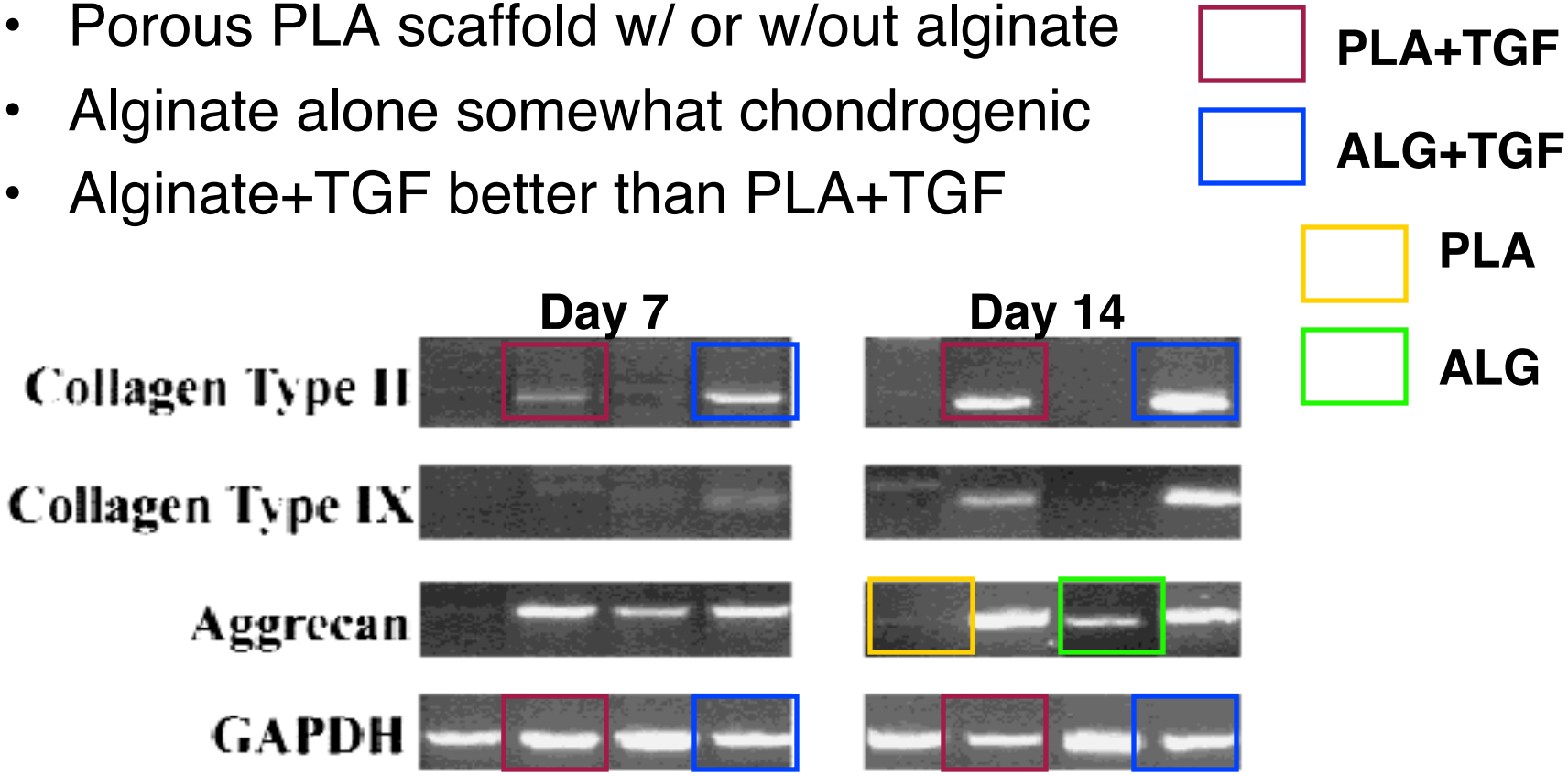




Caterson et al., *J Biomed Mater Res* **57**:394 (2001)

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 gave best results: low apoptosis, very rigid disc
  - Fresh ECM made: 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

Source: Nagai, T., et al. "Characteristics of a Scaffold-Free Articular Chondrocyte Plate Grown in Rotational Culture." *Tissue Engineering Part A* 14, no. 7 (July 2008): 1183-1193.

