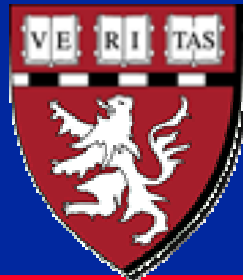




**Massachusetts Institute of Technology  
Harvard Medical School  
Brigham and Women's Hospital  
VA Boston Healthcare System**



**2.785j/3.97J/BEH.411/HST523J**

***IN VITRO* MODELS FOR CELL  
CONTRACTION**

**M. Spector, Ph.D.**

# **SKIN WOUND HEALING *IN VIVO* METHODOLOGY**

- **Non-regenerating, well-defined wound**
- **Evaluate the result**
  - **measure contraction as the decrease in the area of the wound**
  - **scar or regeneration: density and organization of collagen**

Summary: methodology of experiments in vivo

The approach of the skin experiments was to let the experimental animal answer the question as to whether our implants can induce regeneration. The studies were performed in both the guinea pig and the swine. It is important to use a non-regenerating wound model so that there is no ambiguity in interpreting the outcome as regeneration or intact tissue left in the wound bed. In the skin, the full-thickness removal of epidermis and dermis is a non-regenerating wound. An ECM analog with a particular combination of characteristics is grafted into the wound, and, after sufficient time for healing, the tissue in the wound is assessed for contraction and to determine if this tissue is scar or regenerated tissue.

We then modify the matrix characteristics (chemical comp, pore size/orient, and degradation) and repeat the process.

**IV Yannas, et al.**

# SKIN WOUND CONTRACTION

Decrease in wound area with time:

