Mechanotransduction in Stem Cells for Cartilage Tissue Engineering

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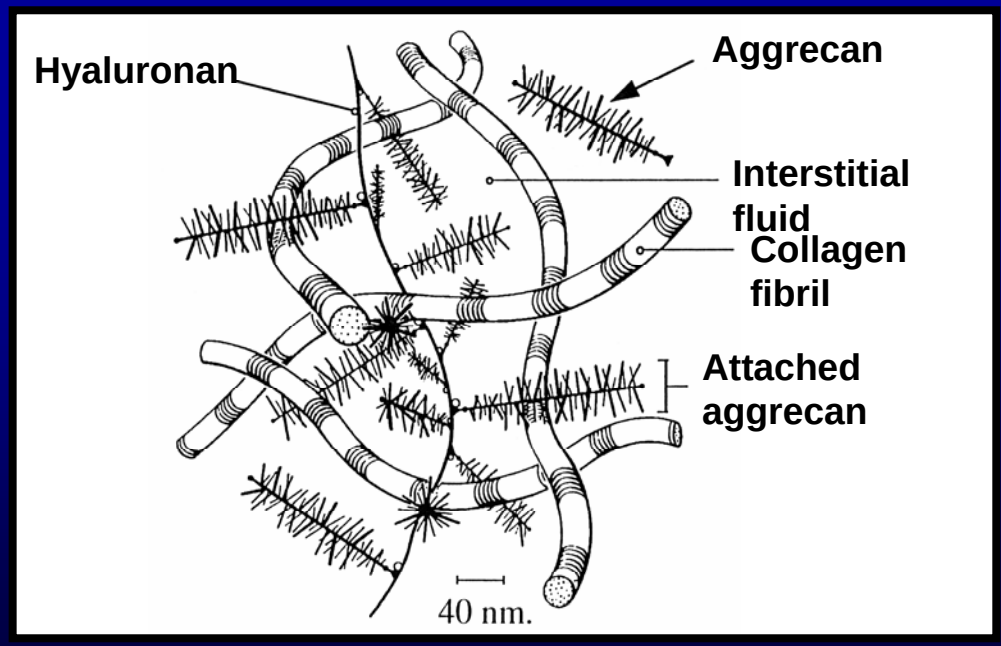
SUNFEST Final Presentation

August 10, 2007

Articular Cartilage Overview

- **Biochemical Composition:**
  - Extracellular matrix
    - Collagen Type II (10%-30% wet wt)
    - Sulfated proteoglycans (3%-10% wet weight)
  - Chondrocytes
- **Function:**
  - Compressive Properties
  - Tensile Properties
  - Fluid Flow



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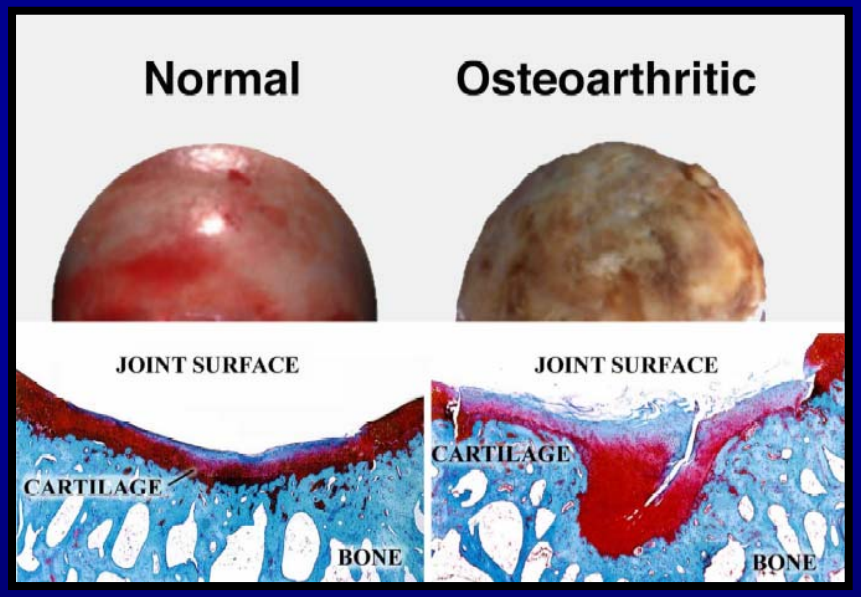
Cartilage Degeneration



Proteoglycan Loss
Collagen Damage

↓ Modulus
↑ Hydraulic Permeability

↑ *Matrix Deformation*
↓ *Fluid Pressurization*

↓ Load-Bearing Capacity

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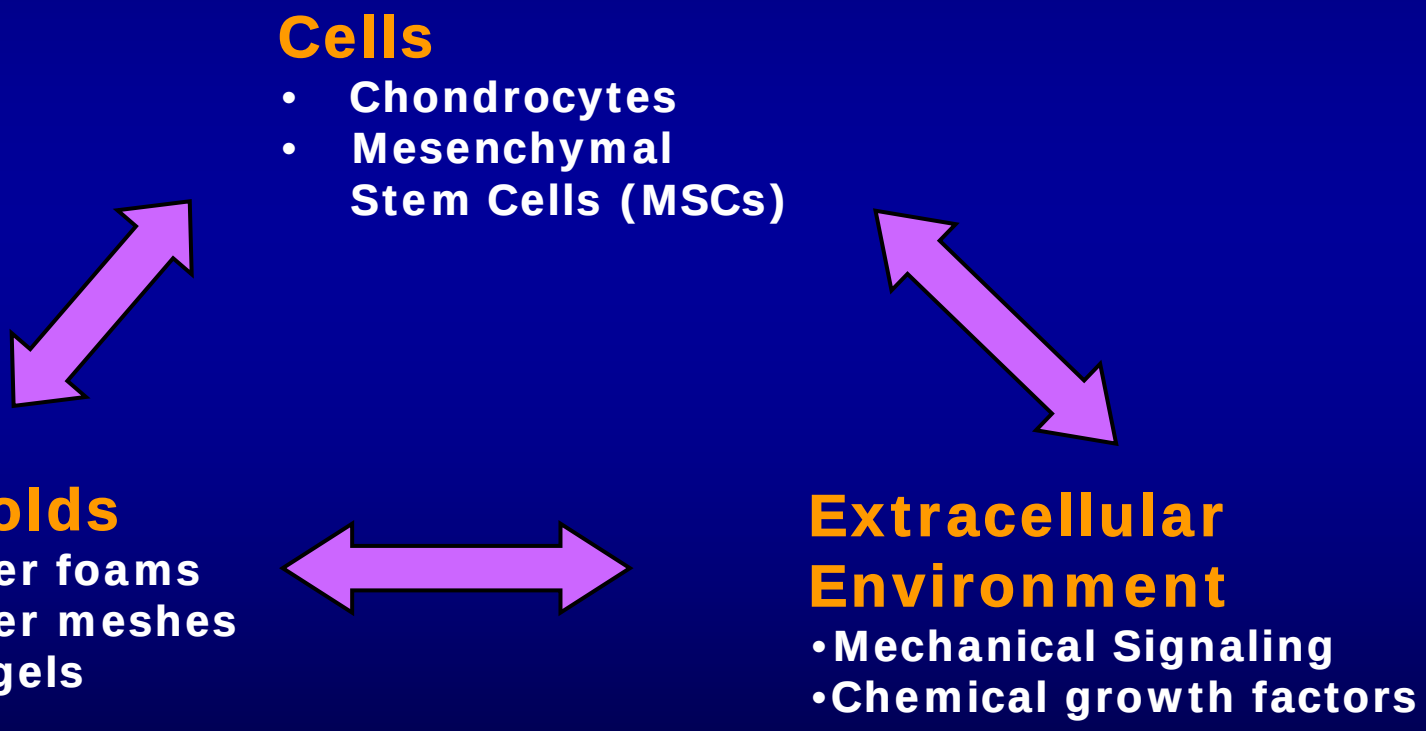
Tissue Engineering Paradigm

"a field…that seeks to develop functional cell, tissue, and organ substitutes to repair, replace or enhance biological function..." [NIH]



