

## **Viscosupplementation: Evidence for a Mechanism of Action**

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Viscosupplementation is a procedure in which a form of hyaluronan, a glycosaminoglycan, is injected into a joint [1]. Three proprietary hyaluronans are approved by the Food and Drug Administration for treatment of knee pain secondary to osteoarthritis.

The mechanism of action initially proposed to explain the effect of hyaluronan on knee pain involved increased lubricity of joint fluid, which was thought to have been impaired during the course of the disease. Nevertheless, early in the development of viscosupplementation it was recognized that the residence time of exogenous hyaluronan was far less than the duration of the time within which relief from pain was found to occur.

A biological process of some type must therefore be responsible for the beneficial effect of hyaluronan that occurs after its removal from the joint.

In the normal joint, cartilage and other joint tissues are continuously being remodeled by means of a complex regulatory system consisting of cytokines and enzymes, produced principally by synovial cells. Osteoarthritis can be thought of as a form of dysregulation of this process in which there is an excess production of proteolytic enzymes, leading to cartilage destruction, inflammation, and pain.

Interleukin-1 $\beta$  (IL-1 $\beta$ ) is one of the major proinflammatory cytokines. We hypothesized that hyaluronan reduced synovial production of proteolytic enzymes, thereby reducing inflammation and pain in the joint.

We previously tested this hypothesis in tissue culture, using an animal synovial cell line. In the present study, the work was extended to the study of synovial cells from patients with osteoarthritis. All experimental procedures were approved by the Institutional Review Board for Human Research at our institution.

Tissues were taken from the suprapatellar pouch and the fat pad of patients who were undergoing total joint arthroplasty, using a full bite of an arthroscopic basket punch (No. 012013, Acufex, Smith & Nephew, Andover, MA). Only relatively flat areas of the synovium were biopsied to minimize variations in the number of

synovial cells attributable to irregularities of the synovial surface. The biopsies averaged approximately 20 mg, 0.12 cm<sup>2</sup>, ±15%. Between 9–12 biopsies were taken from each patient.

Our experimental model consisted of culturing the synovial tissue biopsy in a chemically defined medium that contained no cytokines. IL-1 $\beta$  was added to the medium, and after 24 hours we measured the amount of proteolytic enzyme activity present in the supernatant.

The assay method, which we developed [2], was designed to detect metalloproteinases (MMPs), which are the principal proteolytic enzymes involved in the degradation of joint cartilage. Briefly, after the synovial tissue had been stimulated in culture for 24 hours, the supernatant was recovered and added to 96-well plates that contained a film of collagen, which served as the substrate for the MMPs. There are many different MMPs; our assay was designed to detect mostly MMP1. The reaction was allowed to proceed for a fixed time, after which the amount of collagen remaining in the plate was measured using optical methods, and the enzyme activity was calculated.

We studied the effect of five different hyaluronans (HAs) on the ability of IL-1 $\beta$  to stimulate synovial cells to secrete MMPs. Three of the HAs were approved for viscosupplementation; two others were included in the study to permit us to evaluate the role of molecular weight.

The three proprietary HAs differed in concentration and in the number of injections per course required for clinical efficacy. In this study, the HA concentrations were standardized at 8 mg/mL, which is the concentration of HA in Synvisc, but less than the HA concentration in Hyalgan and Supartz. In addition, we conducted additional studies at 4 mg/mL using Synvisc and Hyalgan.

When synovial tissue was cultured under the conditions we used, the synovial cells spontaneously produced a low level of enzyme activity (first bar). When IL-1 $\beta$  was added a significant increase in MMP activity was observed, as reported previously [3]. We could be certain that it was the synovial cells that produced the MMPs, not the fat cells that were also present in the biopsy, because we conducted other studies (not discussed here) in which we showed that fat cells did not respond to IL-1 $\beta$  [3]. When Hyalgan and IL-1 $\beta$  were added simultaneously, there was a small but statistically significant reduction in the IL-1 $\beta$ -induced MMP activity (third bar). Synvisc, in contrast, almost completely blocked the effect of IL-1 $\beta$ .

The experiment was repeated with three other hyaluronans, namely a low molecular weight HA (0.22 MDa) and two higher molecular weight HAs, one of which was Supartz. We found that only the higher molecular weight HAs were able to block the effect of IL-1 $\beta$ .

In each of the four preceding experiments, there were slight variations in the amount of MMPs that were produced by control biopsies. When the data was corrected for these variations so that the amount of reduction of IL-1 $\beta$ -stimulated activity of each of the HAs could be expressed on a 0-100% scale, we found that the higher molecular weight HAs were effective in blocking the pro-inflammatory consequences of IL-1 $\beta$ , thereby supporting our hypothesis.