Interlude:

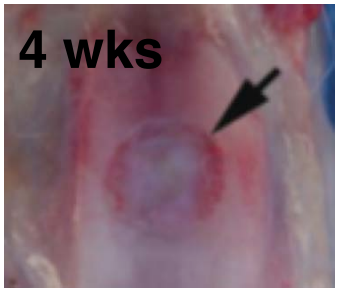
What TE topics would you like to hear more about (list on board)...?

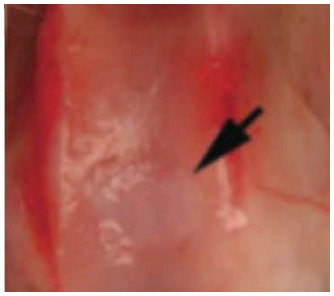
tree kangaroo: cutest animal ever? scientific proof!?

Photo of young tree kangaroo removed due to copyright restrictions.

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



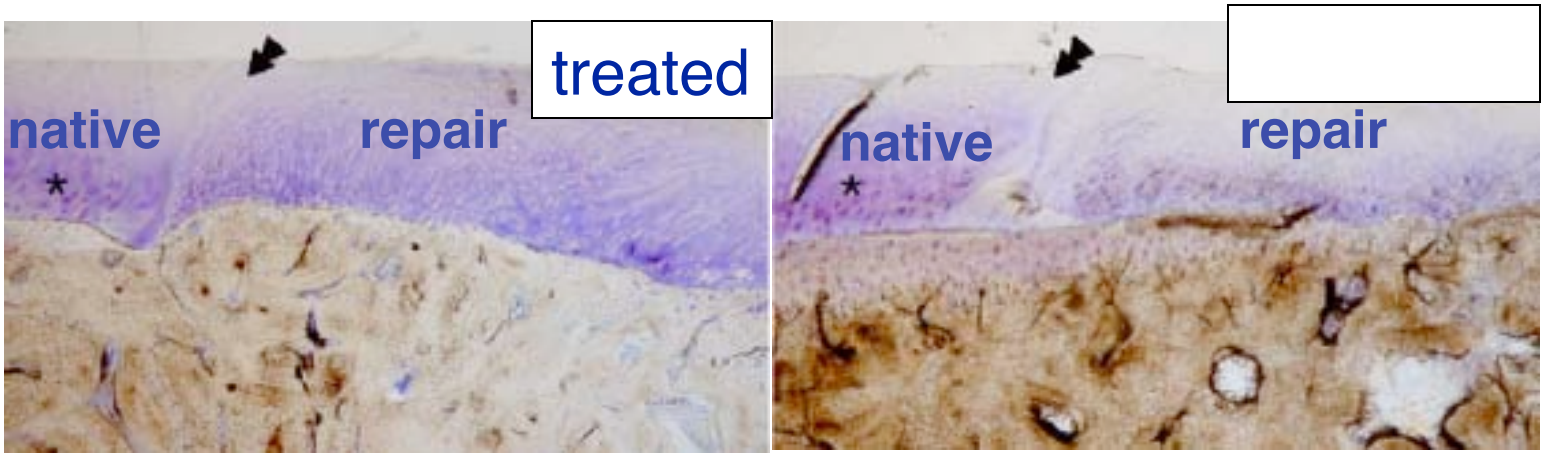


		Grade (Points)				
Group	*Interval Until Animals Were Sacrificed (Wks)*	*Restoration of Osteochondral Architecture*	*Repair Tissue Integration*	*Cellular Morphology*	*Matrix Staining*	*Total Score*
Untreated	4	0.13	0.25	0.00	0.00	1.88
	8	0.63	0.50	0.38	0.13	4.59
	12	1.00	1.13	0.13	0.25	5.63
MSCs only	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

Source: Liu, Y., et al. "Osteochondral Defect Repair with Autologous Bone Marrow-Derived Mesenchymal Stem Cells in an Injectable, in Situ, Cross-Linked Synthetic Extracellular Matrix." *Tissue Engineering* 12, no. 12 (December 2006): 3405-3416. doi:10.1089/ten.2006.12.3405.

