

Mechanistic Basis of Osteoarthritis

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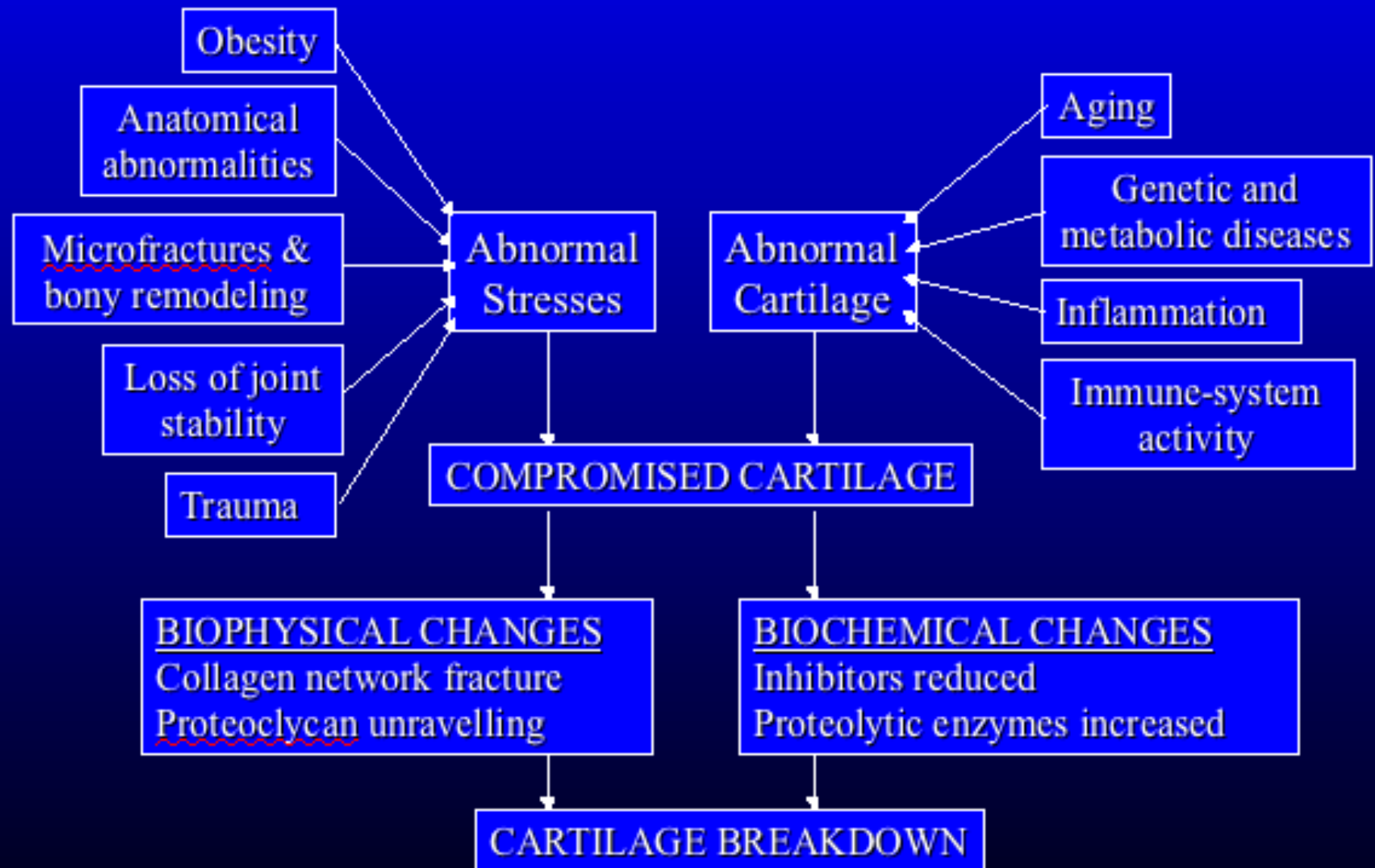
Department of Orthopaedic Surgery