Our observation that high molecular weight HA blocked enzyme production far more effectively than did low molecular weight HA has many possible explanations, one of which is that the effect is due to the viscosity of the HAs, because high molecular weight HAs are more viscous. One initial experimental approach to this question consisted of studying the effects of less concentrated and hence less viscous HA solutions. What we found when we examined the effect of 4 mg/mL of Hyalgan and Synvisc was a significant change in their ability to block cytokine-induced MMP production. At this concentration, we could not detect an effect due to Hyalgan, and the effect of Synvisc was much less than that caused by the higher concentration.

A problem with this experiment from a strictly scientific viewpoint is that both the viscosity and concentration were changed when the HA reduced from 8 to 4 mg/mL. Therefore no insight was provided into the responsible mechanism. From a clinical viewpoint, however, the data is important for the following reason.

Dilution of exogenous HA by residual joint fluid always occurs whenever a patient is treated with viscosupplementation. To examine the extent of this effect, we measured the amount of joint fluid that remained in the knee joint after it had been aspirated. Data was obtained under anesthesia from a consecutive series of 20 patients undergoing total knee replacement for osteoarthritis. A standard aspiration was done using a 21-gauge 3-inch spinal needle, with the knee in extension and the patient supine. In the first 4 patients, the needle was inserted at the location of the standard superolateral arthroscopy portal. In the next 16 patients, an additional aspiration attempt was made with the knee flexed and the needle inserted into the anterolateral compartment from an anterior approach. Then a standard arthrotomy was performed, and the suprapatellar pouch, medial compartment, lateral compartment, and intercondylar notch were observed directly, and any fluid in these areas was aspirated under direct vision.

We found that the average volume of fluid retained in the knee joint was 7.15 mL, range 3–13 mL.

Assume that each knee had been injected with 2 mL of HA of any approved viscosupplement, and that perfect mixing occurs with the joint fluid. The largest HA concentration would occur in patients with the smallest residual volume of synovial fluid, 3 mL, as indicated by the red points. The corresponding HA concentration would

therefore represent the maximum concentration of HA achievable in the joint. The scale on the right side shows the reduction in HA concentration (below the maximum level achievable) for the remaining 18 patients.

It can be seen that residual synovial fluid will normally result in large, uncontrolled variation in exogenous HA concentration in the joint. Thus, regardless of whether the mechanism of action of HA involves concentration or viscosity, it would be reasonable to expect that the clinical efficacy of viscosupplementation could be markedly influenced by the effectiveness with which the joint was aspirated.

In conclusion, our data supports the hypothesis that the mechanism responsible for pain relief in viscosupplementation is an anti-inflammatory effect due to HAs having high molecular weight. Of course, there are other possibilities.

The anti-inflammatory effect of HA depended strongly on its concentration. Considering the normal range of residual joint fluid following aspiration, our observation of the concentration dependence of HA inhibition of MMPs suggested that dilution of the viscosupplement could be an important source of variability in the efficacy of viscosupplementation.

1. Kelly, M.A., Goldberg, V.M., Healy, W.L., Pagnano, M.W. and Hamburger, M.I., Osteoarthritis and beyond: A consensus on the past, present, and future of hyaluronans in orthopedics, in *Orthopedics CME Quiz*. 2003.
2. Kolomytkin, O.V., Marino, A.A., Waddell, D.D., Mathis, J.M., Wolf, R.E., Sadasivan, K.K. and Albright, J.A., IL-1 $\beta$ -induced production of metalloproteinases by synovial cells depends on gap-junction conductance during the early stage of signal transduction. *Am. J. Physiol.* **282**:C1254-C1260, 2002.
3. Marino, A.A., Waddell, D.D., Kolomytkin, O.V., Meek, W.D., Wolf, R.E., Sadasivan, K. and Albright, J., Increased intercellular communication through gap junctions may contribute to progression of osteoarthritis. *Clin. Orthop.* **In Press**, 2003.