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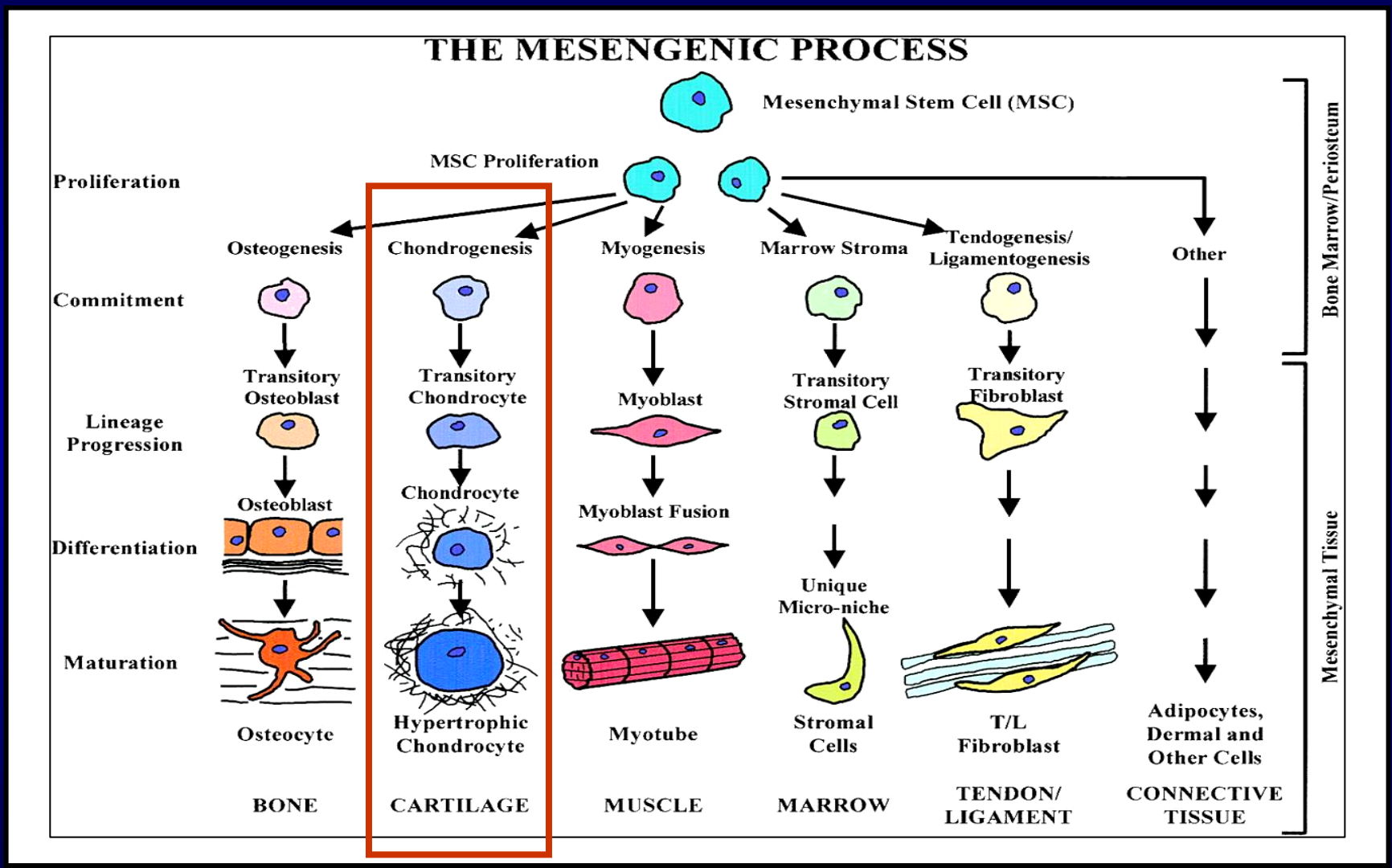
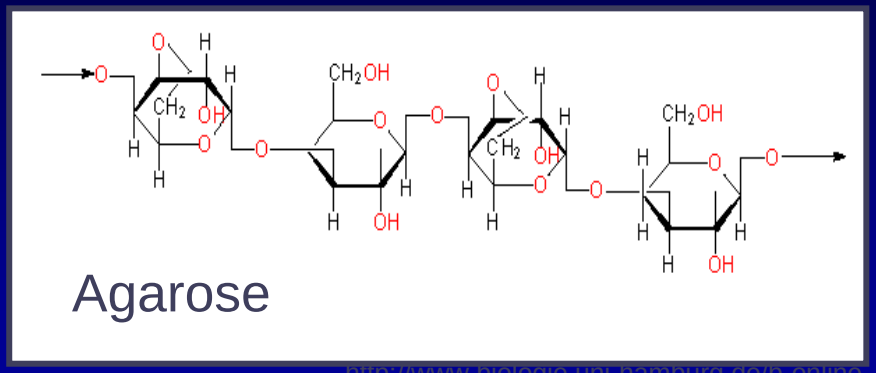
Mesenchymal Stem Cells



Scaffold Design



http://www.biologie.uni-hamburg.de/b-online

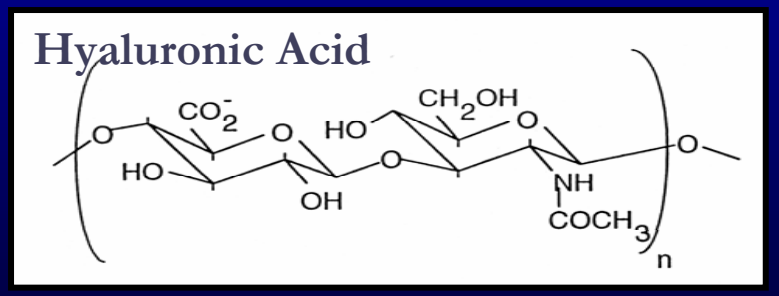
Hyaluronic Acid (HA)

- Photo-crosslinkable polysaccharide hydrogel
- Natural extracellular matrix component – biodegradable
- Successful cell encapsulation

Agarose

- Thermo-crosslinkable hydrogel made of polysaccharides (D- and L- galactose)
- Cellular encapsulation
- Mechanical Properties

- Not biodegradable
- Immunogenic



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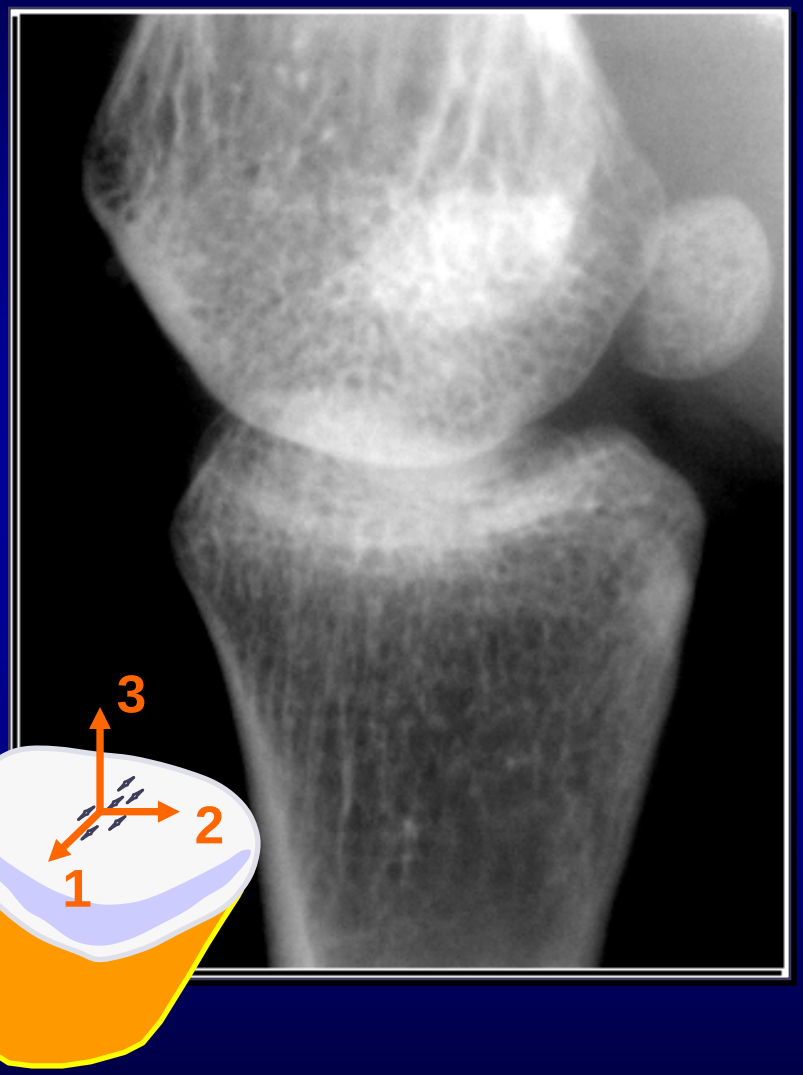
Physiologic Loading of Cartilage



- Compressive Forces
- Sliding Forces

Mechanical conditioning has been shown to improve chondrocyte mechanical properties

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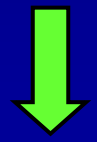
Mechanotransduction

Extracellular Signal

MAP Kinase activation / Signal transduction



Gene Expression Changes

Matrix Composition Biochemical Change

Mechanical Properties (Equilibrium Modulus, Dynamic Modulus, etc.)

