Harvard-MIT Division of Health Sciences and Technology
HST.535: Principles and Practice of Tissue Engineering
Instructor: Alan Grodzinsky

Mechanical Regulation of Chondrocyte Metabolism: Cartilage Tissue Engineering & Molecular Nano-Mechanics

Alan Grodzinsky

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Mechanobiology, Tissue Engineering, and Molecular Nano-Mechanics

- Loading environment in vivo; matrix constituents
- Tissue Engineering case study: cell-seeded self-assembling peptide scaffold for cartilage
- **Mechanobiology:** loading affects transcription, translation, post-translational and biosynthesis of matrix molecules: rate & molecular structure
- Molecular Mechanics: importance of matrix nano-structure & molecular interactions to macro-tissue mechanical properties

JOINT LOADING (Stresses & strains on cartilage)

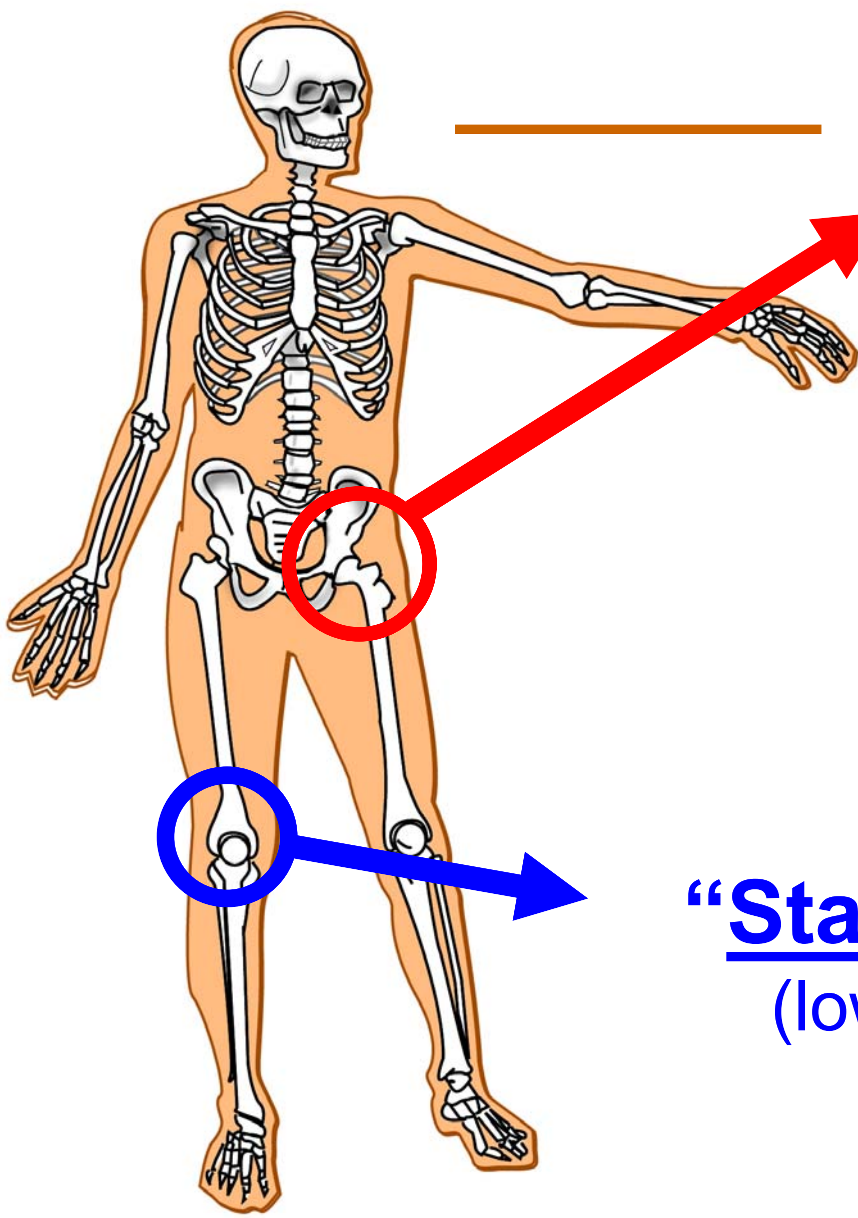


Figure by MIT OCW.

≤ 15-20 MPa Peak Stress

(but only 1-3% "strain")

(Hodge+, PNAS, '86)

"**Static**" Compression **0 → 45%**

(lower compressive stress ~3 MPa applied for 10's min.)

(Eckstein, J Biomech, 2000)

Collagen Fibrils: Resist Tension & Shear

Photos and diagrams removed for copyright reasons.

AGGRECAN: Resists Compression & Fluid Flow

EM:

Buckwalter, Rosenberg 1980's

Photo removed for copyright reasons.

AGGRECAN (2 MDa)

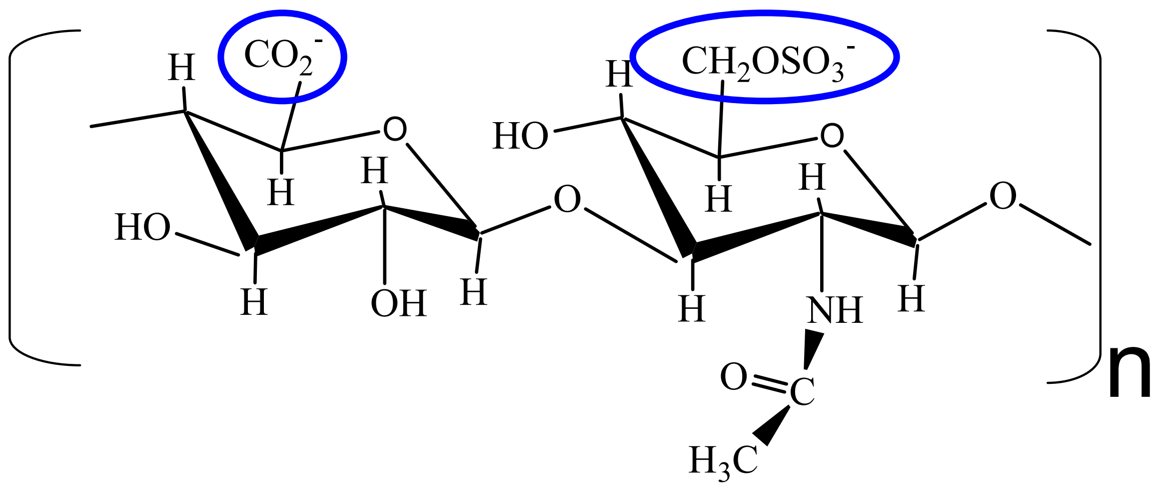
G1 G2 G3

Diagram removed for copyright reasons.

Link protein

Hyaluronan

(–) charged CS-GAGs



Aggregate

(200 MDa)

Tapping Mode AFM

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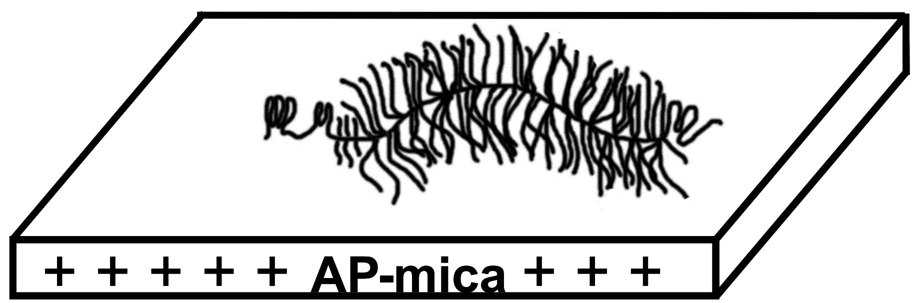
Aggrecan from Bovine Fetal and Mature cartilages (A1A1D1D1) (ambient conditions)



Laurel Ng+, J Struc Biol, 2003

Single Aggrecan

EM:

Buckwalter,
Rosenberg
1980's

Photo removed for copyright reasons.

Photo removed for copyright reasons.

A "polyelectrolyte brush within a brush"

Aggregate

Ng+, J Struc Biol, 2003

- Aggrecan in cartilage is ~10x more dense
- Aggrecan & other (ECM) continually made by cells

Photos removed for copyright reasons.

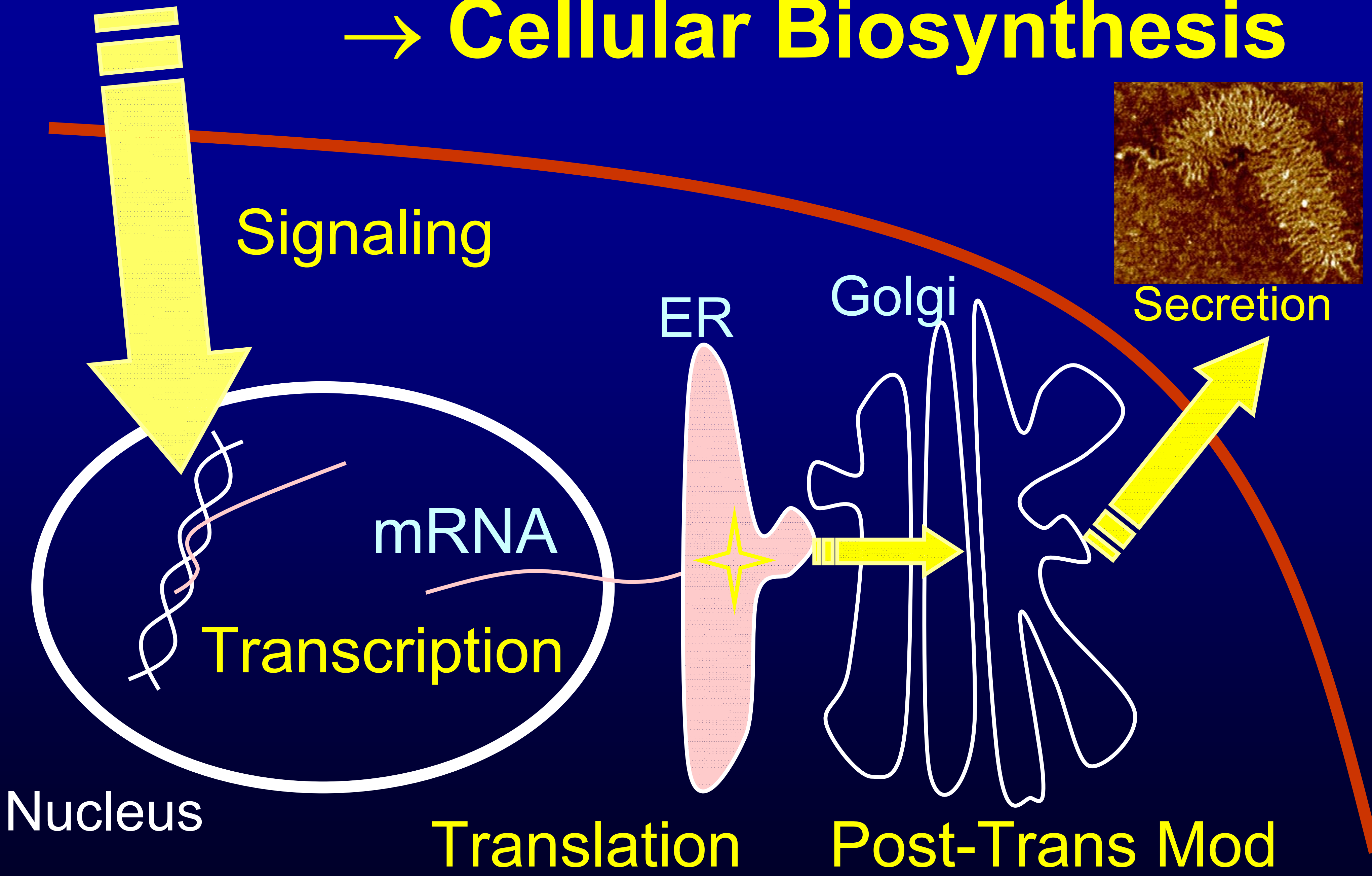
Dense monolayer on mica, tapping mode AFM, **(Ng, J Struc Bio '03)** (collaboration with Christine Ortiz, Anna Plaas, John Sandy)

Photo removed for copyright reasons.

A very talented tissue engineer

Chondrocyte in native cartilage

Mechanical & Biological Factors → Cellular Biosynthesis



Molecular Self Assembly of aggrecan-aggregate outside cell in dense matrix

Photo removed for copyright reasons.

That’s normal cartilage….

Degradation of aggrecan/collagen ECM is a hallmark of Osteoarthritis

Building up functional ECM, despite normal catabolic turnover, is hallmark of Tissue Engineering

Photo removed for copyright reasons.
Cover of “MosaicPlasty Osteochondral Grafting: Technique Guide” by Hangody, L. et al.

Injury → Focal Defect:

Surgical Approaches to Repair:

- Cell Transplantation
- Mosaic Plasty
- Microfracture
- Drilling...

Photos removed for copyright reasons.

Early Stage Osteoarthritis:

Loss of sulfated GAG

Photo removed for copyright reasons.

"End Stage" at Joint Replacement

>0.5 million per year in USA

Tissue Engineering: Mechanobiology and Molecular Nano-Mechanics

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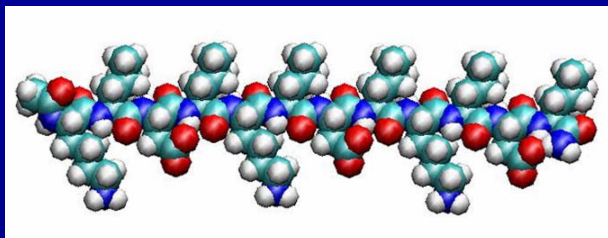
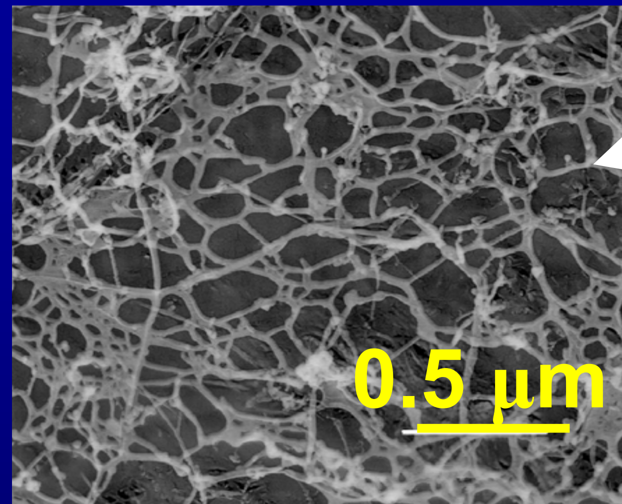
"Tissue Engineering" using Self-Assembling Peptide Gel Scaffold **(Kisiday+, PNAS, 2002)**



$(KLDL)_3$

w/o cells

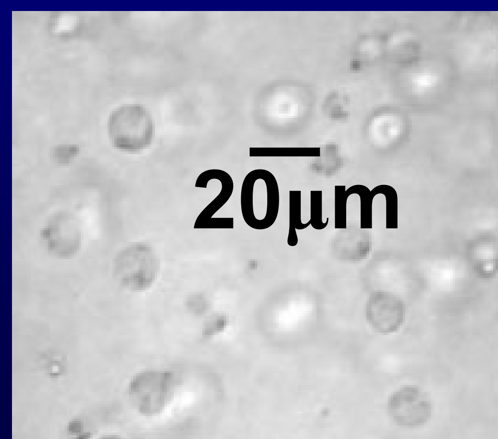


Fiber:
10-20 nm diam.