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 – why?
- Early damage can promote later disease
- Osteoarthritis pathology
  - PG and collagen loss, PG size ↓
  - ↑ water content, ↓ strength
  - chondrocyte death
- Symptoms
  - loss of mobility
  - pain

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

Treatments for cartilage damage

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

Public domain image
(Wikimedia commons)

S.W. O'Driscoll. *J Bone Joint Surg* **80**:1795 (1998)
S. Poitras, et al. *Arth Res Ther* **9**:R126 (2007)
C.M. Revell & K. A. Athanasiou. *Tissue Eng Pt B-Rev* **15**:1 (2009)

Cutting edge of treatment

- Cell-based therapies on the market (e.g., Carticel)
- Scaffold-based approaches in trials (e.g., NeoCart, INSTRUCT)

Figure 21: **Injecting Carticel under periosteal patch**

2. Tissue Production
Cells grow on a patented 3D matrix in a tissue engineering processor under conditions that simulate those in the body. >

3. NeoCart Implant
NeoCart has the characteristics of native articular cartilage.
<

Many clinical trials are ongoing

Found 137 studies with search of: cartilage

Hide studies that are not seeking new volunteers.

Rank	Status	Study
1	Suspended	Cartilage Autograft Implantation System (CAIS) for the Repair of Knee Cartilage Through Cartilage Regeneration Conditions: Articular Cartilage Injury; Osteochondritis Dissecans Intervention: Device: Cartilage Autograft Implantation System
2	Recruiting	AS902330 in Cartilage Injury Repair (CIR) Condition: Isolated Cartilage Injury of the Knee Interventions: Drug: AS902330; Other: Placebo
3	Completed	The Objectives of the Cartilage Repair Registry is to Report Long Term Efficacy and Safety of Cartilage Repair Procedures in Registry Patients. Conditions: Articular Cartilage; Cartilage Diseases Intervention: Biological: Carticel (autologous cultured chondrocyte) implantation
4	Recruiting	Study to Compare the Efficacy and Safety of Cartistem® and Microfracture in Patients With Knee Articular Cartilage Injury or Defect Conditions: Cartilage Injury; Osteoarthritis Interventions: Biological: Cartistem; Procedure: Microfracture treatment
5	Completed	Effects of CHONDRON (Autologous Chondrocytes) With Ankle Cartilage Defect Condition: Articular Cartilage Defects of Ankle Joint Intervention: Procedure: autologous cartilage Implantation
6	Recruiting	Evaluation of the CR Plug (Allograft) for the Treatment of a Cartilage Injury in the Knee. Condition: Knee Injury Intervention: Procedure: cartilage repair with allograft plug
7	Recruiting	Autologous Transplantation of Mesenchymal Stem Cells (MSCs) and Scaffold in Full-thickness Articular Cartilage Conditions: Knee Cartilage Defects; Osteoarthritis Intervention: Biological: Bone marrow derived mesenchymal stem cells
8	Completed	MRI Markers of Cartilage Damage in Knee With Osteoarthritis Condition: Osteoarthritis, Knee Intervention: Other: Magnetic Resonance Imaging
9	Recruiting	Post Market Study of DeNovo NT, Natural Tissue Graft

← FGF

← MSCs

Screenshot from www.clinicaltrials.gov, May 2010

Lecture 6: conclusions

- Both *in vitro* and *in vivo* models of cartilage repair can reveal valuable insights, but have different strengths.

- Cell-based therapies have come to market for cartilage TE, and scaffold-based therapies are on the horizen.

Next time: Atissa on presenting with a partner.

Lecture 8: special topics in TE.

20.109 Laboratory Fundamentals in Biological Engineering

Spring 2010