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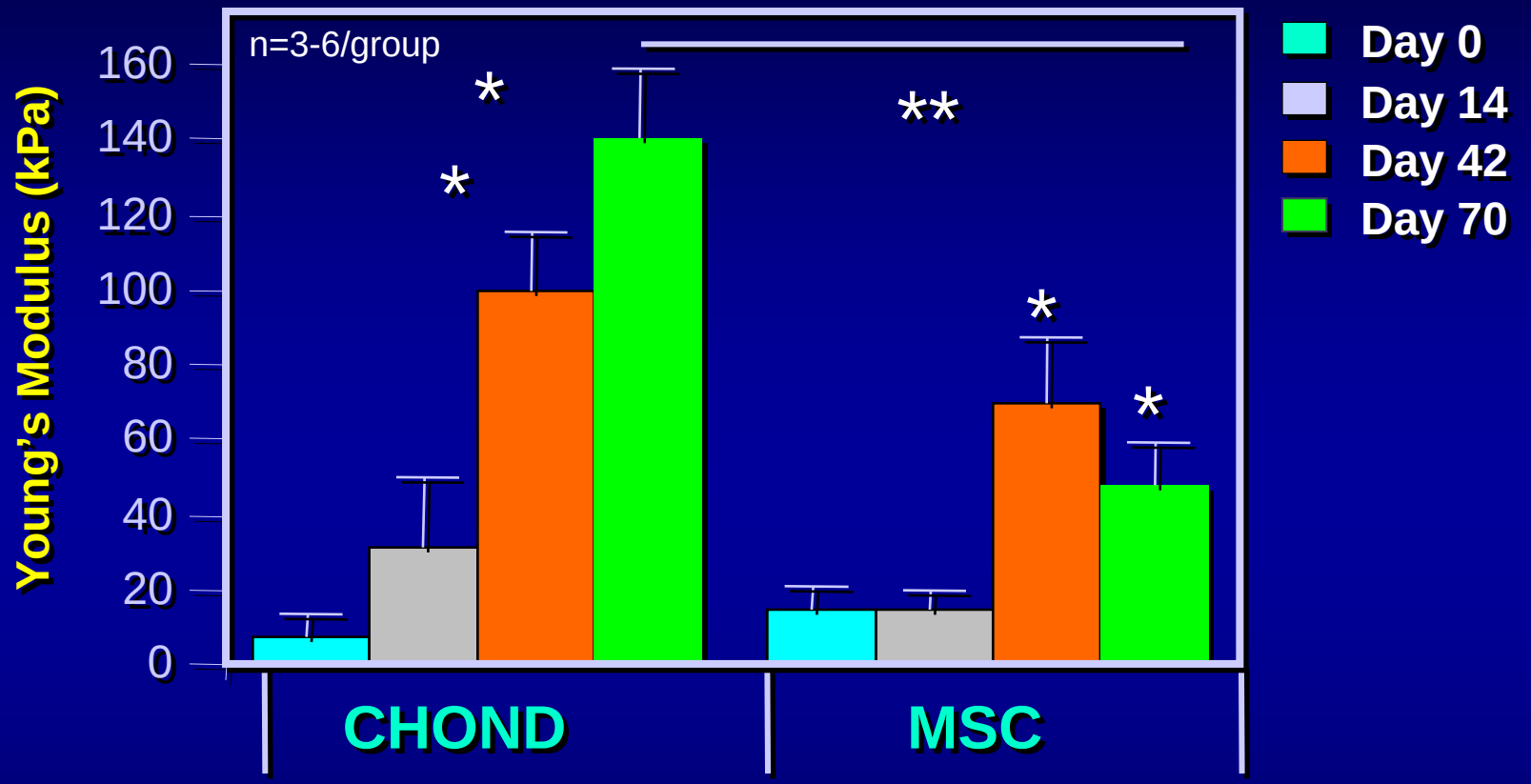
*p<0.05 vs. day 0, **p<0.05 (day 70)

Chondrogenesis in 3-D Culture



- MSC-laden constructs increase in mechanical properties with time
- Young's Modulus of MSC-laden constructs << that of chondrocyte-laden constructs

Mauck+ 2006

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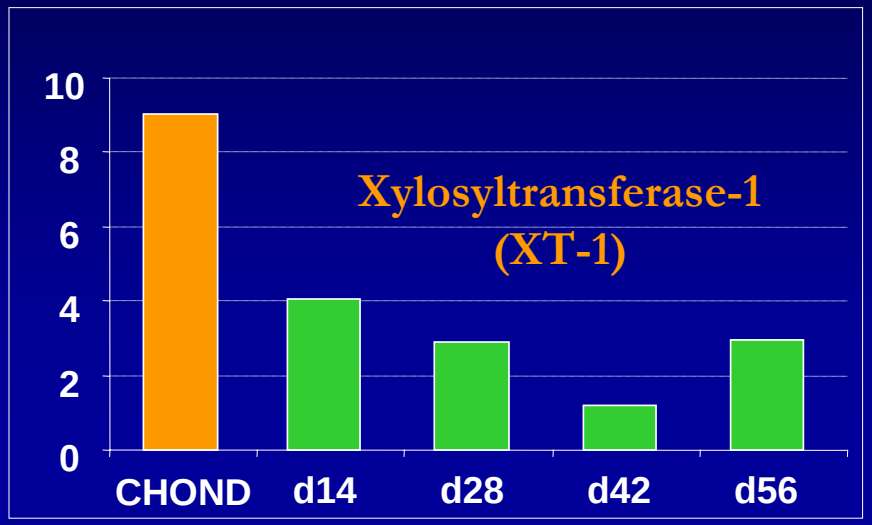
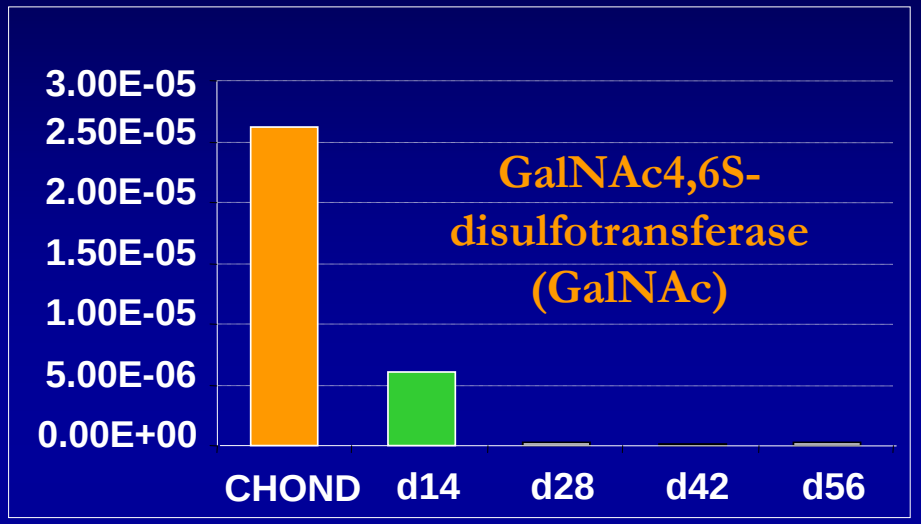
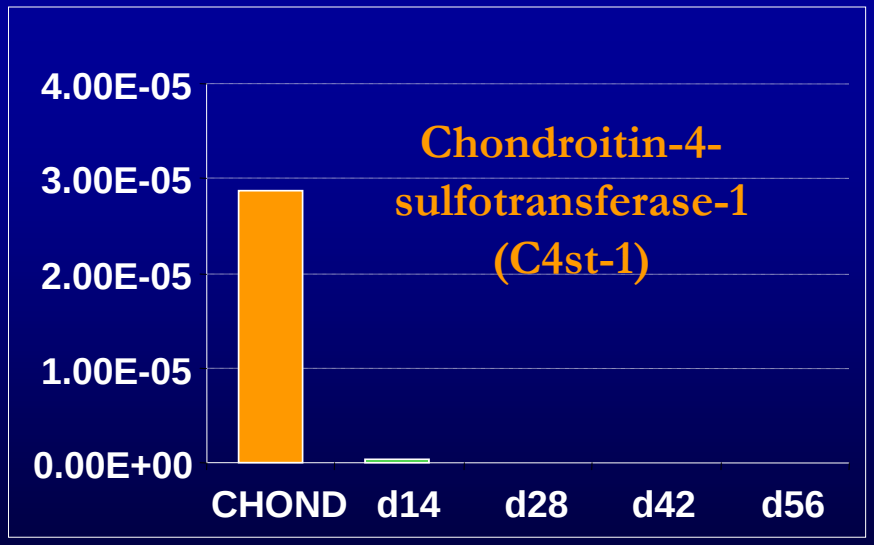
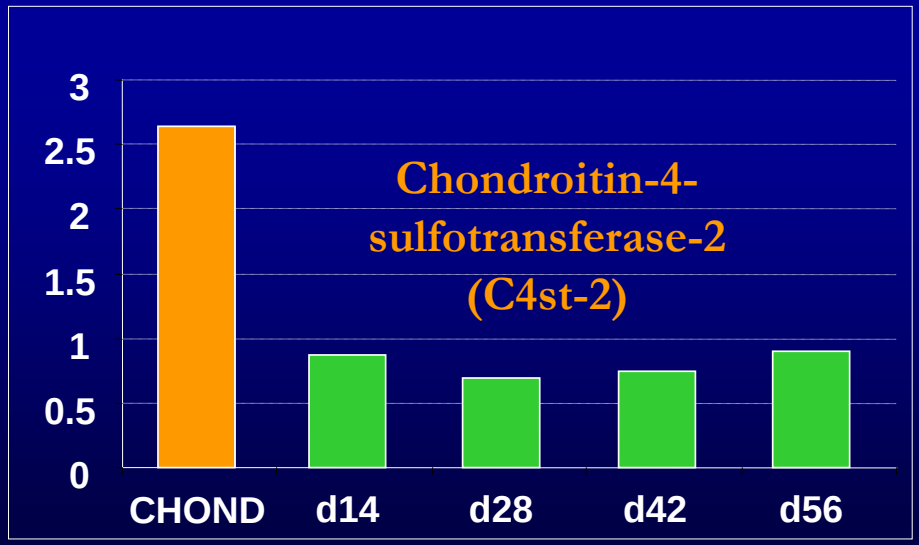
Target Gene Expression Results









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Hypothesis and Rationale:

Hypothesis: Compressive loading of MSC-seeded scaffolds leads to upregulation of matrix biosynthetic genes in the short term and increases in mechanical properties in the long term.