# **Viscosupplementation: Evidence for a Mechanism of Action**

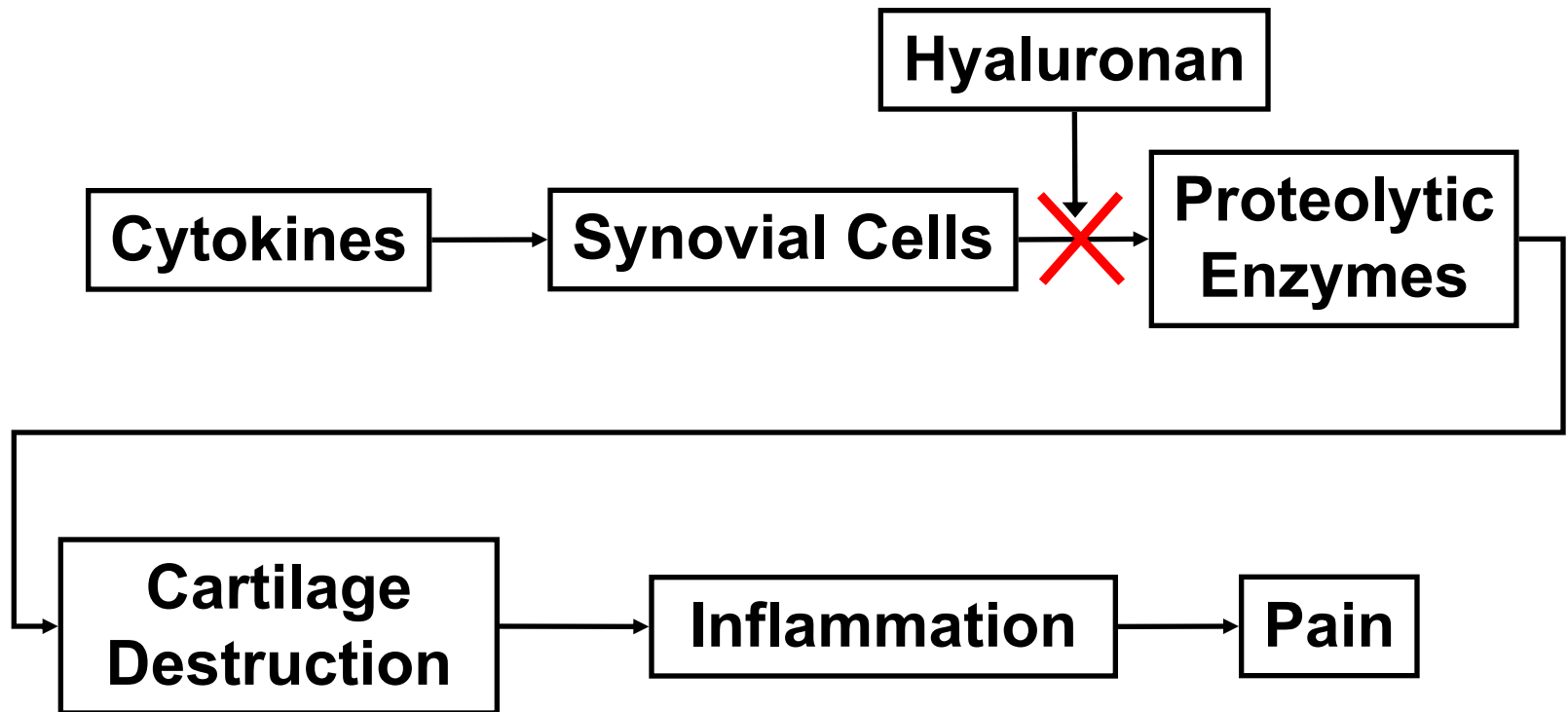
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# Viscosupplementation

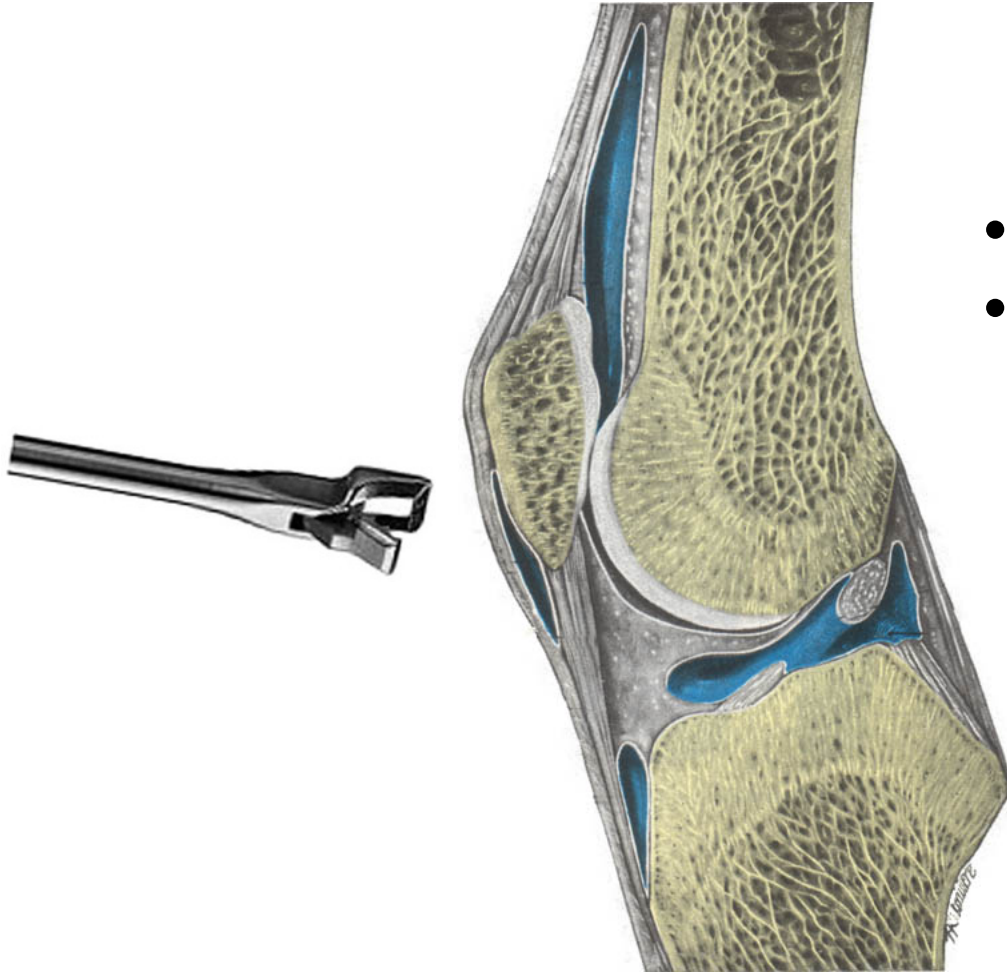
- Exogenous hyaluronan for pain
- Biophysical mechanisms
- Biological mechanisms

