

# **EFFECT OF HYALURONANS ON CYTOKINE-INDUCED METALLOPROTEINASE ACTIVITY SECRETED BY SYNOVIAL LINING CELLS FROM PATIENTS WITH OSTEOARTHRITIS**

**Dunn, S., Waddell, D.D., Kolomytkin, O.V., Marino, A.A.**

Department of Orthopaedic Surgery,  
Louisiana State University Health Sciences Center,  
Shreveport, Louisiana  
amarino@lsuhsc.edu

Viscosupplementation using hyaluronans (HAs) is approved for treating pain due to osteoarthritis (OA). One possible mechanism is the reduction in expression of metalloproteinases (MMPs), cartilage-destroying enzymes that are produced in response to proinflammatory cytokines. We hypothesized that the HAs reduced IL-1-induced MMPs secreted by synovial lining cells in OA patients, and tested the hypothesis using synovial biopsies from patients having total knee replacement for OA, Kellgren-Lawrence Grade IV. All experimental procedures were approved by the Institutional Review Board for Human Research at our institution.

The tissues were taken from the suprapatellar pouch and the fat pad using the full bite of an arthroscopic basket punch. The biopsies averaged 20 mg ( $0.12 \text{ cm}^2$ )  $\pm$  15%. Up to twelve biopsies were obtained from each patient.

The HAs studied were Hyalgan (0.5–0.7 MDa); Supartz (0.6–1.2 MDa); Synvisc (6.0 MDa); and two non-clinical HAs (Lifecore, 0.22 MDa and 1.4 MDa). The clinical HAs were supplied in PBS and were transferred to Neuman-Tytell media (NTM) by dialysis, performed at 5°C for one week. The non-clinical HAs were obtained as solids and dissolved directly in NTM. All HA solutions were adjusted to a concentration of 8 mg/mL.

The tissues were incubated at 37°C for 24 hours in 200  $\mu$ L NTM containing various combinations of IL-1 (100 pg/mL) and HAs. The MMP activity of the supernatant (principally MMP-1 and MMP-3) was then measured using collagen film as a substrate, after MMP activation by 1 mM p-amino phenylmercuric acetate. Further details are given elsewhere (1).

The results, expressed in percent (mean  $\pm$  SE) as an inhibition of the MMP response induced by IL-1 (considered as 100%) were: 0.22 MDa HA,  $94.8 \pm 3.1$  (N=4); Hyalgan,  $85.8 \pm 1.4$  (N=5); 1.4 MDa,  $28.4 \pm 1.4$  (N=5); Supartz,  $9.0 \pm 2.2$  (N=4); Synvisc,  $2.3 \pm 2.5$  (N=5). The inhibition of IL-1 induced MMPs caused by each of the HAs was significant at  $P < 0.05$ . Control experiments indicated that none of the HAs affected MMP production in the absence of IL-1 (data not shown).

HAs blocked IL-1-induced MMP activity produced by synovial lining cells. The inhibition occurred via a process that may have depended on HA molecular weight, because inhibition was generally greater with increasing HA molecular weight.

The results established that HAs can inhibit cytokine-induced MMP activity, as hypothesized, suggesting that the mode of action of HA might be due to reduced MMP activity, with concomitant reduction in the pain caused by inflammation secondary to the destruction of cartilage by MMPs.

(1) Kolomytkin, O.V., Marino, A.A., et al. *Am. J. Physiol.* 282:C1254–C1260, 2002.

Supported by Genzyme.