Rationale:

- Mechanical signals are relevant developmentally
- Changes in gene expression and chondrogenic expression in chondrocytes after mechanical signaling
- Target genes shown to be mechanically sensitive in chondrocytes

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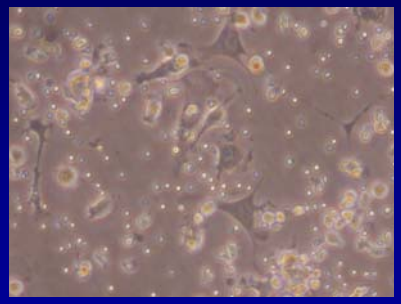
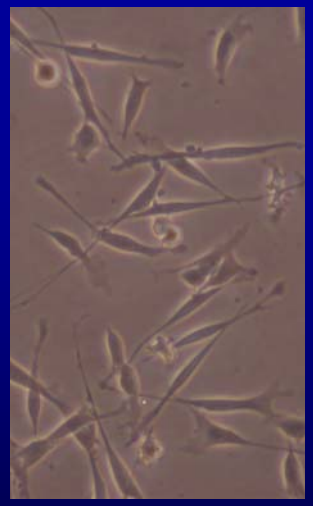
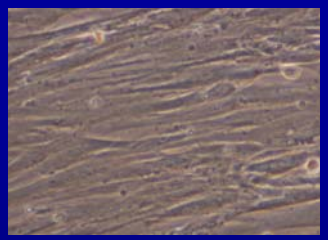
3D Hydrogel Culture

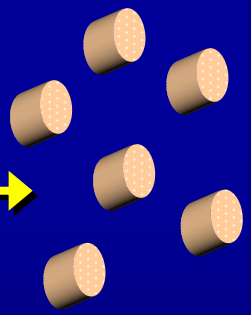


2% Agarose (@45°C) or 2% Hyaluronic Acid:
20 x 10^6 cells/ml

Free Swelling Culture







Disks:
2.25 X Ø 5.0 mm

Chondrogenic Medium (CM-/CM+) (+ TGF-β3)

Bovine MSC Harvest and Expansion

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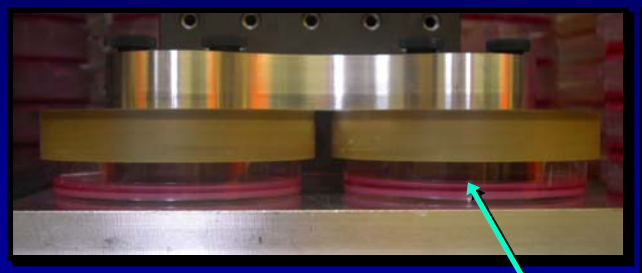
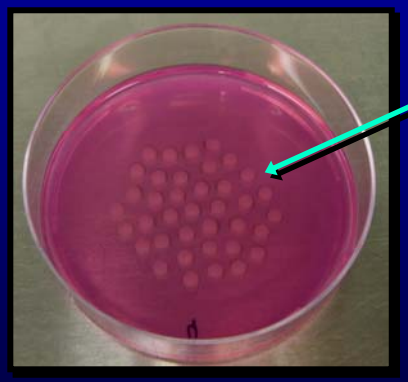
Dynamic Compression Bioreactor

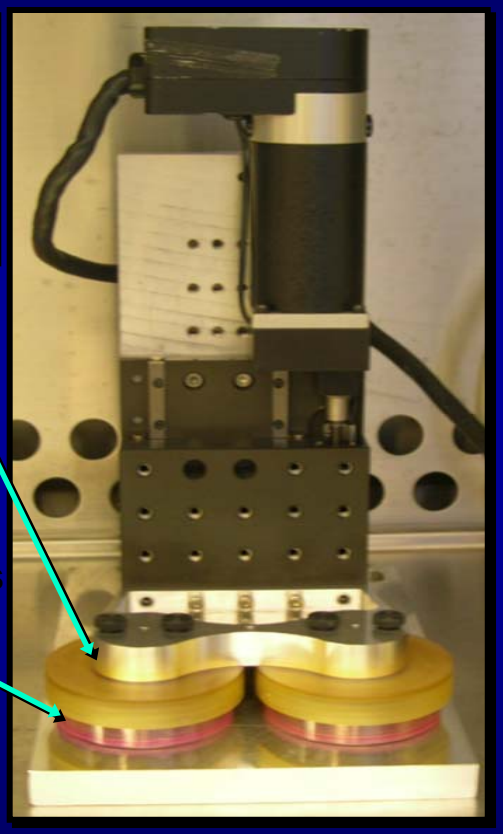


Dynamic Compression Bioreactor
(10% deformation, sinusoidal waveform)

Loading Plate

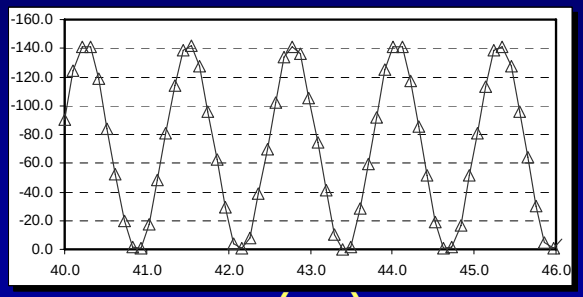
Constructs in Petri Dish





Bioreactor Displacement

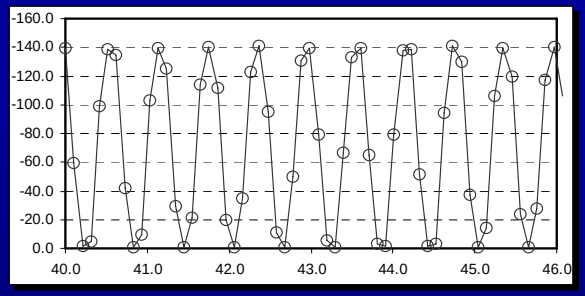
1.0 Hz



(microns)

(sec)

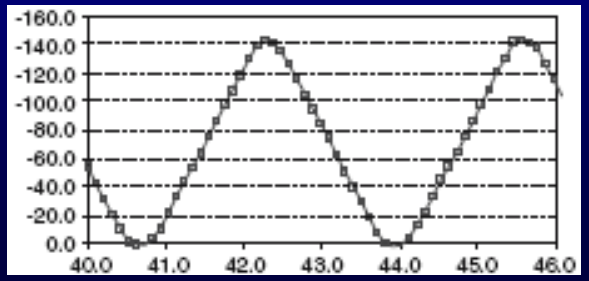
3.0 Hz



(microns)

(sec)

0.33 Hz



(microns)

(sec)

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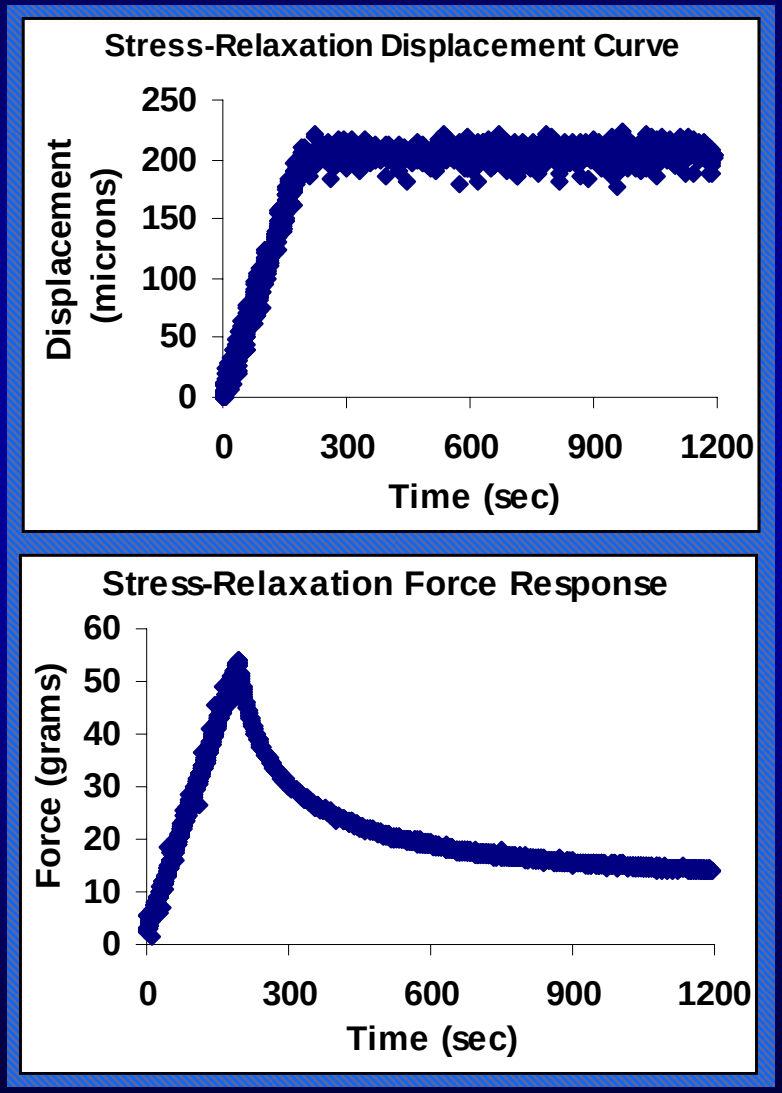
Methods