# Osteoarthritis



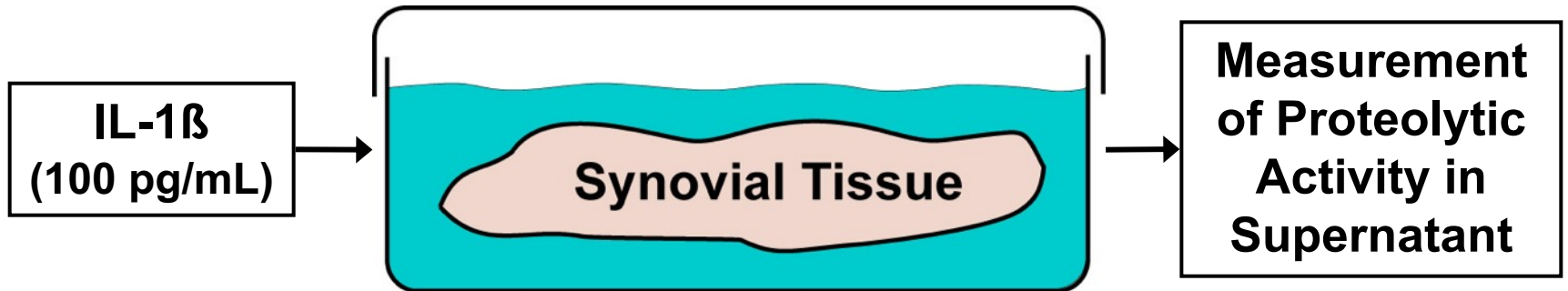
Hypothesis: Hyaluronan blocks enzyme production

