

Spatial Resolution of Chondrocyte Response to Mechanical Signals for Cartilage Tissue Engineering

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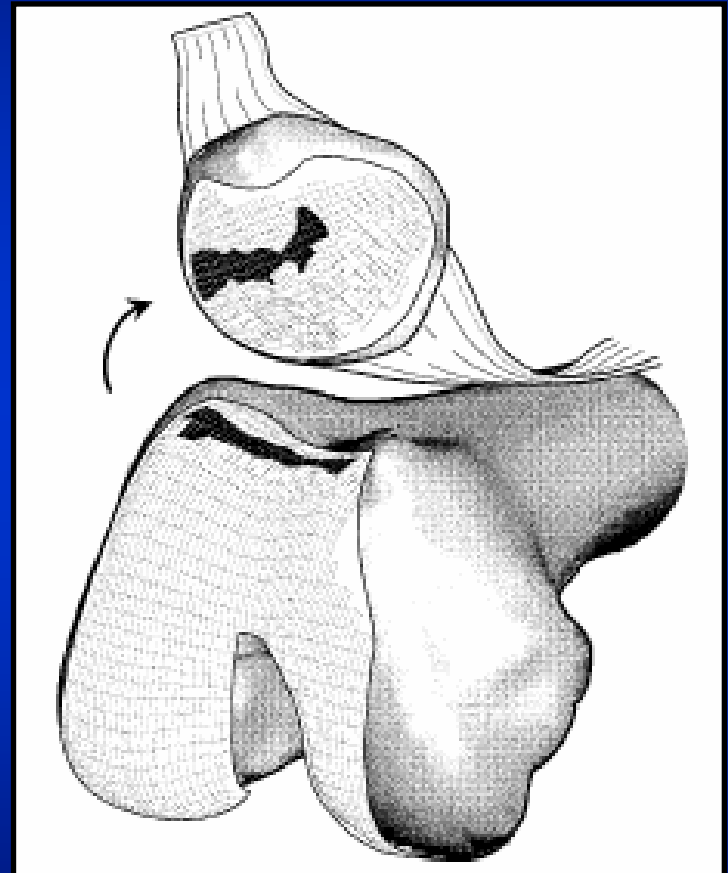
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Cartilage

Cartilage → Support

- Transmits and distributes loads
- Composed of **extracellular matrix** and **chondrocytes**
- Dynamic environment → tissue constantly remodeled
- Avascular → limited repair of damaged tissue



Osteoarthritis (OA)



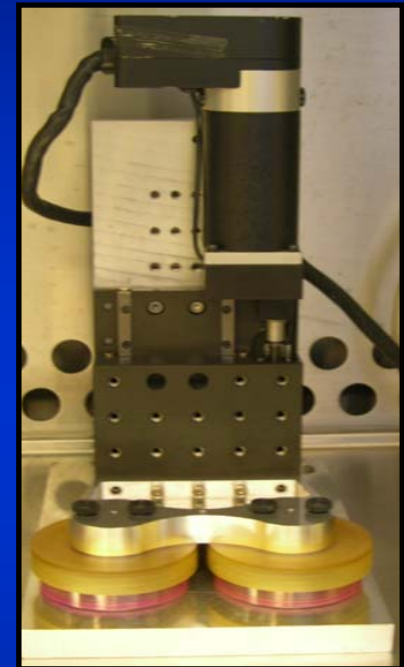
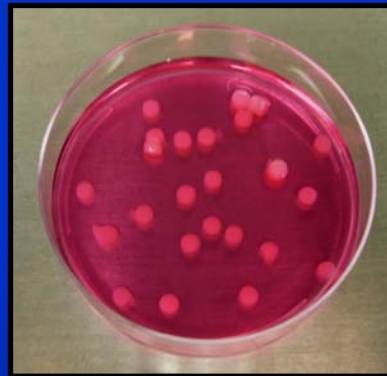
- Affects ~21 million Americans
- Characterized by cartilage tissue degradation
- Causes stiffening of joints, pain, loss of joint function

Solution?

Tissue Engineering

Goal: Restore the function of damaged organs by producing new tissue

- Combine cells with scaffolds
- Stimulate tissue growth by loading with bioreactors



The functional properties of tissue-engineered constructs are often much less than that of native tissue.