LSUHSC - Shreveport

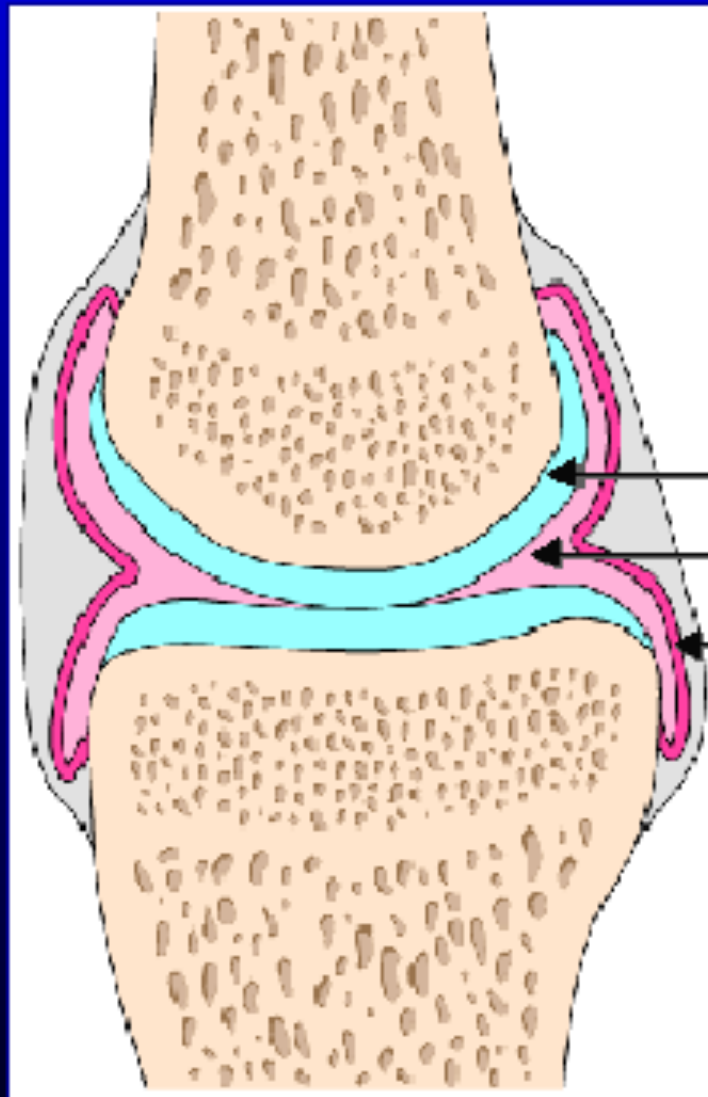
Summary

- Arthritis is ...
- Gap junctions are ...
- Approach
- Results
- Evaluation

Factors Involved in Osteoarthritis

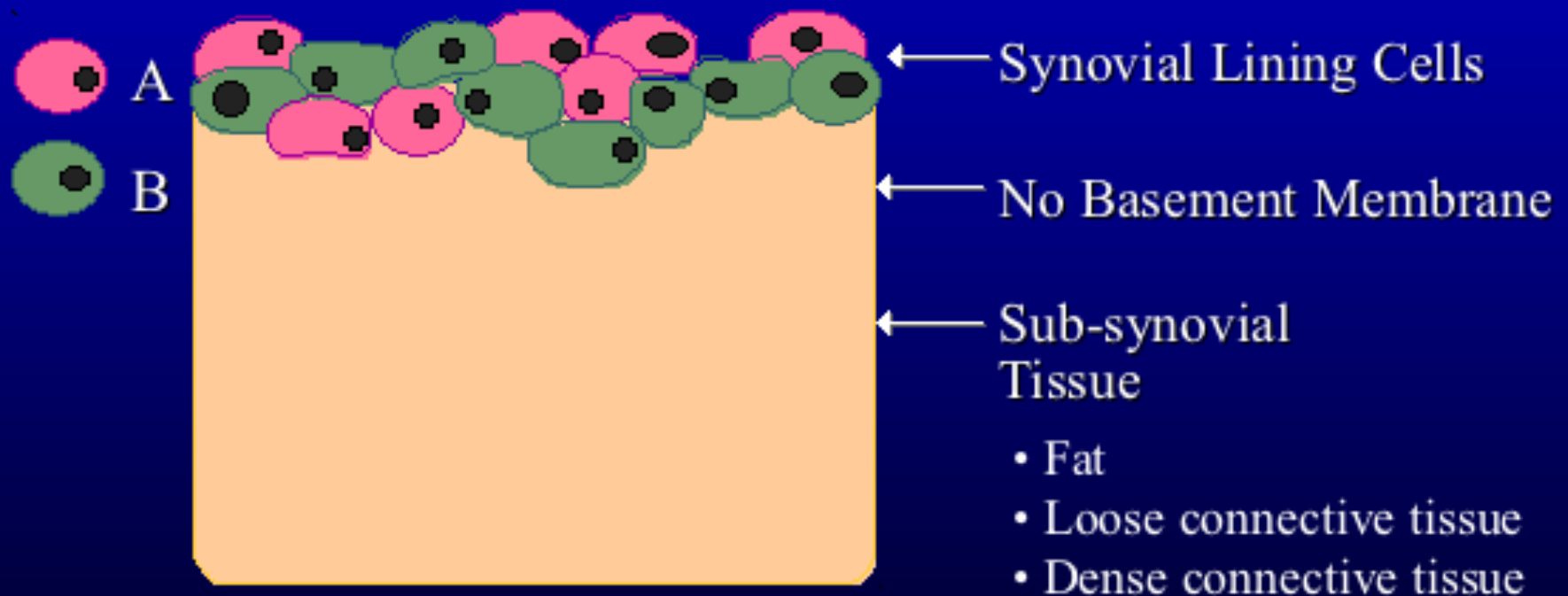


Generic Synovial Joint

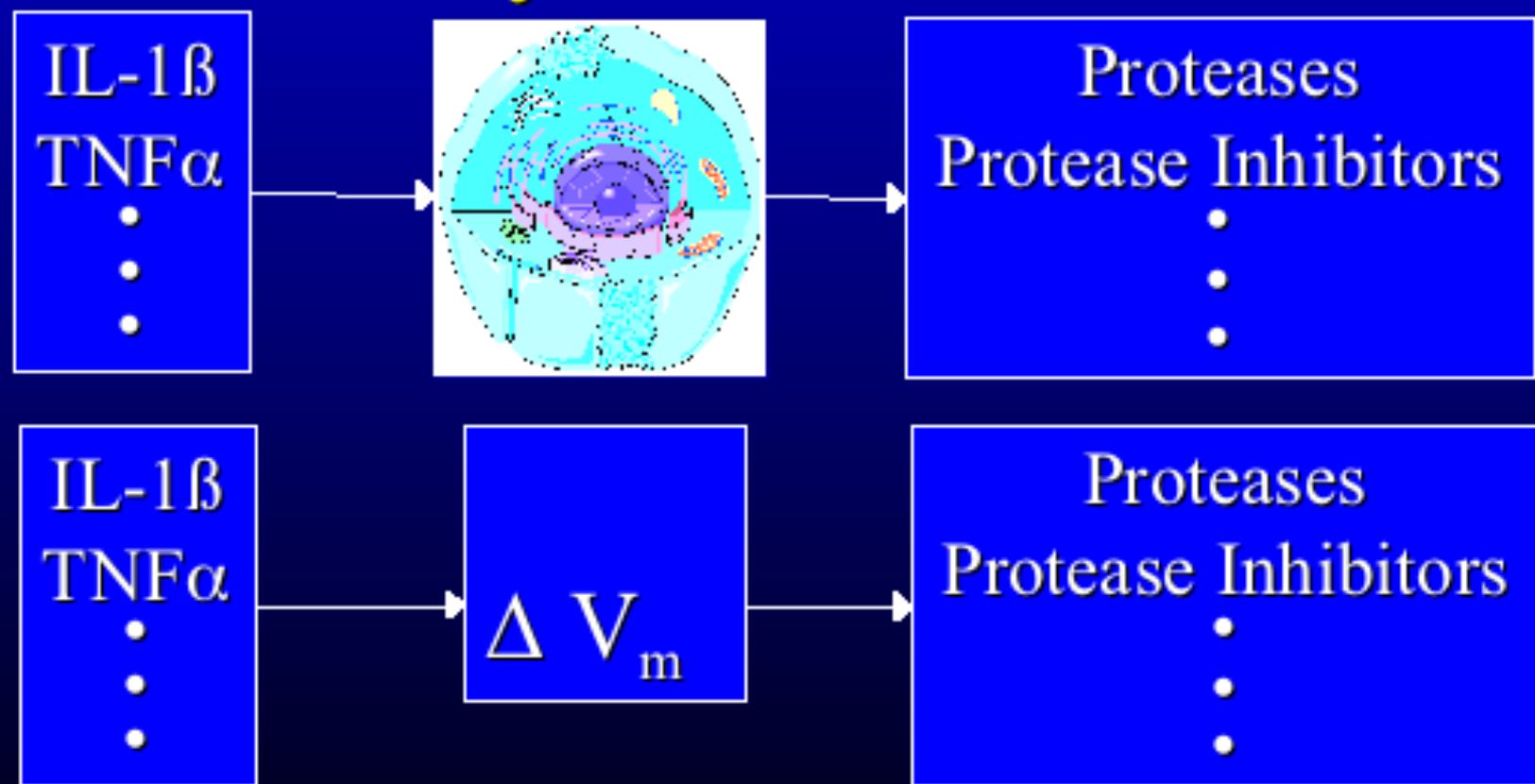


Cartilage
Synovial fluid
Synovium

Organization of Synovium

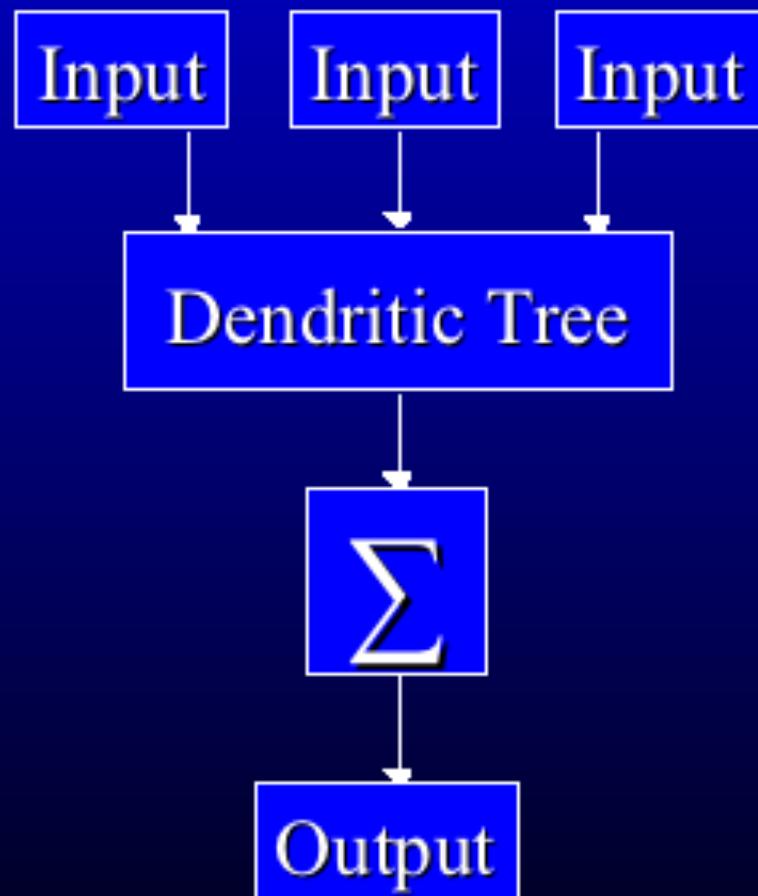


Hypothetical Role of Membrane Potential (V_m) in Regulation of Synovial Cells



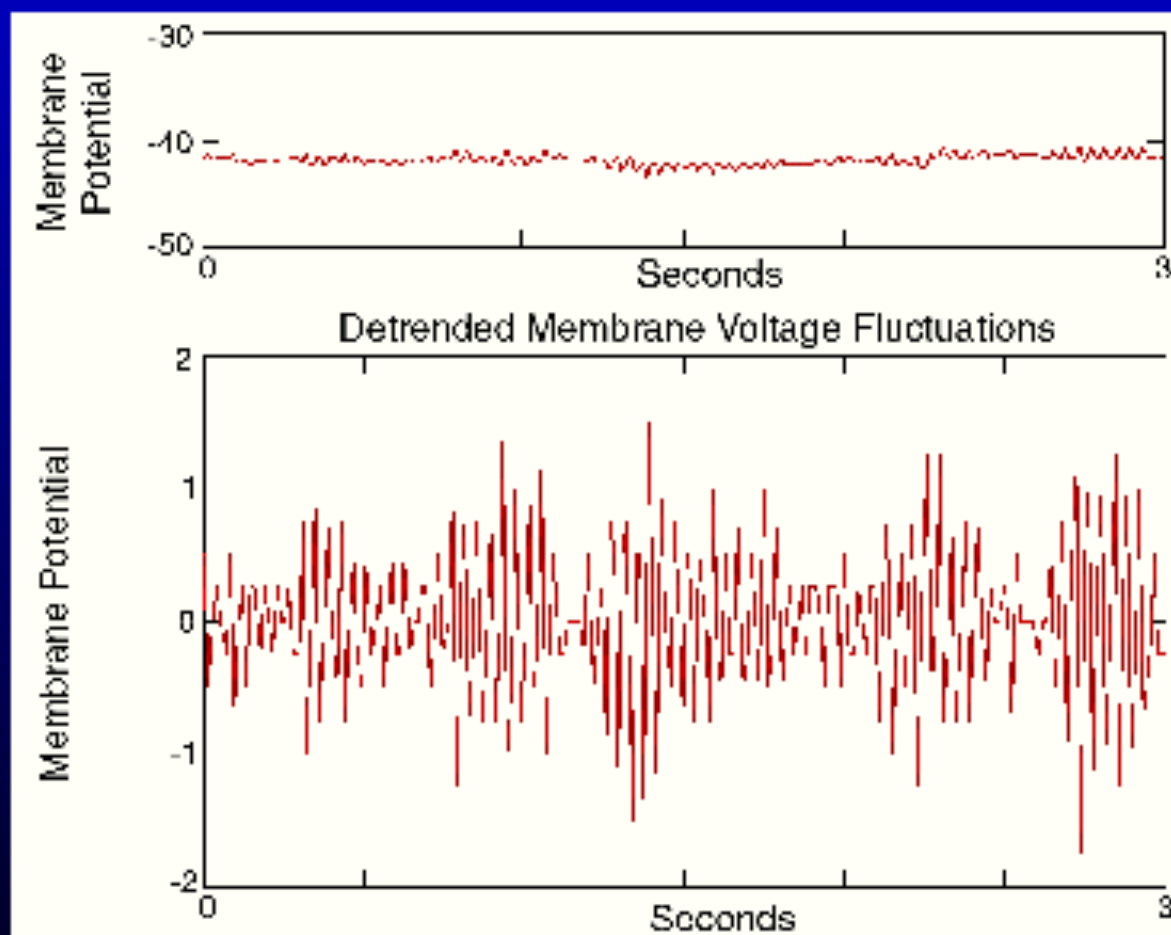
Why V_m ?