(gel= 0.4% solid)

Self-assemble with cells

bovine chondrocytes

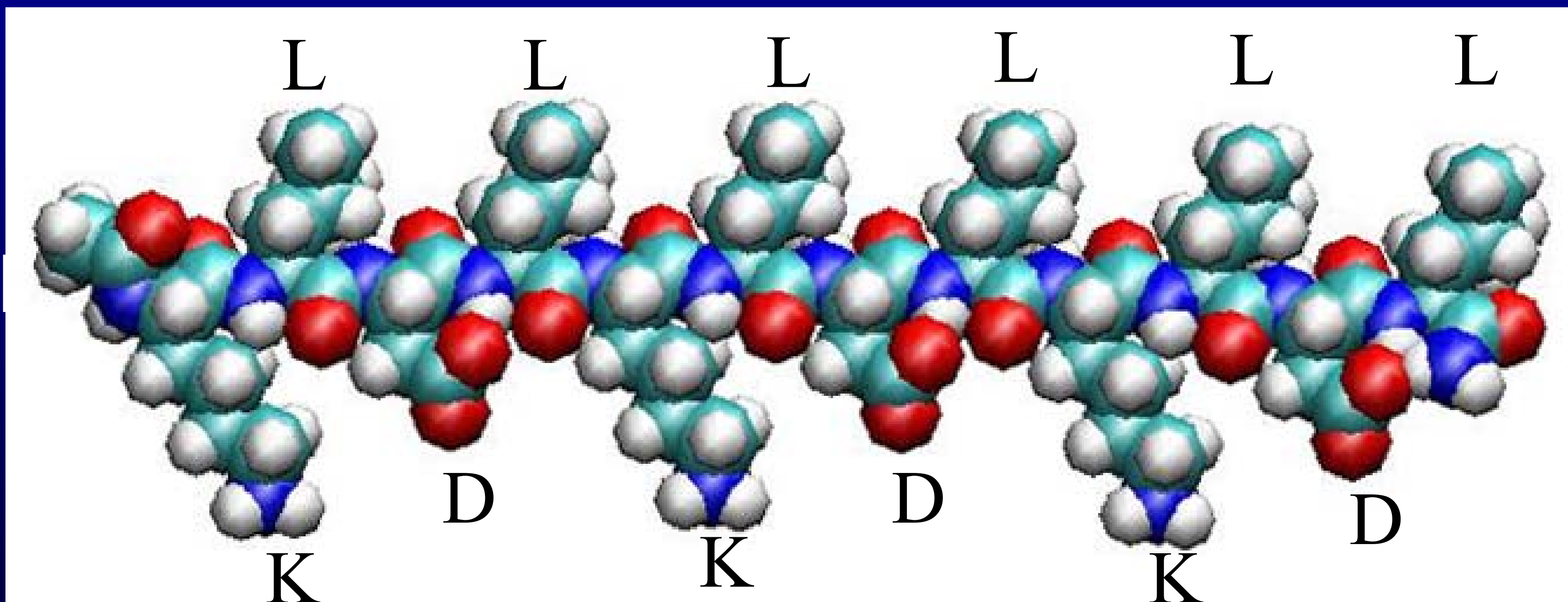


$30x10^6$ cells/ml

1 cm

Cartilage Tissue Engineering Using Self-Assembling Peptides

- Alternating hydrophobic/hydrophilic side groups

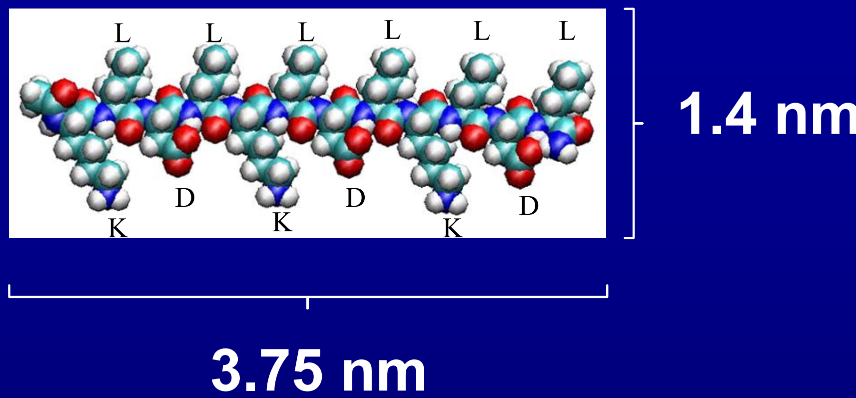


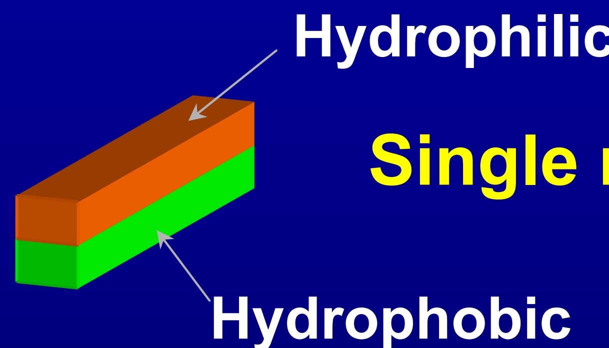
- Experimental Sequence - “KLD12”

[lysine (**K**) - leucine (**L**) - aspartate (**D**) - leucine (**L**)]$_3$

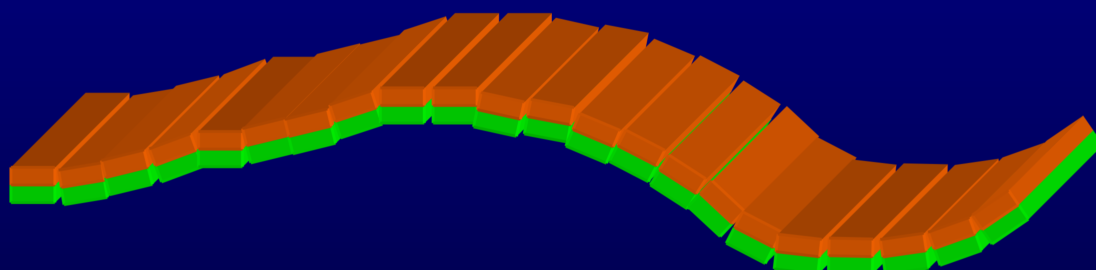
Peptide Nanofiber Formation

[lysine (**K**) - leucine (**L**) - aspartate (**D**) - leucine (**L**)]**3**



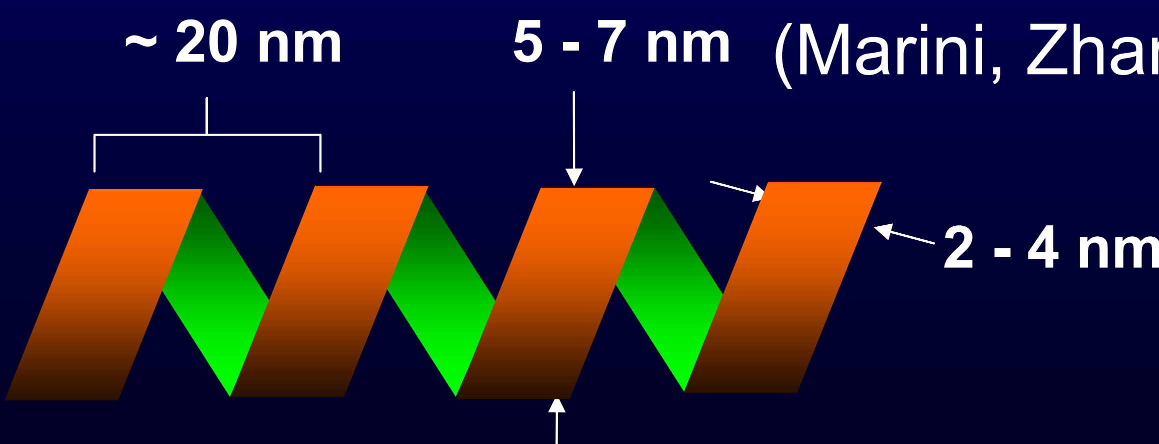


Single molecule



β-sheet tape: single molecule thick

(Marini, Zhang+ 2002)



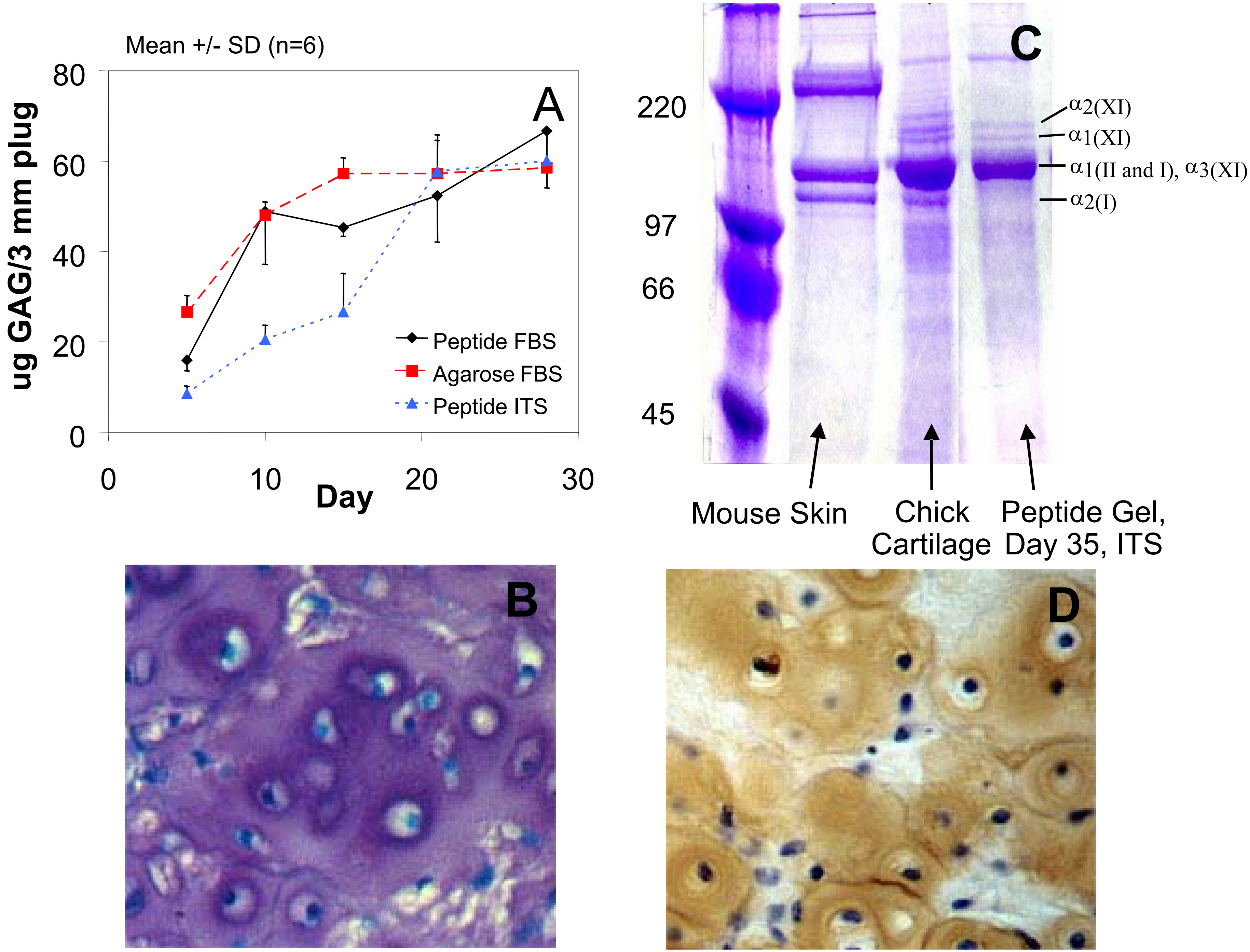
Twisted tape

Photo of first page of this article
- removed for copyright reasons.

Watson & Crick
Nature,
April 2, 1953

"Molec. Struc. of
Nucleic Acids"

GAG Accumulation   Type II Collagen



Compressive Stiffness of Peptide-NeoTissue

(Kisiday, PNAS '02)

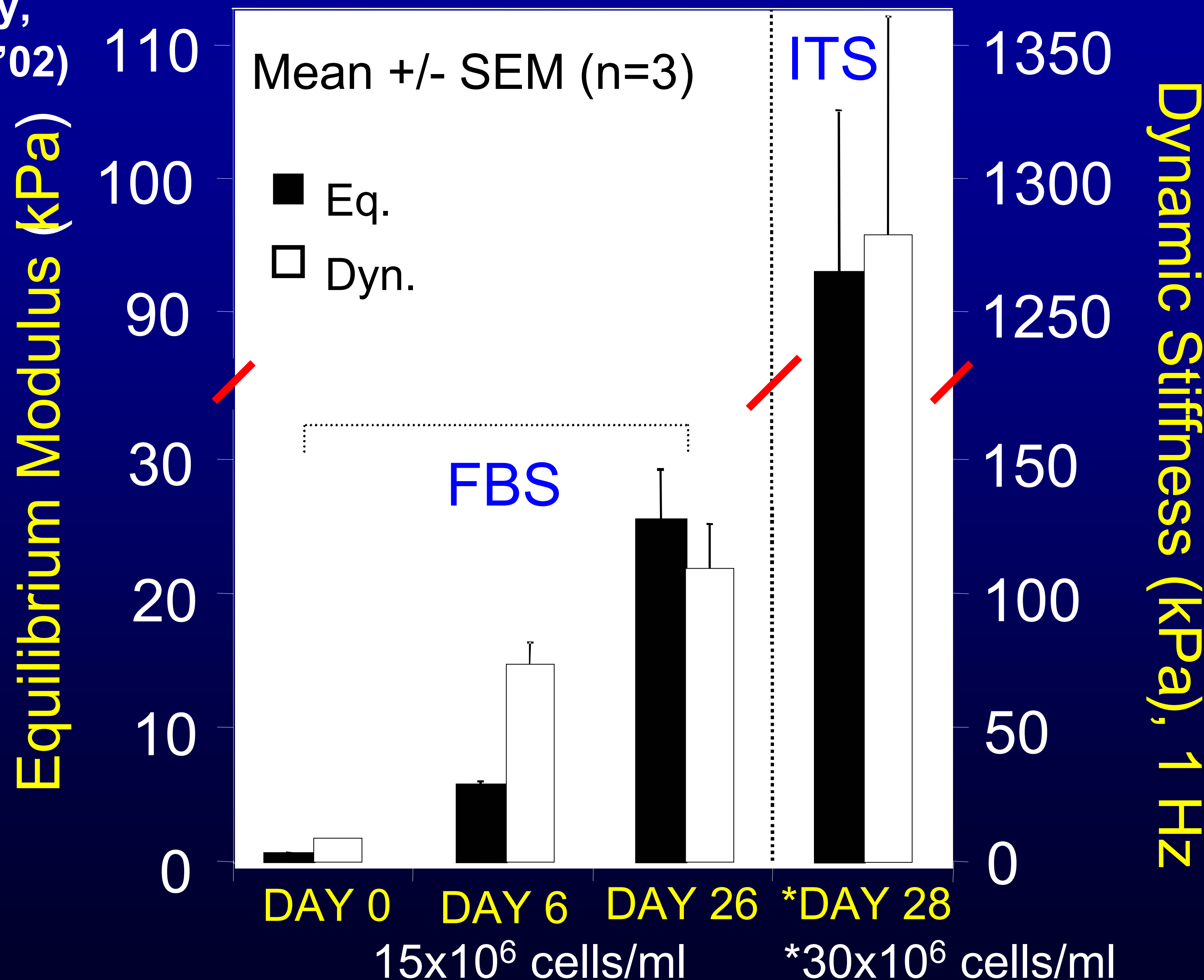


Photo removed for copyright reasons.
Bicyclist, with their knee highlighted.