Solution?

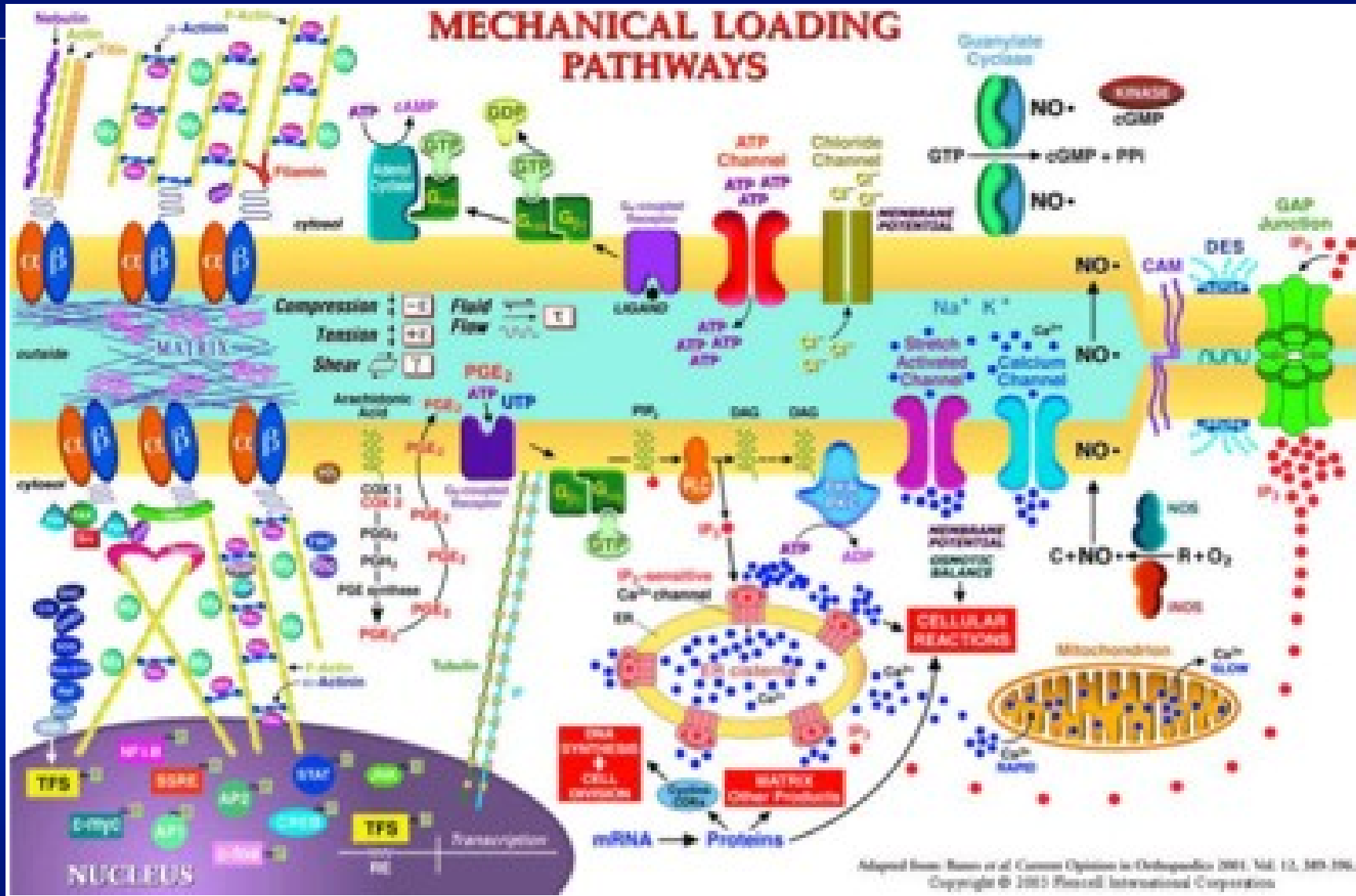
Mechanotransduction?