1. Analogy with Excitable Cells

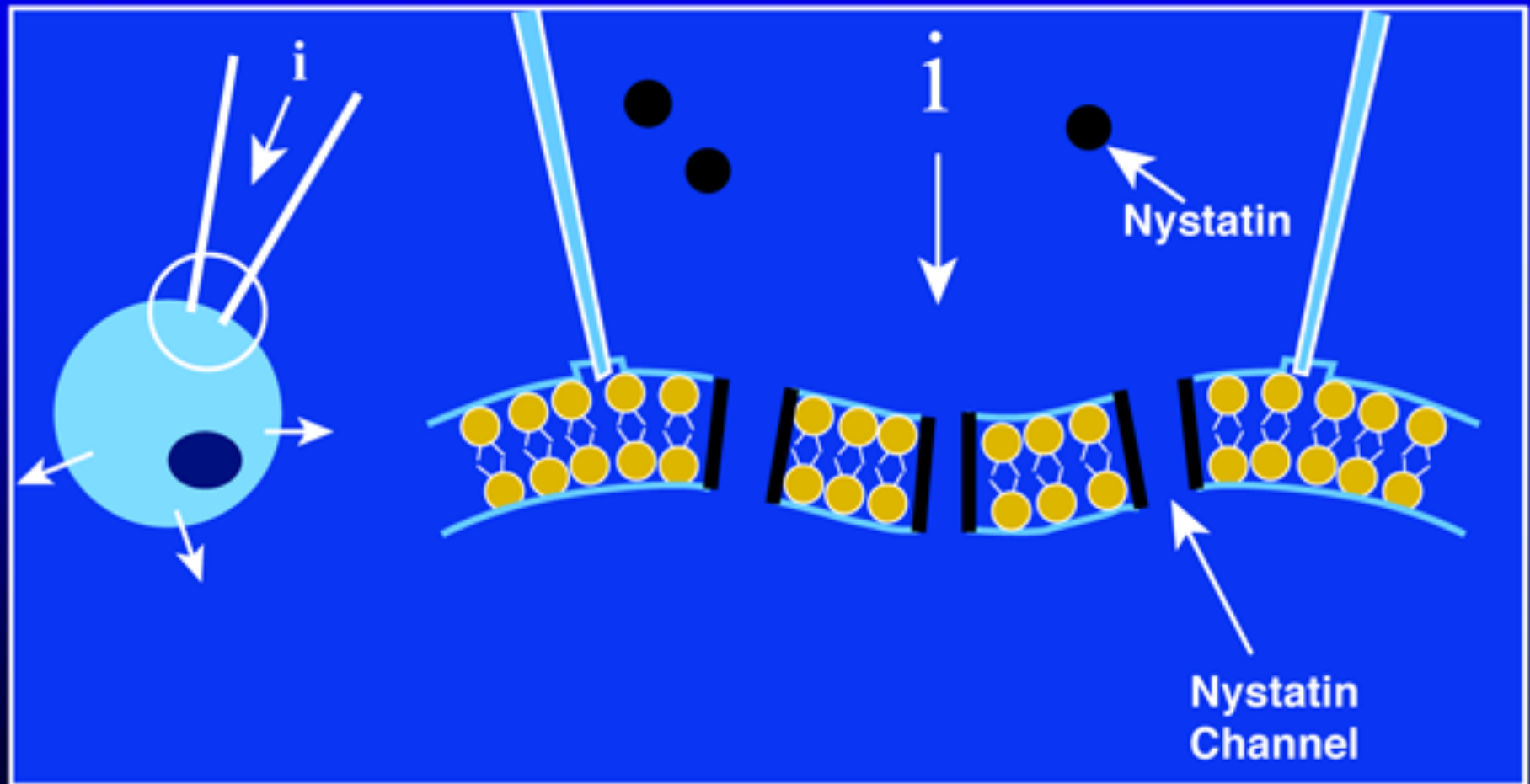


Why V_m ?