How do Cells Respond to Joint Loading in normal and tissue engineered cartilage?

~ 1 Cycle / sec

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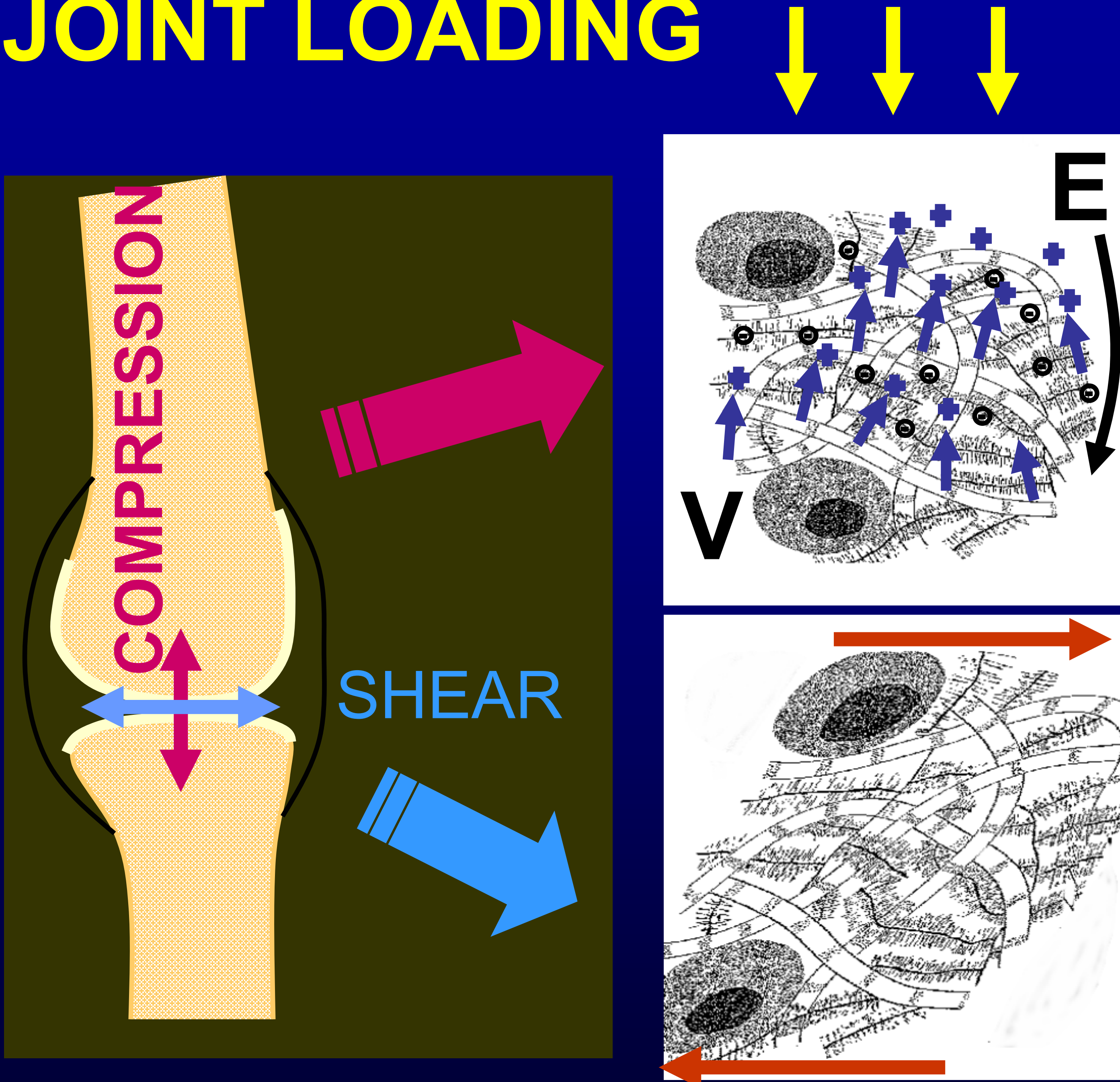
JOINT LOADING

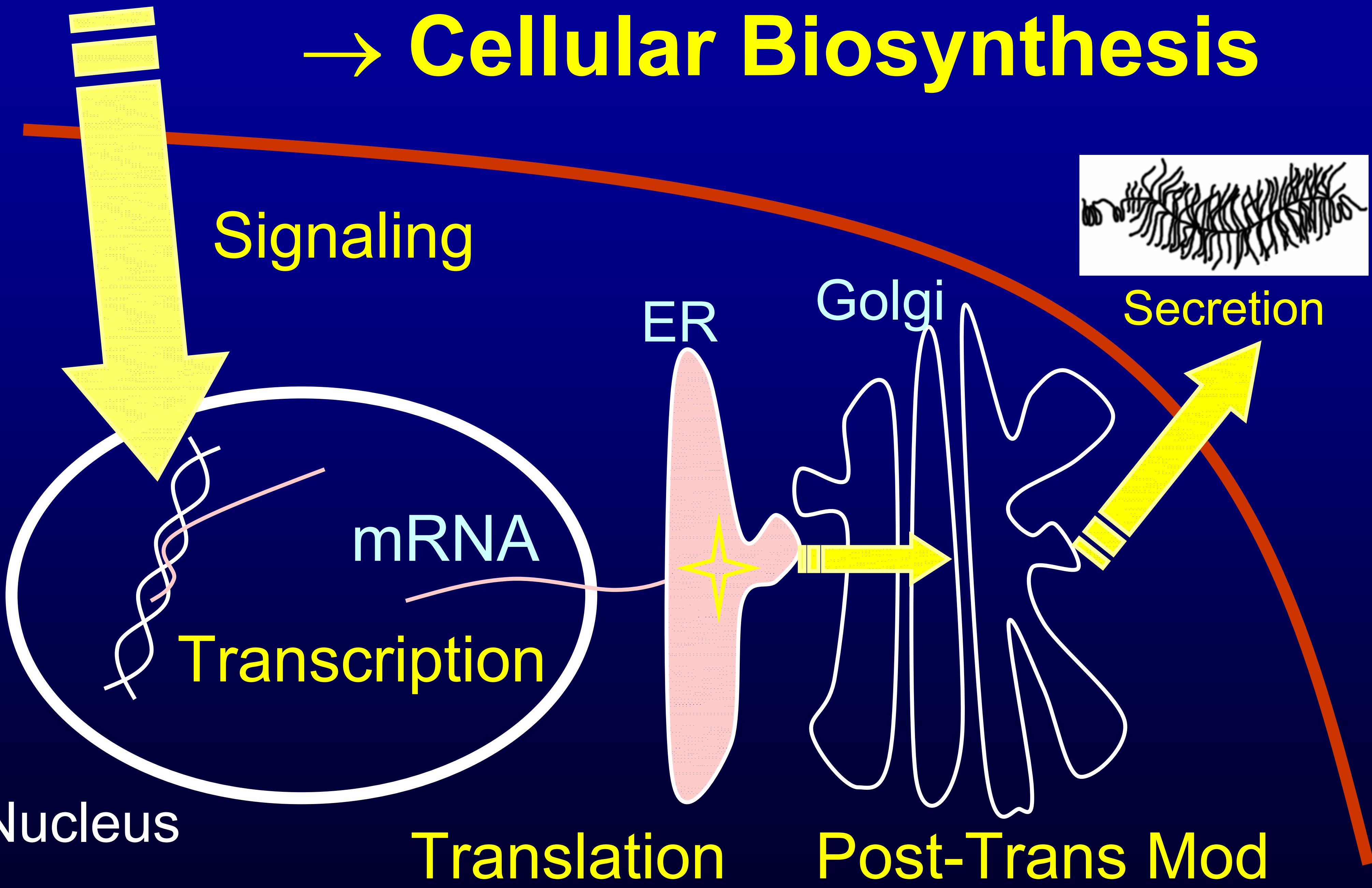


Physical Signals:

- Cell & Matrix Deformation
- Fluid Flow
- Pressure Grad
- Streaming Potentials

Transport of Growth factors, cytokines, nutrients

Physical & Biological Factors → Cellular Biosynthesis



Cartilage Explants & Tiss Eng Constructs

Photo removed for copyright reasons.

Frank +
J Biomech
2000

IN VITRO STUDIES

- **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.,* 1993; Giori *et al*., 1993; Sah *et al*., 1996; 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; ……..

Dynamic Compression: Stimulates Synthesis & Augments Transport of Soluble Factors

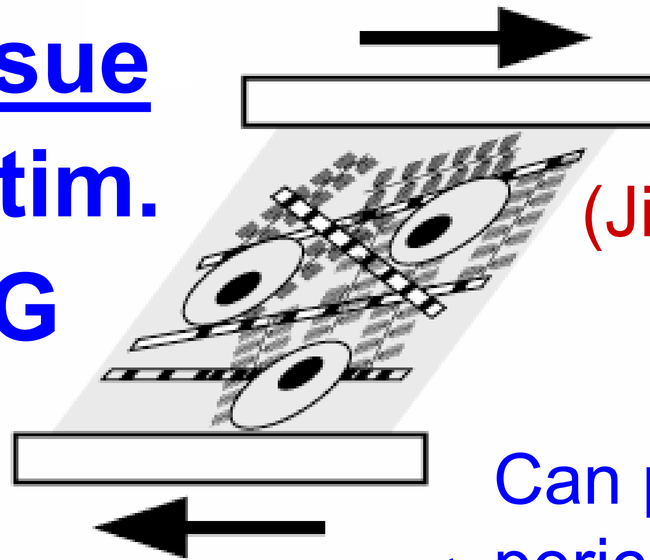
Native Cartilage Explants

(Bonassar et al., JOR, 2001)

Biosynthesis ^{125}**I-IGF-1 Transport**

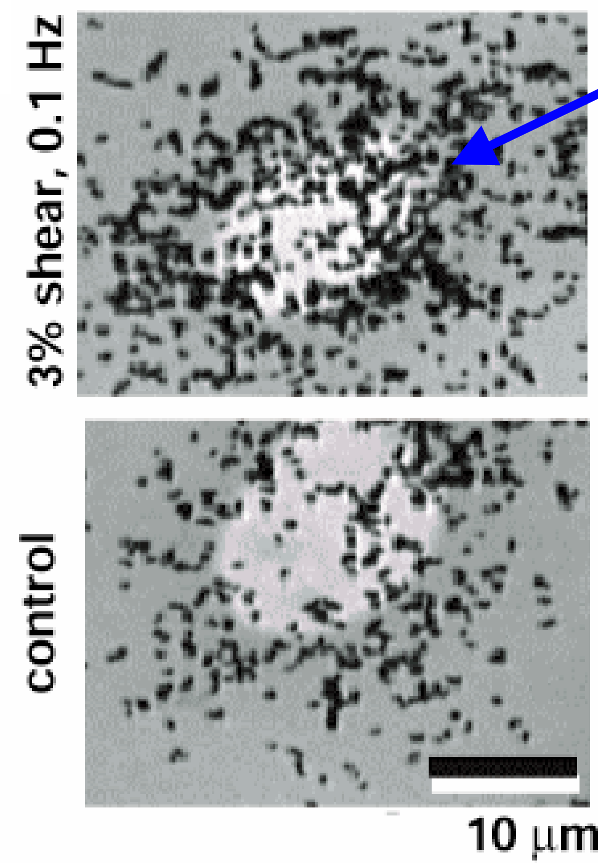
Graphs removed for copyright reasons.

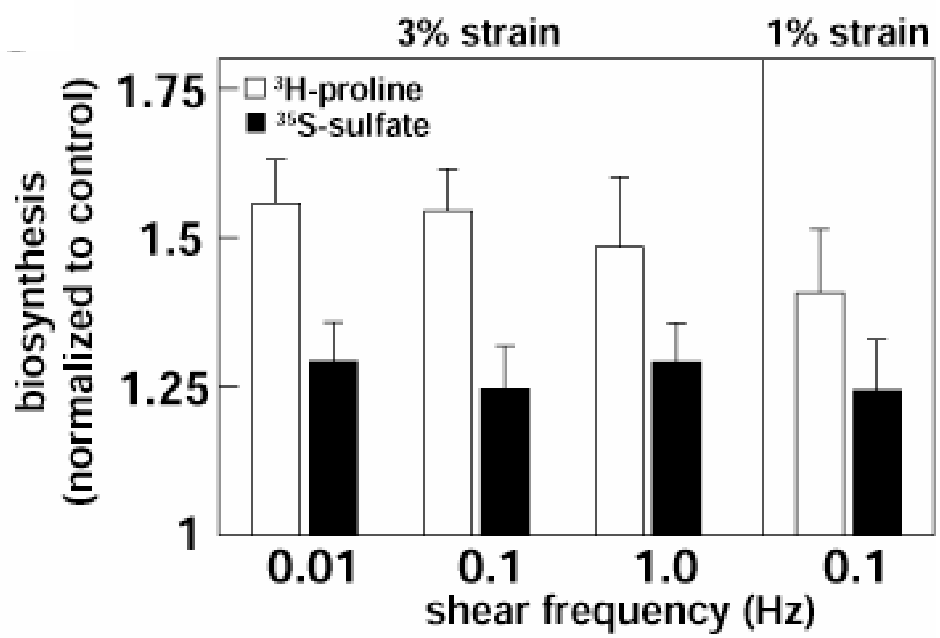
Dynamic Tissue Shear: can stim. collagen > PG synthesis



(Jin et al., ABB, ’01, ‘03)

Can preferentially augment pericellular matrix (Hunziker)





Dynamic Compression & Culture

(Kisiday+, J Biomech, 2004; Tissue Eng, 2004, in press)

Dynamic compression:

- Frequency: 1 Hz
- Static offset: 5%
- Sinusoidal amplitude: 2.5%
- Alternate Day Loading
- (45min on / 5hr-15min off) X4