# **Effect of Hyaluronans on Cytokine-Induced Metalloproteinase Activity**

**Sharon Dunn**

**David D. Waddell**

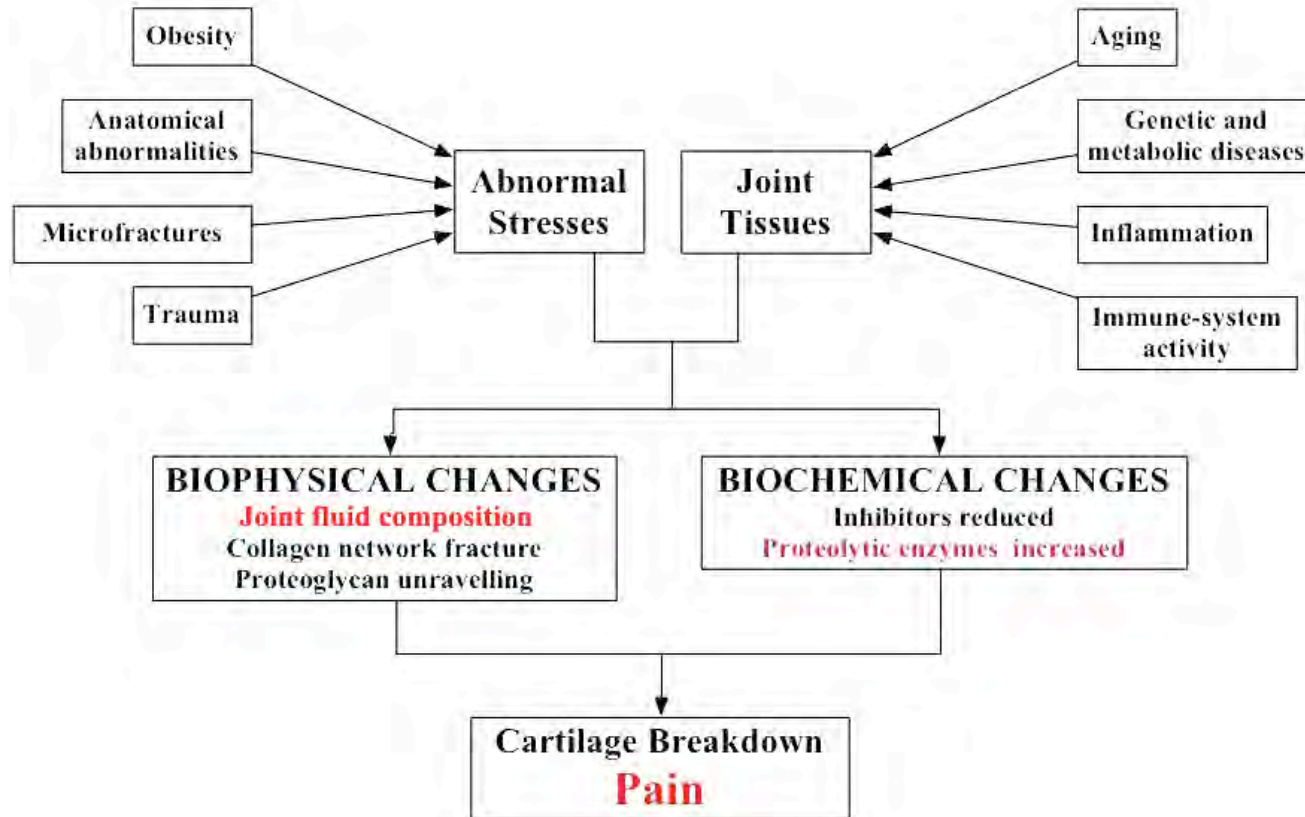
**Oleg V. Kolomytkin**

**Andrew A. Marino**

# Summary

- Effects of hyaluronan (HA) on IL-1 $\beta$ -induced matrix metalloproteinases (MMPs) produced by synovial lining cells
- HA antagonizes MMPs
- Role of molecular weight and concentration
- Significance for viscosupplementation
- Possible HA mechanism: gene arrays

