Cartilage TE: from \textit{in vitro} and \textit{in vivo} models to the clinic

Module 3, Lecture 6

20.109 Spring 2011
Lecture 5 review

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

- Compare qPCR and end-point RT-PCR as gene expression assays.
Topics for Lecture 6

- Proteoglycan assay
- qPCR analysis
- Cartilage TE *in vitro*
- Cartilage TE *in vivo*
- Cartilage TE in the clinic
Proteoglycan content assay

- DMMB cationic dye binds (-) groups on PGs
- GAG sulfates detected at pH 1.5-3.0
- Alginate carboxyls detected at pH 2.0-3.0
- Complex causes short-lived $A_{595}$ peak reduction
- Figure shows PG standard in presence of alginate

qPCR cycling

• Melt DNA, activate hot start enzyme, 10 min at 95 °C
• 40 PCR cycles: melt (15 sec at 95 °C); anneal/extend
• Anneal/extend <=1 min at 60 °C
  – 2-step cycling often sufficient (short products)
  – take single fluorescence snapshot at end
• Melting curve
  – slowly heat to 95 from 60 °C
  – continuously measure fluorescence

*Image from Roche manual*
qPCR threshold cycle $C_T$

- Initial cycles used to set baseline
- $C_T = \text{intensity} > > \text{background}$

- Two main ways to calculate $C_T$
  - $2^{\text{nd}}$ derivative maximum
    - each $C_T$ identified by largest $\Delta$ slope
  - Fit points
    - all $C_T$s identified by same threshold
    - linear regression in log phase
    - recommended for our analysis type

*From Roche manual*

*Roche, LightCycler 480 Operator’s Manual, software version 1.5*
T/R qPCR data

Raw fluorescence vs cycle #

Log scale with noise threshold
qPCR relative expression analysis

• Relative gene expression analysis
  – Control for cDNA amount with reference (e.g., 18S rRNA)
  – Expression change relative to a control (e.g., fresh cells)

• E is amplification efficiency for that primer set

\[
\text{ratio} = \frac{(E_{\text{target}})^{\Delta C_{\text{P target}}(\text{control-sample})}}{(E_{\text{ref}})^{\Delta C_{\text{P ref}}(\text{control-sample})}}
\]

qPCR primer set standard curves

• Slope indicates primer amplification efficiency
  – $E = 10^{(-1/slope)}$
  – $E = 2$ for slope = -3.3

• Measure samples over 3-5 logs, in triplicate
Detection limit for change

2-fold change detectable but $C_T$ error/scatter may overlap
Optimizing primer concentration

First experiment – too high [primer]

No-template controls give primer-dimer product!

Later experiment

High [cDNA] sample oddly shaped

Great replicate agreement and flat controls (green)

No-template controls give primer-dimer product!
Interlude

Lecture 8: your choice of TE topics (list on board)

Which one is cuter? Tree kangaroo or human baby?
Chondrogenesis *in vitro*

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

Recent Grodzinsky lab work shows merits of peptide gels

CN II expression of stem cells increases in agarose, RAD, and KLD gels over 5-10 days

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?

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)?

![Image of tissue samples showing native and treated repair compared to untreated repair.](image-url)
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


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)

Cutting edge of treatment

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

Found 186 studies with search of: cartilage

<table>
<thead>
<tr>
<th>Rank</th>
<th>Status</th>
<th>Study</th>
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<tbody>
<tr>
<td>1</td>
<td>Recruiting</td>
<td>Cartilage Autograft Implantation System (CAIS) for the Repair of Knee Cartilage Thro</td>
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<td>Conditions: Articular Cartilage Injury; Osteochondritis Dissecans</td>
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<td>Interventions: Procedure: Microfracture; Procedure: Cartilage Autograft Im</td>
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<tr>
<td>2</td>
<td>Active, not recruiting</td>
<td>Evaluation of an Acellular Osteochondral Graft for Cartilage LESions Pilot Trial</td>
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<tr>
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<td>Condition: Articular Cartilage Injury</td>
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<td>Interventions: Device: Kensey Nash Corp. Cartilage Repair Device;</td>
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<tr>
<td>3</td>
<td>Recruiting</td>
<td>AS902330 in Cartilage Injury Repair (CIR)</td>
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<td>Condition: Isolated Cartilage Injury of the Knee</td>
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<td>Interventions: Drug: AS902330; Other: Placebo</td>
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<td>4</td>
<td>Completed</td>
<td>The Objectives of the Cartilage Repair Registry is to Report Long Term Efficacy and Safety of Cartilage Repair Pr</td>
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<tr>
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<td>Conditions: Articular Cartilage; Cartilage Diseases</td>
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<td></td>
<td>Intervention: Biological: Cartilec (autologous cultured chondrocyte) in</td>
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<td>5</td>
<td>Recruiting</td>
<td>Study to Compare the Efficacy and Safety of Cartistem® and Microfracture in P</td>
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<tr>
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<td>Conditions: Cartilage Injury; Osteoarthritis</td>
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<td></td>
<td>Interventions: Biological: Cartistem; Procedure: Microfracture treatm</td>
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<tr>
<td>6</td>
<td>Not yet recruiting</td>
<td>Follow-Up Trial Comparing BST-CarGel and Microfracture in Repair of Articular Cartilage Lesions in the Knee</td>
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<tr>
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<td>Condition: Articular Cartilage Lesions in the Knee</td>
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Screenshot from www.clinicaltrials.gov, May 2011

- scaffold + own tissue
- scaffold alone
- drug alone
- cord blood stem cells in gel
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 horizon.

Next time: Atissa on presenting with a partner; research proposal open discussion.

Lecture 8: special topics in TE.