$$A = A_0 e^{-kt}$$

**A,** area

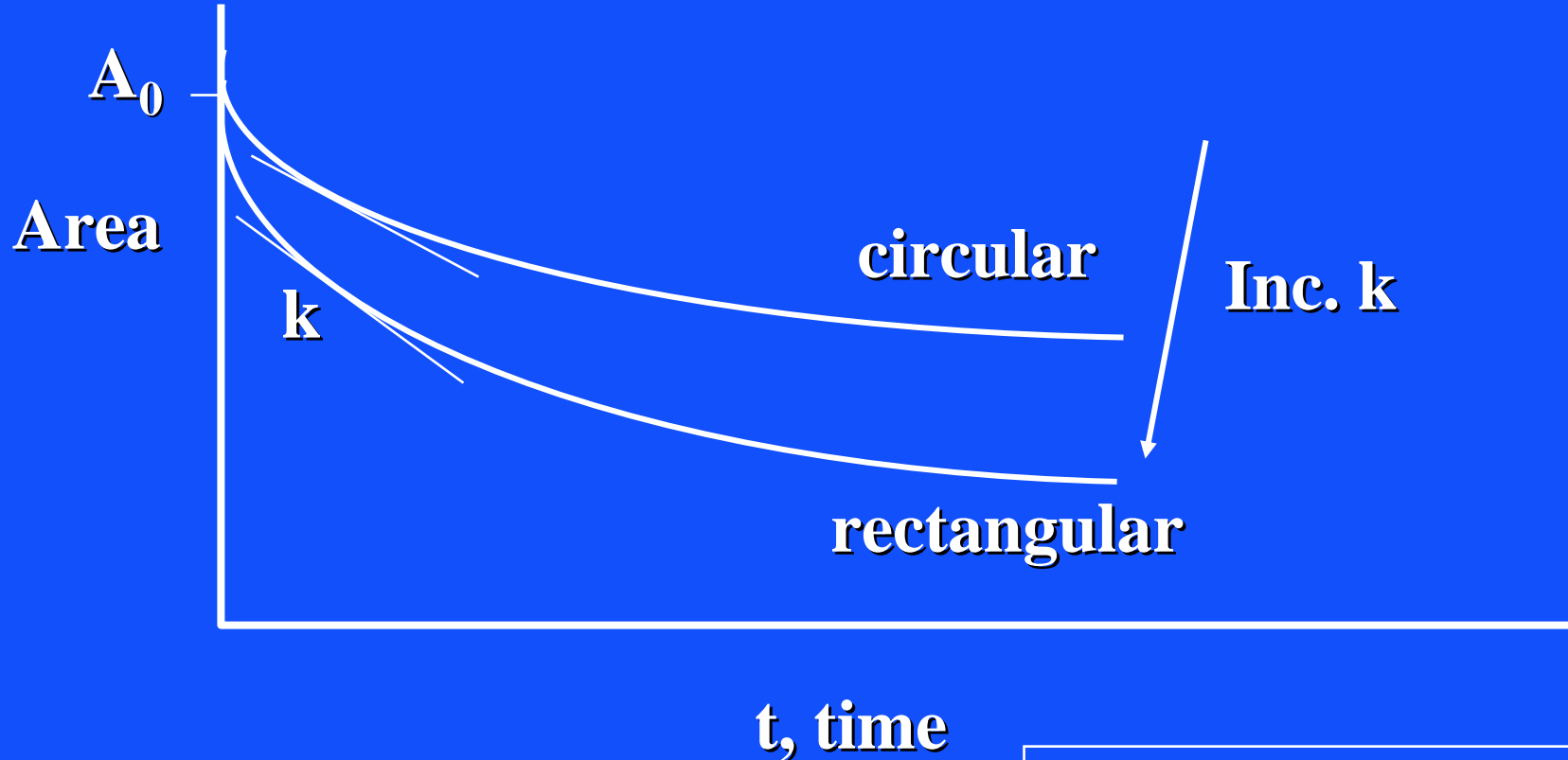
**A<sub>0</sub>,** initial area

**t,** time

**k,** “specific rate of contracture”; amount by which a unit area of wound decreases per day; uniform for rectangular and triangular wounds and lower for circular wounds

# SKIN WOUND CONTRACTION

$$A = A_0 e^{-kt}$$



# ***IN VITRO* MODELS FOR CELL CONTRACTION**

- **Cells seeded into a preformed collagen-GAG sponge**
  - Measure dimensional change
  - Cell force monitor
- **Cells wrinkling a flexible (silicone) membrane**
  - Measure % cells wrinkling the membrane
- **Cells cast in a collagen gel (lattice)**
  - Measure the dimensional change

# COLLAGEN-GAG MATRICES

## Analogs of Extracellular Matrix

- Type I (bovine tendon)
- Type II (porcine)
- Chondroitin 6-sulfate

Photo removed for  
copyright reasons.

Photo removed for  
copyright reasons.

- Freeze-dried
- Dehydrothermally cross-linked

**IV Yannas, et al. PNAS, 1989**

# Canine articular chondrocytes in a collagen-GAG matrix synthesize type II collagen (red) but they also contract the matrix

**15 Days in Culture**

Photo removed for copyright reasons.

**Type II collagen (red)  
immunohistochemistry**

Our laboratory has investigated the use of porous type I and type II collagen matrices for in vitro and in vivo articular cartilage tissue engineering for several years. To briefly summarize some previous findings, it has been found that ....

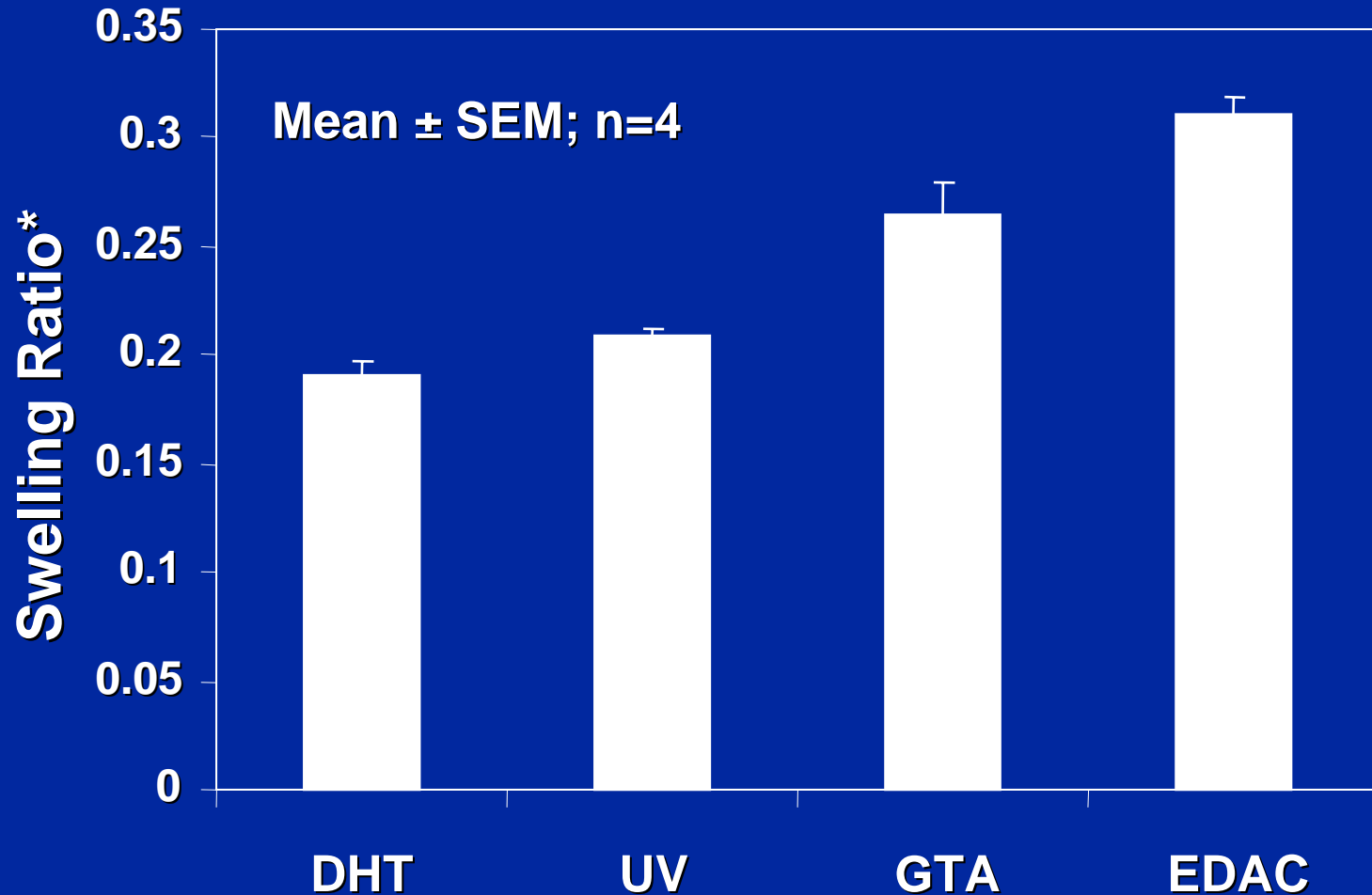
As shown in this matrix seeded with third passage canine chondrocytes and cultured for 15 days and stained for type II collagen.