Mechanotransduction: Cellular mechanism to convert mechanical stimuli to biochemical signals

- Elucidate mechanotransduction pathways →
Enhance functionality of tissue-engineered constructs

Question: How do chondrocytes respond to extracellular stimuli?

MECHANICAL LOADING PATHWAYS

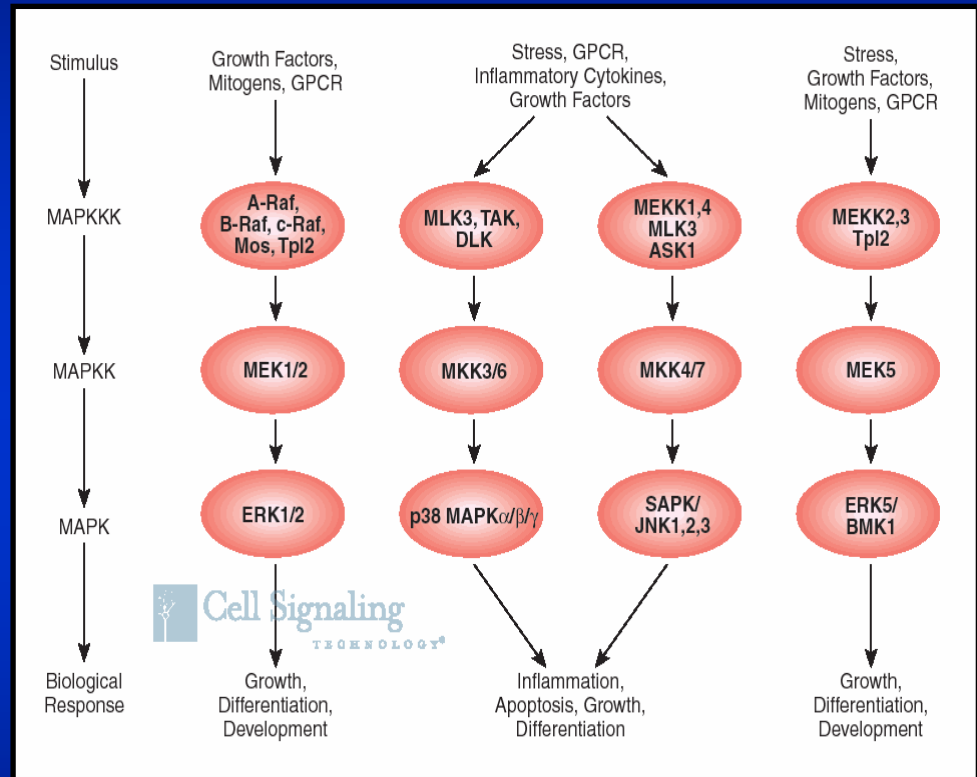


Adapted from: Bass et al. Current Opinion in Cell Biology 2001, Vol. 13, 389-396.
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MAPKs

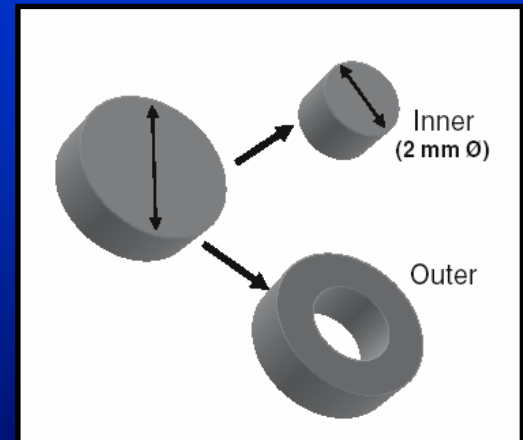
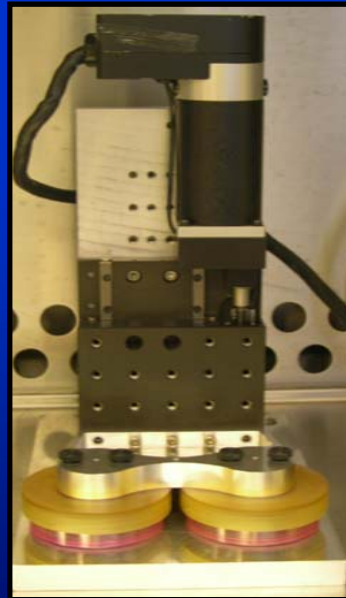
Mitogen-activated Protein Kinases (MAPKs)