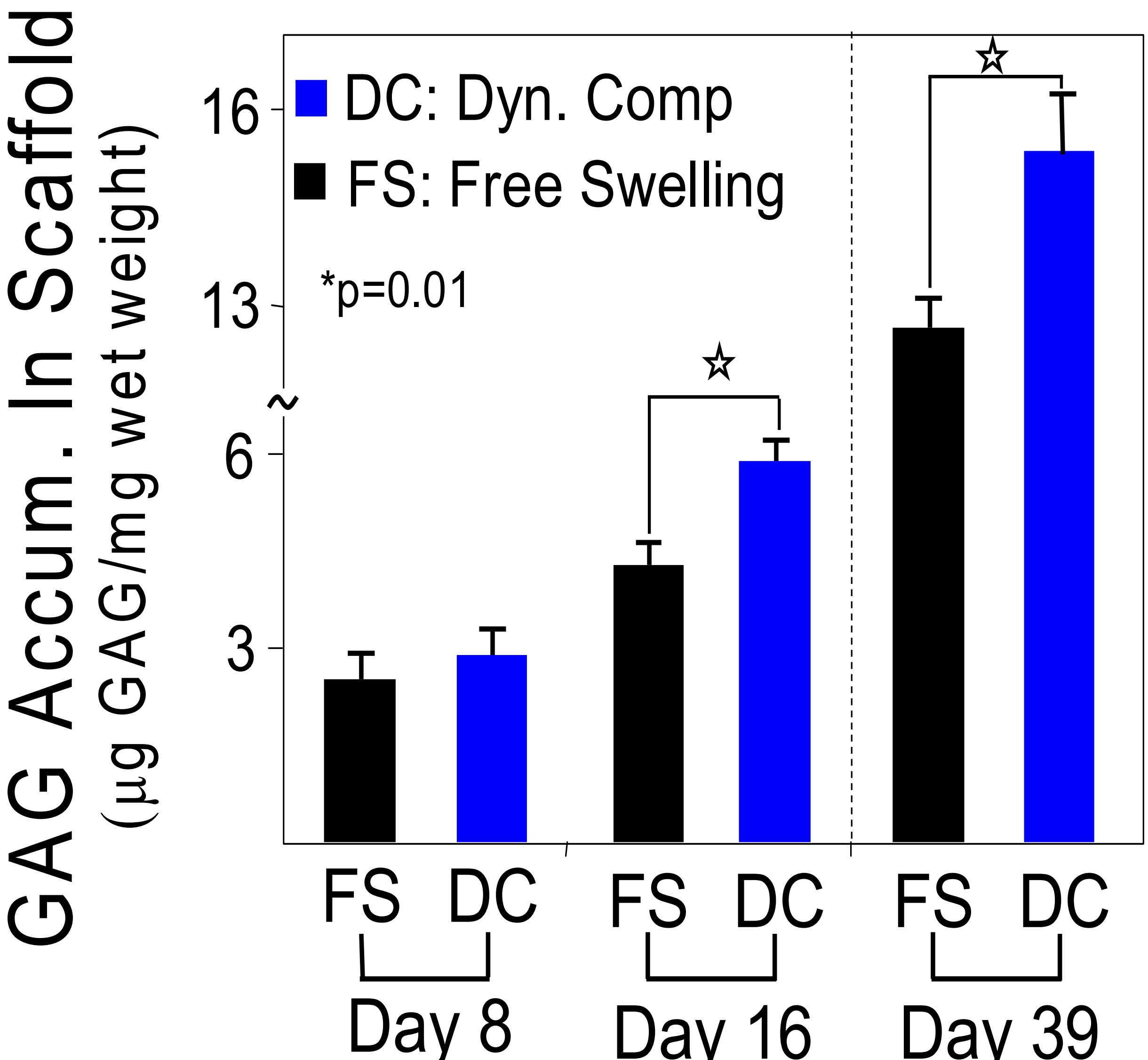
Photo removed for copyright reasons.

Culture medium:

- DMEM + 1% ITS + 0.2% FBS
- Changed every other day

chamber

Dynamic Comp



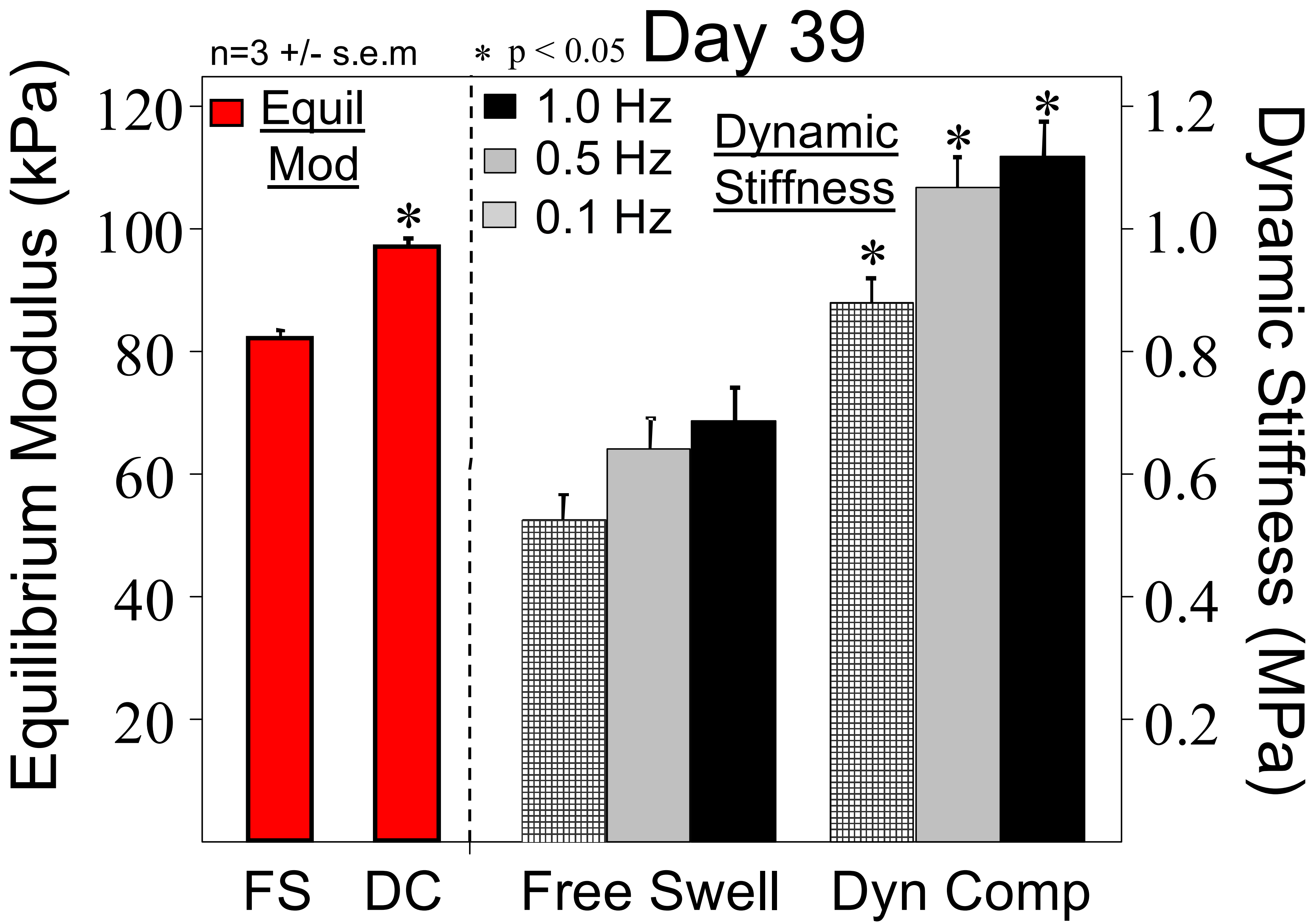
Day 0 GAG: 8.5 μg/mg ww

(Kisiday+, J Biomech, 2004)

Photos removed for copyright reasons.

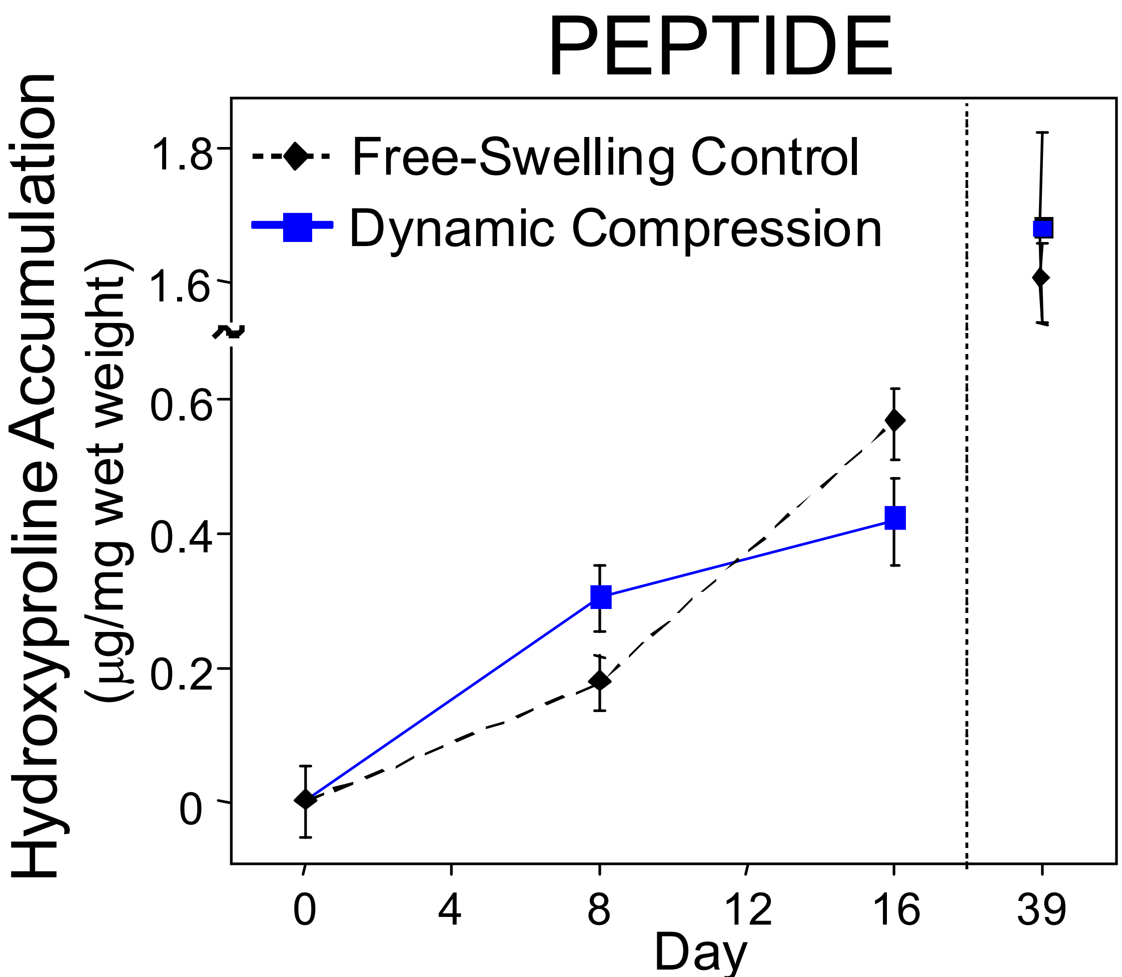
Free Swelling Control

Compressive stiffness is sensitive to Aggrecan content



Problems: (a) Mechanobiology

Type II Collagen Accumulation is not enhanced by dynamic compression.....