2. Fractal Dimension



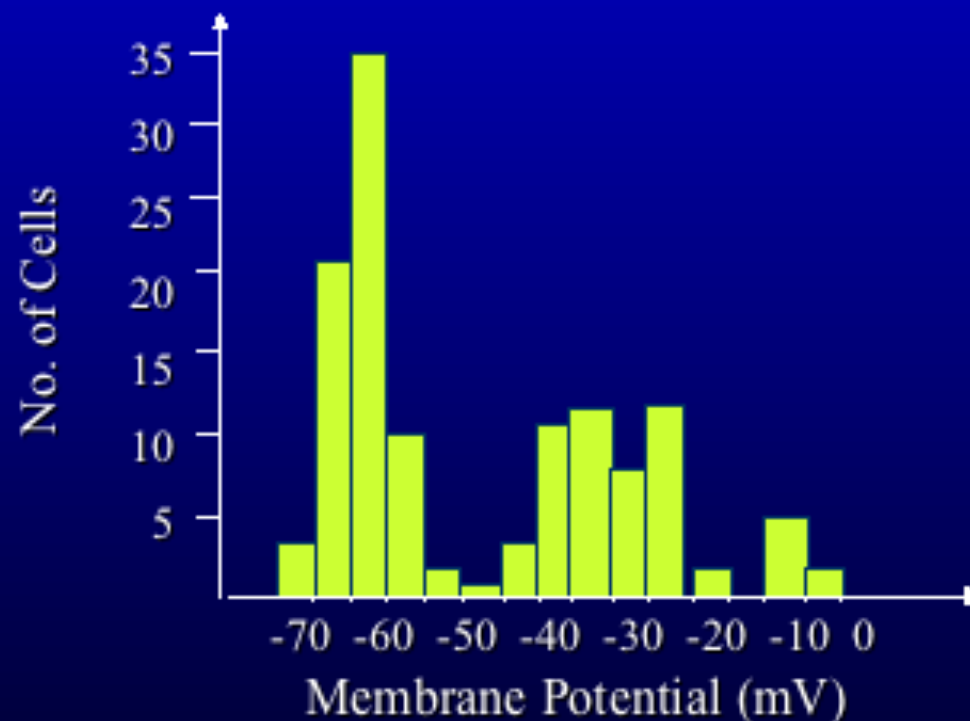
Nystatin Patch-Clamp



Initial Measurements

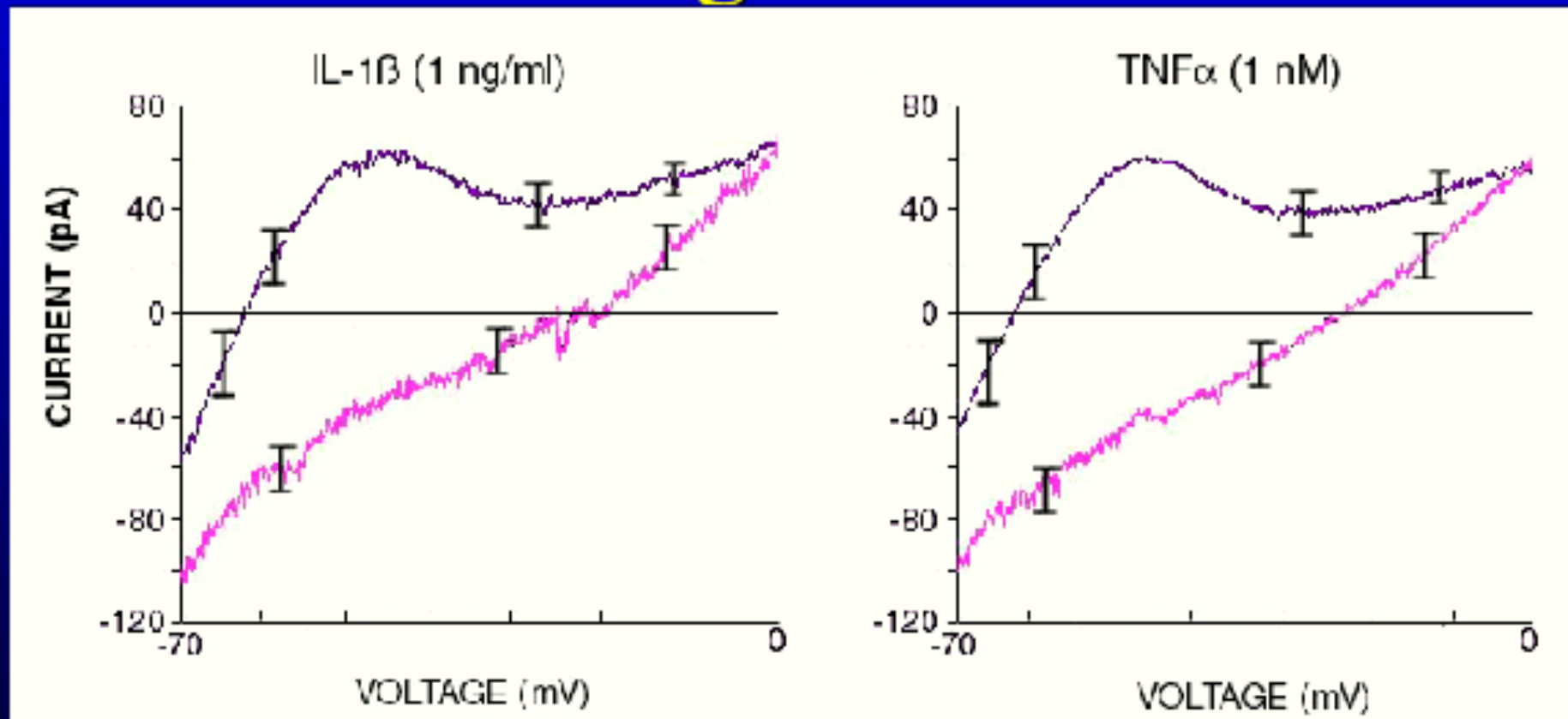
Cell Membrane Potential

- Aggregated HIG-82 cells (rabbit synovial cells)



- In isolated cells, Membrane Potential ~ 0

Effect of Cytokines on Current-Voltage Curves



Initial

10 minutes after addition of cytokine

Mechanism of IL-1 β Effect

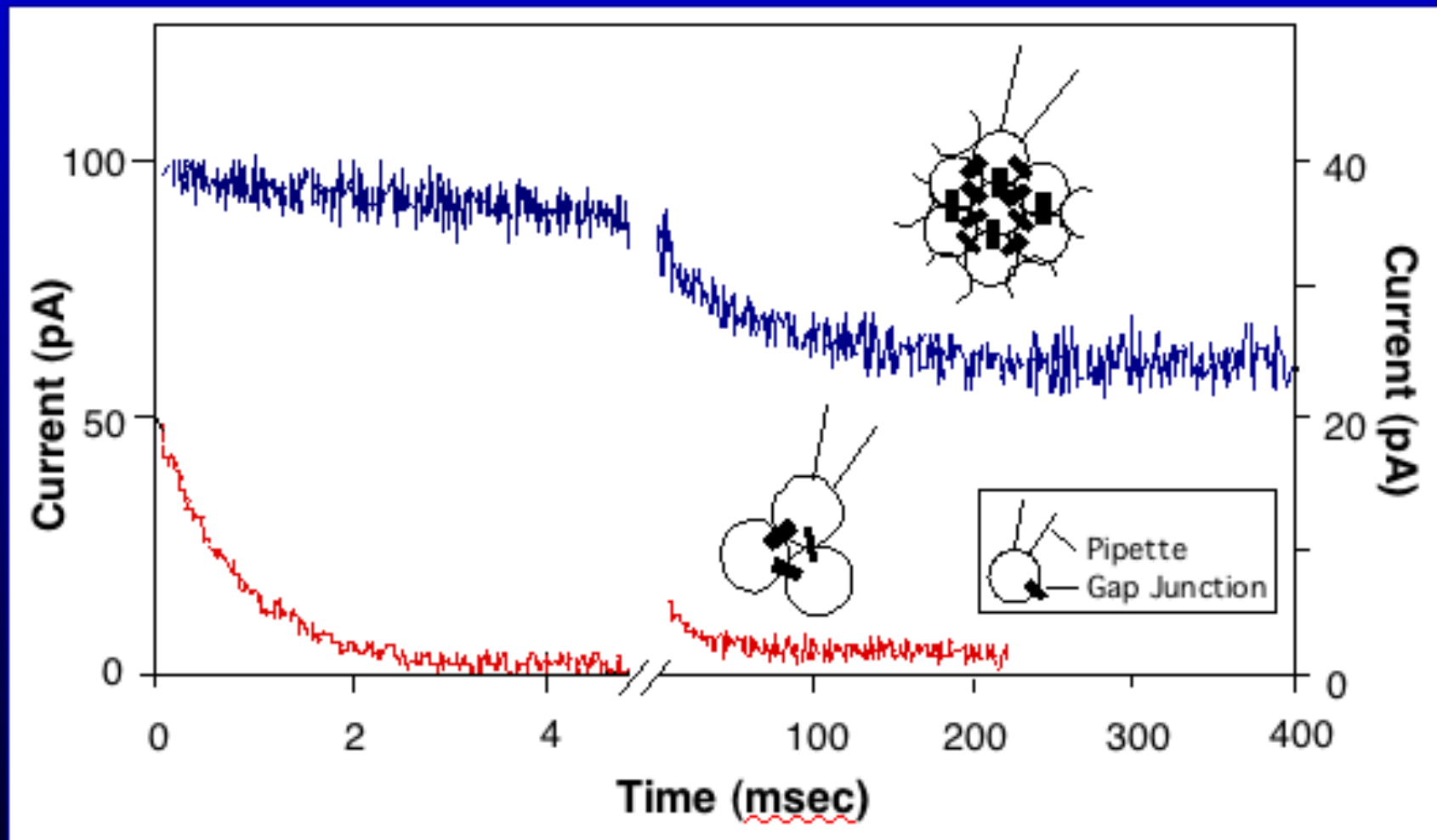
SUBSTANCES	REVERSAL POTENTIAL (mV)	
	BEFORE	AFTER
1 ng/ml IL-1 β	-69 \pm 2.0	-29* \pm 3.5
1.5 μ M PMA	-66 \pm 2.3	-68 \pm 1.0
1 ng/ml IL-1 β and 1.5 μ M PMA	-65 \pm 2.4	-39* \pm 7.0
3 μ M BIS	-70 \pm 1.0	-71 \pm 1.0
1 ng/ml IL-1 β and 1.5 μ M BIS	-69 \pm 1.0	-67 \pm 3.2
10 μ M Ca ²⁺ ionophore	-67 \pm 1.6	-66 \pm 1.9
0.25 μ M PMA and 5 μ M Ca ²⁺ ionophore	-70 \pm 1.3	-30* \pm 8.2
50 μ M nifedipine	-68.5 \pm 1.6	-70.5 \pm 1.0
10 μ M verapamil	-65 \pm 4.8	-63 \pm 5.3
1 ng/ml IL-1 β and 50 μ M nifedipine	-68 \pm 1.6	-65.5 \pm 4.1
1 ng/ml IL-1 β and 10 μ M verapamil	-68 \pm 1.3	-60 \pm 4.8

*P < 0.05

Activation of PKC and Ca²⁺ Influx
necessary and sufficient

Am. J. Physiol. 276:C9, 1999.