# Surgical Biopsy Procedure

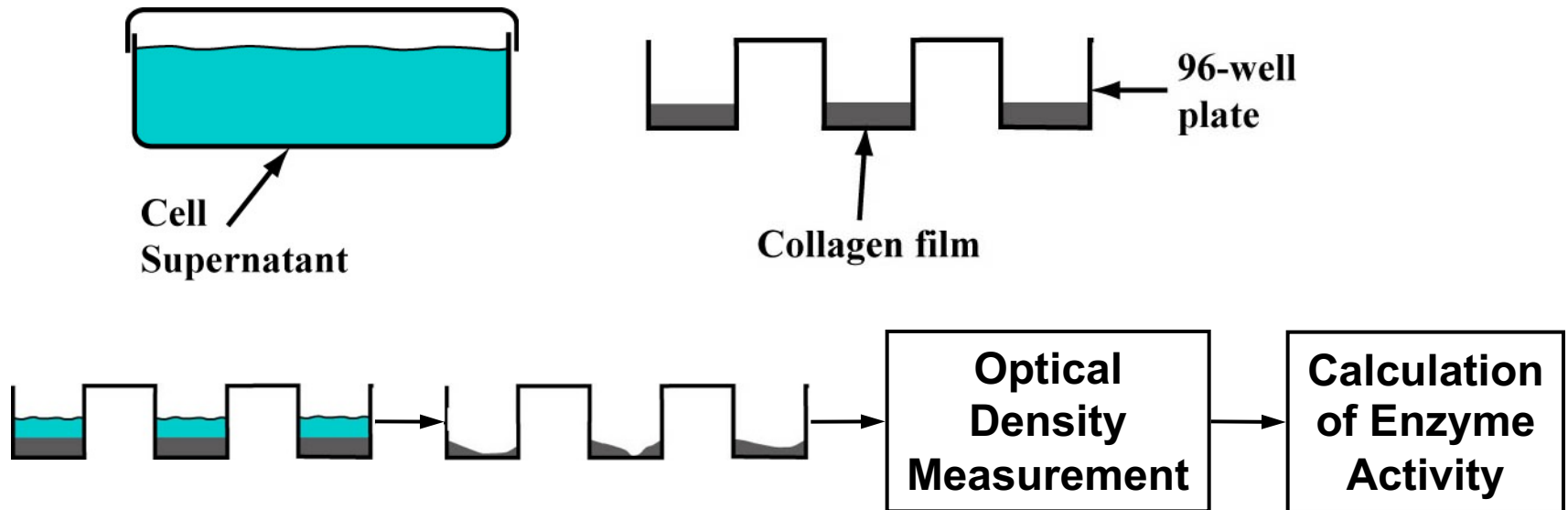


- 20 mg, 0.12 cm<sup>2</sup> ( ±15%)
- 9-12 biopsies per patient

# Experimental Model



# Assay Method for Metalloproteinases (MMPs)

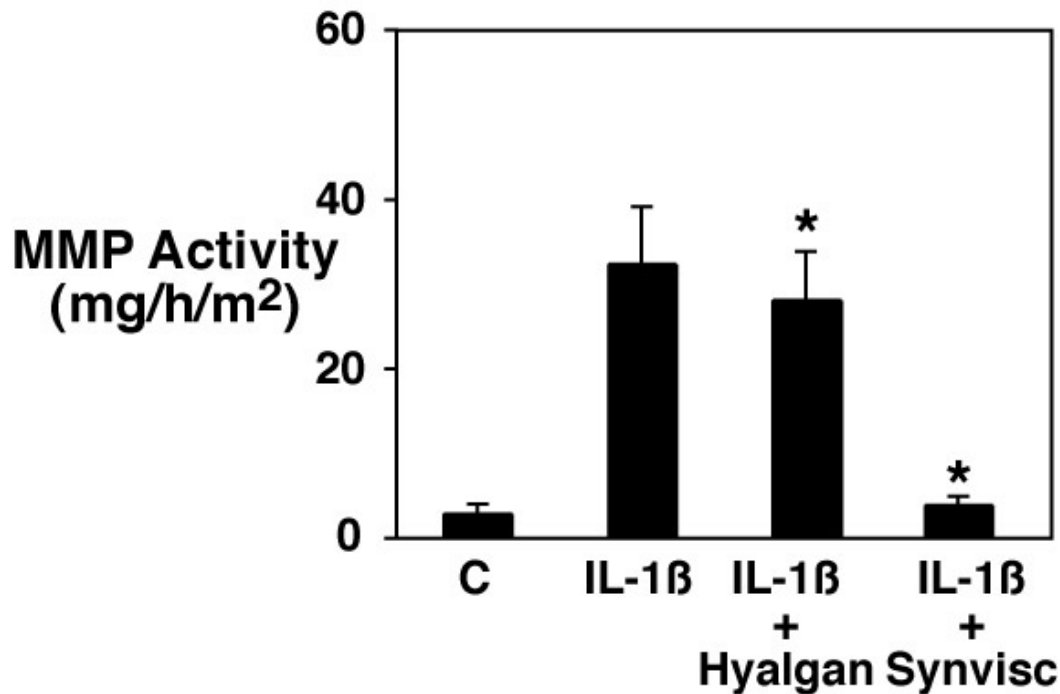


- Mean of triplicate measurements per condition per patient

# Hyaluronans Studied

<b><u>PROPRIETARY NAME</u></b>	<b><u>SOURCE</u></b>	<b><u>MOLECULAR WEIGHT (MDa)</u></b>	<b><u>CONCENTRATION (mg/mL)</u></b>	
			<b><u>Clinical</u></b>	<b><u>This Study</u></b>
<b><i>Synvisc</i></b>	<b>Genzyme</b>	<b>6</b>	<b>8</b>	<b>8, 4</b>
<b>—</b>	<b>Lifecore</b>	<b>1.4</b>	<b>—</b>	<b>8</b>
<b><i>Supartz</i></b>	<b>Seikagaku</b>	<b>0.6-1.2</b>	<b>10</b>	<b>8</b>
<b><i>Hyalgan</i></b>	<b>Fidia</b>	<b>0.5-0.7</b>	<b>10</b>	<b>8, 4</b>
<b>—</b>	<b>Lifecore</b>	<b>0.2</b>	<b>—</b>	<b>8</b>