- Family of biochemical molecules → sense stimuli and respond with cascade of events
- Key members:
 - ERK1/2
 - p38 MAPK
 - SAPK/JNK



J1-3

- Agarose disks ($2.25 \times \text{Ø } 5.0 \text{ mm}$) seeded with bovine chondrocytes at $30 \times 10^6 \text{ cells/ml}$
- Disks separated into inner core and outer annulus regions after loading
- 3 replicates (J1-3)

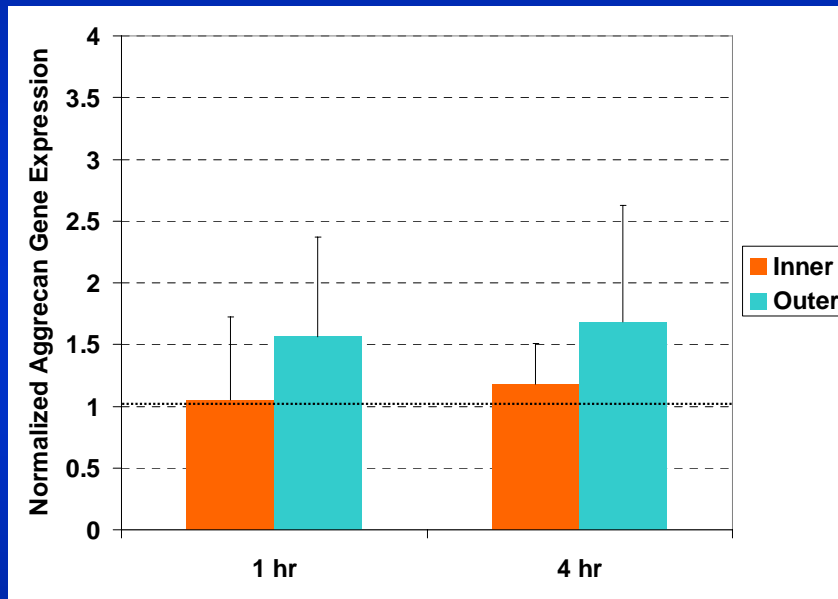


Gene Expression Analysis

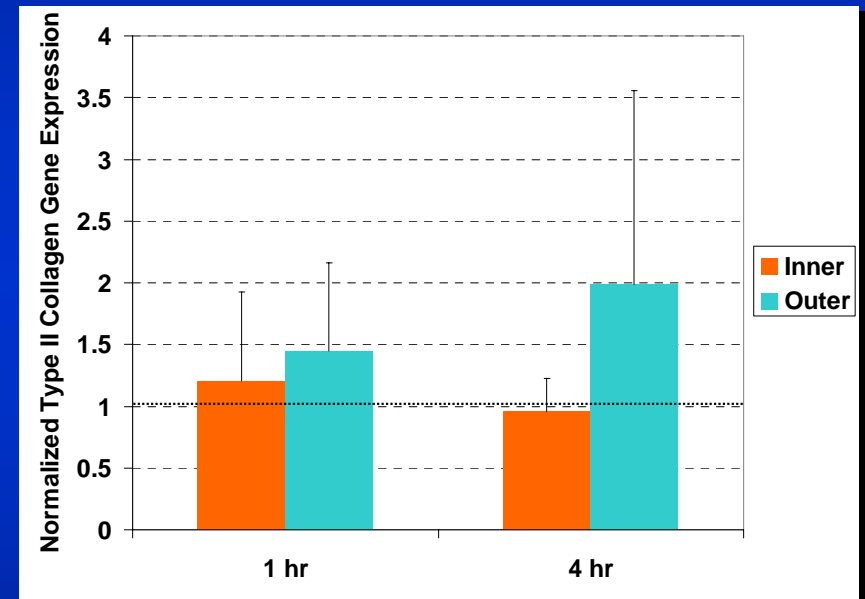
1. RNA Extraction
2. cDNA Synthesis
3. Real-time PCR
 - Aggrecan
 - Type II Collagen