- Mechanical Properties – Equilibrium Modulus
  - Creep testing
  - Stress relaxation
- Biochemistry:
  - GAG content
  - Collagen Content
  - DNA content
- Gene Expression –
  - TRIZOL extraction
  - cDNA synthesis
  - RT-PCR
- Histology



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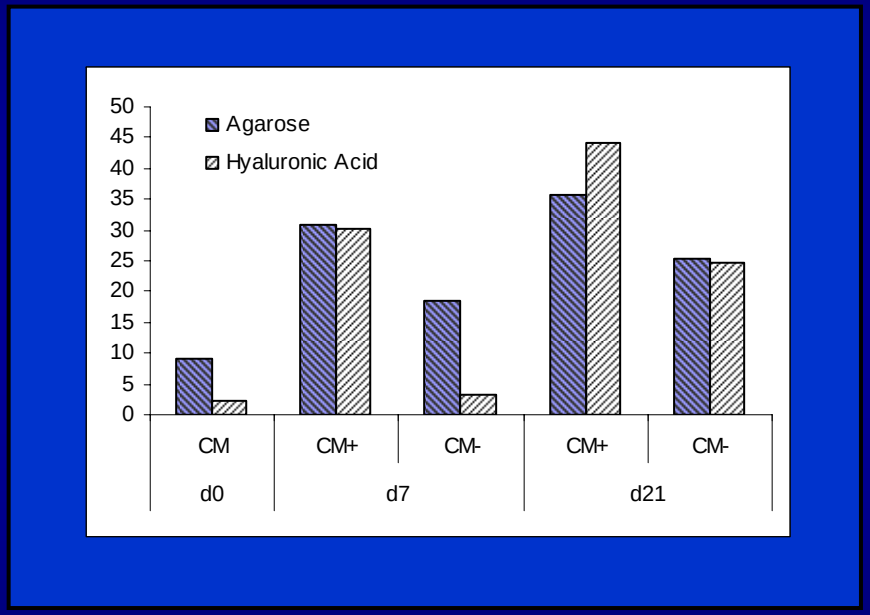
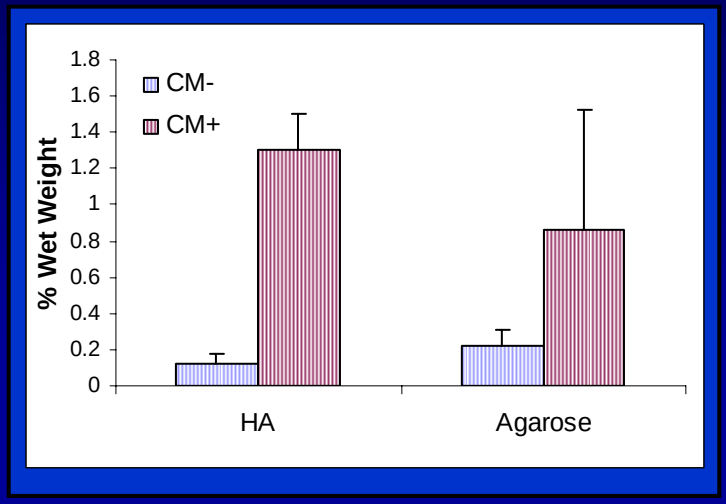
RX2: Encapsulation of MSCs in Hyaluronic Acid Scaffold Promotes Cell Growth and Chondrogenesis

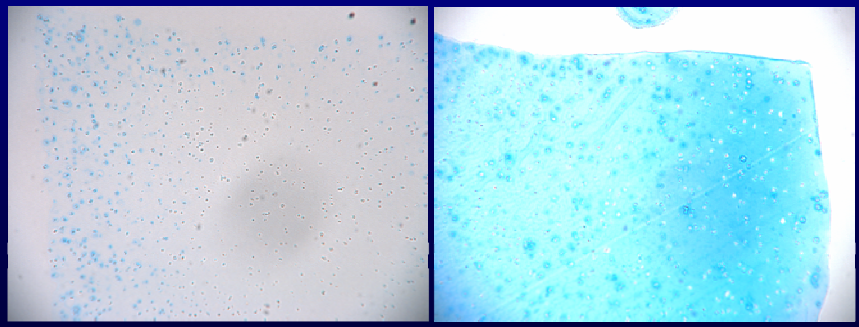


MTT Assay

3-(4, 5-Dimethylthiazol-2-yl)-2, 5 – diphenyltetrazolium bromide) –



GAG Quantification on day 21



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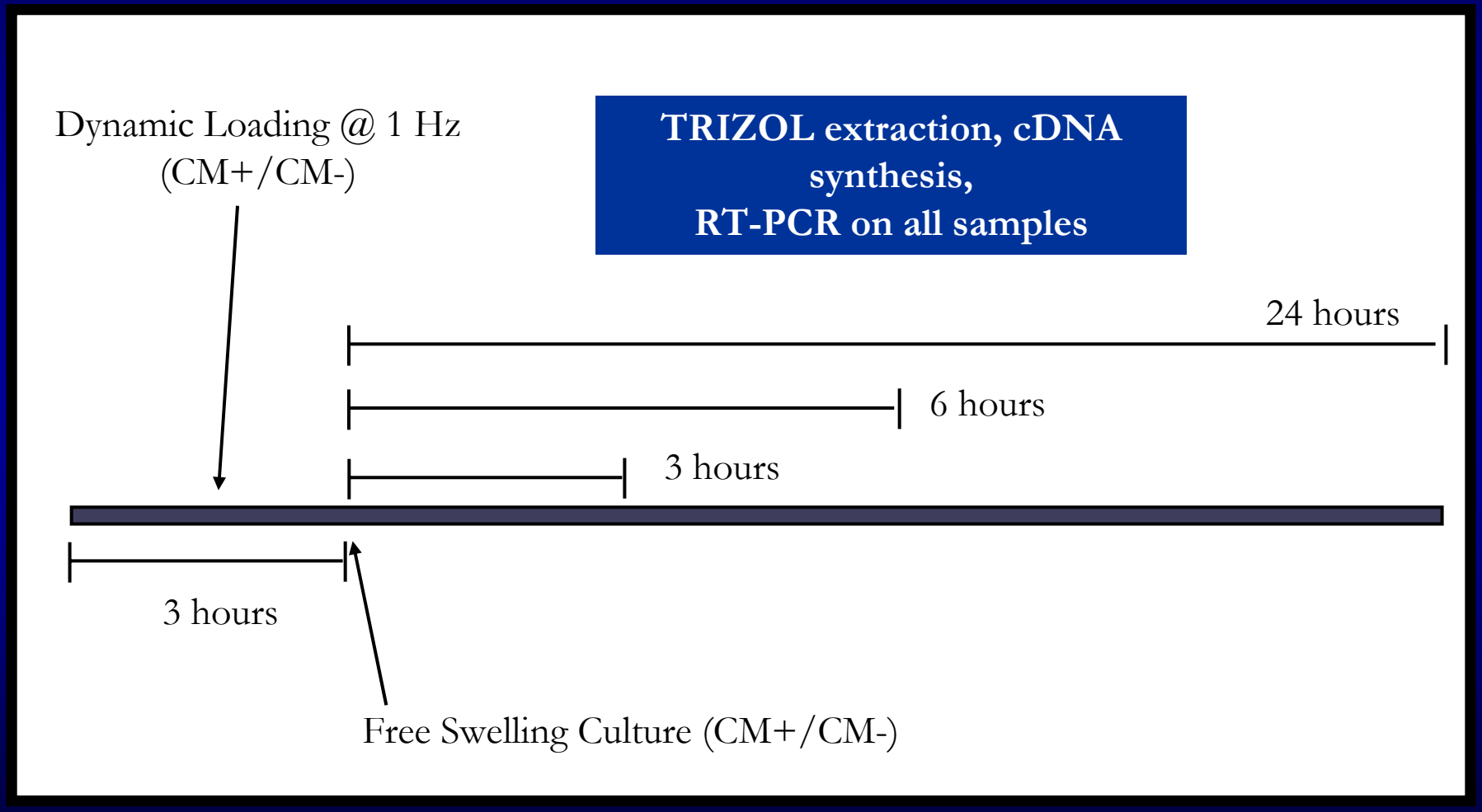
RX3, RX5: Short Term Gene Expression in MSC-laden HA Constructs