However, it is also known that the passaged chondrocytes also display ....

Additionally, we have seen that levels of biosynthetic activity...

**CR Lee, *et al*, *Biomat*.  
2001;22:3145**

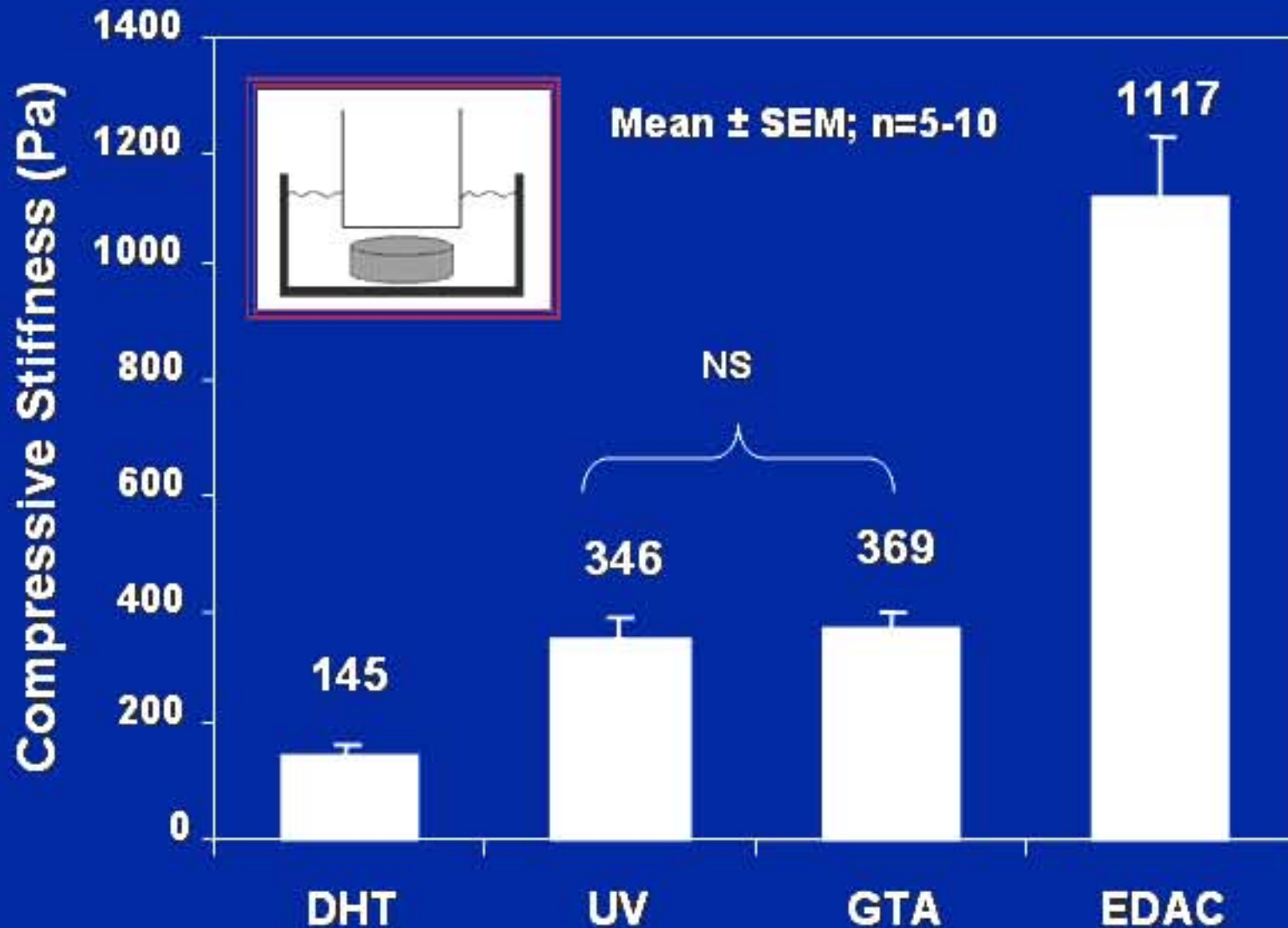
# Cross-link Density



\*Swelling ratio  $\sim$  cross-link density [Weadock, *et al.*, 1983]  
All groups significantly different;  $p < 0.05$



