Chondrocyte Response to Mechanical Loading: Cartilage Tissue Engineering and Molecular Nanomechanics

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Motivation: Progression of Degradation of Cartilage ECM in OA... Can Focal Injuries be repaired?
CARTILAGE Extracellular Matrix

Proteoglycans
- Aggrecan
- Versican
- Link protein
- Biglycan (DS-PG1)
- Decorin (DS-PGII)
- Epiphycan (DS-PGIII)
- Fibromodulin
- Lumican
- Perlecan
- SZP/Lubricin

Proteins
- COMP (Thrombospondin-5)
- Thrombospondin-1 and -3
- CMP (Matrilin-1)
- Matrilin-3
- CILP
- C-type lectin
- Fibronectin
- PRELP
- Chondroadherin
- Tenascin-C
- Fibrillin
- Elastin
- gp-39/YKL-40
- Matrix gla protein/ MGP
- Pleiotrophin
- Chondromodulin-I/SCGP
- Chondromodulin-II
- CD-RAP
- Growth factors
- Chondrocalcin
- PARP
- Lysozyme
- Phospholipase A2
- Proteinases and inhibitors

Collagen Types (% of total collagen)
- Type II (75% foetal, >90% adult)
- Type III (>10% in adult human articular)
- Type IX (covalently fibril-associated collagen, 10% foetal, 1% adult)
- Type X (hypertrophic cartilage only)
- Type XI (fibril template, 10% foetal, 3% adult)
- Type VI (chondron basket, microfilaments <1%)
- Type XII/XIV (non-covalently fibril-associated collagens)
- Type XIII (transmembrane)
Mediators and Cellular Changes involved in Cartilage Matrix Degeneration in OA
(Mary Goldring and Steven Goldring, J Cell Phys, 2007)
Disc Extracellular Matrix Composition
(Peter Roughley, Spine, 2004)

<table>
<thead>
<tr>
<th>Collagens</th>
<th>Proteoglycans</th>
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<tr>
<td><strong>Fibrillar</strong></td>
<td><strong>Aggregating</strong></td>
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<tr>
<td>Type I</td>
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<tr>
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<tr>
<td>Type III</td>
<td>Hyaluronan</td>
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<td>Type V</td>
<td>Link protein</td>
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<td>Type XI</td>
<td>Fibril-associated</td>
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<tr>
<td><strong>Fibril-associated</strong></td>
<td></td>
</tr>
<tr>
<td>Type IX</td>
<td>Decorin</td>
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<tr>
<td>Type XII</td>
<td>Biglycan</td>
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<tr>
<td>Type VI</td>
<td>Pericellular</td>
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<tr>
<td>Type X</td>
<td>Perlec an</td>
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• **Cartilage Tissue Engineering**: A Case Study

• **Molecular Mechanics, a Paradigm**: Why do cells in compression make aggrecan? Quantifying Aggrecan Nano-molecular Structure-Function

• **Mechanobiology**: Chondrocyte Gene Expression and Biosynthesis respond sensitively to Mechanical Loading & Injury

• **Cartilage Tissue Engineering**: Summary Example using MPCs in Self Assembling Peptide Scaffolds
Case Study:

Response of Chondrocytes in Self-Assembling Peptide Gel Scaffold to “Anabolic” Dynamic Mechanical Compression
Self-Assembling Peptide Gel Scaffold

(Kisiday+, PNAS, 2002;  J Biomech '04;  Tiss Eng ‘05)

Peptide: 12 amino acid -KLDLKLDLKLDLKLDL-

(Lys Leu Asp)$_4$

Bovine chondrocytes
Equine, bovine MPCs

30x10$^6$ cells/ml

Fiber: 10-20 nm diam. (gel= 0.4% solid)

Self-assemble with cells

K

L

D

w/o cells

0.5 $\mu$m

20 $\mu$m

1 cm
Self-Assembling Peptide Nanofiber Family

(Shuguang Zhang, 1995+)

- Peptides self-assemble into scaffolds for 3D tissue engineering.
- Self-assembly occurs at normal osmolarity and at physiological pH.

Twisted beta-sheet tape

Beta-sheet helical tape
Extracellular Matrix Synthesis In Vitro
Kisiday+, PNAS 2002

• Bovine calf chondrocytes secrete proteoglycans over time

• Equil. & Dynamic compressive modulus approach 1/4 that of native tissue after ~4 wk

• Collagen synthesis is predominantly type II (electrophoresis of extracted collagens, immunohistochemistry)

• Cell division increased the density of viable cells ~80% over the first 9 days of culture
Compressive Stiffness of Peptide-NeoTissue

(Kisiday, PNAS '02)

Equilibrium Modulus (kPa)

Dynamic Stiffness (kPa), 1 Hz

Mean +/- SEM (n=3)

- Eq.
- Dyn.