J1-3. Real-time PCR



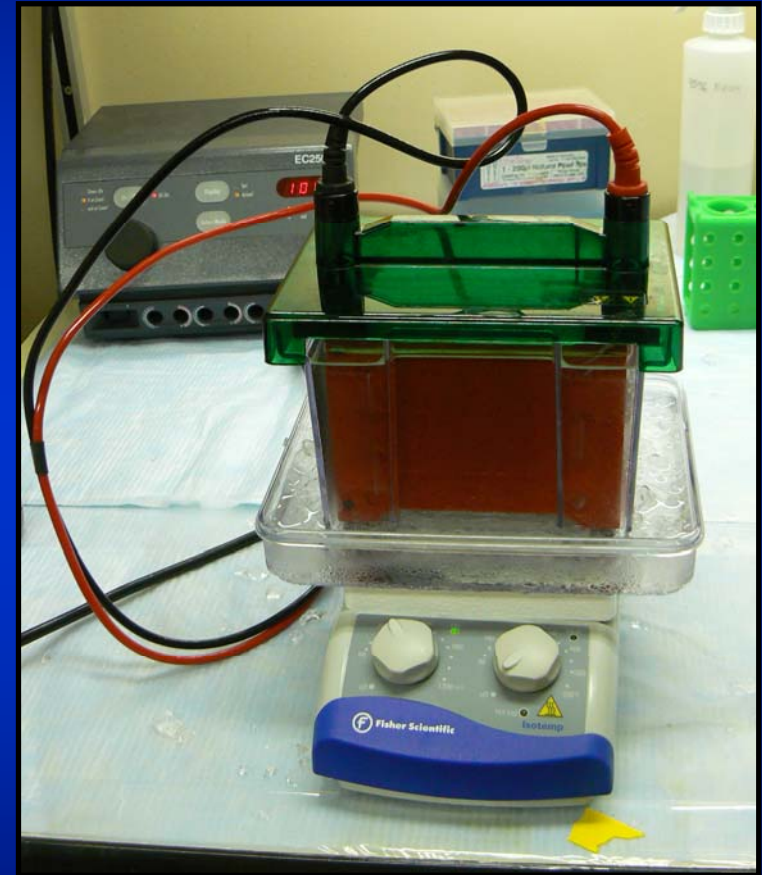
Aggrecan



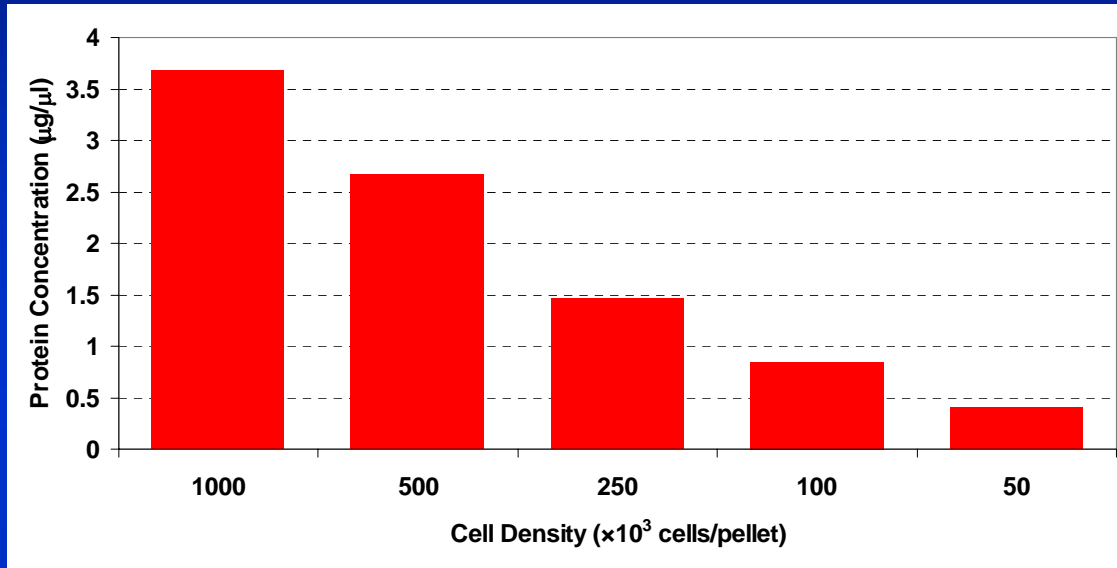
Type II Collagen

Western Blotting

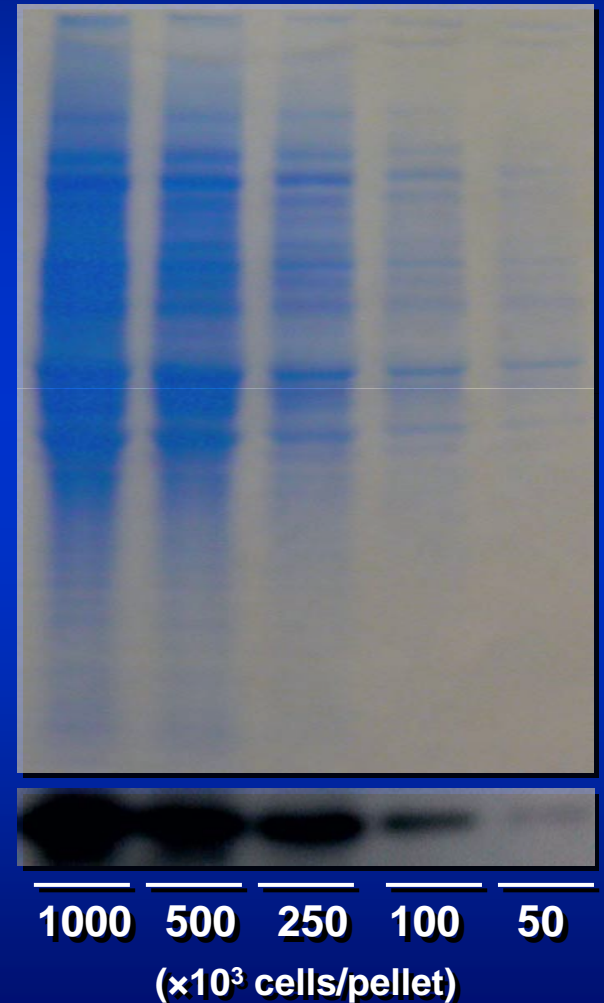
1. Cell Lysis and Protein Extraction
2. Protein Quantification by BCA Assay
