Mechanotransduction in Stem Cells for Cartilage Tissue Engineering

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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
Cartilage Degeneration

Proteoglycan Loss
Collagen Damage

↓ Modulus
↑ Hydraulic Permeability

↑ Matrix Deformation
↓ Fluid Pressurization

↓ Load-Bearing Capacity
Tissue Engineering Paradigm

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

Cells
- Chondrocytes
- Mesenchymal Stem Cells (MSCs)

Scaffolds
- Polymer foams
- Polymer meshes
- Hydrogels

Extracellular Environment
- Mechanical Signaling
- Chemical growth factors
Mesenchymal Stem Cells

THE MESENGENIC PROCESS

Proliferation
- Mesenchymal Stem Cell (MSC)
  - MSC Proliferation

Commitment
- Osteogenesis
  - Transitory Osteoblast
    - Transitory Osteoblast
      - Myoblast Fusion
        - Myotube
          - Unique Micro-niche
            - T/L Fibroblast
              - TENDON/LIGAMENT
                - Adipocytes, Dermal and Other Cells

Lineage Progression
- Chondrogenesis
  - Transitory Chondrocyte
    - Chondrocyte
      - Hypertrophic Chondrocyte
        - CARTILAGE

Differentiation
- Osteoblast
  - Osteocyte
    - BONE

Maturation
- Marrow Stroma
  - Tendogenes/Ligamentogenesis
    - Other

Marrow/Poistosteum
- Mesenchymal Tissue
Scaffold Design

**Agarose**

- Thermo-crosslinkable hydrogel made of polysaccharides (D- and L- galactose)
- Cellular encapsulation
- Mechanical Properties
  - Not biodegradable
  - Immunogenic

**Hyaluronic Acid (HA)**

- Photo-crosslinkable polysaccharide hydrogel
- Natural extracellular matrix component – biodegradable
- Successful cell encapsulation
Physiologic Loading of Cartilage

Mechanical conditioning has been shown to improve chondrocyte mechanical properties

- Compressive Forces
- Sliding Forces
Mechanotransduction

Extracellular Signal

MAP Kinase activation / Signal transduction

Gene Expression Changes

Matrix Composition Biochemical Change

Mechanical Properties (Equilibrium Modulus, Dynamic Modulus, etc.)
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
Target Gene Expression Results

- **Xylosyltransferase-1 (XT-1)**
  - CHOND: d14 > d28 > d42 > d56

- **GalNAc4,6S-disulfotransferase (GalNAc)**
  - CHOND: d14 > d28 > d42 > d56

- **Chondroitin-4-sulfotransferase-1 (C4st-1)**
  - CHOND: d14 > d28 > d42 > d56

- **Chondroitin-4-sulfotransferase-2 (C4st-2)**
  - CHOND: d14 > d28 > d42 > d56
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
3D Hydrogel Culture

2% Agarose (@45°C) or 2% Hyaluronic Acid:
20 x 10⁶ cells/ml

Disks:
2.25 X Ø 5.0 mm

Bovine MSC Harvest and Expansion

Free Swelling Culture

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

Penn
Dynamic Compression Bioreactor

Dynamic Compression Bioreactor
(10% deformation, sinusoidal waveform)

Loading Plate

 Constructs in Petri Dish

Bioreactor Displacement

1.0 Hz

3.0 Hz

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

Dynamic Loading @ 1 Hz (CM+/CM-)

3 hours

3 hours

Free Swelling Culture (CM+/CM-)

24 hours

6 hours

TRIZOL extraction, cDNA synthesis, RT-PCR on all samples
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
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?
RX1: Long Term Mechanical Loading

Agarose and HA Hydrogels

Dynamic Loading (3 hrs/day)

0 days

Free Swelling Culture (CM+)

21 days

Free Swelling Culture (CM+/CM-)

3 Hz

1 Hz

0.33 Hz

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

**Equilibrium Moduli - Agarose**

- CM- (light blue bars)
- CM+ (dark black bars)

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<th>Time</th>
<th>Moduli (kPa)</th>
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<td>d0</td>
<td>10 ± 2</td>
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<tr>
<td>d21</td>
<td>20 ± 3</td>
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<tr>
<td>FS</td>
<td>50 ± 5</td>
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<tr>
<td>3 Hz</td>
<td>40 ± 4</td>
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<tr>
<td>1 Hz</td>
<td>30 ± 3</td>
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<tr>
<td>0.33 Hz</td>
<td>25 ± 2</td>
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**Equilibrium Moduli - Hyaluronic Acid**

- CM- (light blue bars)
- CM+ (dark black bars)

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Matrix Biosynthesis Genes are Upregulated in Response to Long Term Loading

**Agarose Long Term**

- **C4st-1**

  - CM-
  - CM+

**HA Long Term**

- **C4st-2**

  - CM-
  - CM+
Matrix Biosynthesis Genes are Upregulated in Response to Long Term Loading

Agarose Long Term

HA Long Term

XT-1

GalNAC
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
Dynamic Loading Decreases GAG Biosynthesis in Agarose Constructs

![Graph showing Agarose GAG Content](image)
HA Hydrogels Show Negligible Changes in Biochemical Composition in Response to Dynamic Loading

- Hyaluronic Acid GAG Content

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