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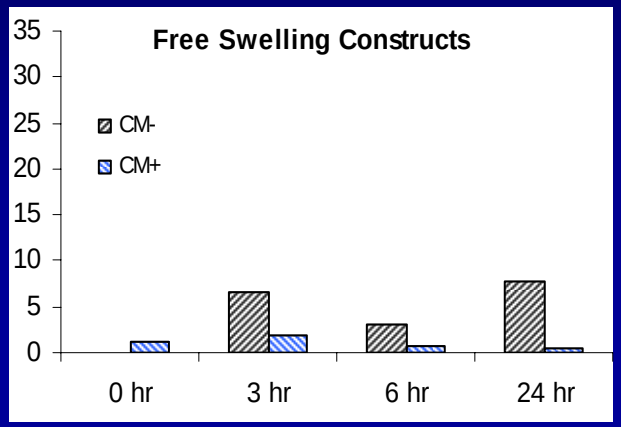
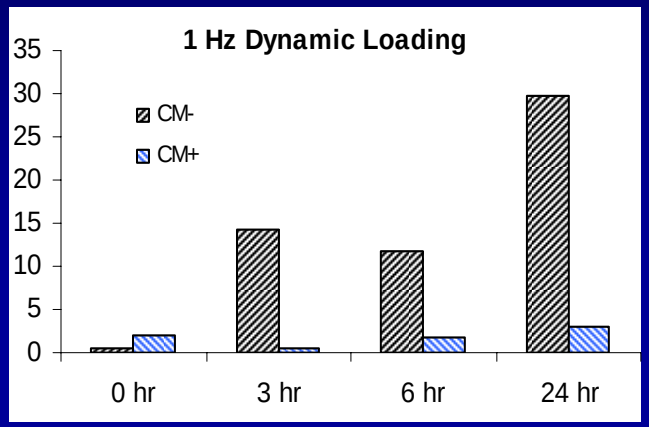
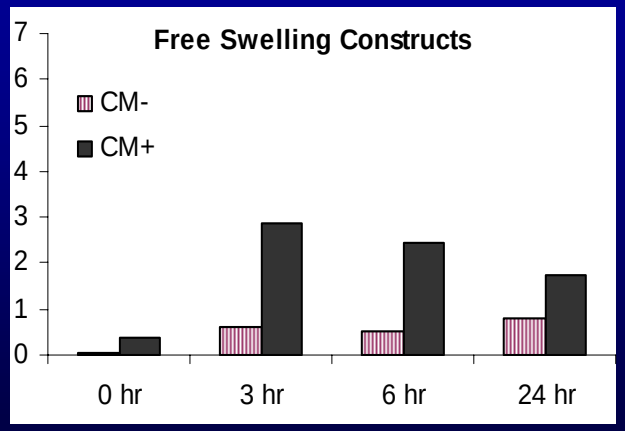
RX3, RX5: Matrix Biosynthesis Genes are Upregulated in Response to Short-Term Loading

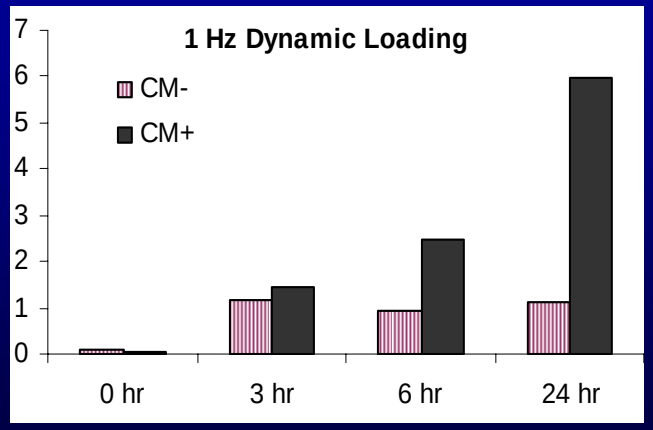




Mechanical upregulation of GalNAc is enhanced in absence of TGF-b3

C4st-1 upregulation is enhanced in presence of TGF-b3





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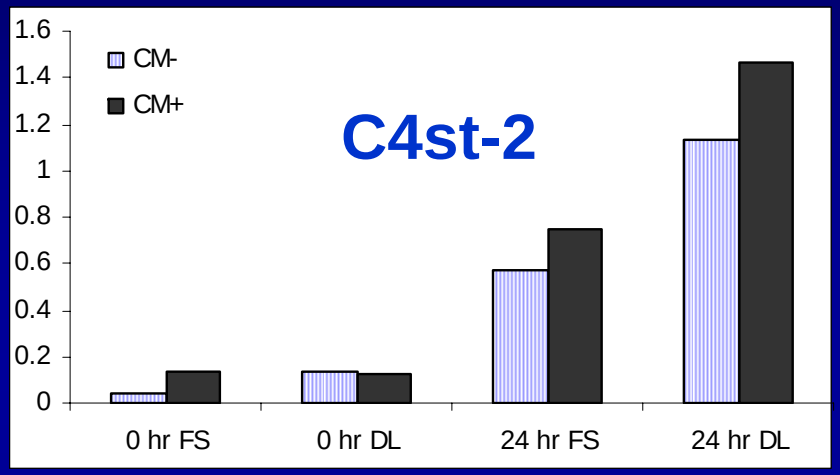
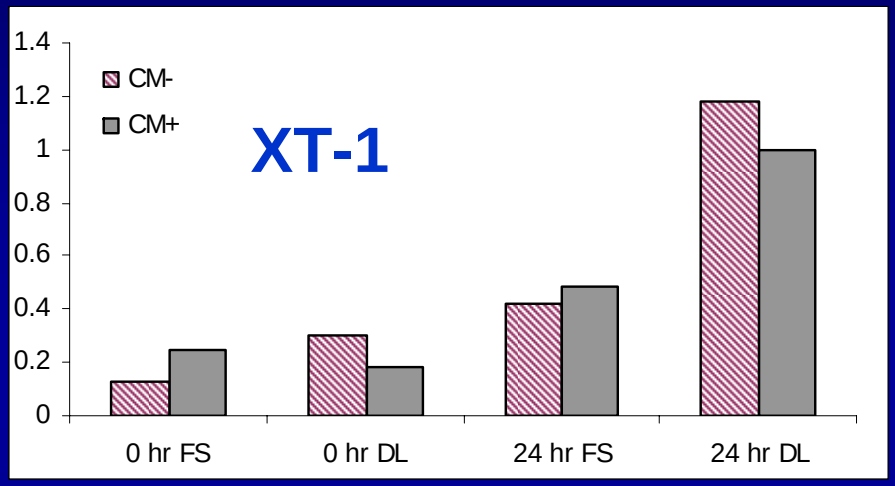
RX3, RX5: C4st-2, XT-1 are Upregulated in Response to Short-Term Loading





Question:

Does increased gene expression translate to long-term improvements in mechanical properties?

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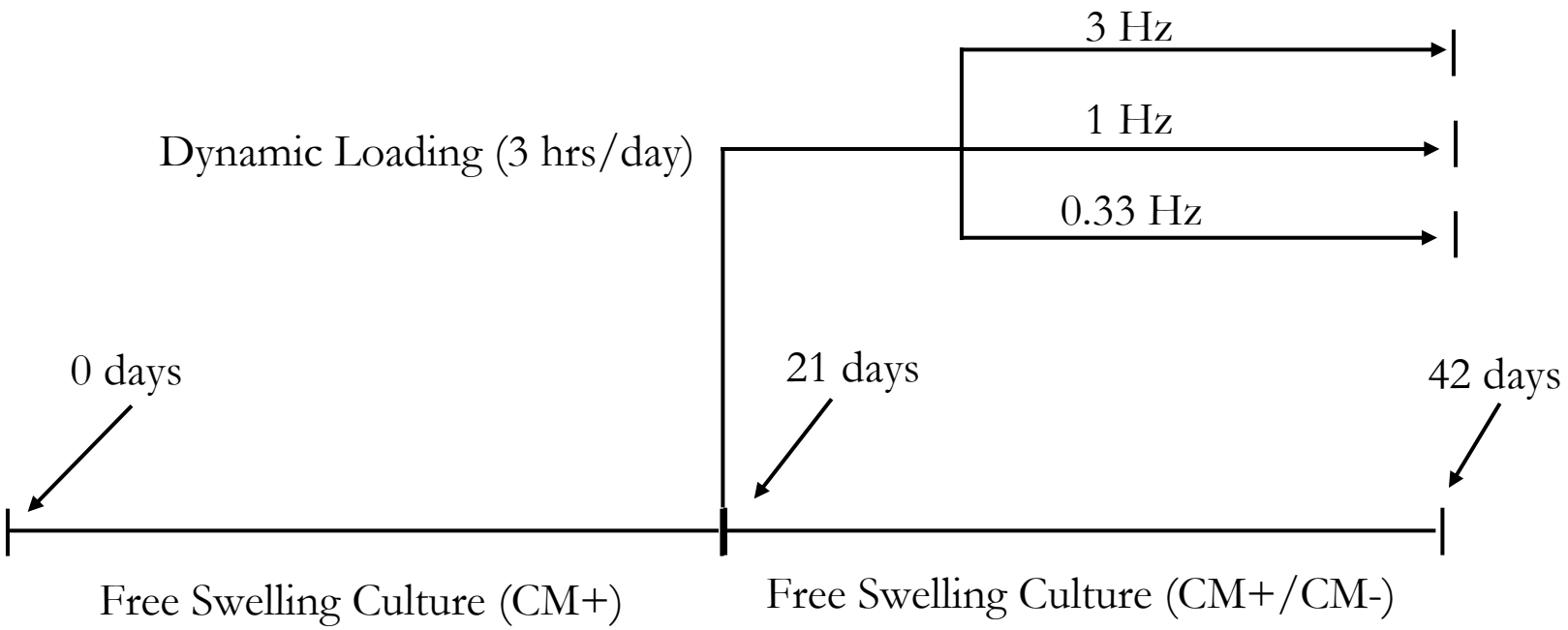
RX1: Long Term Mechanical Loading