# Factors Involved in Osteoarthritis



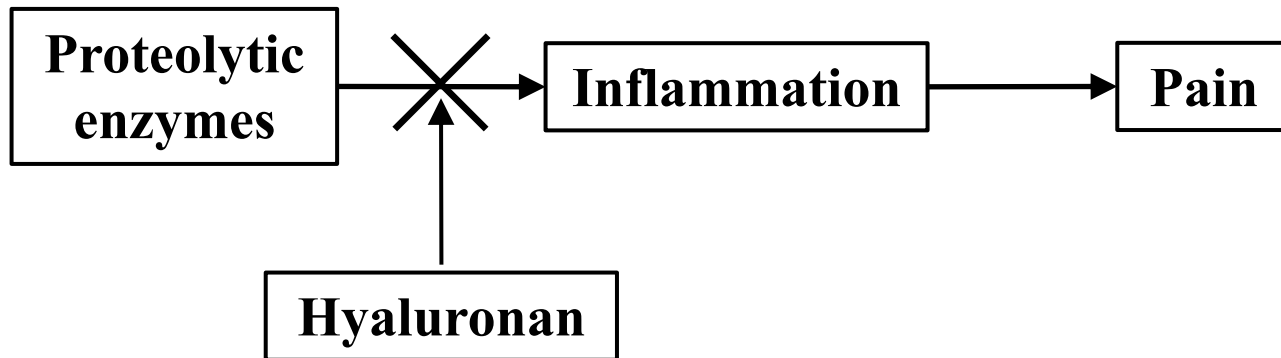
# Viscosupplementation

## Exogenous Hyaluronan for Pain

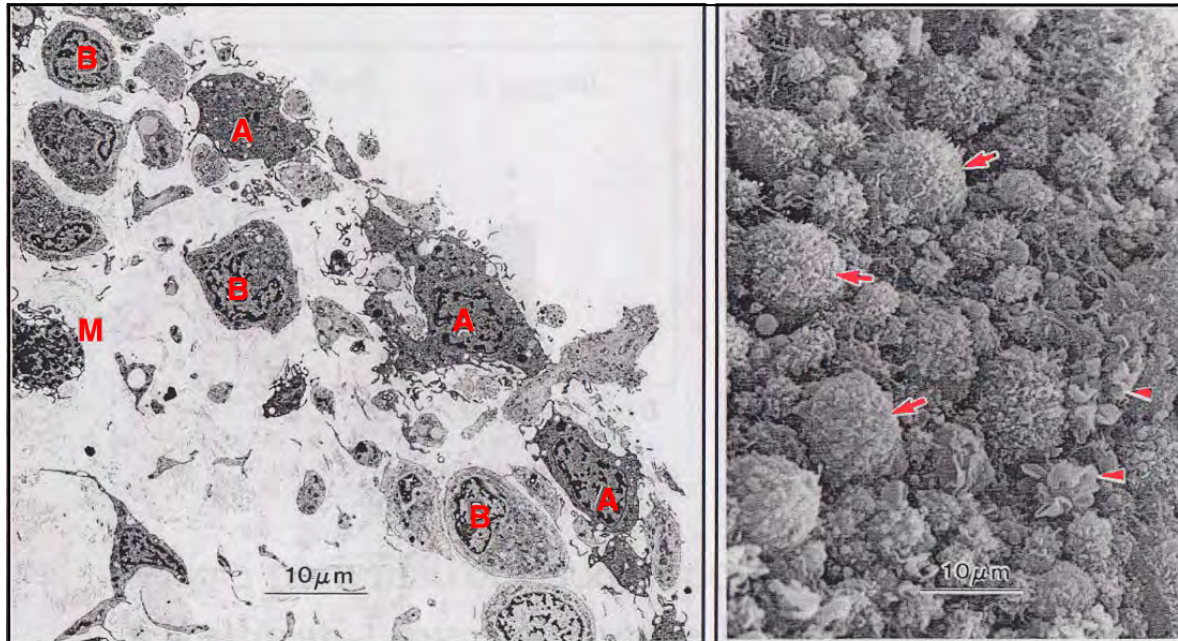
- Biophysical versus biomechanical mechanisms
- Direct versus indirect effects

# Hypothesis

Exogenous hyaluronan antagonizes production of proteolytic enzymes by synovial cells