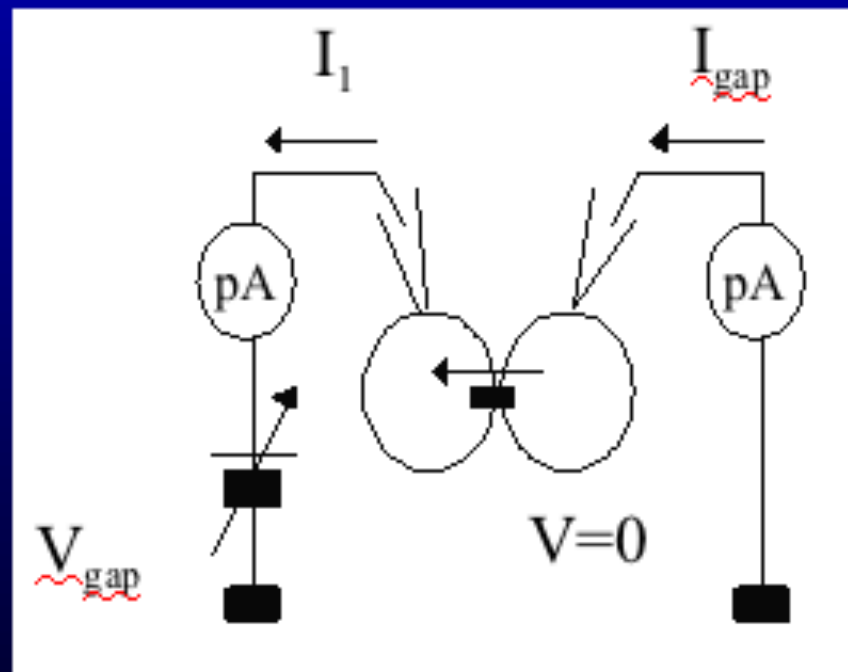
Discovery of Gap Junctions



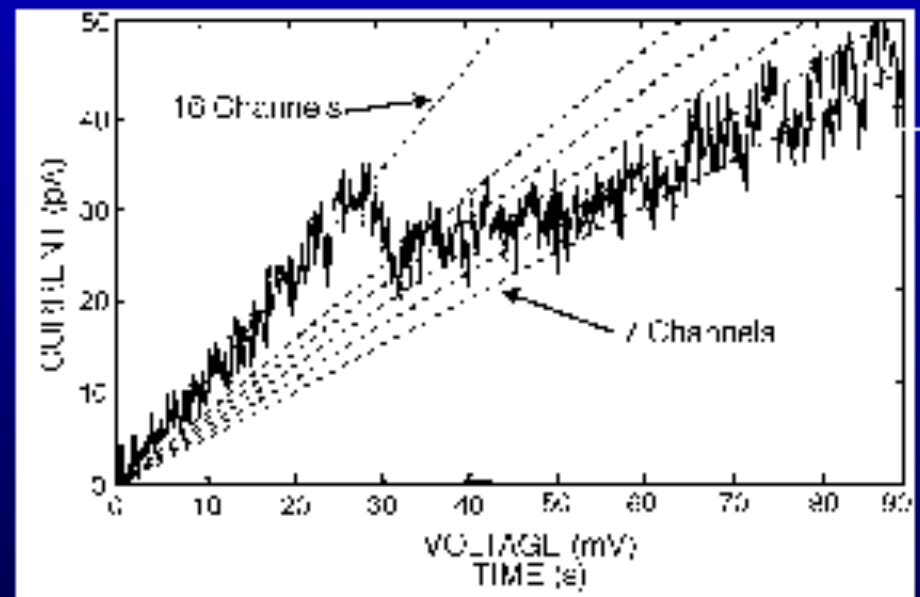
Am. J. Physiol. 273:R1822, 1997.

Current Through Gap Junctions

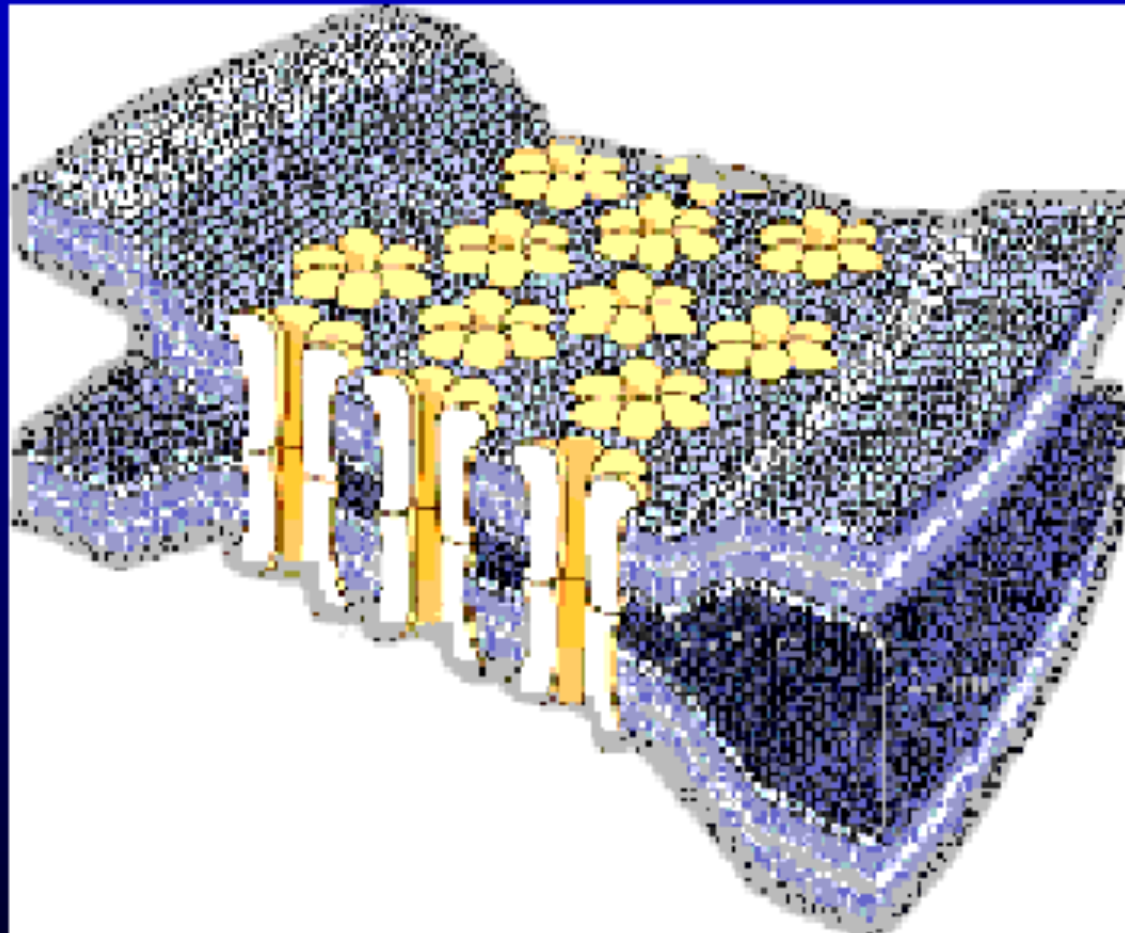
Circuit for Measurement of Gap Junctions



Current



Organization of Gap Junctions



Mammalian Connexins

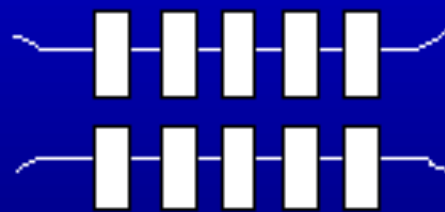
<u>CONNEXIN</u>	<u>TISSUE</u>
Cx26	Liver, Pancreas, Endometrium
Cx30	Brain, Skin
Cx30.3	Skin
Cx31	Skin, Placenta
Cx31.1	Skin
Cx32	Liver, Kidney, Pancreas
Cx33	Testes
Cx37	Endothelium, Lung, Ovary
Cx40	Endothelium, Smooth muscle, Myocardium, Lung
Cx43	Epithelium, Heart, Uterus, Connective tissue, Brain
Cx45	Kidney, Skin
Cx46	Lens
Cx50	Lens