# Compressive Stiffness



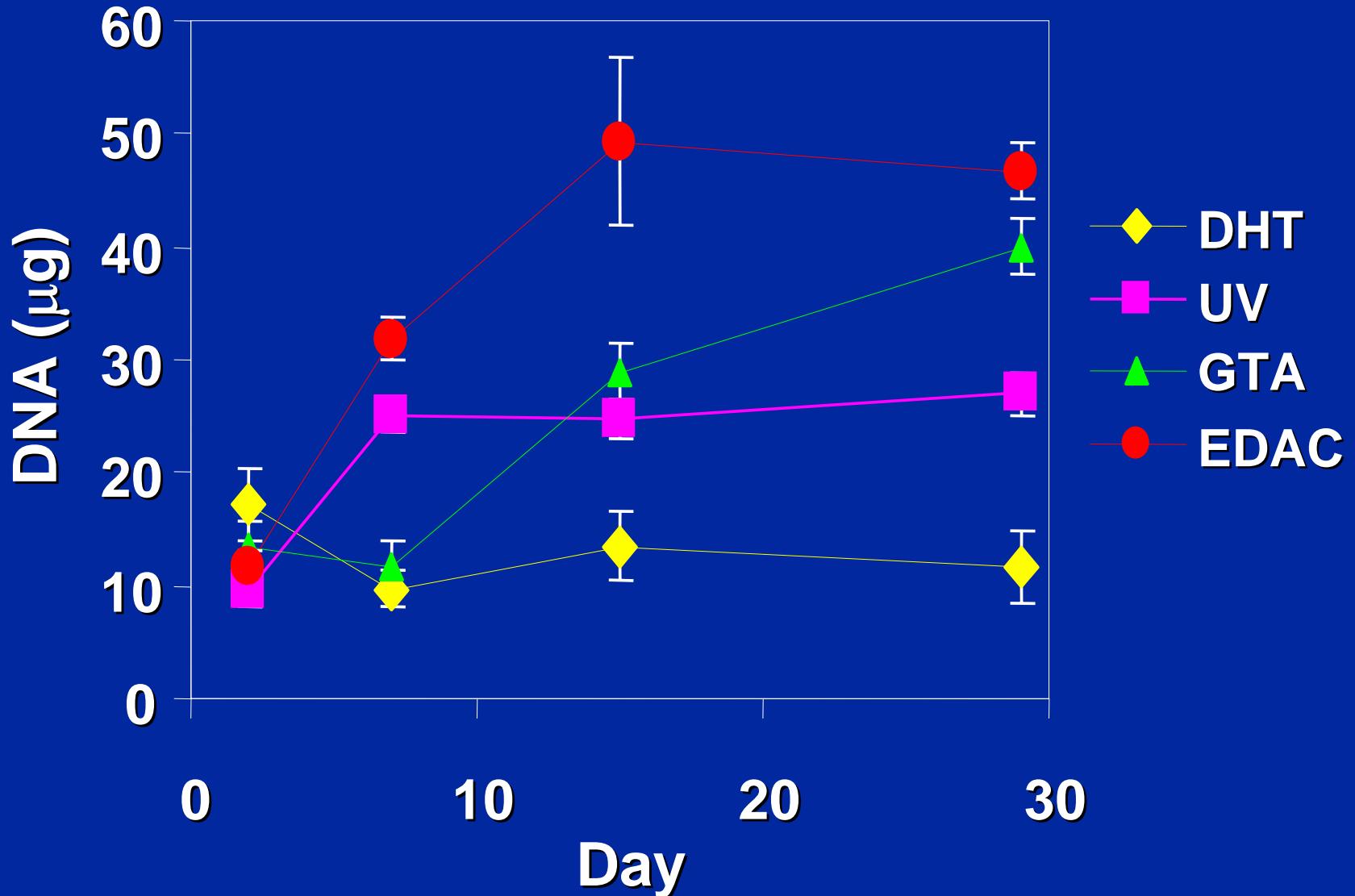
# Adult canine articular chondrocytes (passage 3) contract a type I collagen-GAG matrix, reflected in the decrease in diameter

Graph removed for  
copyright reasons.