# Organization of Synovium



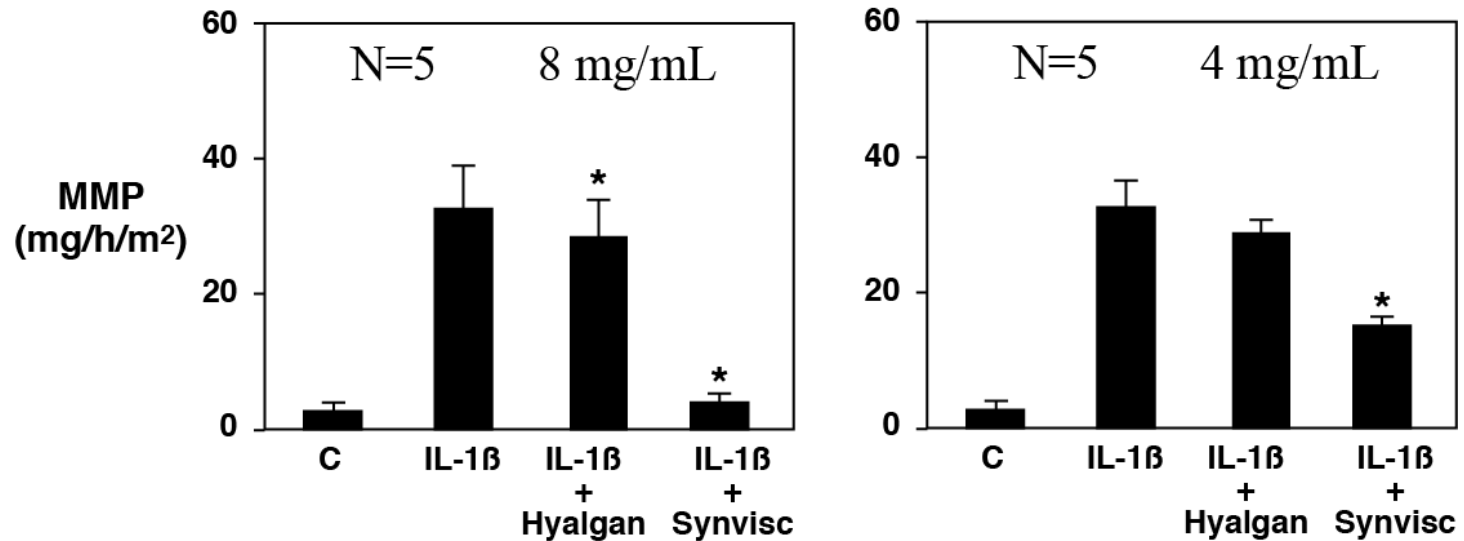
**Type A: Macrophage**

**Type B: Secretory**

**M: Mast Cell**

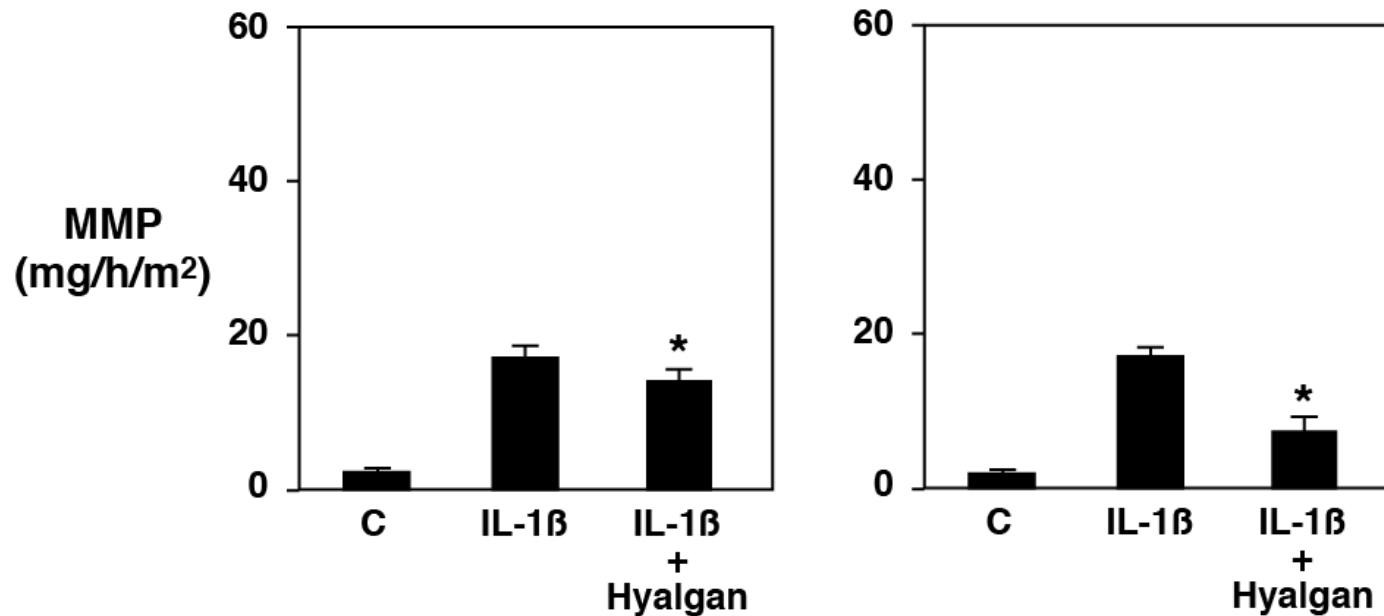