Connexon Complexity

Connexins

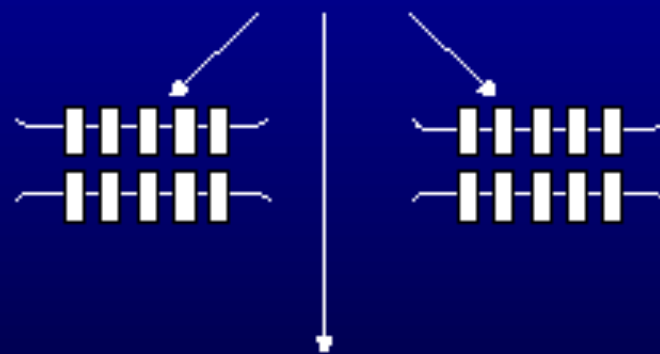


Connexon



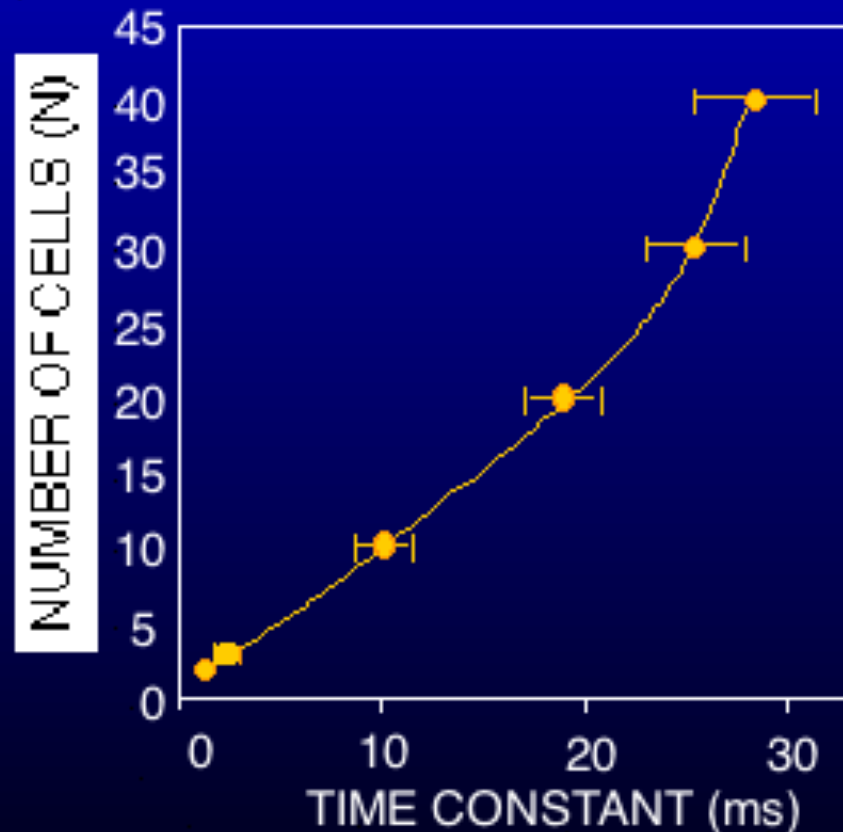
Cell No. 1

Cell No. 2

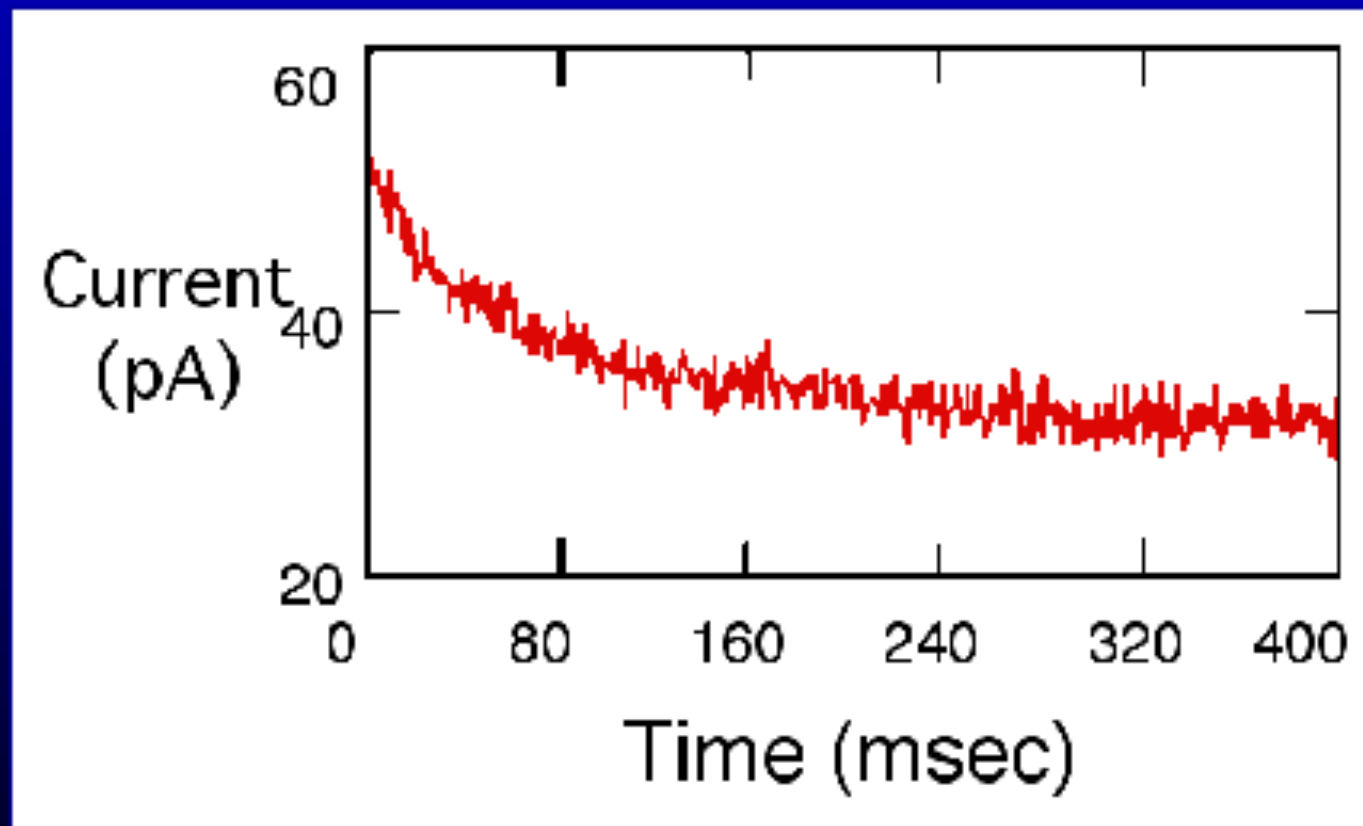


12 Subunits x 13 Proteins
= 10^{10} combinations
or = 10^{50} permutations

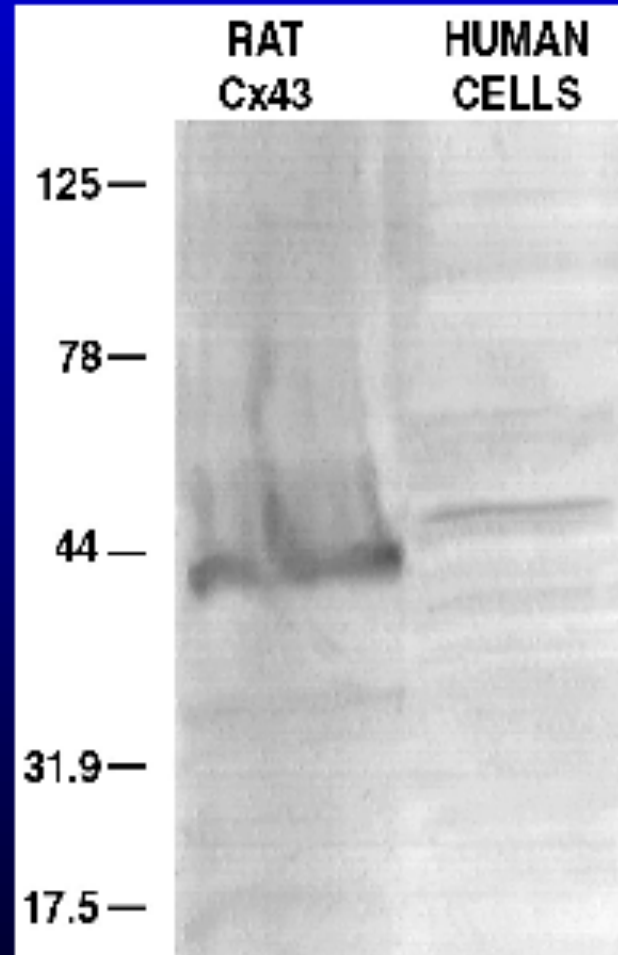
Relation between the Time Constant for Current Decay in HIG-82 Cells and Aggregate Size in Culture