3. SDS-PAGE (polyacrylamide gel electrophoresis) and Electrophoretic Transfer
4. Gel Staining
5. Antibody Probing and Protein Detection



MSC1. Western Blotting

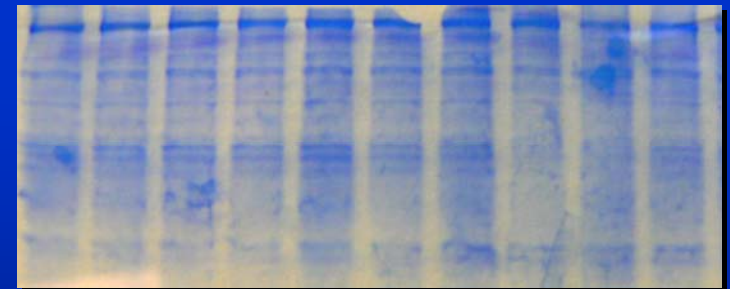
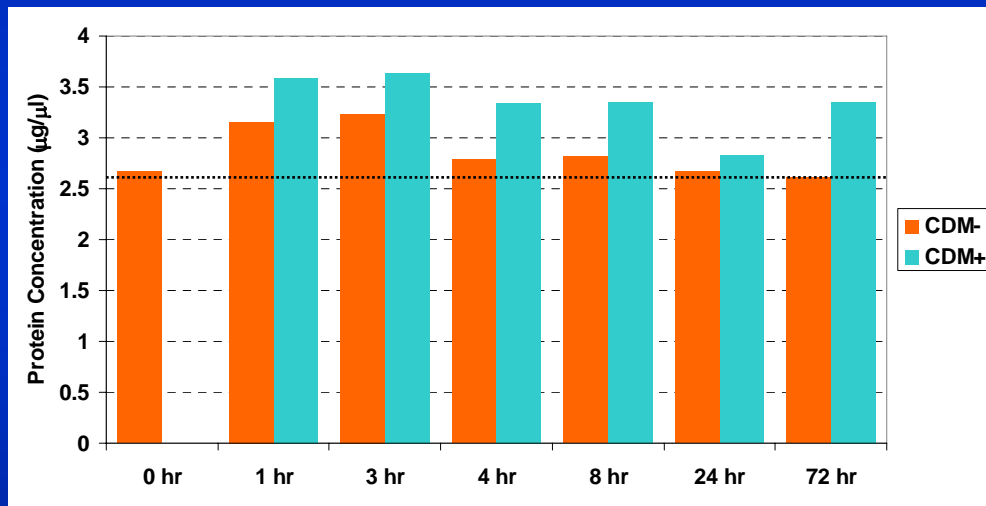