Agarose and HA Hydrogels



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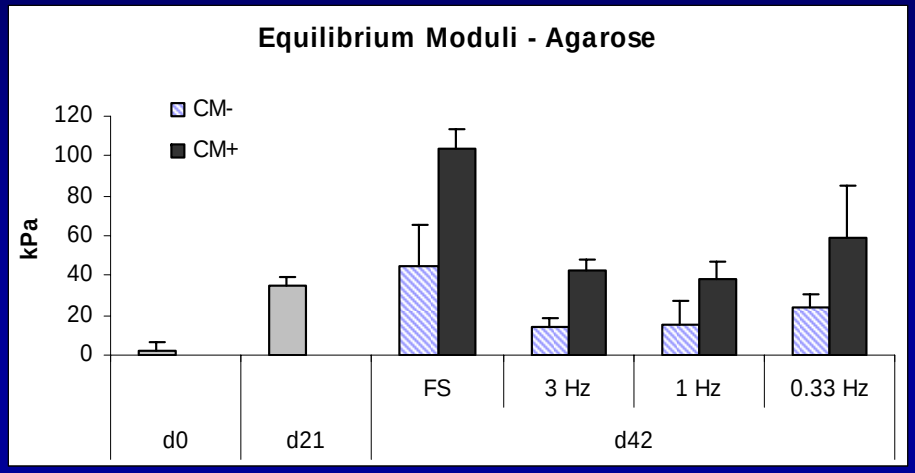
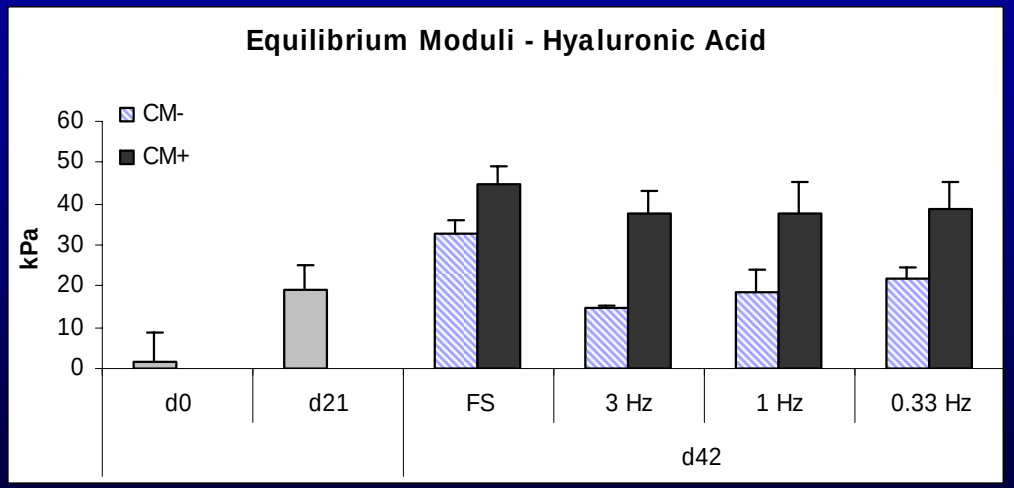
Dynamic Loading Causes Decrease in Equilibrium Moduli in Agarose and HA Constructs





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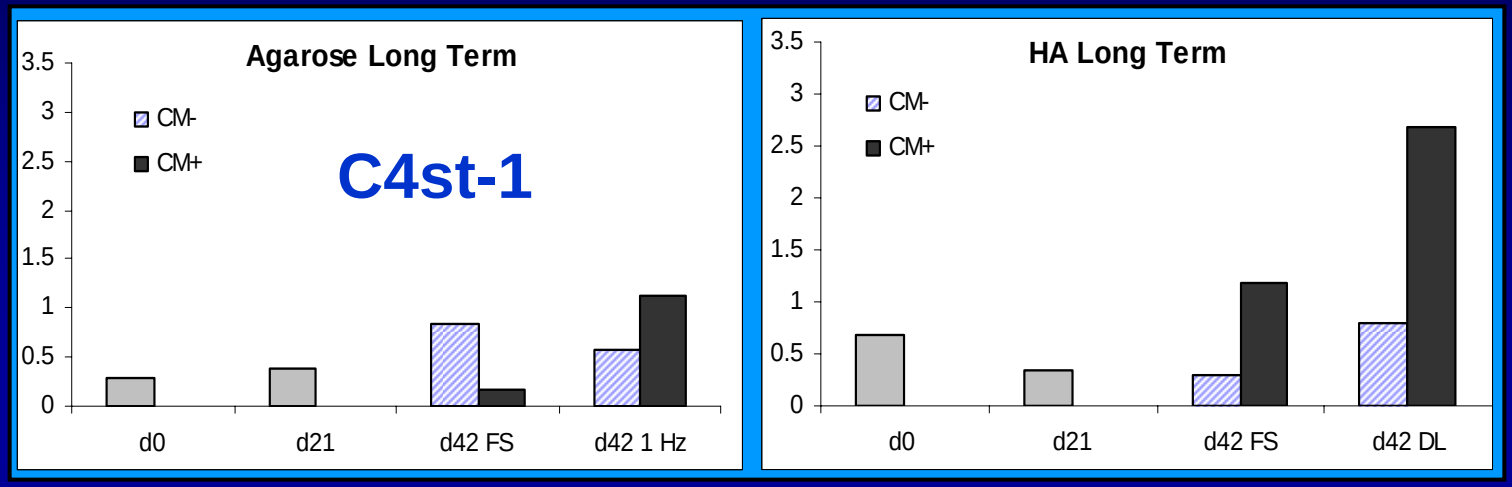
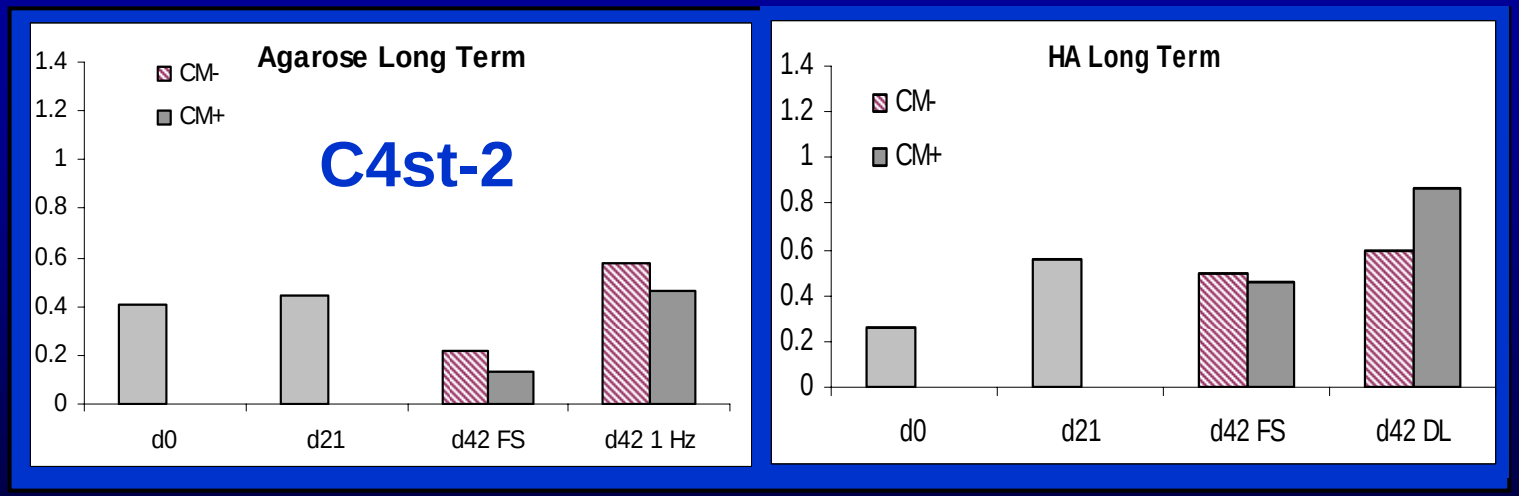
Matrix Biosynthesis Genes are Upregulated in Response to Long Term Loading