Highly x-link.,  
high modulus

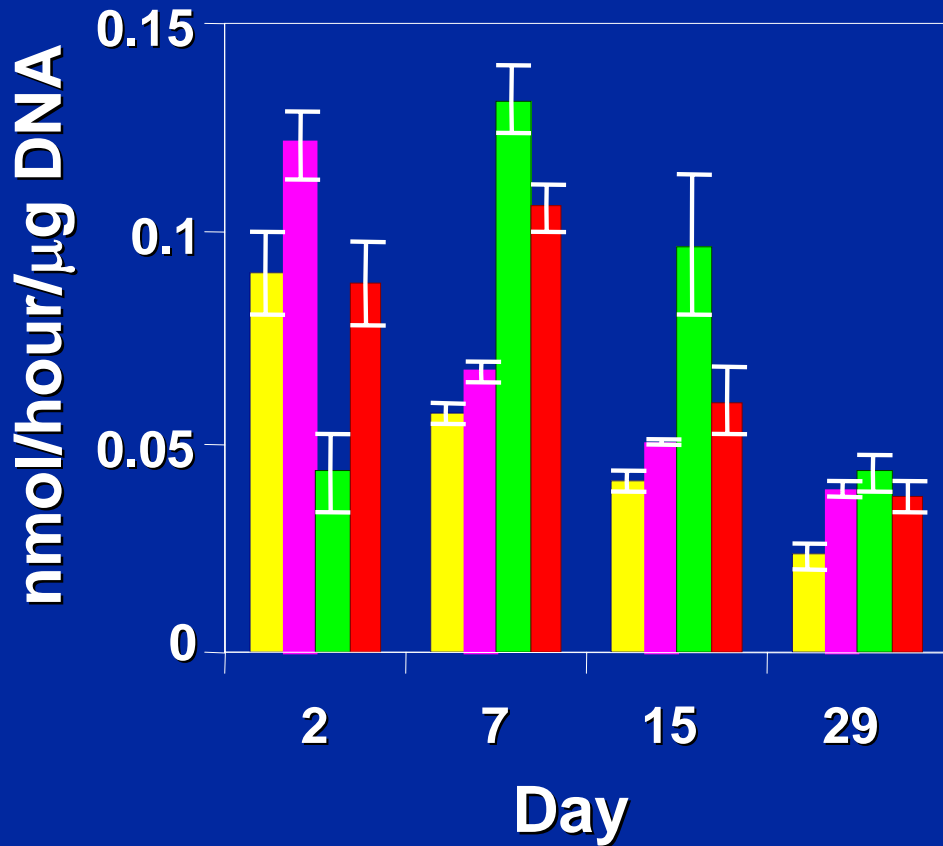
Little x-link.,  
low modulus

# Chondrocyte Proliferation Correlation with Contraction?

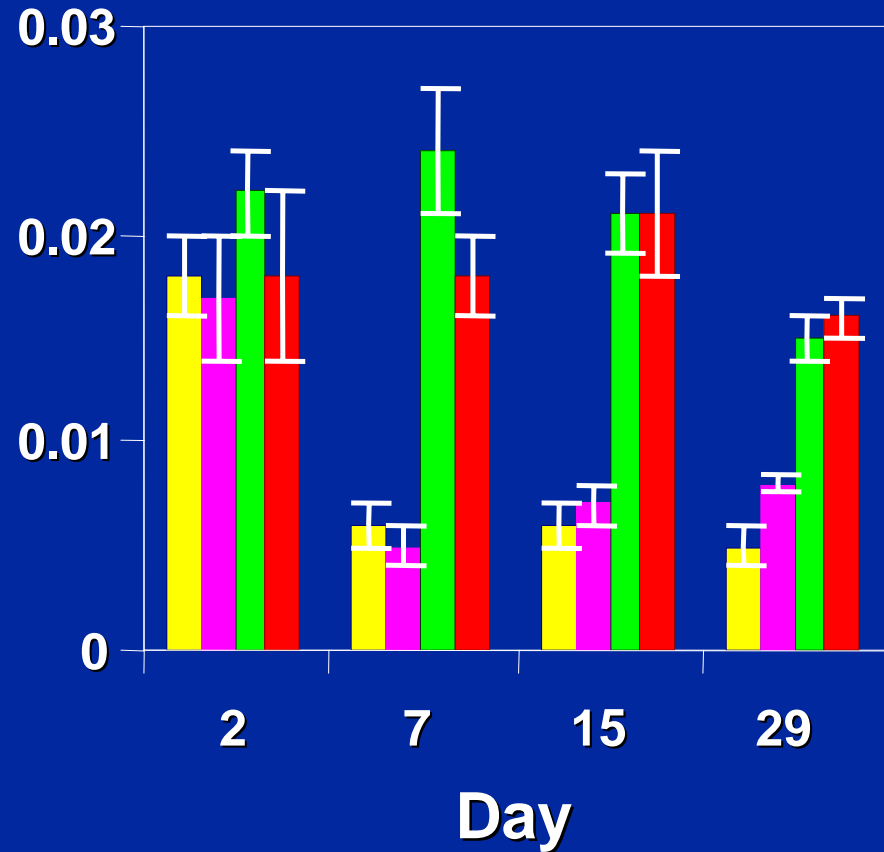


# Chondrocyte Biosynthesis Correlation with Contraction?

## Proline Incorporation



## Sulfate Incorporation



■ DHT

■ UV

■ GTA

■ EDC

# Osteoblastic cells (MC3T3-E1) contracting a collagen-GAG matrix

Pores compressed as specimens  
decrease in size (no evident dissolution)

Photo removed for  
copyright reasons.