- Mesenchymal stem cells (MSCs) pelleted at various cell densities
- Probed for BiP/GRP78



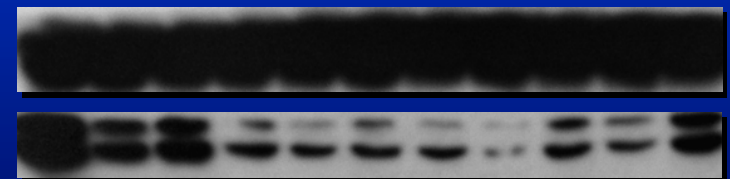
MSC2. Chondrogenesis Timescale

- MSCs pelleted at 250×10^3 cells/pellet, cultured in CDM-/+ , and collected at various time points (0, 1, 3, 4, 8, 24, 72 hr)
- Probed for total and phospho ERK1/2



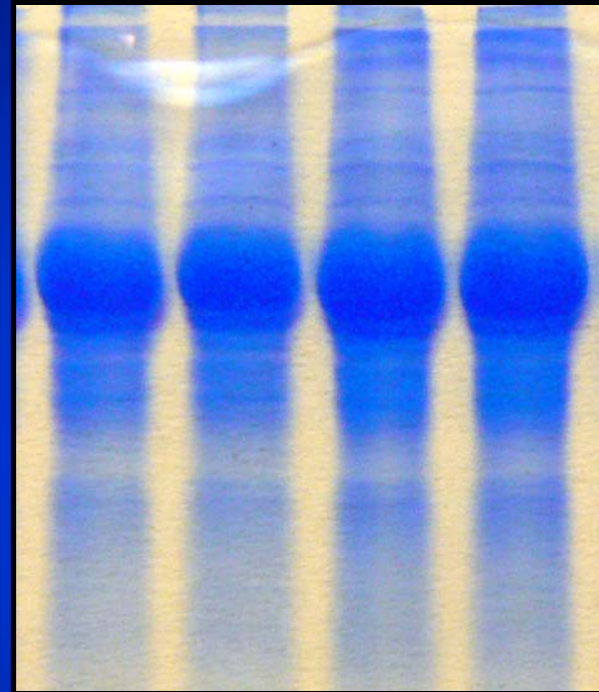
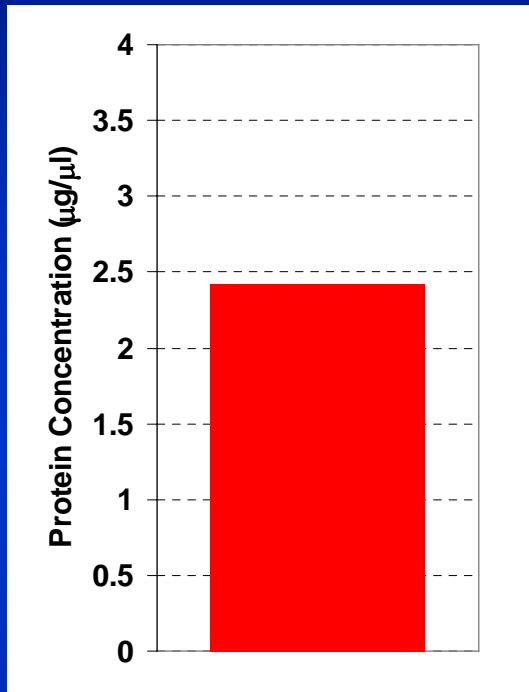
Total ERK1/2