# Effect of HA on Stimulated MMP Production from OA Synovial Tissue



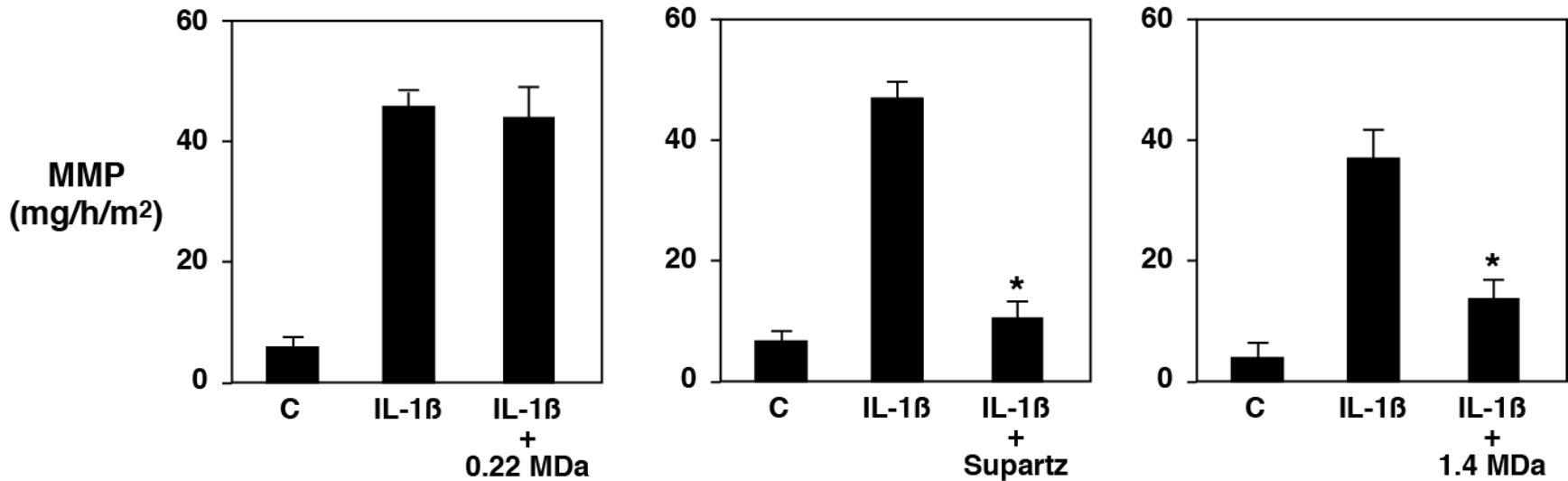
- N=5 patients for each experiment
- Kellgren-Lawrence grade IV OA
- \*P < 0.05, paired t test