Transient Current Response of a Synovial Lining Cell in Tissue

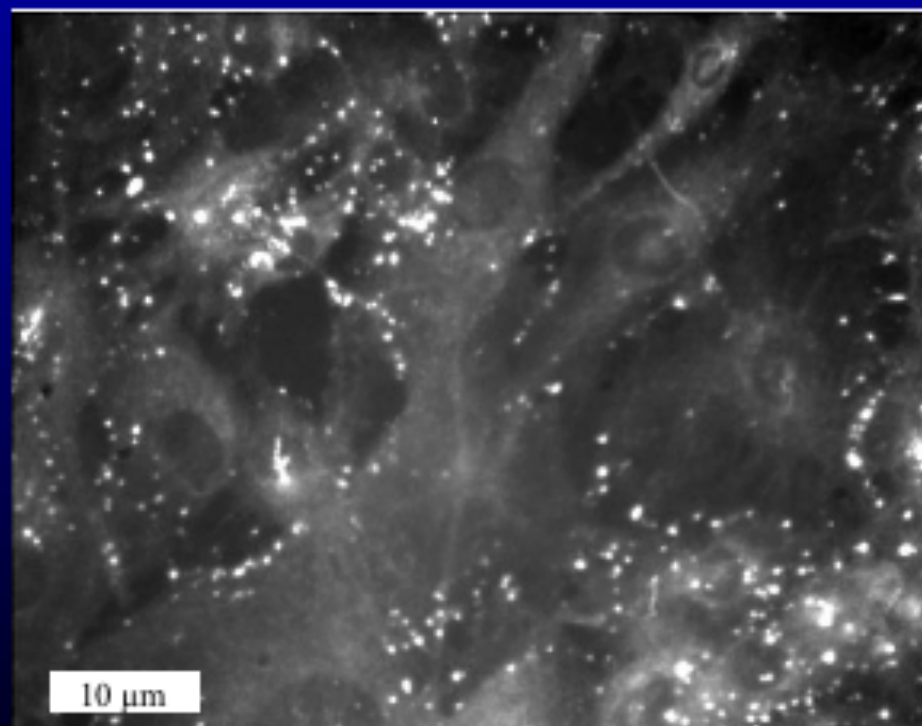


Western Blot Analysis



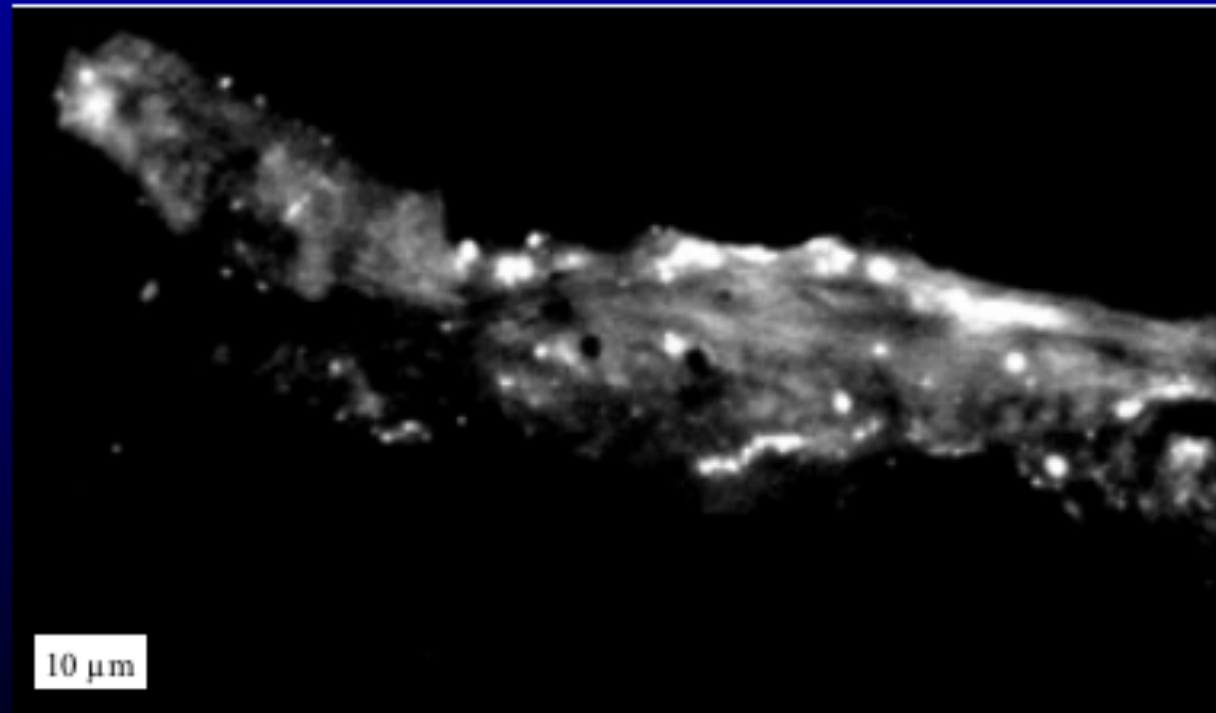
Immunohistochemical Detection in Cultured Human Synovial Cells