**1 wk**

Photo removed for  
copyright reasons.

**2 wk**

Photo removed for  
copyright reasons.

**4 wk**

**C. Menard, *et al.*,  
*Biomat.* 2000;21:1867**

# MC3T3-E1 Cells in a Collagen-GAG Matrix, 4 wks

Photo removed for  
copyright reasons.

**C. Menard, *et al.*,  
*Biomat.* 2000;21:1867**

# LINEAGE OF THE ARTICULAR CHONDROCYTE

## Progenitor Cells\*

- 
- Bone marrow stromal cell (mesenchymal stem cell)
  - Pericyte ?
  - Smooth muscle cell ?
  - Synovial cell ?
- \* All express SMA
-

**Marrow Stromal Cells in  
Monolayer Culture (7 days)  
SMA IH (>90% SMA+)**

**Neg. control**

Photos removed for  
copyright reasons.

**Lapine, p2**

**D. Cai, *et al.*  
*Tiss. Engr.* 7;829-841(2001)**

**Canine, p1**



# Mesenchymal Stem Cell-Seeded Matrices

Graph and two photos  
removed for  
copyright reasons.

**D. Cai, *et al.***  
***Tiss. Engr.* 7;829-841 (2001)**

# **CORRELATION OF CONTRACTION WITH SMA CONTENT**

## **Regulation of SMA Expression**

- **Time in culture**
- **Selected growth factors**
  - **TGF- $\beta$ 1 increases SMA expression**

# **Western blot evaluation of the SMA content of human articular chondrocytes with time in culture**

Diagram and graph removed for copyright reasons.

**B. Kinner and M. Spector**  
***JOR* 2001;19:233-24**

**Does contraction increase with increasing SMA?**

**Passage 7 cells that contain more SMA cause 40% more contraction of the collagen-GAG matrix**



Graph removed for copyright reasons.

Graph removed for copyright reasons.

**Subtract contraction of non-seeded matrix and normalize to cell number**

**B. Kinner and M. Spector  
*JOR* 2001;19:233-24**

# SMA Western Blots

## *Articular Cartilage*

Photo removed for  
copyright reasons.

## *Meniscus*

Photo removed for  
copyright reasons.

# **SMA WESTERN BLOT RESULTS**

## **Human Articular Chondrocytes**

Graph removed for  
copyright reasons.

# Cell Force Monitor

Diagram removed for copyright reasons.  
See Freyman, T.M., I.V. Yannas, R. Yokoo, and L.J. Gibson. "Fibroblast contraction of a collagen-GAG matrix." *Biomaterials* 22 (2001) 2883-2891.