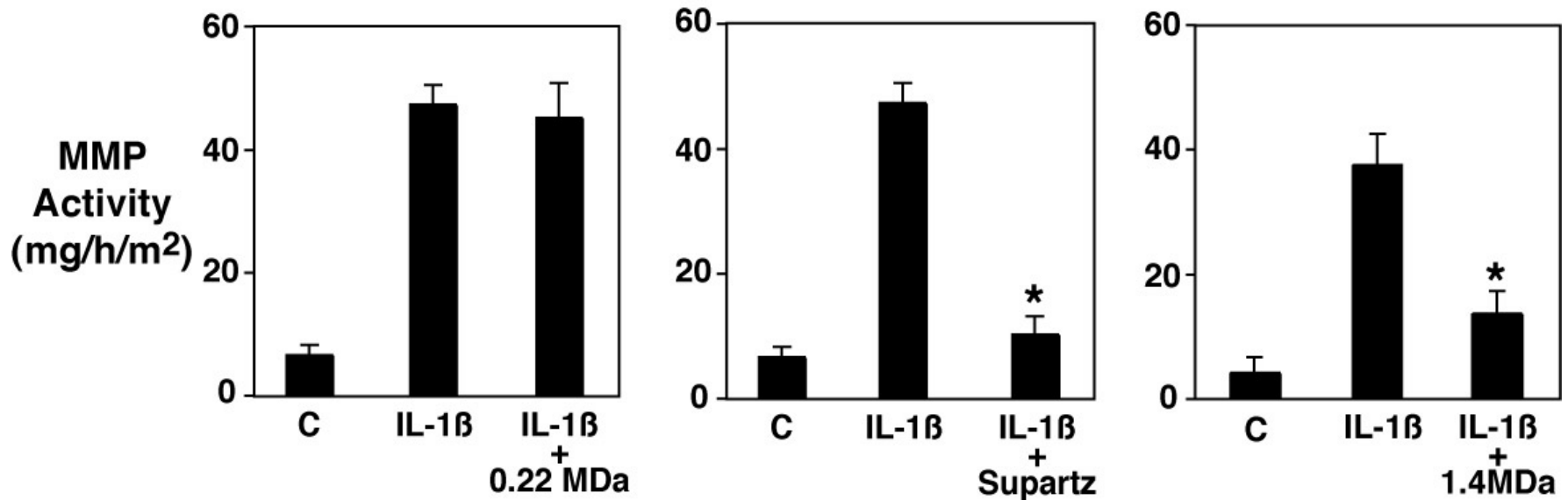
# Effect of Hyalgan and Synvisc on Stimulated MMP Production from Synovial Tissue