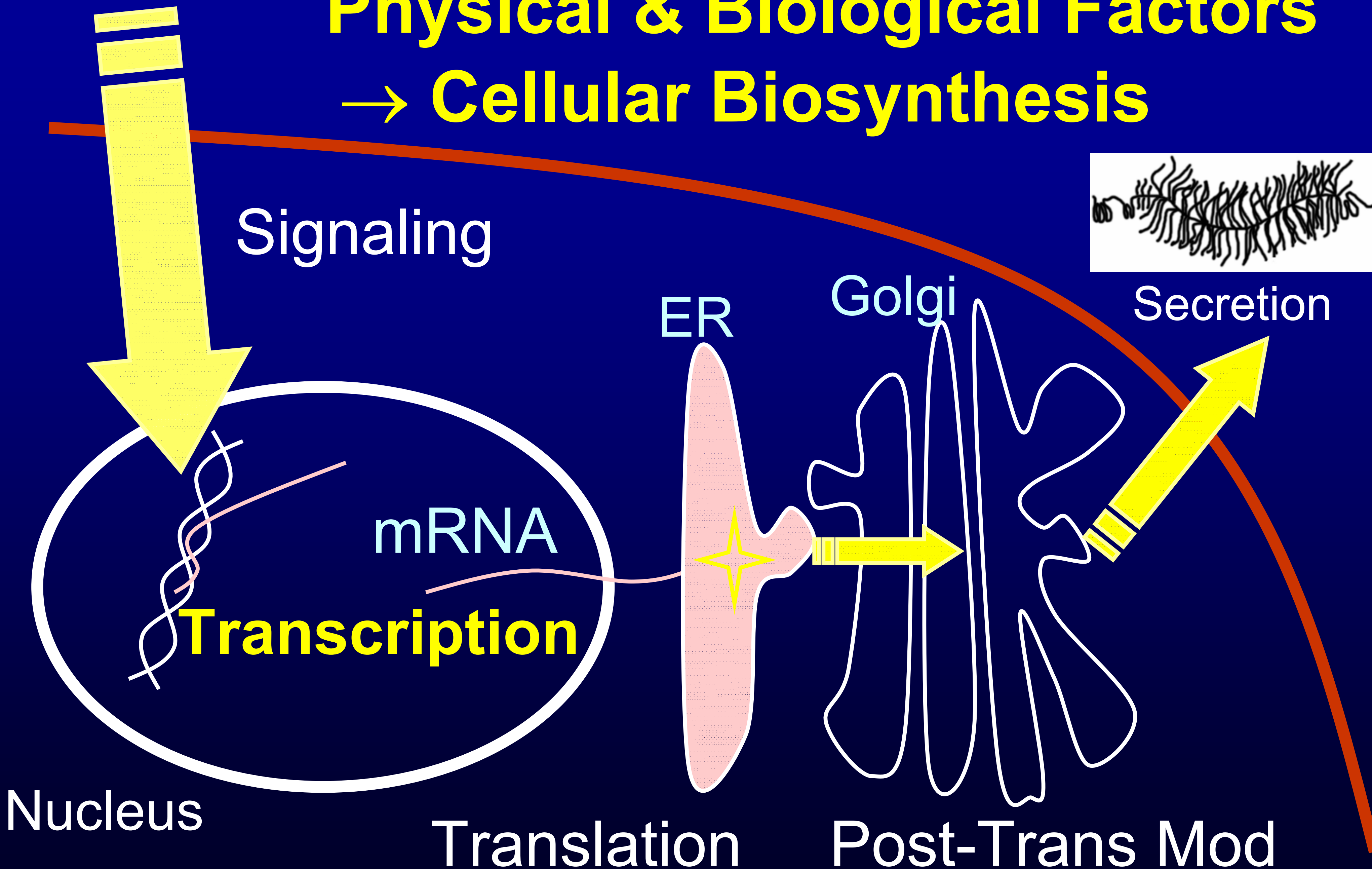


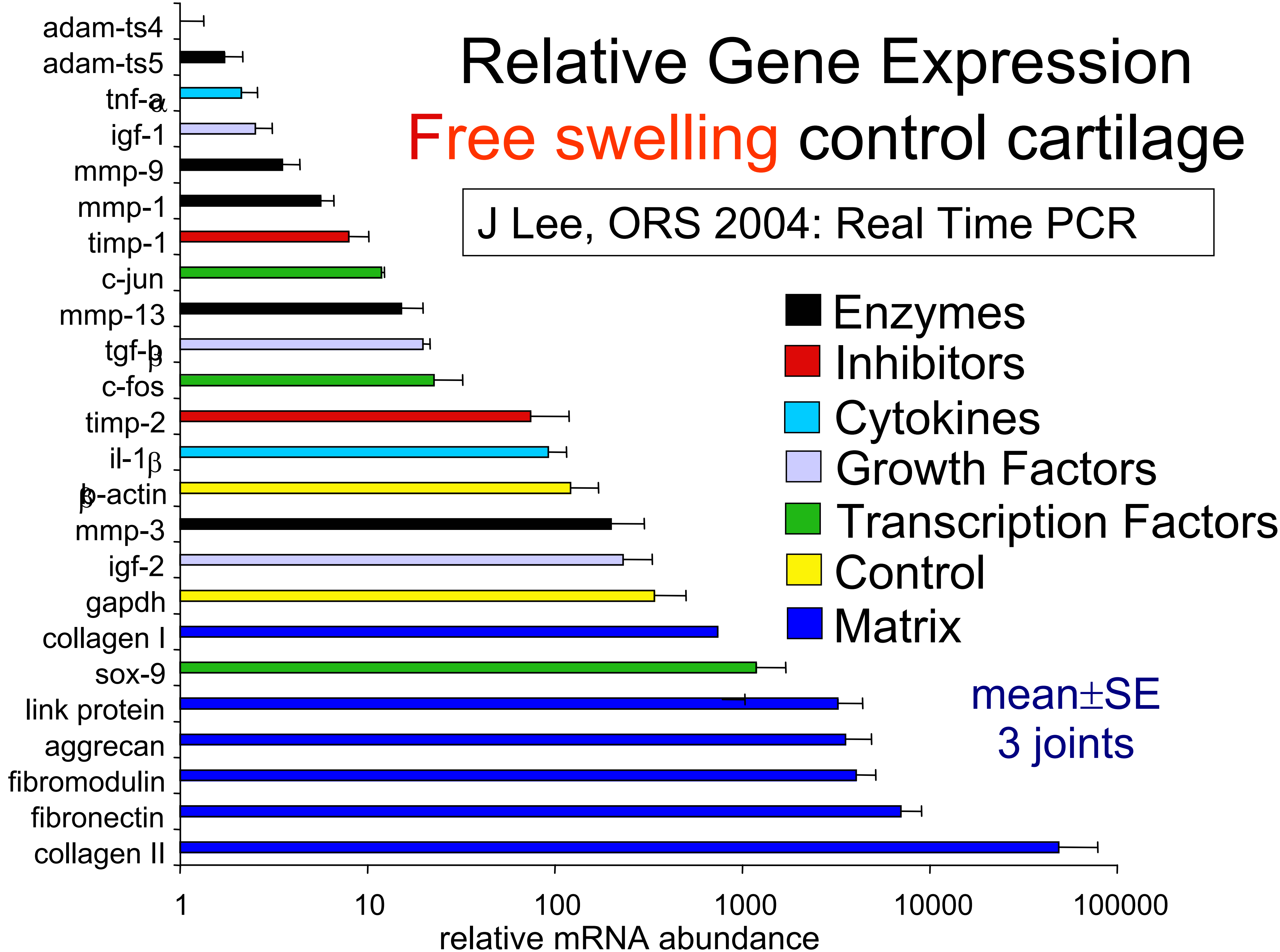
(Kisiday+, J Biomech, 2004)

.....Now trying shear !

2 more examples:

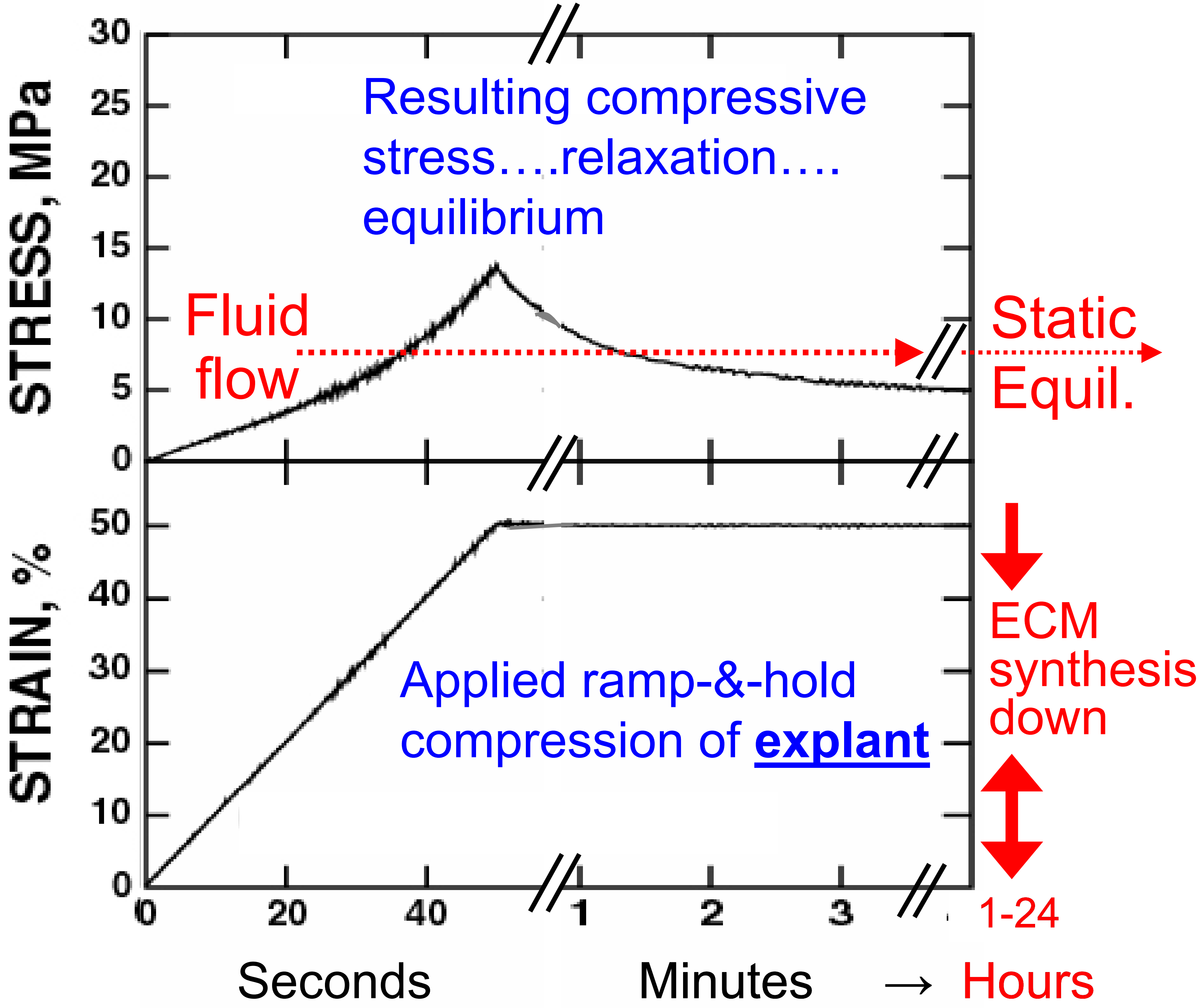
Physical & Biological Factors
→ Cellular Biosynthesis





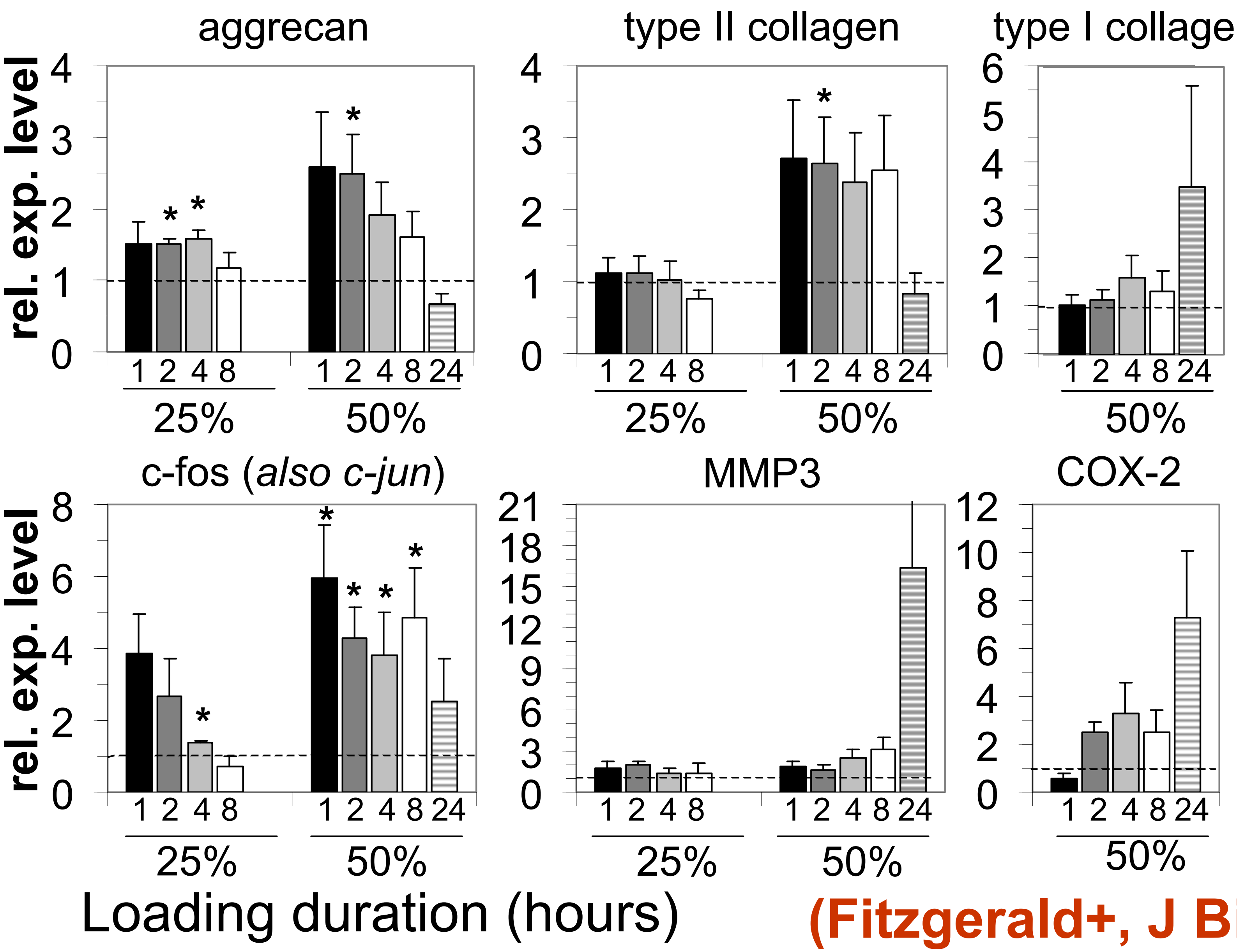
Relative Gene Expression
Free swelling control cartilage
J Lee, ORS 2004: Real Time PCR
Enzymes
Inhibitors
Cytokines
Growth Factors
Transcription Factors
Control
Matrix
mean±SE
3 joints
adam-ts4
adam-ts5
tnf-α
igf-1
mmp-9
mmp-1
timp-1
c-jun
mmp-13
tgf-β
c-fos
timp-2
il-1β
β-actin
mmp-3
igf-2
gapdh
collagen I
sox-9
link protein
aggrecan
fibromodulin
fibronectin
collagen II
1
10
100
1000
10000
100000
relative mRNA abundance

Effects of compression: Chondrocyte gene express.



Static Compression for 1, 2, 4, 8, 24 hr → Induces gene transcription

Real Time RT-PCR ABI 7900HT Applied Biosystems



Matrix proteins transiently upregulated but **suppressed by 24hrs**

Proteases upregulated by 24hrs

free-swell expression level = 1

(Fitzgerald+, J Biol Chem, 04)

Main Expression Trends
(Clustering & Principle Comp Anal)

Four graphs removed for copyright reasons.

Centroid 1

aggrecan, collagen II, cfos, cjun

Centroid 2

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

Centroid 3

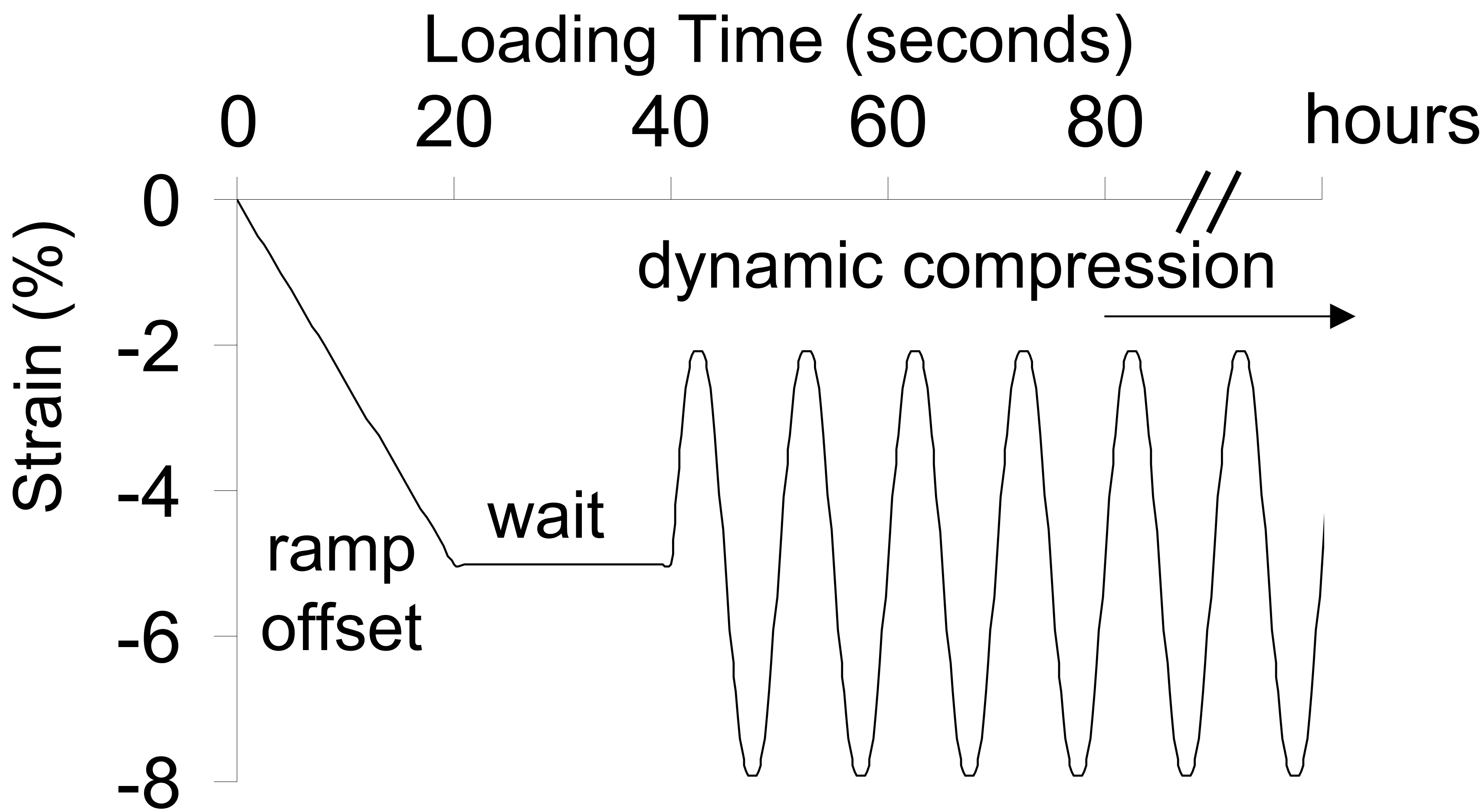
MMP3, MMP9, MMP13, TIMP1, ribosomal 6-P, collagen1