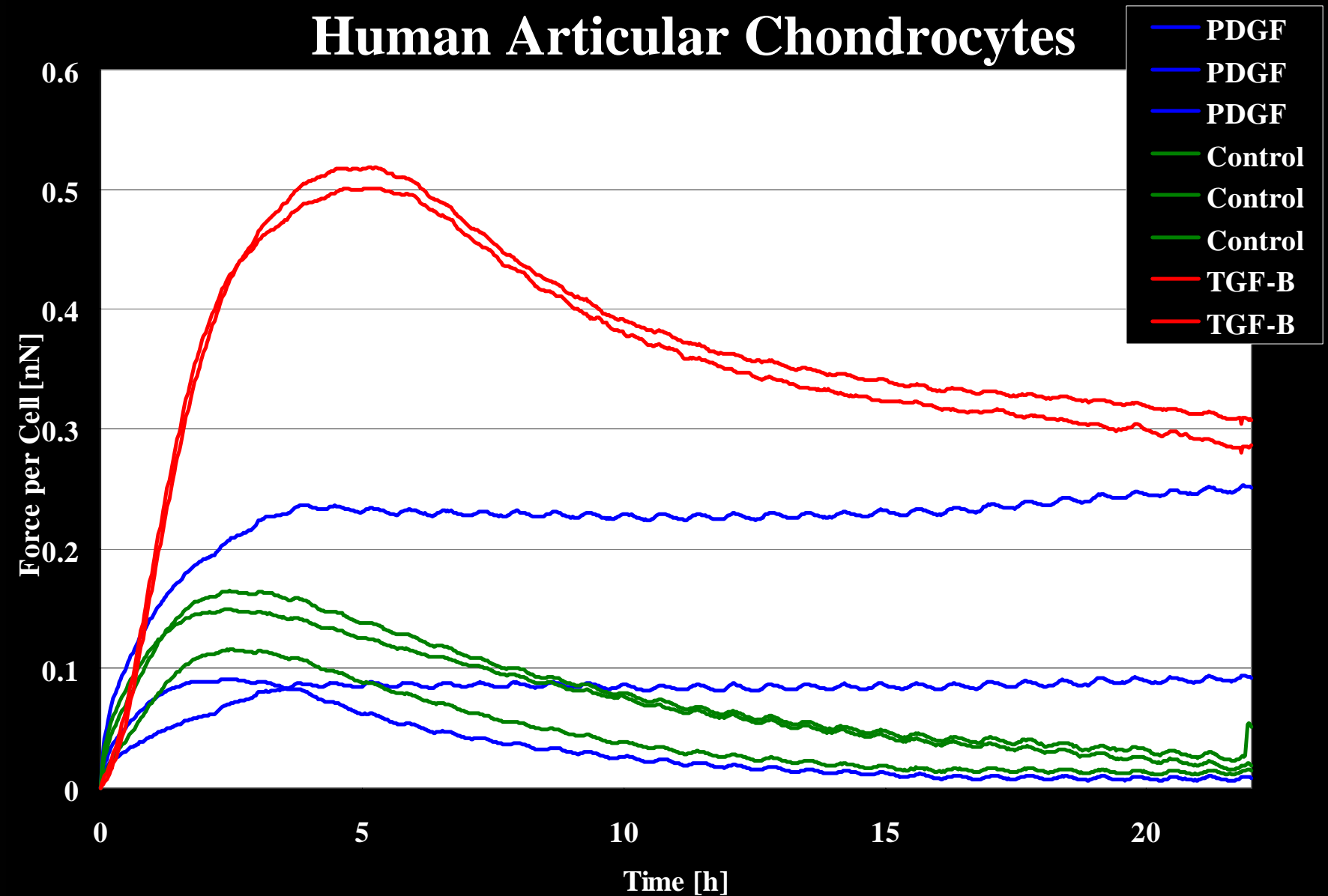
# Adult Canine Articular Chondrocytes Cell Force Monitor

Graph removed for  
copyright reasons.



# Cell Force Monitor Results

## Human Articular Chondrocytes



# ***IN VITRO* MODELS FOR CELL CONTRACTION**

- **Cells seeded into a preformed collagen-GAG sponge**
  - Measure dimensional change
  - Cell force monitor
- **Cells wrinkling a flexible (silicone) membrane**
  - Measure % cells wrinkling the membrane
- **Cells cast in a collagen gel (lattice)**
  - Measure the dimensional change