FBS

ITSMean +/- SEM (n=3)

*30x10^6 cells/ml

*DAY 28

15x10^6 cells/ml

DAY 0

DAY 6

DAY 26

DAY 28

Equilibrium Modulus (kPa)

0

10

20

30

40

50

60

70

80

90

100

110

120

130

140

150

160

170

180

190

200

DAYS

0

6

26

28

*10^6 cells/ml
Add Mechanobiology: Apply dynamic compression to enhance neo-tissue synthesis & functionality
(Kisiday+, J Biomech, 2004; Tissue Eng, 2005)

Dynamic compression:
- Frequency: 1 Hz
- Static offset: 5%
- Sinusoidal amplitude: 2.5%
- Alternate Day Loading
- (45min on / 5hr-15min off) X4
Dynamic Comp $\rightarrow$ GAG $\uparrow$ $\rightarrow$ stiffness $\uparrow$

GAG Accum. In Scaffold ($\mu$g GAG/mg wet weight)

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<td>Day 8</td>
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<td>2</td>
<td>6</td>
<td>3</td>
<td>13</td>
<td>16</td>
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*p=0.01

Free Swelling Control

(Kisiday+, J Biomech, 2004)
(Kisiday+ Tissue Eng, 2005)
Cartilage Repair Strategy

• **Autologous Cells:** *Marrow derived Progenitors*
  – Chondrogenesis capable
  – Easily harvested and expanded
  – Minimize risk of disease & immunogenicity

• **Scaffold:** *Self-assembling peptide hydrogel*
  – Potential for minimally-invasive delivery & *in situ* assembly
  – Promotes chondrogenesis of MPCs

• **Equine model:**
  – Biomechanics similar to human
  – Horses get Osteoarthritis
  – CSU: Cartilage defect repair model
Many Questions:

• What is mechanical environment of construct in the joint, and how do the cells respond to mechanical loading after implantation?

• Are the emerging tissue-level & molecular level mechanical properties sufficient?

• What is fine structure of the ECM molecules produced? Are their molecular mechanical properties sufficient for success?
Real Time In Vivo Cartilage Contact Deformation

Ankle Joint

Guoan Li+ (MGH), J Biomech, 2008

Combined Dual-Orthogonal Fluoroscopic + MR Imaging
In Vivo Ankle Cartilage Creep Deformation

(Guoan Li+, J Biomech, ‘07; JOR ‘08)

healthy males age 32-42

Time (seconds)

Peak Strain (%)
Equilibrium Mechanical Properties of Normal Articular Cartilage

**Compression:**
- Equilibrium Mod: ~1 MPa
- Dynamic Comp Stiffness ~10x higher at 1Hz

**Tension:** 20 - 50 MPa

**Shear:** 0.2 - 0.5 MPa
Cartilage Stiffness & Fluid Permeability in “Dynamic Confined Compression”
Dynamic Compression & Streaming Potentials: “Poroelastic Electromechanical Behavior”

(Frank+ ’87)
Mow+, ’80; Eisenberg+ ‘87; Huyghe + ‘95, ‘02; Lai + 1991; Soulhat+, ‘99
A very talented “tissue engineer”

Chondrocyte in native cartilage
Rabbit Articular Cartilage

(E. Hunziker)

1 mm
Collagen Fibrils: Resist Tension & Shear
AGGRECAN: Resists “Static” Compression & Fluid Flow (“Dynamic” Compression)

EM:
Buckwalter, Rosenberg 1980’s

AGGRECAN (3 MDa)

G1 G2

Link protein

Hyaluronan

(−) charged CS-GAGs

Aggregate (300 MDa)
Molecular Structure of Aggrecan: Tapping Mode Atomic Force Microscopy

Aggrecan from Bovine Fetal and Mature cartilages (in ambient conditions)

(collaboration with Christine Ortiz, Anna Plaas, John Sandy)
Aggrecan Monomer
(Bovine Fetal Epiphyseal)
(Laurel Ng+, J. Struc Biol, 2003)

EM:
Buckwalter, Rosenberg
1980’s

…why does the cell make such a complicated, charged molecule to resist compressive loads??
(cells in cartilage, disc, tendon, many other tissues)
Mechanical & Biological Factors Regulate Cellular Biosynthesis

- DNA
- mRNA
- Transcription
- Nucleus
- Translation
- ER
- Golgi
- Post-Trans Mod
- Secretion
- Signaling
Aggreccan structure varies with age, loading, species, health/disease in native tissue

(Laurel Ng+, J. Struc Biol, 2003)

(Fetal Bovine)  (Mature Bovine)

• Is it mechanically functional & optimal in long run?
• Which is made in tissue engineered constructs?
GAG-GAG spacing ~ Elec. Debye length!!