Centroid 4

ADAMTS4, ADAMTS5, TIMP3, fibronectin, HSP70, TGFβ, COX-2

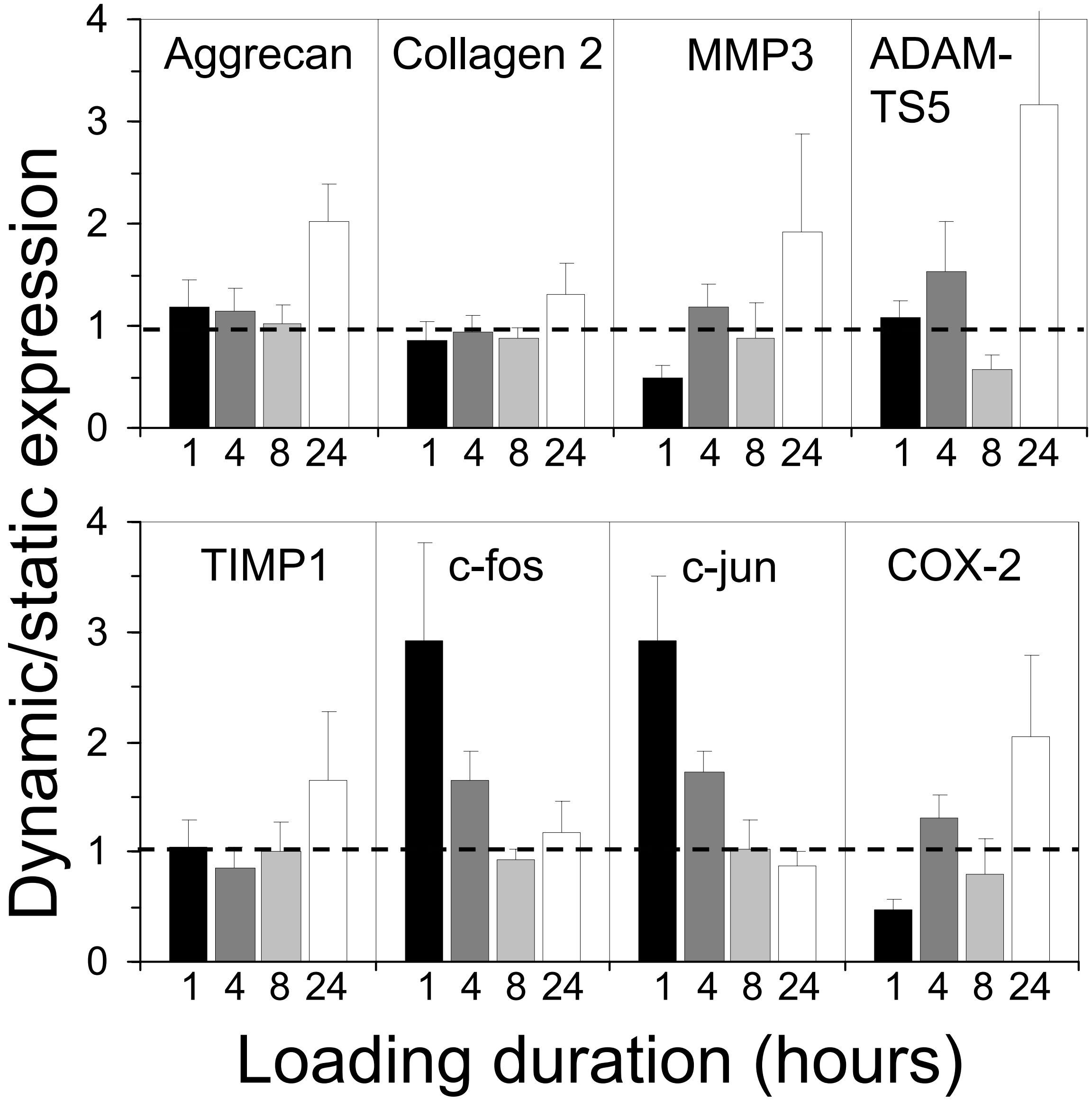
Effect of Dynamic Compression: 3% strain amplitude at 0.1 Hz

(known to stimulate PG and protein synthesis)



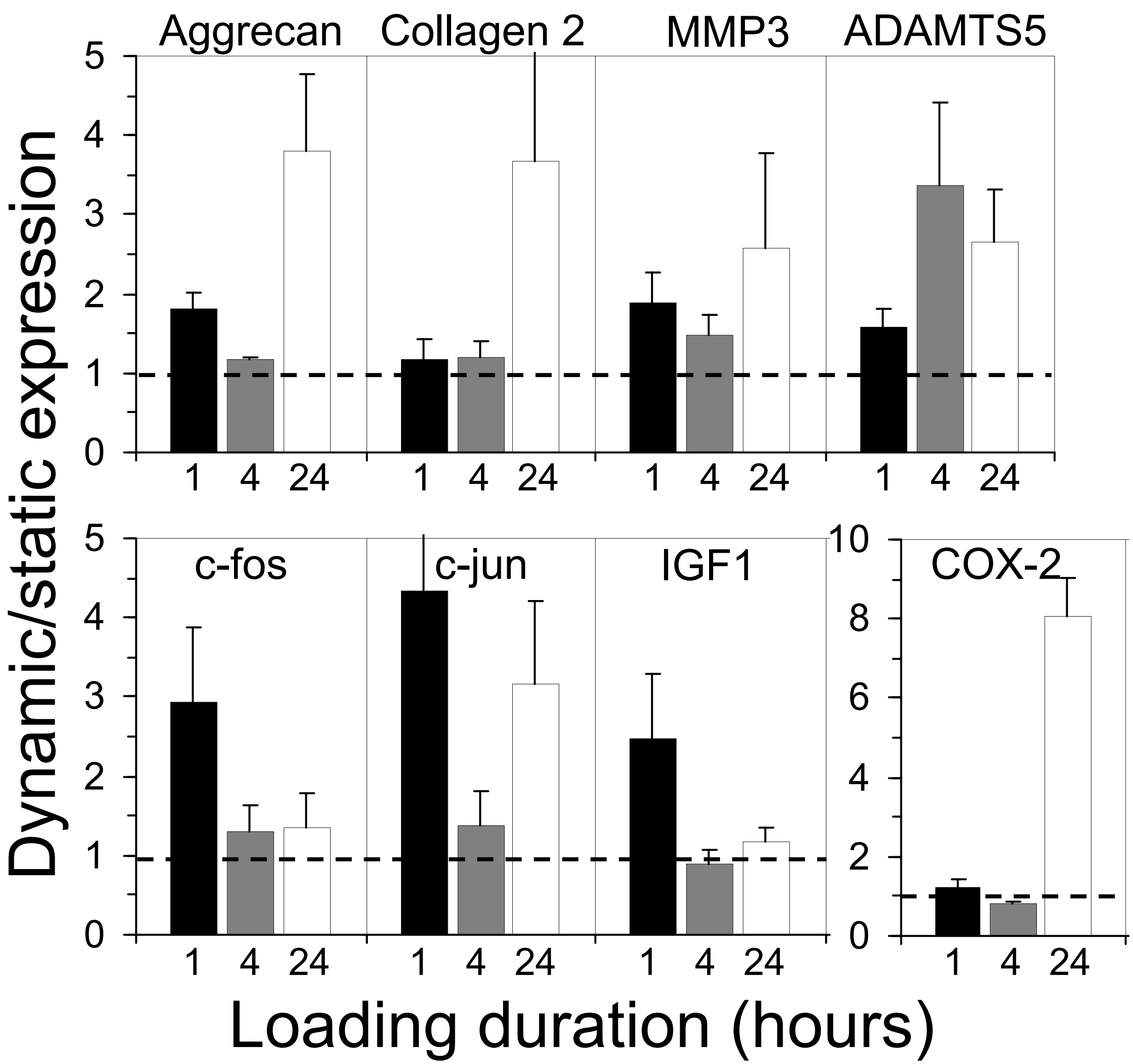
(Fitzgerald+, ORS, 2004)

3% Dynamic Compression at 0.1Hz: Effect on mRNA



- Matrix proteins follow different trend, increased with loading duration.
- Proteases increasing with duration (same as static).
- Transcription factors same trend as static but reduced amplitude.
- 5% static control = 1

3% Dynamic Tissue Shear at 0.1Hz: Effect on mRNA



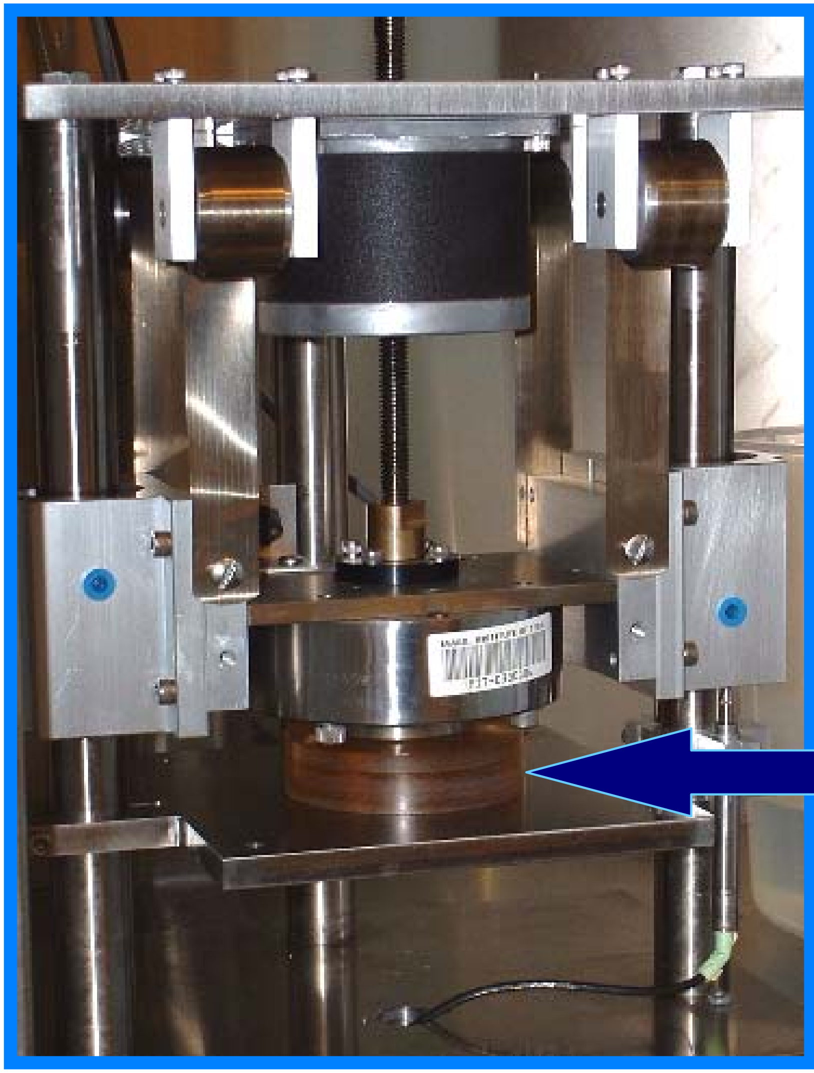
- **Shear also increased expression** of matrix proteins (~50% greater than dynamic compression).
- Gene regulation occurs in the absence of fluid flow.
- 0% static control level = 1

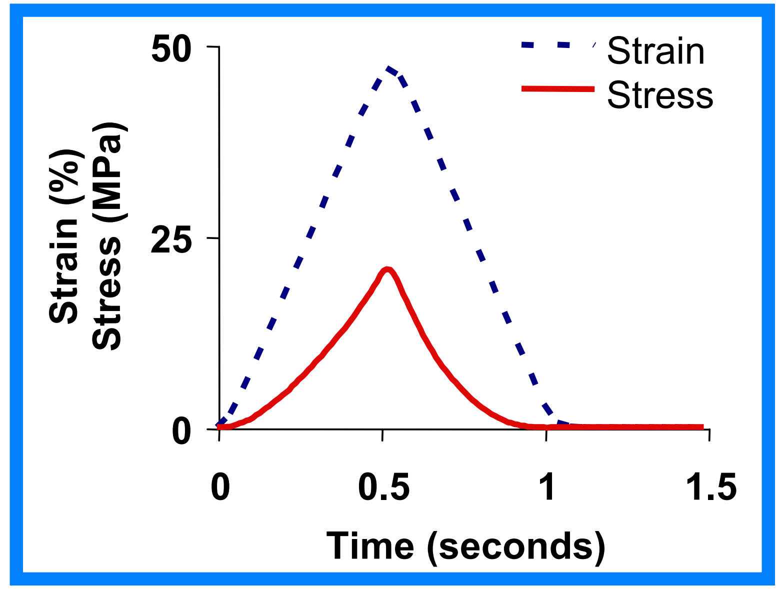
80% of torn ACL (knee injuries) progress to OA in 14 years

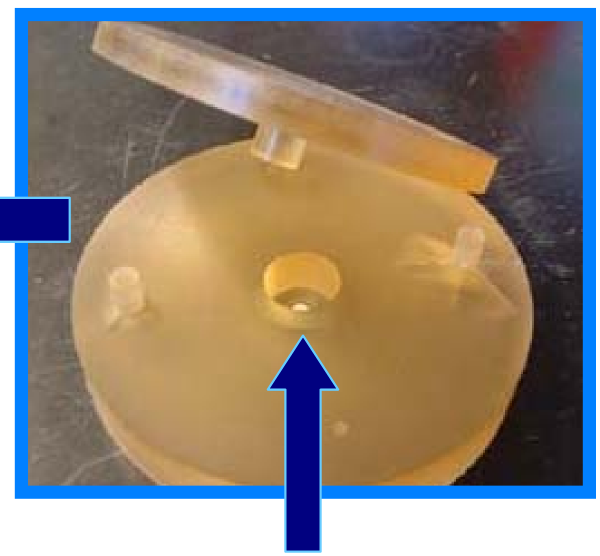
Photo removed for copyright reasons.
Basketball player lying on the court with a torn ACL.