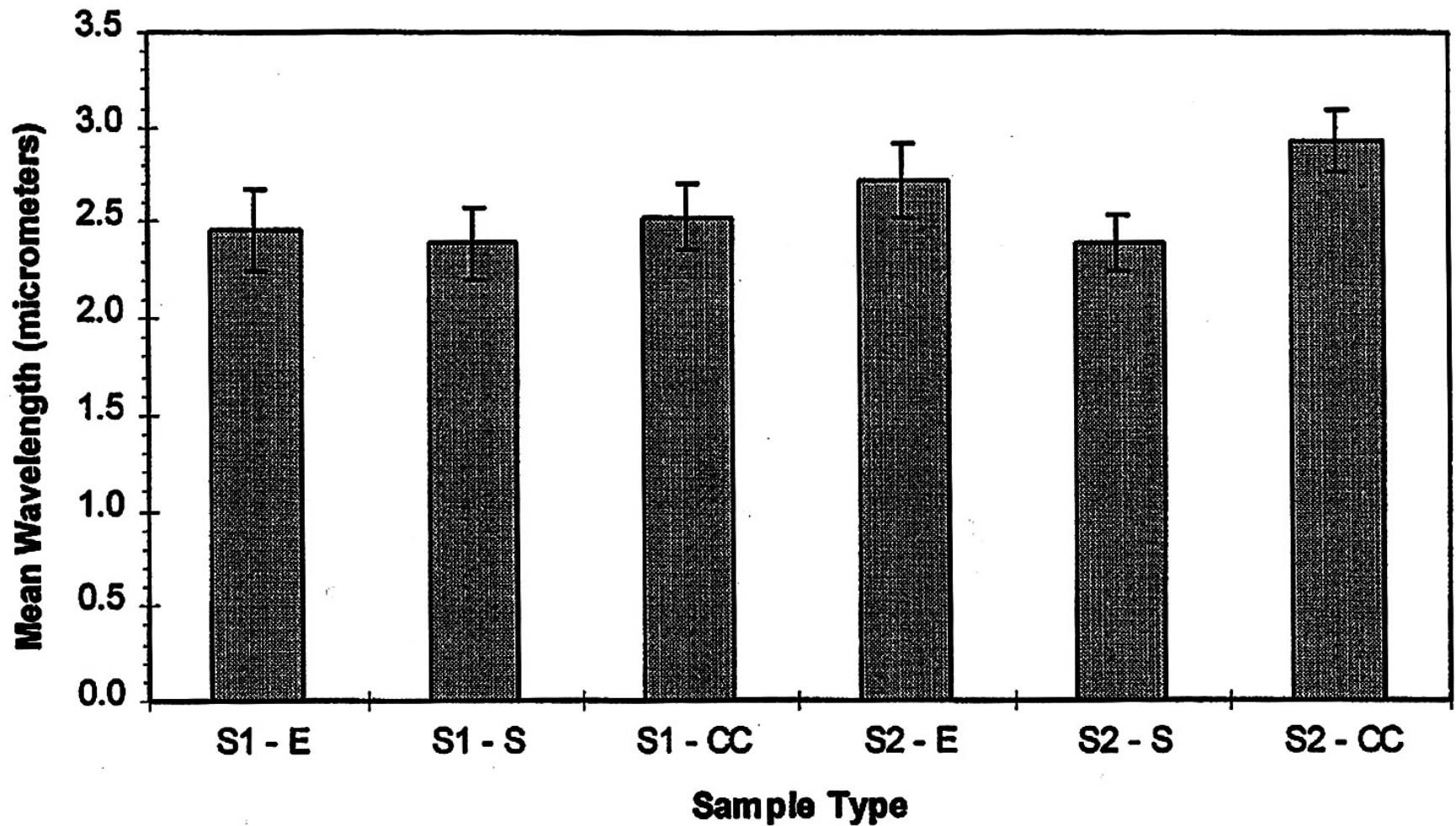


# Fibroblasts on Silicone Membranes





# Fibroblast Wrinkling of a Silicone Membrane



E, elongated  
S, stellate  
CC, cell-cell

L. Suh, *et al.*

# Lung Fibroblasts on a Silicone Membrane

**SMA (red)**

Photos removed for copyright reasons.  
See Figure 2 in Hinz, B. et al. "Alpha-Smooth Muscle Actin  
Expression Upregulates Fibroblast Contractile Activity."  
*Mol. Biol. Cell* 12:2730-2741 (2001).

**F-actin**

**Overlay  
SMA yellow**

# Wrinkling of a Silicone Membrane by Fibroblasts Correlates with SMA

Graphs removed for copyright reasons.

See Figure 1 in Hinz, B. et al. "Alpha-Smooth Muscle Actin Expression Upregulates Fibroblast Contractile Activity."  
*Mol. Biol. Cell* 12:2730-2741 (2001).

Graph removed for copyright reasons.

See Figure 3 in Hinz, B. et al. "Alpha-Smooth Muscle Actin Expression Upregulates Fibroblast Contractile Activity."  
*Mol. Biol. Cell* 12:2730-2741 (2001).

# ***IN VITRO* MODELS FOR CELL CONTRACTION**

- **Cells seeded into a preformed collagen-GAG sponge**
  - Measure dimensional change
  - Cell force monitor
- **Cells wrinkling a flexible (silicone) membrane**
  - Measure % cells wrinkling the membrane
- **Cells cast in a collagen gel (lattice)**
  - Measure the dimensional change



# **COLLAGEN GEL CONTRACTION ASSAY**

- **Cells cast in gelatin.**
- **Gel can be fixed to the culture dish or free-floating.**
- **In one assay, the gel is fixed to the dish for 5 days and then released and the dimensional change recorded.**

# Fibroblasts from Dupuytren's Contracture in a Collagen Gel

Graphs removed for  
copyright reasons.

# Fibroblasts Cast in a Collagen Gel (Lattice)

Figure removed for copyright reasons.

See Figure 4 in Hinz, B. et al. "Alpha-Smooth Muscle Actin Expression Upregulates Fibroblast Contractile Activity."  
*Mol. Biol. Cell* 12:2730-2741 (2001).

# Fibroblasts in Collagen Gels for 5 days: IH

SMA, red; F-actin, green in A, B, D, E; ED-A FN, green C, F; nuclei, blue

Photo removed for copyright reasons.

See Figure 5 in Hinz, B. et al. "Alpha-Smooth Muscle Actin Expression Upregulates Fibroblast Contractile Activity."

*Mol. Biol. Cell* 12:2730-2741 (2001).

# ***IN VITRO* MODELS FOR CELL CONTRACTION**

- **Cells seeded into a preformed collagen-GAG sponge**
  - Measure dimensional change
  - Cell force monitor
- **Cells wrinkling a flexible (silicone) membrane**
  - Measure % cells wrinkling the membrane
- **Cells cast in a collagen gel (lattice)**
  - Measure the dimensional change

# CONTRACTILE CONNECTIVE TISSUE CELLS

- Express SMA *in vivo*
- Capable of contracting collagen-GAG matrices *in vitro*
- SMA-positive cells retain differentiated phenotype
- SMA trait derived from the stem cell
- Amount of contraction correlated with the SMA content
- SMA and contraction up-regulated by TGF- $\beta$ 1
- Roles have yet to be determined, but may be both positive and negative