Fetal

Length\textsubscript{contour} = 41 \pm 7 \text{ nm}
GAG Spacing = 3.2 \pm 0.8 \text{ nm}

Bovine

32 \pm 5 \text{ nm}
3.2 \pm 0.8 \text{ nm}

Adult

4.4 \pm 1.2 \text{ nm}

(Debye Length \sim 1 \text{ nm at physiological ionic strength})
OUTLINE

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• **Mechanobiology**: Chondrocyte Gene Expression and Biosynthesis respond sensitively to Mechanical Loading & Injury

• **Cartilage Tissue Engineering**: Summary Example using MPCs in Self Assembling Peptide Scaffolds
AFM – Contact mode imaging  
(in fully hydrated state)

constant normal load

Tip scan rate $\sim 60 \, \mu m/s$

$R_{tip} \sim 50 \, nm$
Nanosized Tip interacts with
2-4 aggrecan

$R_{tip} \sim 2.5 \, \mu m$
Colloidal Tip interacts with
$>1,000$ aggrecan
AFM – Contact mode imaging (in fully hydrated state)

Aggrecan Height Image of Brush Patterned onto Au-coated chip via “micro-contact printing” (1 aggrecan per ~25 x 25 nm)

Dean+, Macromol 2005

(a)
**Human:** Newborn aggrecan is stiffer

- Stress-strain curves calculated from the micro-size tip force-displacement data using Derjaguin approximation

![Stress-strain curves](image)

Hsu-Yi Lee: ORS, 2008
Why does the cell make Aggrecan??

![Stress vs. Strain Graph](image)

- Stress (MPa)
- Strain

0.1M NaCl

< 4-5 Debye lengths

GAG
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How do Mechanical Forces affect Expression and Synthesis in normal (cartilage) tissue and in engineered (cartilage) constructs?

~ 1 Cycle / sec
JOINT LOADING Hypothesis

Mechanical Signals:
- Cell & Matrix Deformation
- Fluid Flow
- Pressure Grad
- Streaming Potentials
- Transport of Growth factors, cytokines, nutrients
Experimental Details

Bovine calf femoropatellar groove

Cartilage bone cylinders: 9mm diameter

Cartilage slices: 9mm x 1mm

Cartilage disks: 3mm x 1mm
Cartilage Explants & Tiss Eng Constructs
Chondrocytes in Cartilage

- **Static Compression**: Inhibits ECM Biosynthesis

- **Moderate Dynamic Compression and Dynamic Tissue Shear**: Can Stimulate ECM Biosynthesis

Palmoski and Brandt, 1984; Gray et al., 1988; Sah et al., 1989; Urban et al., 1993; Parkkinen et al., ‘93; Giori et al., ‘93; Sah et al., 1996; Quinn, ’98; Hering, 1999; Buschmann et al., 1999; Smith et al., 2000; Bonassar et al., 2000; Hung et al., 2000; Guilak et al., 2000; Jin et al., 2001, 2003; others…
**Dynamic Compression: Stimulates Synthesis & Augments Transport of Soluble Factors**

Native Cartilage Explants

(Bonassar et al., JOR, 2001)

**Aggrecan synthesis**

![Chart showing proteoglycan synthesis](chart1.png)

<table>
<thead>
<tr>
<th>ng/ml IGF-1</th>
<th>0% compression</th>
<th>3% @ 0.1 Hz</th>
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<tbody>
<tr>
<td>0</td>
<td>0.2</td>
<td>0.6</td>
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<tr>
<td>300</td>
<td>0.4</td>
<td>0.8</td>
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48 hrs

**125I-IGF-1 Transport**

![Chart showing IGF-1 transport](chart2.png)

Tissue [IGF-1] (CPM)/[CPM]*

<table>
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<th>Time, hr</th>
<th>Control</th>
<th>2% @ 0.1 Hz</th>
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<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>12</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>24</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>36</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>48</td>
<td>2.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

τ = 12.6 hr

τ = 6.0 hr

n = 8 +/- SD
Dynamic Tissue Shear: uniformly stimulates aggregcan, collagen synthesis

Shear: ~No pressure gradients or relative fluid flow $\rightarrow$

Hypothesis: matrix deformation $=$ physical stimulus

Pericellular matrix

(Jin et al., 2001, 2003)
Relative Gene Expression

Free swelling bovine cartilage

Lee+, Arth Rheum 2005: Real Time PCR

[Graph showing relative mRNA abundance of various genes and proteins]
Effects of compression: Chondrocyte gene express.