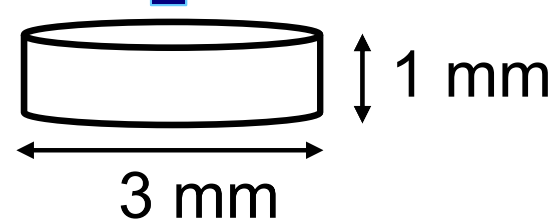
Scientific American, 2000

Injurious Compression



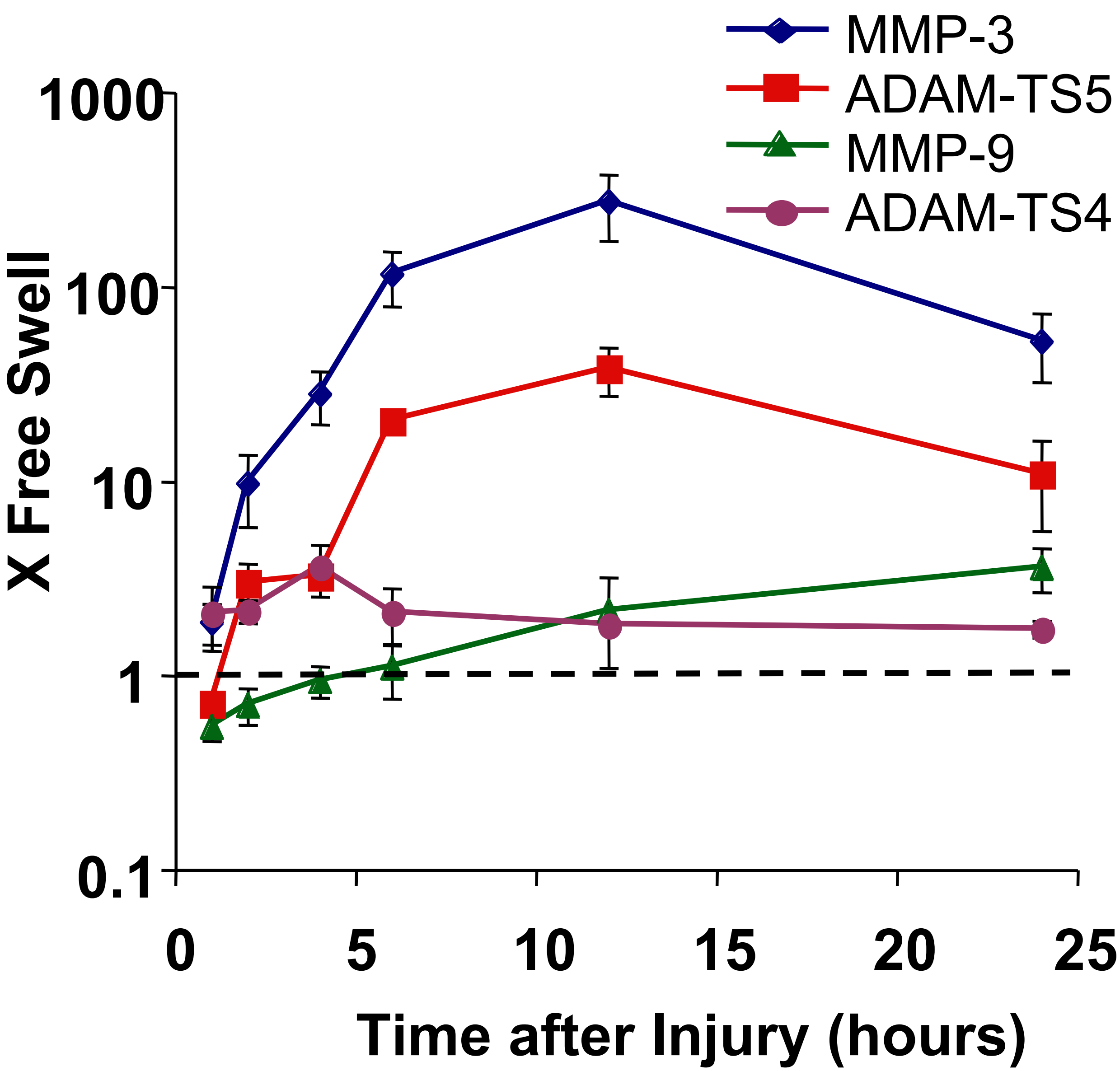


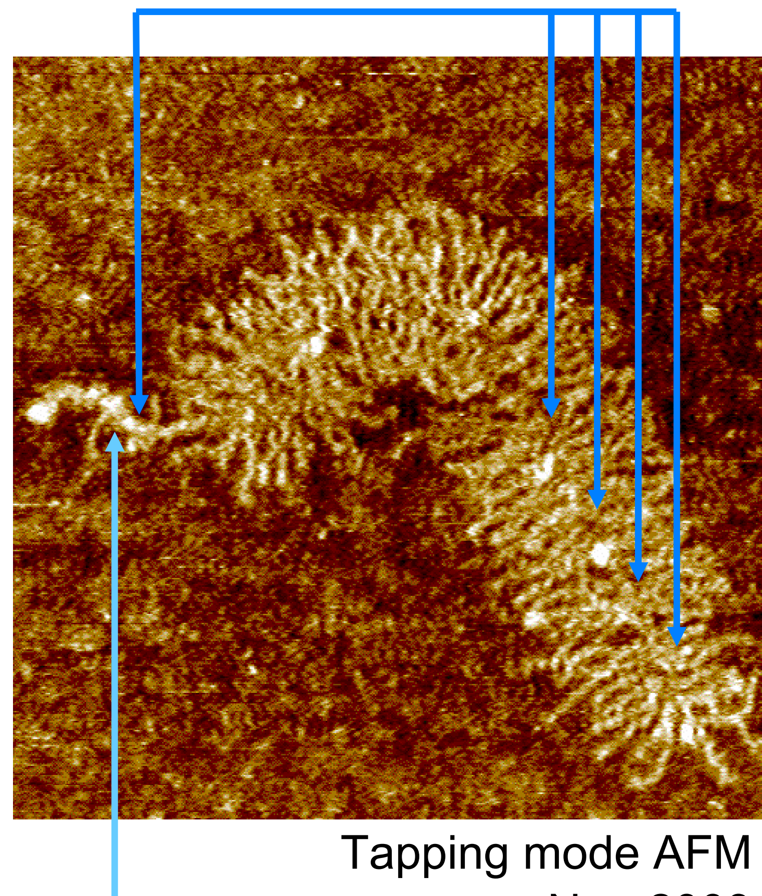




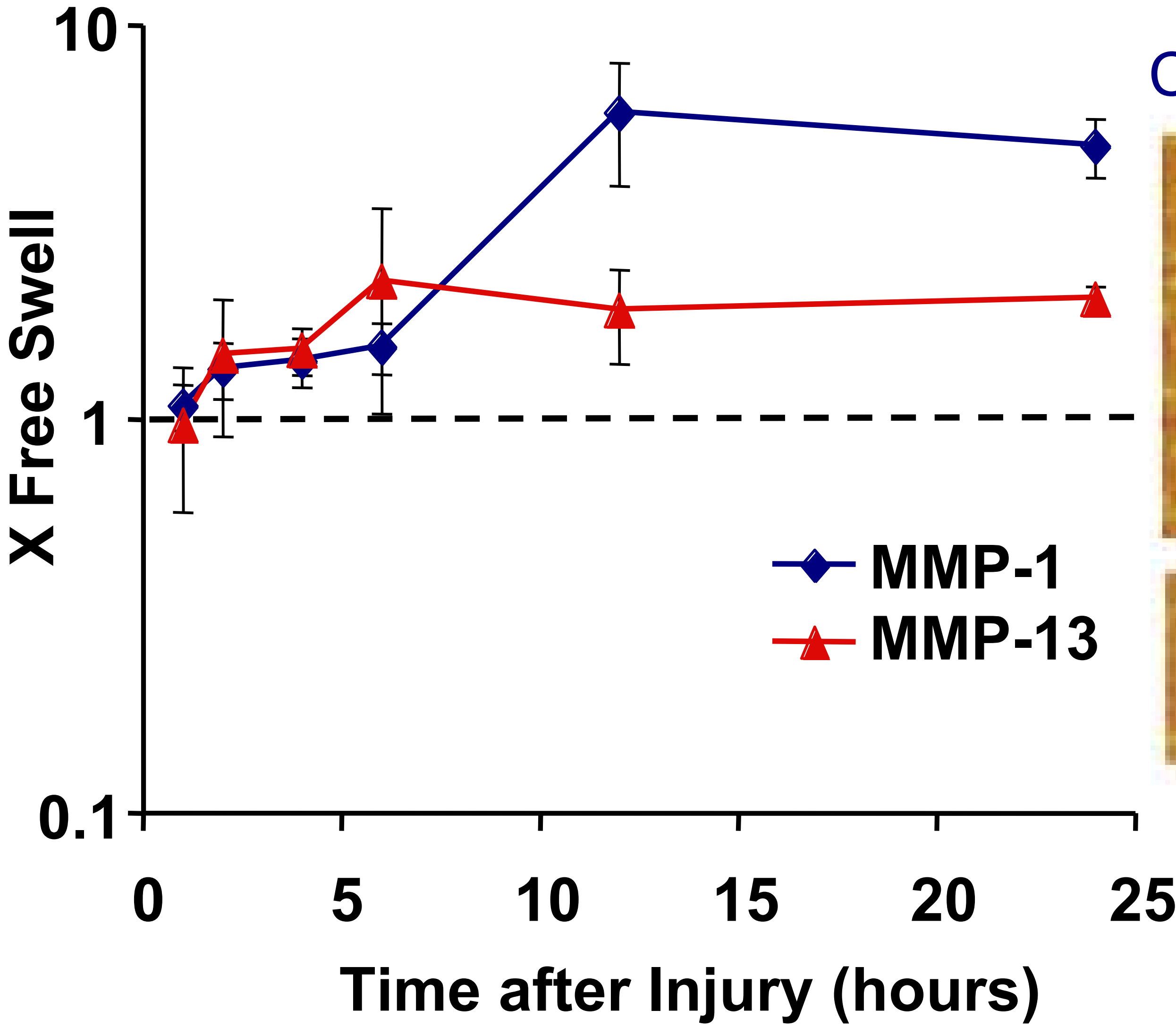
Injury: 50% strain
1/s strain rate

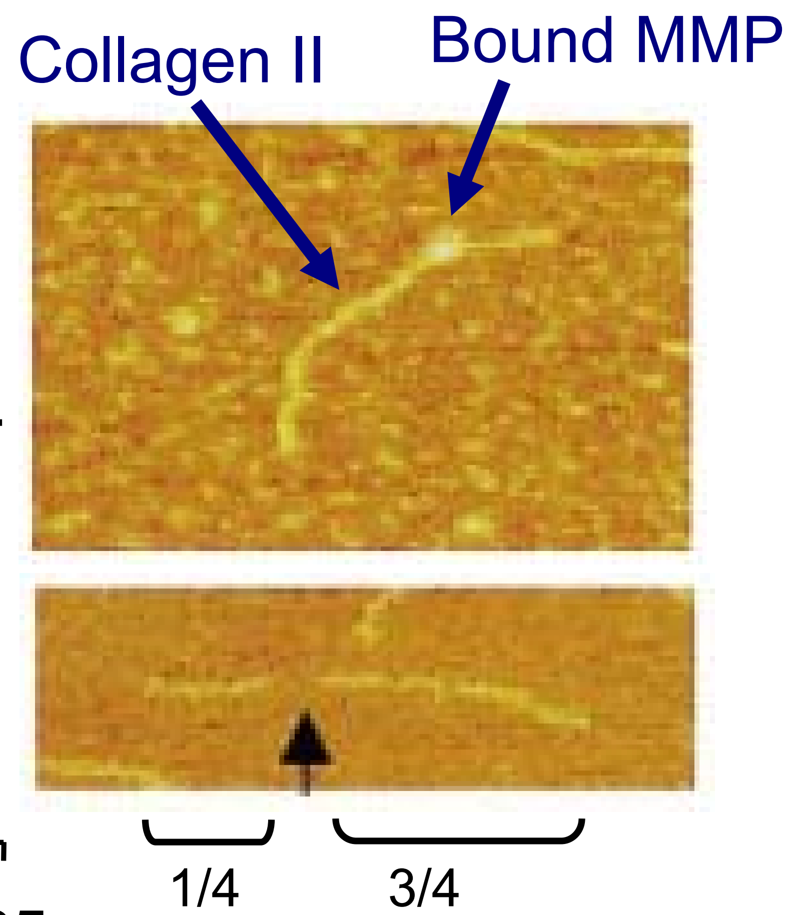
Proteolytic Enzymes





Collagen Degrading Enzymes





Tapping mode AFM
Sun+ 2000

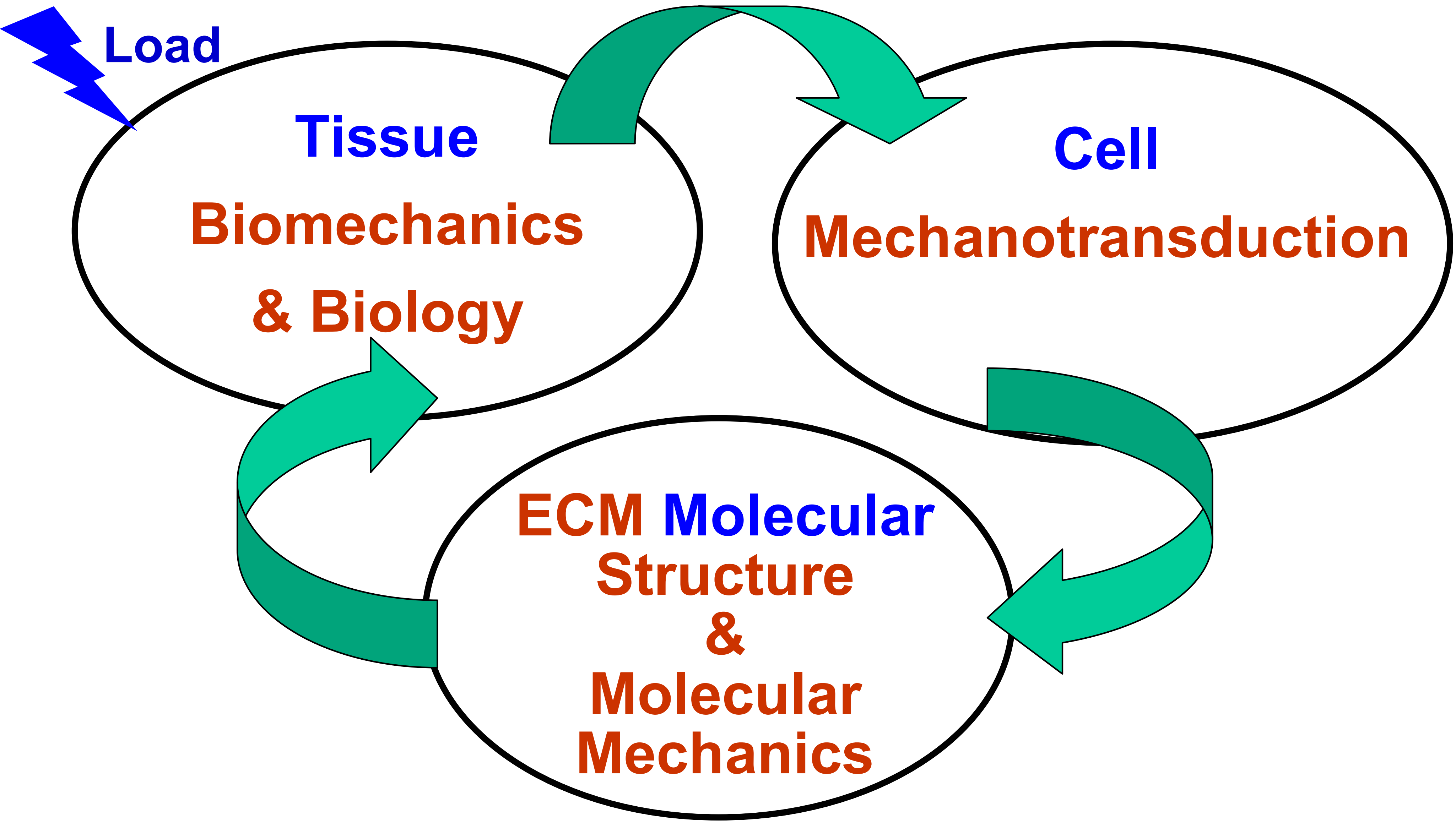
Summary: Changes in Chondrocyte Biosynthesis & Gene Expression in response to "Loading" of cartilage explants:

…Appear to be very sensitive to the specific parameters of "loading"

Different effects of:

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

Cartilage Tissue Engineering: Mechanobiology & Nano-Mechanics



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(b) Molecular Nano-Mechanics:

Photo removed for copyright reasons.

50 nm

-OR-

Photo removed for copyright reasons.