- **8 mg/mL**
- N=5 patients
- Grade IV OA
- \*P<0.05, paired t test



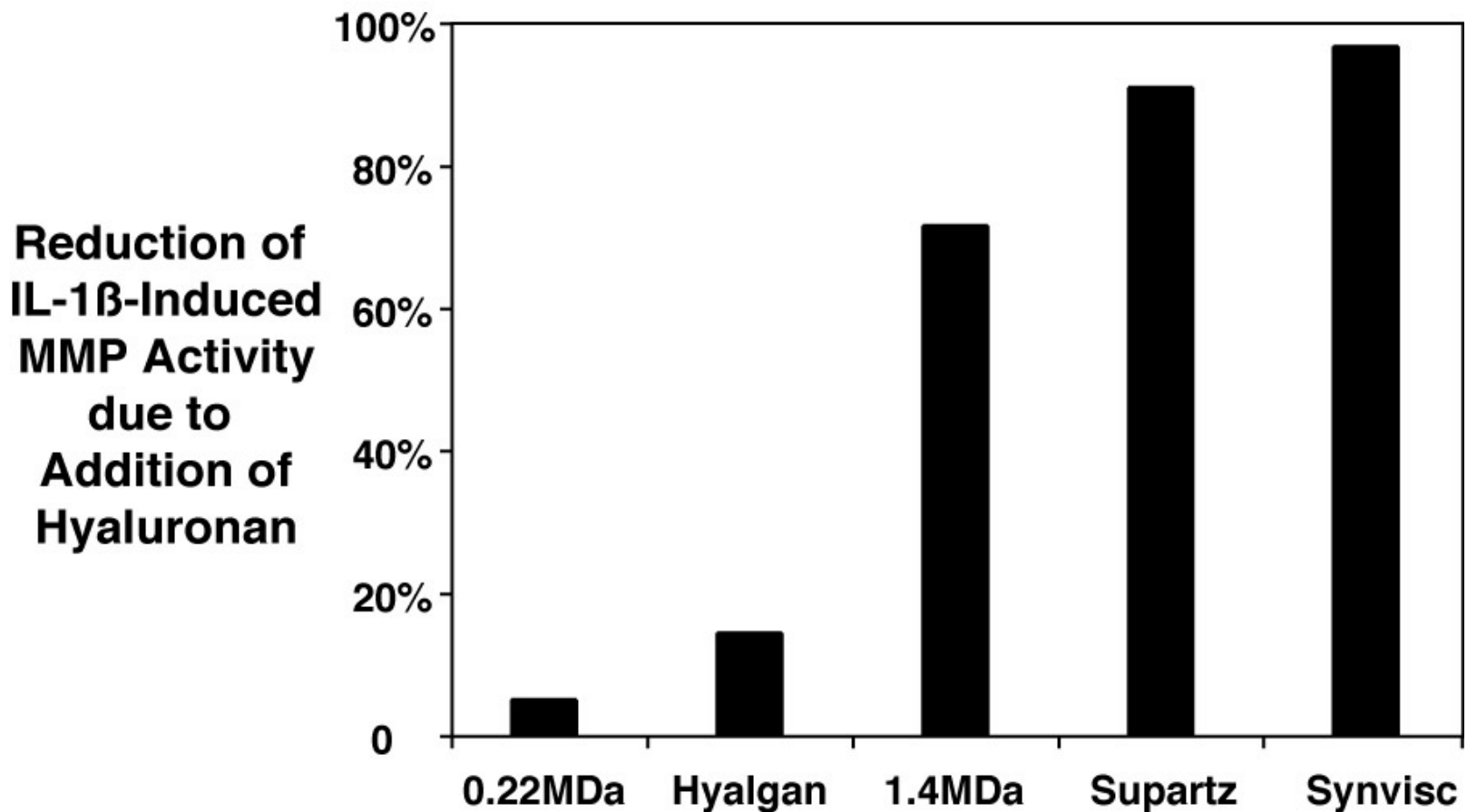
# Effect of Hyaluronans on Stimulated MMP Production from Synovial Tissue



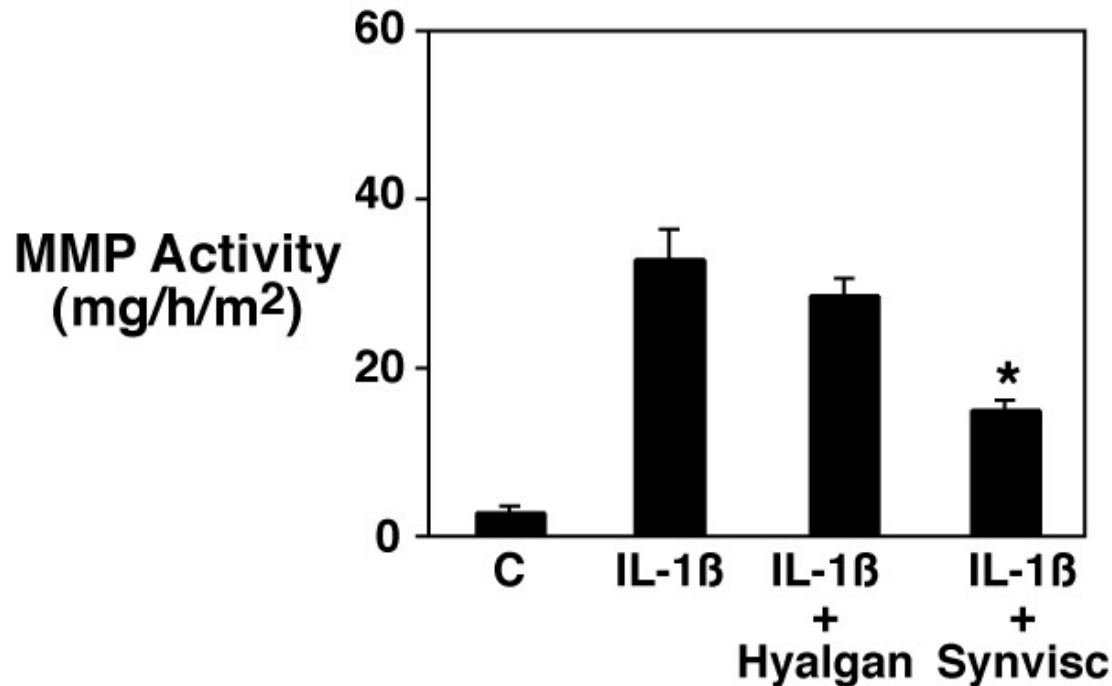
- 8 mg/mL
- N=5 patients in each experiment