- Resulting compressive stress, relaxation, equilibrium
- Fluid flow
- Applied ramp-&-hold compression of explant
- Static Equil.
- ECM synthesis down by 1 hour
- 1-24 Hours
"Systems Biology Approach": Clusters of Co-expressed Genes Compression → Multiple time dependent expression patterns & involves Intracellular Calcium and Cyclic AMP

Centroid 1

Centroid 2

Centroid 3

Centroid 4

Main Expression Trends

(Fitzgerald+ J Biol Chem, 2004)

aggrecan, collagen II, cfos, cjun

link protein, MMP-1, TIMP2, sox9, fibromodulin, MAPk1

MMP3, MMP9, MMP13, TIMP1, ribosomal 6-P, collagen1

ADAMTS4, ADAMTS5, TIMP3, fibronectin, HSP70, TGFβ, COX-2
Mechano-Induced Gene Expression Trends
→ PCA + K-means Clustering (“Systems Biol.” Approach)
( Fitzgerald, J Biol Chem, 2006 )

Ramp-&-hold transient → static compression

\[
\begin{align*}
\text{Group 1: Aggrecan, Collagen II,} \\
\text{Link, Fibromodulin Fibronectin}
\end{align*}
\]

\[
\begin{align*}
\text{Group 2: MMP3, MMP9,} \\
\text{MMP13 ADAMTS4, TNF}\alpha, \\
\text{COX-2, Type I Collagen}
\end{align*}
\]
MAPK signaling (ERK1/2; p38) is a key component in the mechanotransduction pathway.

ERK, p38 activated by compression & shear; Blocking ERK (U0126) and p38 (SB203580) suppresses mechanically induced changes in gene expression.

(Fitzgerald and Jin, J Biol Chem, 2008)
Mechanotransduction Pathways

- Integrin signaling pathways
- MAPKs (ERK1/2, SAPk, p38, JNK)
- Intracellular Calcium
- cAMP
- Injurious compression & proinflammatory pathways
Chondrocyte Biosynthesis & Gene Expression in response to “Loading” is extremely sensitive to the specific parameters of “loading”

- Static Compression
- Dynamic Compression
- Dynamic Shear
- Injurious Compression
Injurious Compression

Injury: 50% strain
1/s strain rate

Strain (%)
Stress (MPa)

Time (seconds)

0 0.5 1 1.5

50
25
0

Injurious Compression

Injury: 50% strain
1/s strain rate
Little or no effect on expression of Matrix Molecules

(Lee+ Arthritis Rheum, 2005)
But…Injury → Increased Expression of Aggrecan & Collagen Degrading Enzymes

- MMP-3 (blue)
- ADAM-TS5 (red)
- MMP-9 (green)
- ADAM-TS4 (purple)

X Free Swell

Time after Injury (hours)

0 5 10 15 20 25

(MMP sites)

341

 aggrecanase sites (ADAM-TS)

1545, 1714, 1819, 1919

(Lee+ Arthritis Rheum, 2005)

(Tapping mode AFM)

Ng+ 2003

Caterson+ 2000
Collagen Degrading Enzymes

![Graph showing the relationship between Time after Injury (hours) and X Free Swell for MMP-1 and MMP-13 enzymes.](image)

- **MMP-1**
- **MMP-13**

**Collagen II Bound MMP**

*(Lee+ Arthritis Rheum, 2005)*

**Tapping mode AFM Sun+ 2000**
# Acknowledgements

## Graduate Students

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
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<tbody>
<tr>
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<td>Mat Sci</td>
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<tr>
<td>Hsu-Yi Lee</td>
<td>EECS</td>
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<td>Sangwon Byun</td>
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<td>Diana Chai</td>
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<td>Yi Sui</td>
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<td>Krishna Swaminathan</td>
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<td>Emily Florine</td>
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## Collaborators

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<tr>
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<tr>
<td>Christine Ortiz</td>
<td>MIT</td>
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<td>Anna Plaas</td>
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<td>John Sandy</td>
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<td>John Kisiday</td>
<td>Colo. St. U.</td>
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<td>Dave Frisbie</td>
<td>Colo. St. U.</td>
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## Recent Students on Project

<table>
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<tr>
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<tr>
<td>Prof. Delphine Dean</td>
<td>Clemson</td>
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<td>Prof. Joonil Seog</td>
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<td>Titan</td>
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<td>Dr. Lin Han</td>
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## Research Staff

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<tr>
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<tr>
<td>Han-Hwa Hung</td>
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## Funding

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