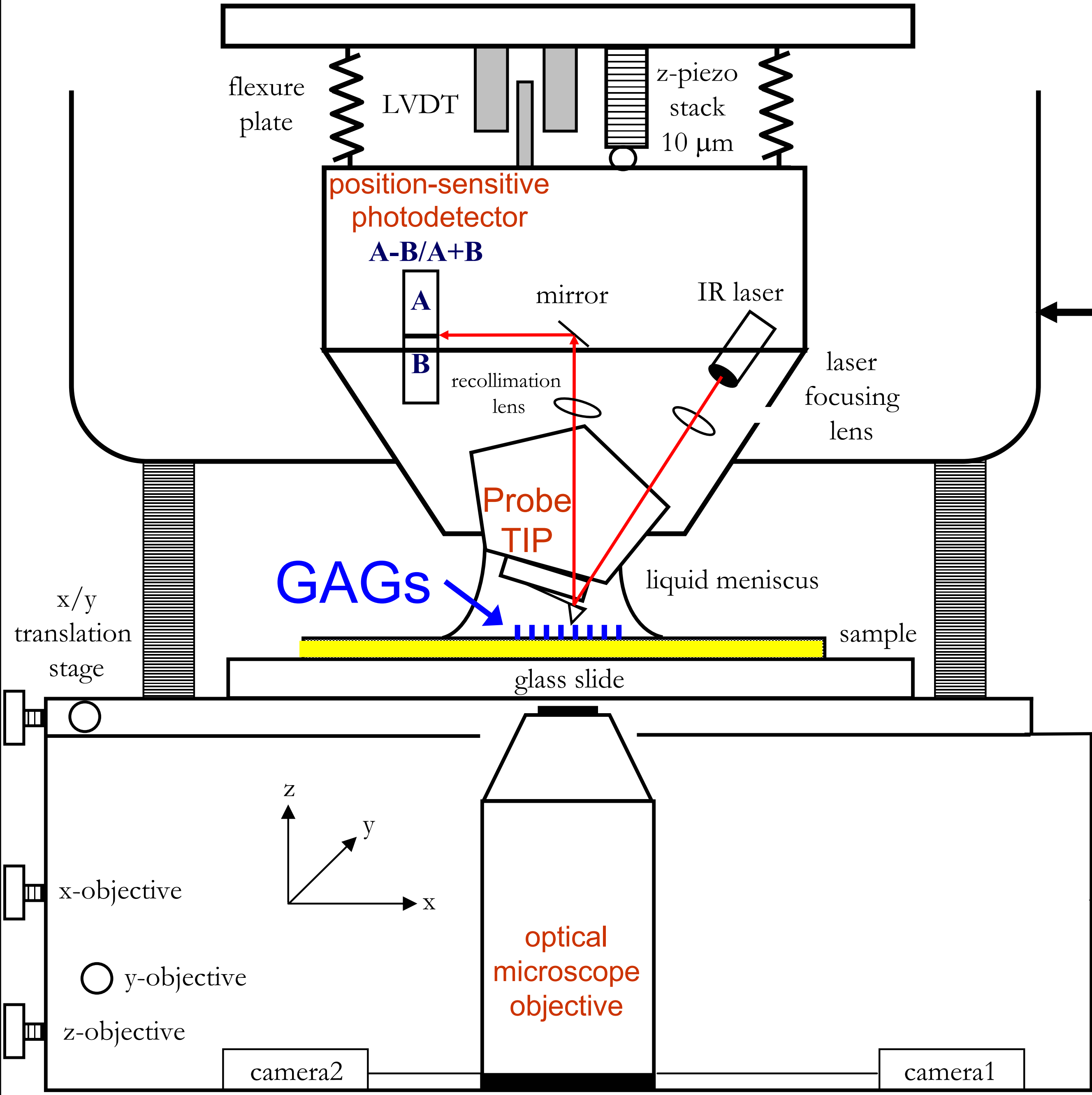
50 nm

What aggrecan structure is synthesized in the tissue engineered construct ???

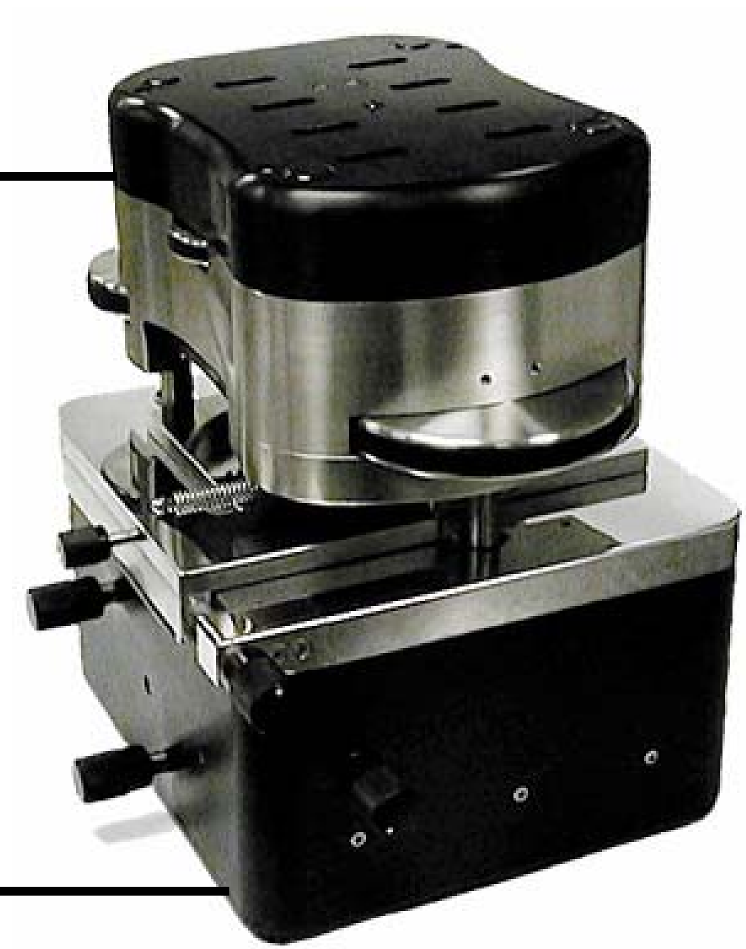
Is it mechanically optimal & functional in long run?

Molecular Mechanics Readout:

Molecular Force Probe

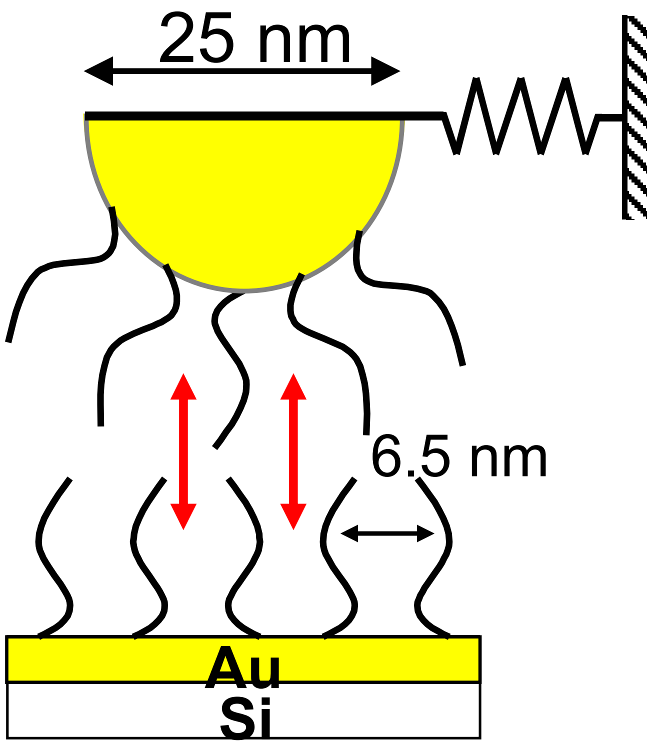


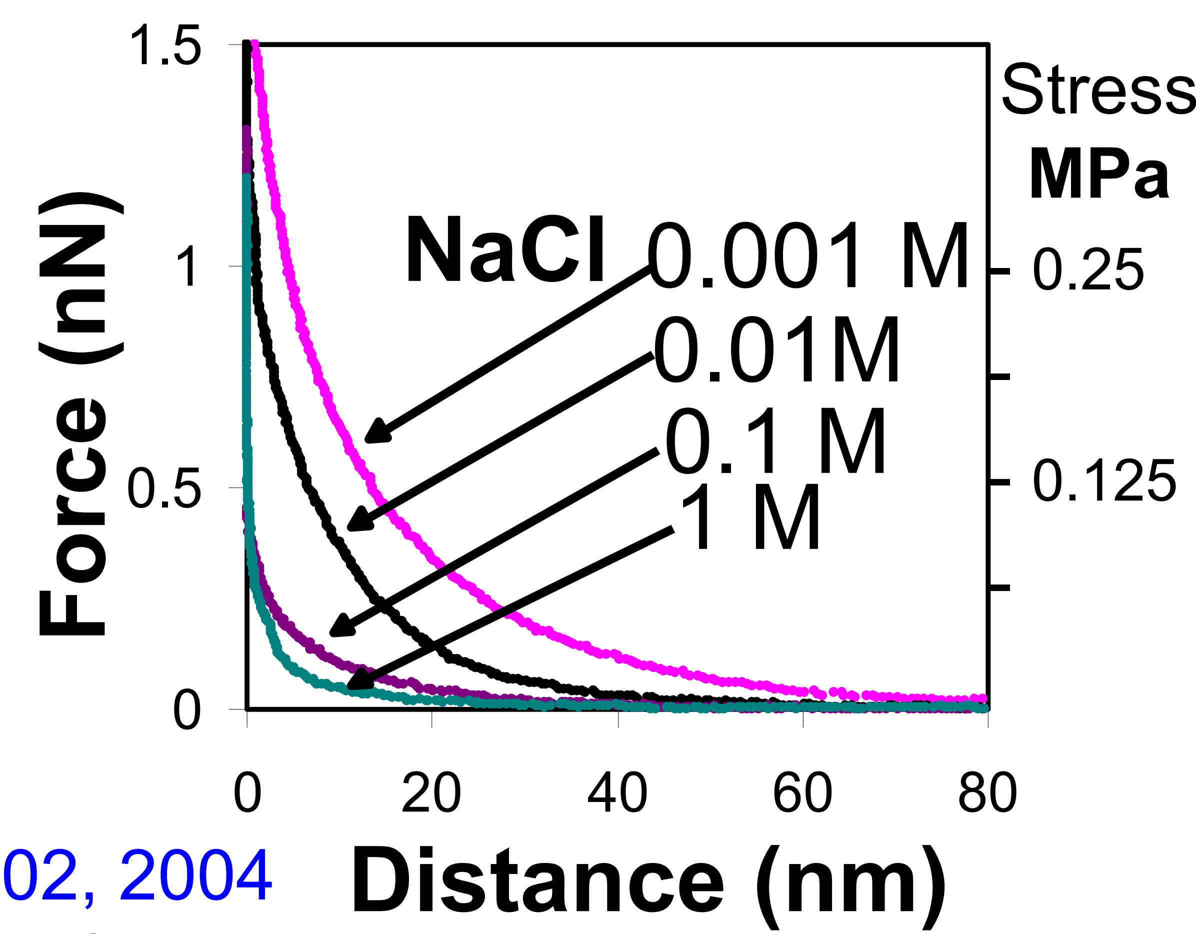
Resolution (in fluids) :
Force~5 pN
Distance~ 0.3 Å



with Prof C. Ortiz

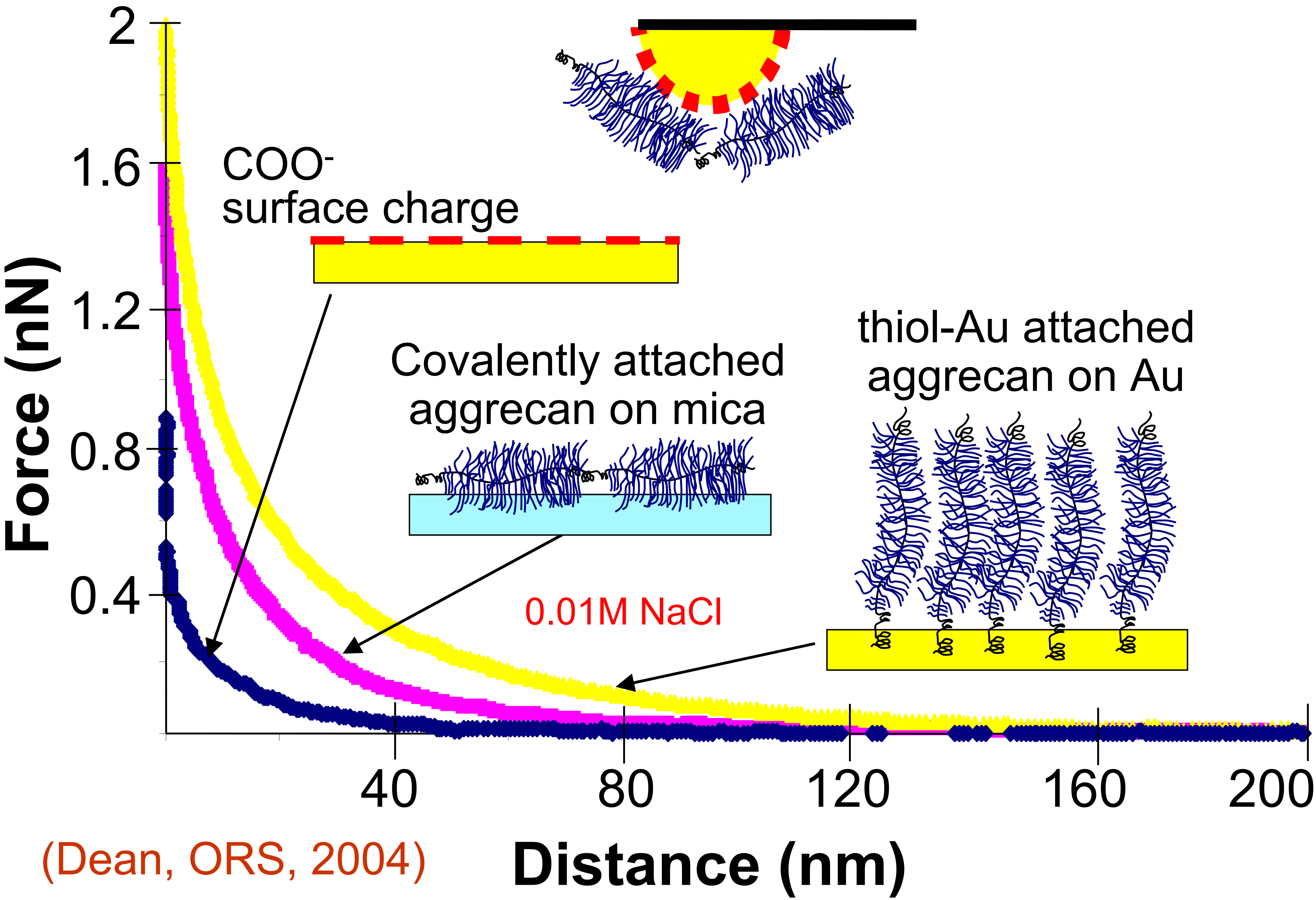
Electrical Repulsive Force between CS-GAGs on Tip and Substrate Of AFM - Molecular Force Probe





Seog +, Macromolecules, 2002, 2004
J Biomech, 2004 (in press)

ECM Molecular Mechanics



(Dean, ORS, 2004)

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