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: 2\textsuperscript{nd} half

1. Enzymatic digestion
   \hspace{1cm} \textbf{Test for collagen proteins (by ELISA)}

2. EDTA-citrate dissolution

\begin{itemize}
  \item Purify mRNA from cells \rightarrow \text{Amplify collagen cDNAs} \rightarrow
  \item Compare collagen I and II transcript levels, normalized to GAPDH
\end{itemize}
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)
Day 5-7: protein analysis by ELISA

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

“blocking” step also needed

**Indirect ELISA**
- bind antigen
- add 1° antibody
- wash
- add 2° antibody, wash

**Sandwich ELISA**
- bind capture antibody
- add antigen
- wash
- add detection antibody, wash
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

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)
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

*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

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

Chondrogenesis in vitro

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

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?

Cells and scaffolds *in vivo*

- Stem cells and/or injectable natural matrix (gelatin/HA) in rabbit knee defects
- Matrix and cells both contributed; synergy

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4 wks

12 wks
Large animal *in vivo* model

- 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


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)

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