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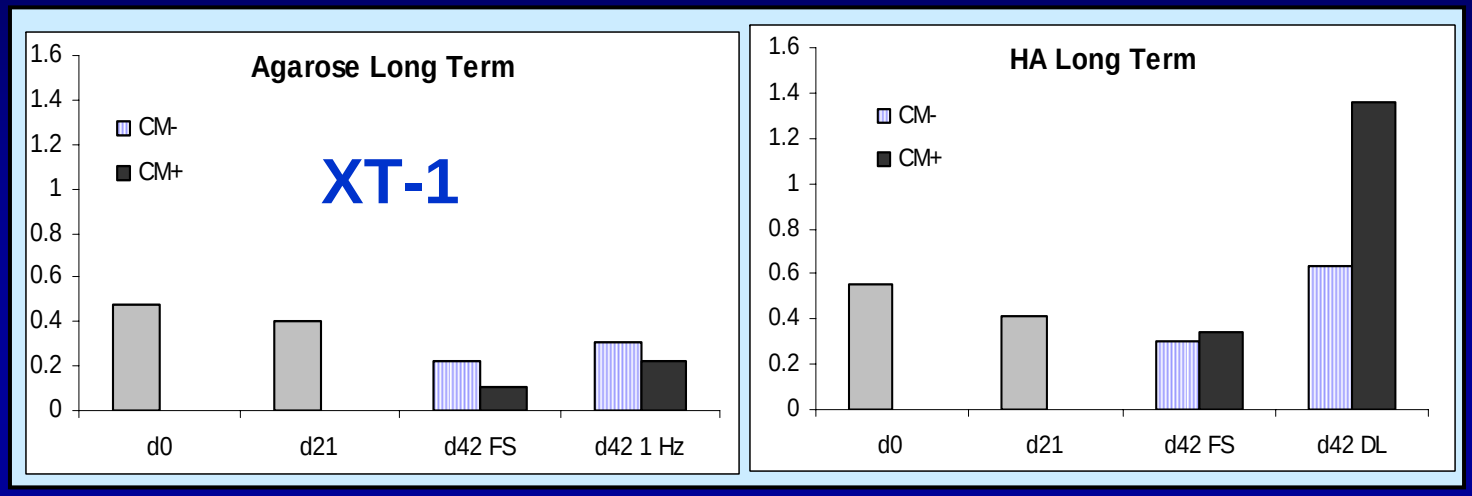

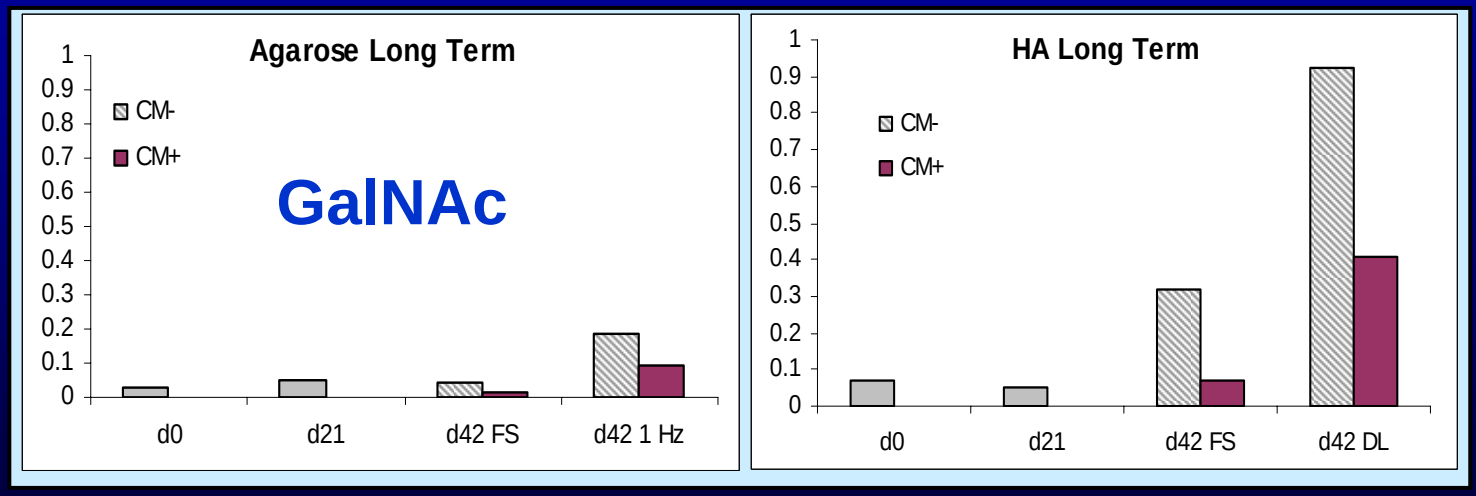

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Discussion

- Hyaluronic Acid is a viable alternative scaffold promoting cell proliferation and chondrogenesis
- Matrix biosynthetic genes C4st-1, 2, XT-1, and GalNAc are mechanically sensitive in MSCs
- Dynamic loading causes upregulation of biosynthetic genes
- Increases in gene expression levels do not translate to mechanical and biochemical improvements in the long term

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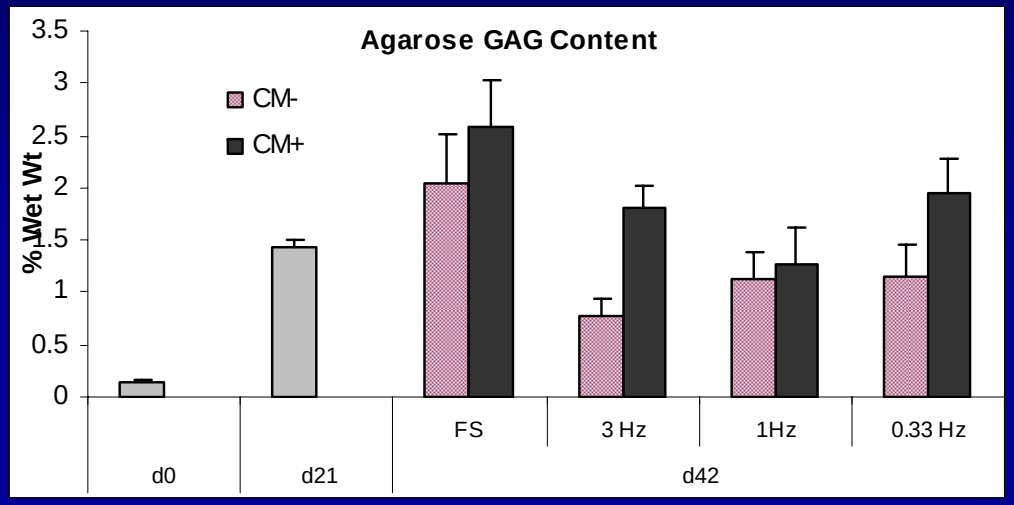
Thank You

Dr. Robert Mauck
Alice Huang
Isaac Erickson
Dr. Meira Yeger-McKeever
Brendon Baker
Nandan Nerurkar
Burdick Lab
Ashwin Nathan
Ashley Stein

NSF
SUNFEST

Dynamic Loading Decreases GAG Biosynthesis in Agarose Constructs



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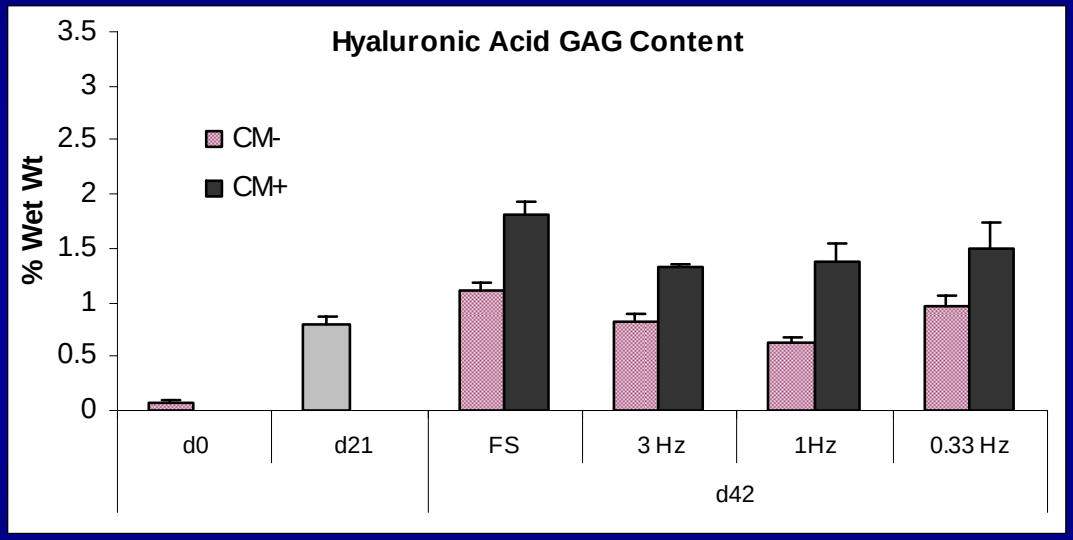
HA Hydrogels Show Negligible Changes in Biochemical Composition in Response to Dynamic Loading



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