# Effect of HA on Stimulated MMP Production from Control Synovial Tissue



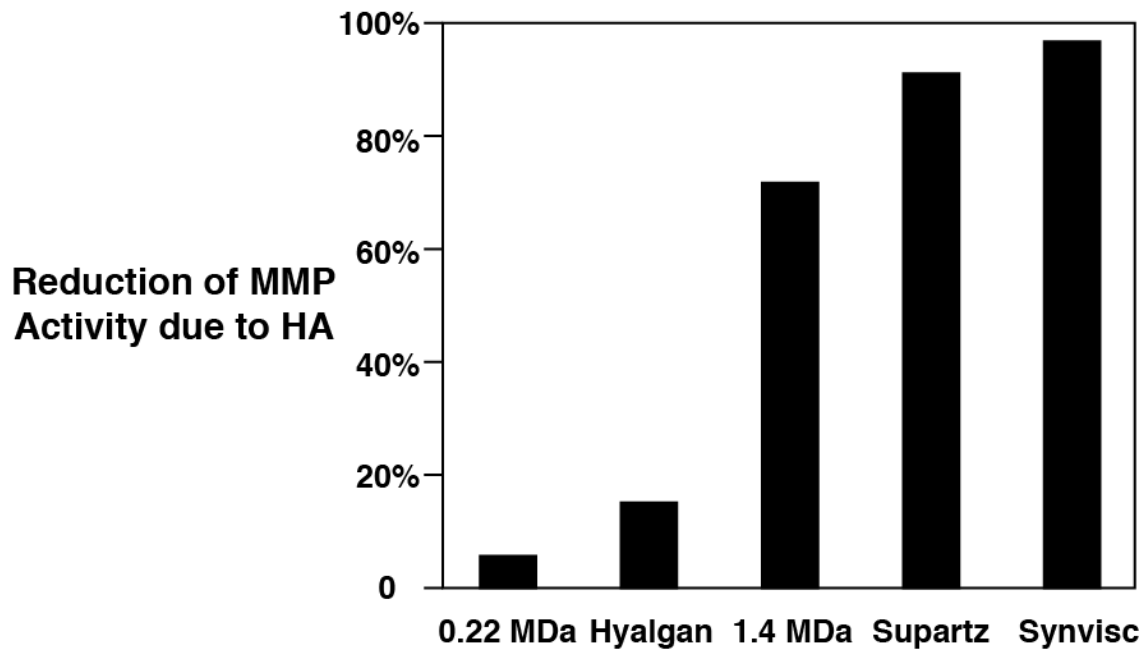
- N=5 patients for each experiment
- No OA
- \*P < 0.05, paired t test

# Effect of HA Molecular Weight on Stimulated MMP Production from OA Synovial Tissue



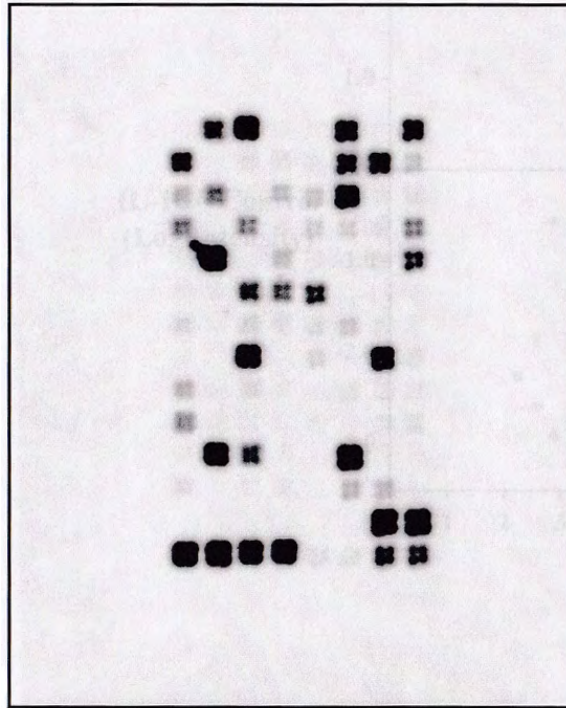
- N=5 in each experiment
- \*P < 0.05, paired t test