Phospho ERK1/2



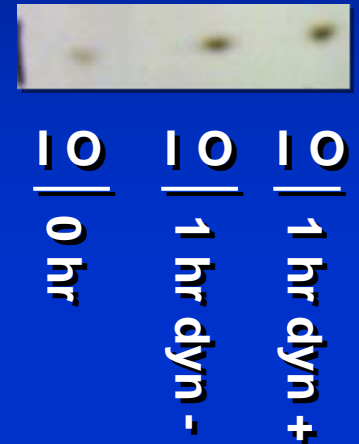
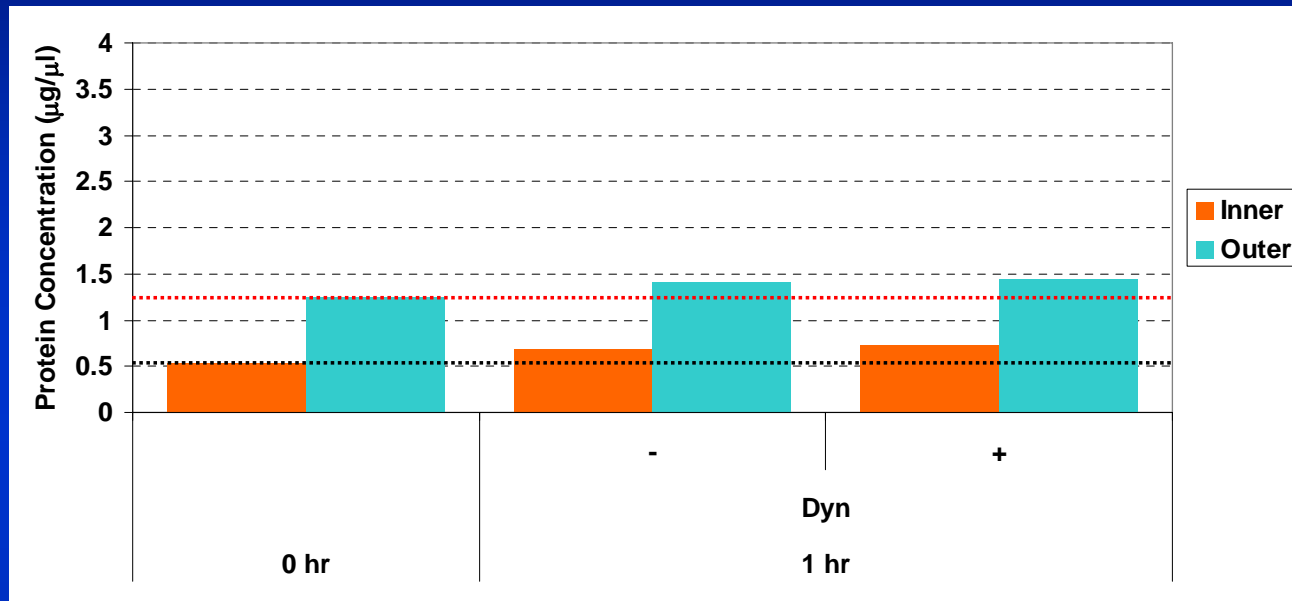
- + - + - + - + - +
0 1 3 4 8 24

Agarose Disks – Western Blotting



- Protein extraction from agarose disks (3 per sample)
- Presence of new bands in stained gel

J1. Western Blotting – Total p38 MAPK



- Probed for total p38 MAPK
- Only bands for outer samples appear on blot → sufficient protein present in outer samples only

Conclusions

Real-time PCR

- Aggrecan and type II collagen gene expression vary between the inner and outer regions
- Gene expression does not vary significantly by region between one- and four-hour loading times

Western Blotting

- Band densities correlated with protein concentrations
- Protocol developed to extract protein from agarose constructs

Future Directions

- Repeat studies with more samples
- Finish Western blotting of phospho and total MAPKs
- Repeat studies with different pre-culture times
- Investigate MSC response to mechanical signals

Question: How does knowledge of chondrocyte mechanotransduction improve current cartilage tissue engineering efforts?

Acknowledgments

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