- Grade IV OA
- \*P<0.05, paired t test

# Relative Ability of Hyaluronans (8 mg/mL) to Block Stimulated MMP Production from Synovial Tissue

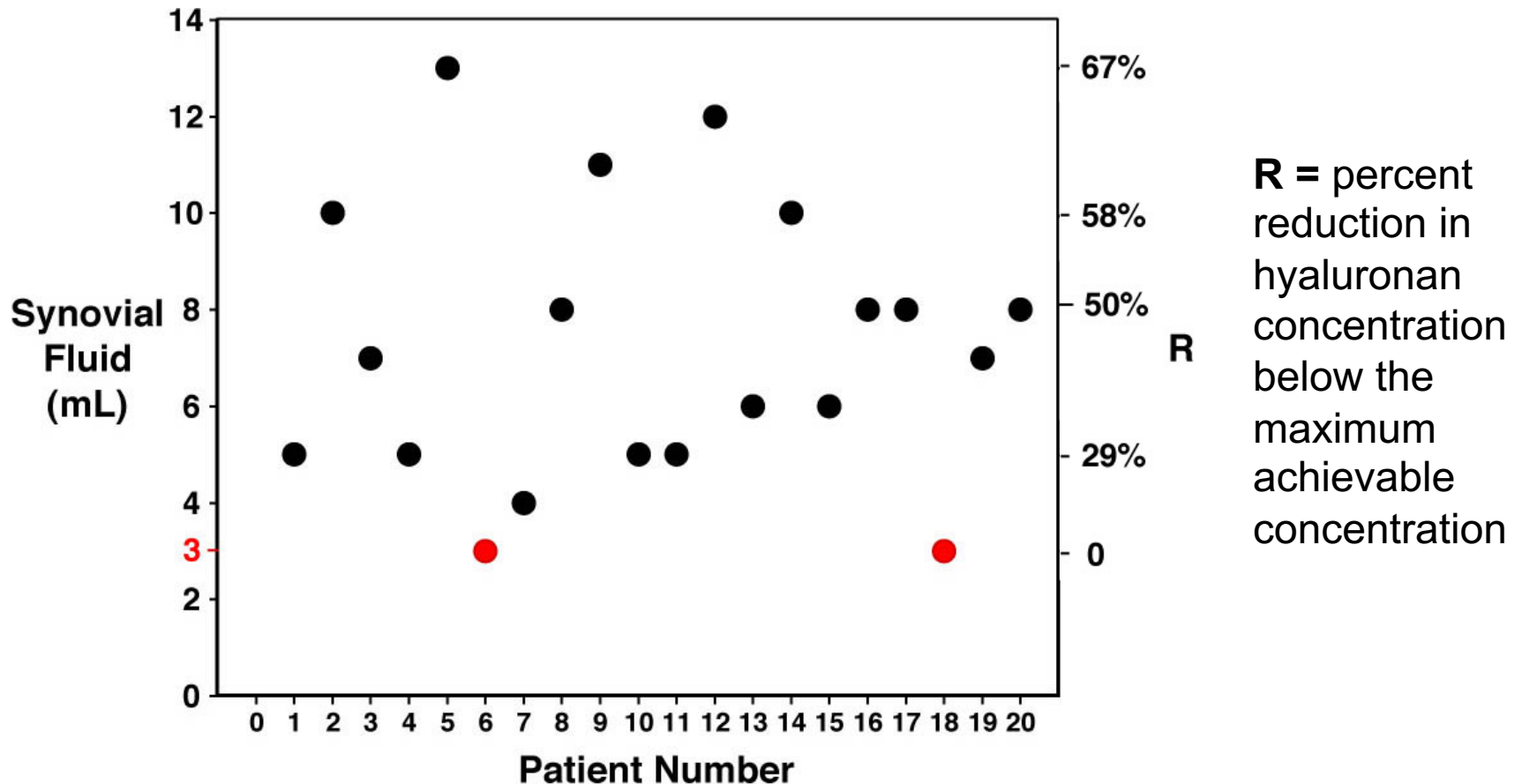


# Effect of Hyalgan and Synvisc on Stimulated MMP Production from Synovial Tissue



- 4 mg/mL
- N=5 patients
- Grade IV OA
- \*P<0.05, paired t test

# Residual Synovial Fluid in Patients with Grade IV Osteoarthritis



# Conclusion

- High MW hyaluronans inhibit cytokine-induced MMPs
- Possible mechanism for clinical efficacy
- Strong dependence on HA concentration
- Possible explanation for clinical variability