# Relative Ability of HAs (8 mg/mL) to Block IL-1 $\beta$ -Induced MMP Production from OA Tissue

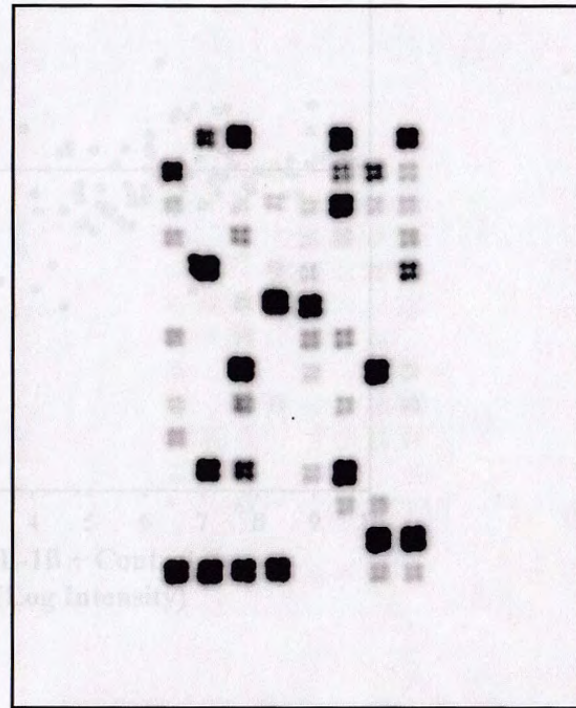


# Signal Transduction Gene Array

Rabbit Synovial Fibroblasts

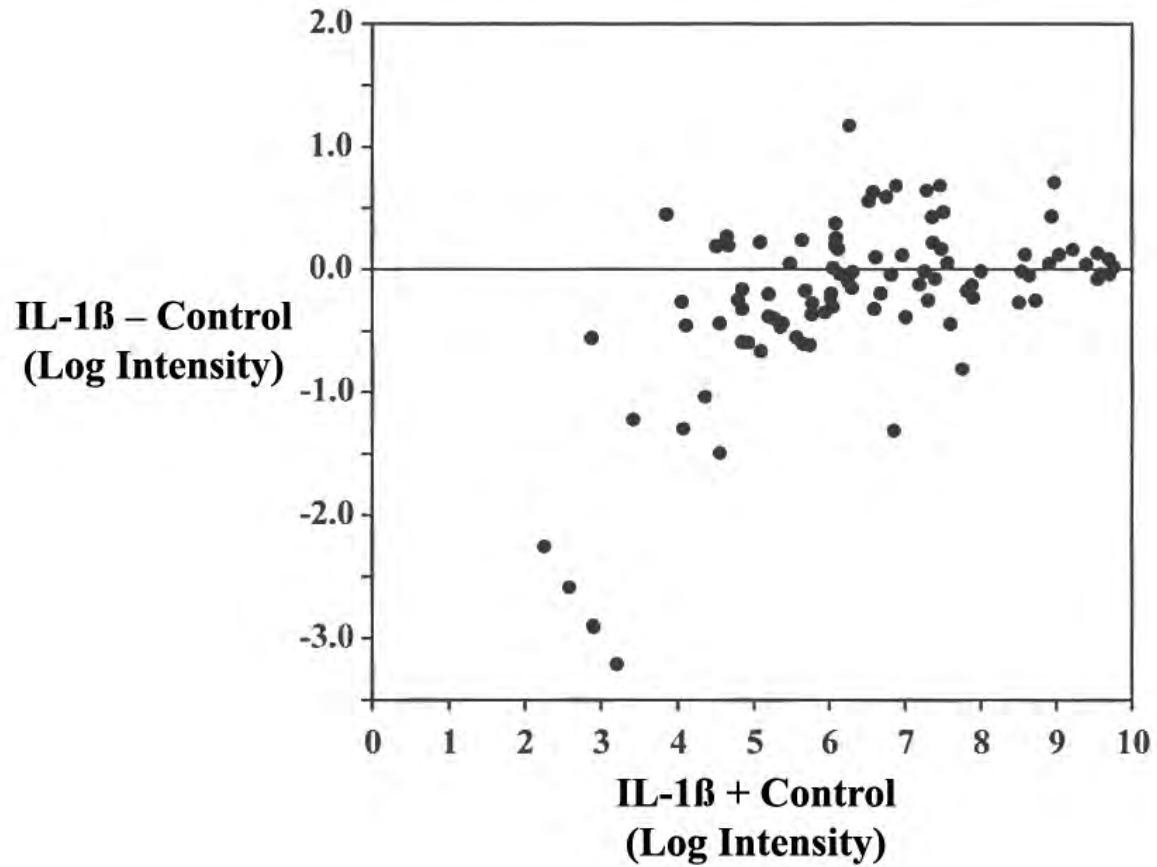


**Control**



**IL-1 $\beta$**

# DNA Array



# Conclusion

- High MW, 8 mg/mL, inhibits cytokine-induced MMPs
- Possible explanation for viscosupplementation efficacy (indirect effect)
- Strong dependence on HA concentration
- Many possible pathways for effect of HA