Connexin43

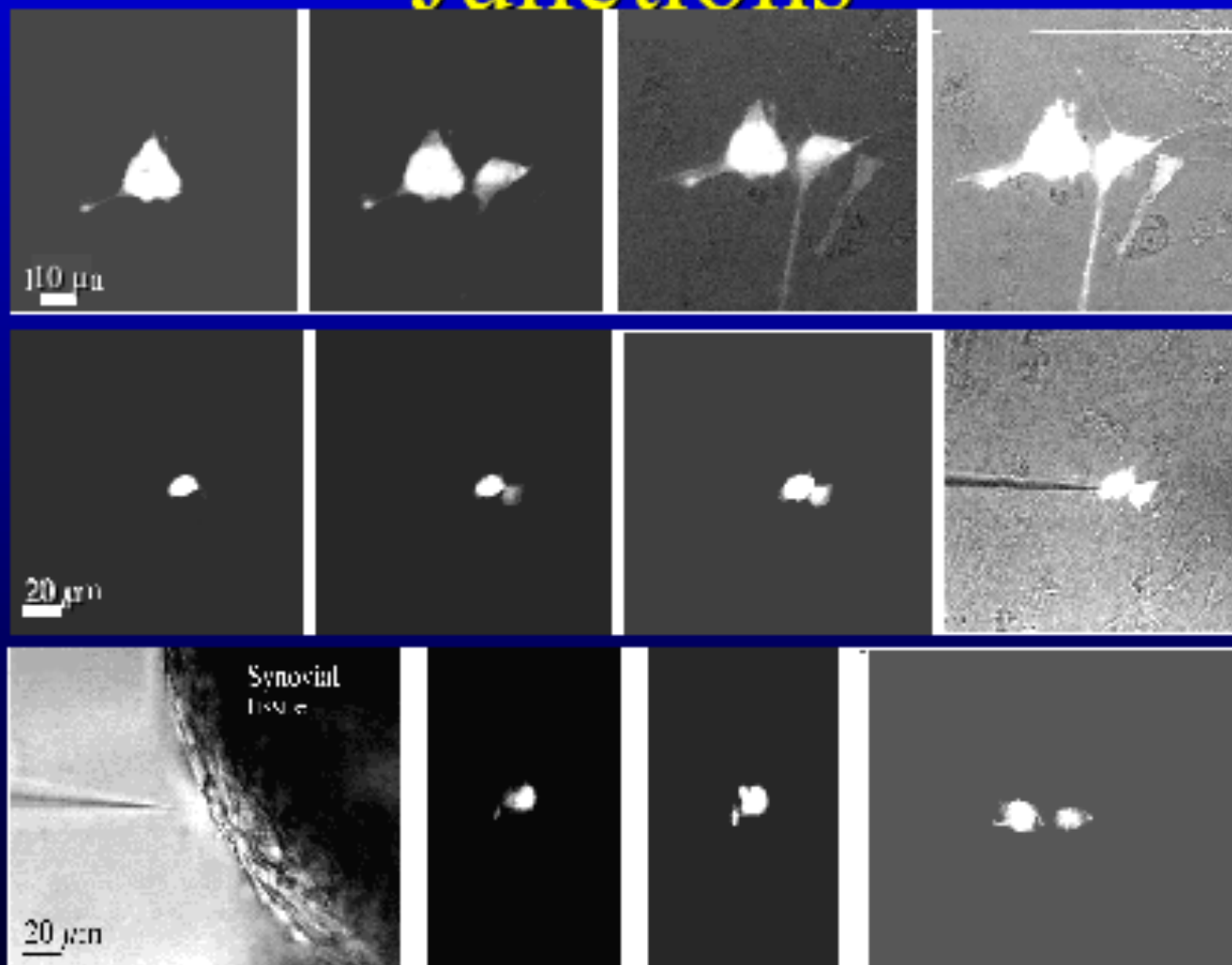


Immunohistochemical Detection in Cryosections of Human Synovial Tissue

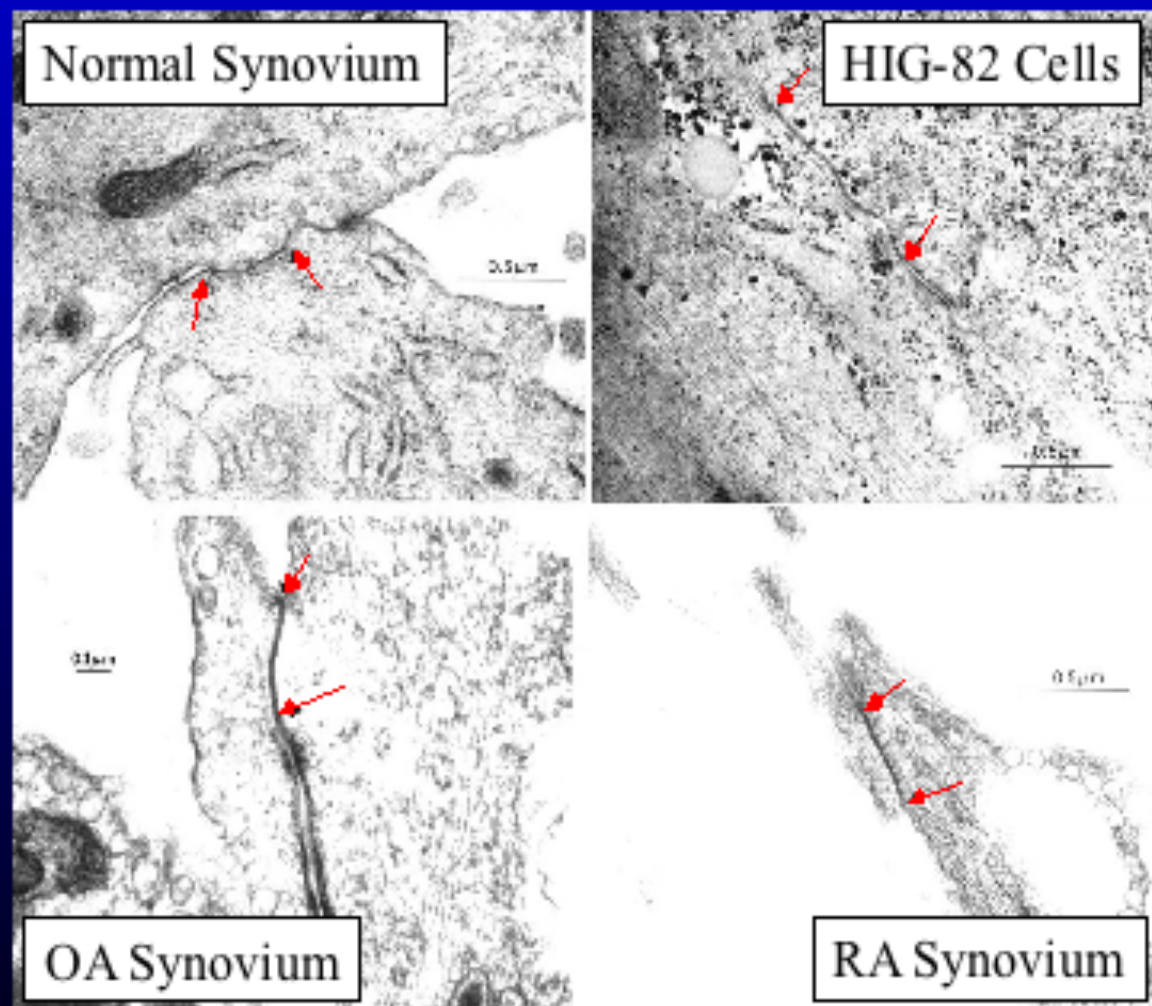
Connexin43



Dye Transfer Through Gap Junctions



Gap Junctions in Human Synovium and between HIG-82 Cells

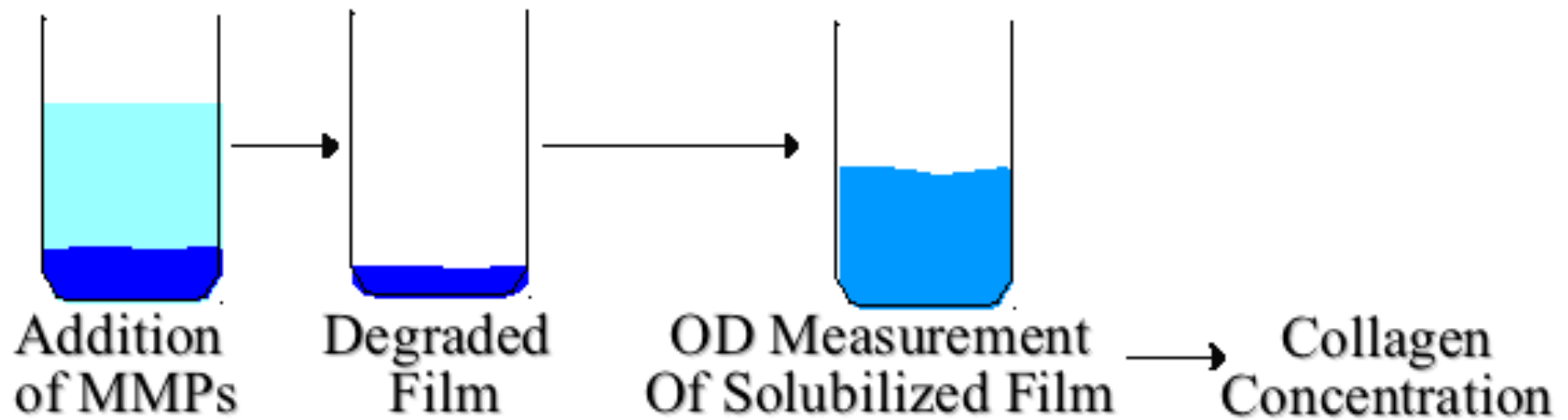
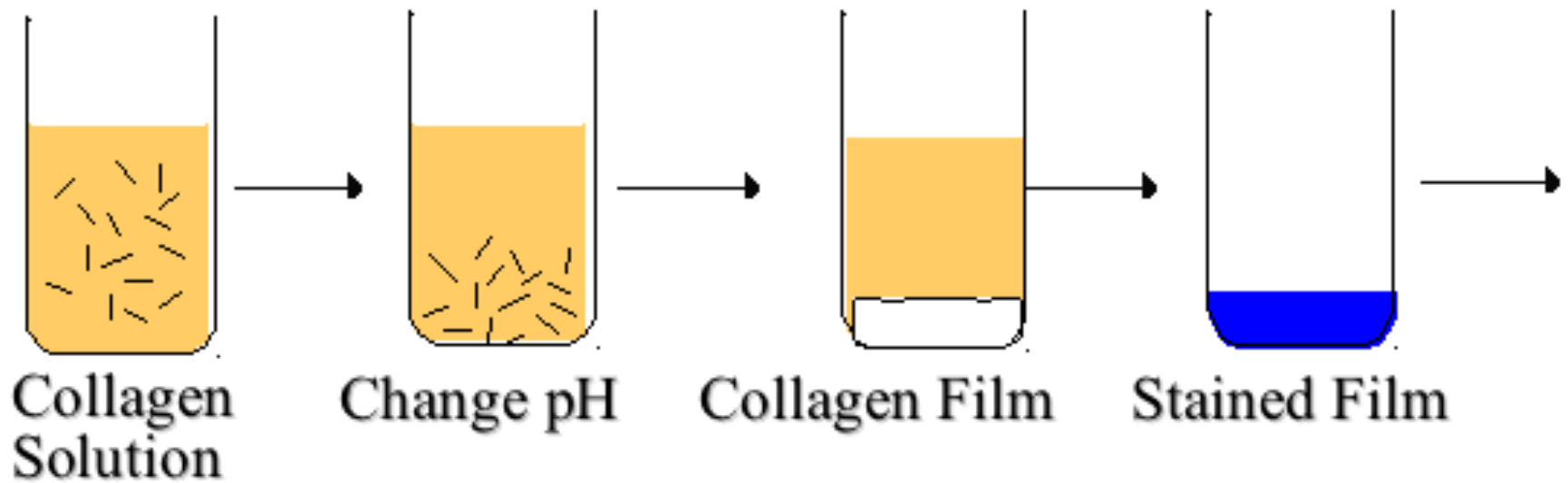


Gap-Junction Plaques in Normal and Arthritic Human Synovia

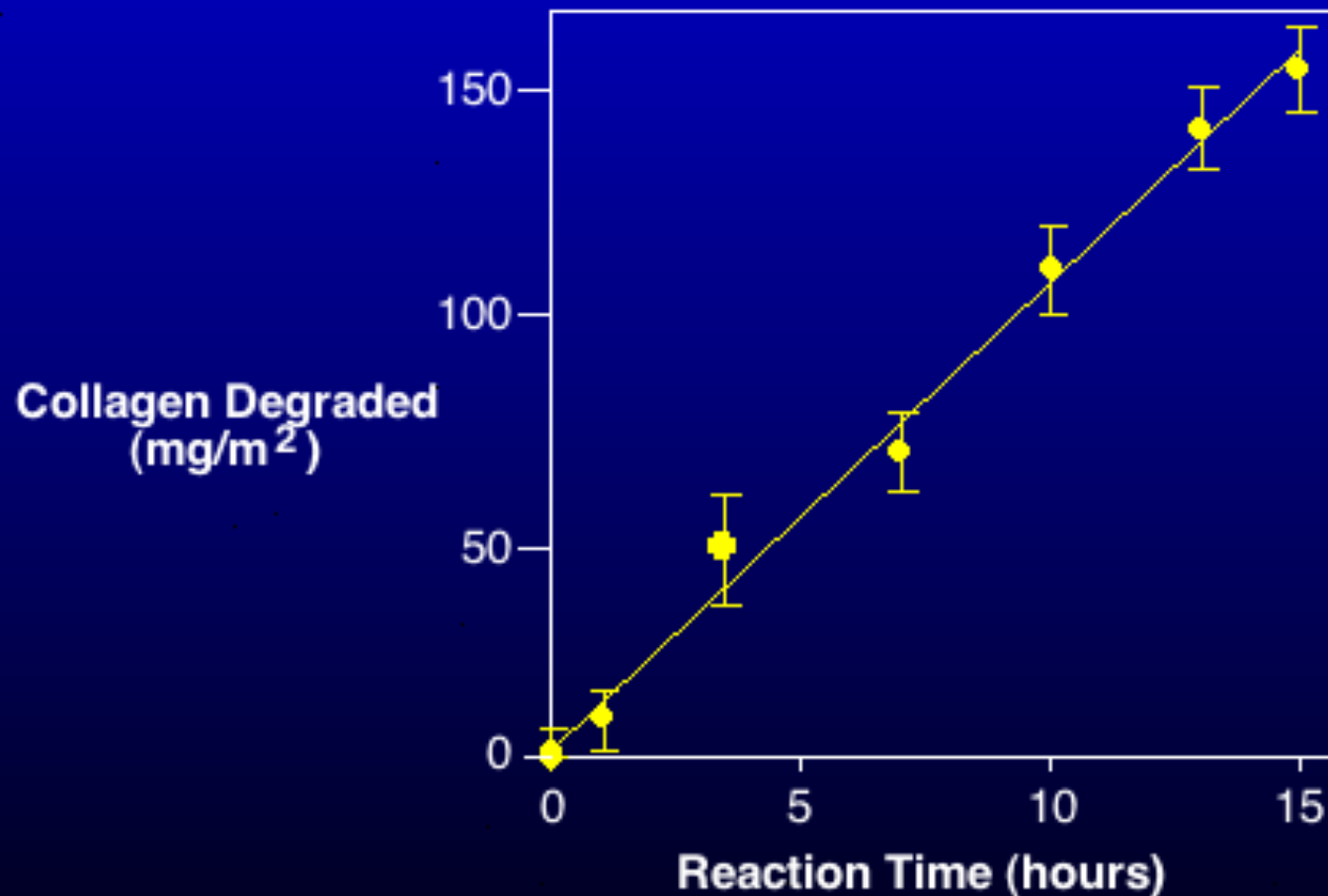
SPECIMEN		No. PLAQUES	
<u>No.</u>	<u>DIAGNOSIS</u>	<u>per 100 CELLS</u>	<u>MEAN ± SD</u>
1	Normal	1.43	
2	"	0.40	
3	"	1.11	
4	"	0.00	
5	"	0.62	0.71 ± 0.57
1	Osteoarthritis	3.72	
2	"	1.26	
3	"	3.61	
4	"	3.65	
5	"	7.52	
6	"	6.35	* 4.35 ± 2.24
1	Rheumatoid Arthritis	3.98	
2	"	2.04	
3	"	9.56	
4	"	1.30	* 4.22 ± 3.74

* P < 0.05

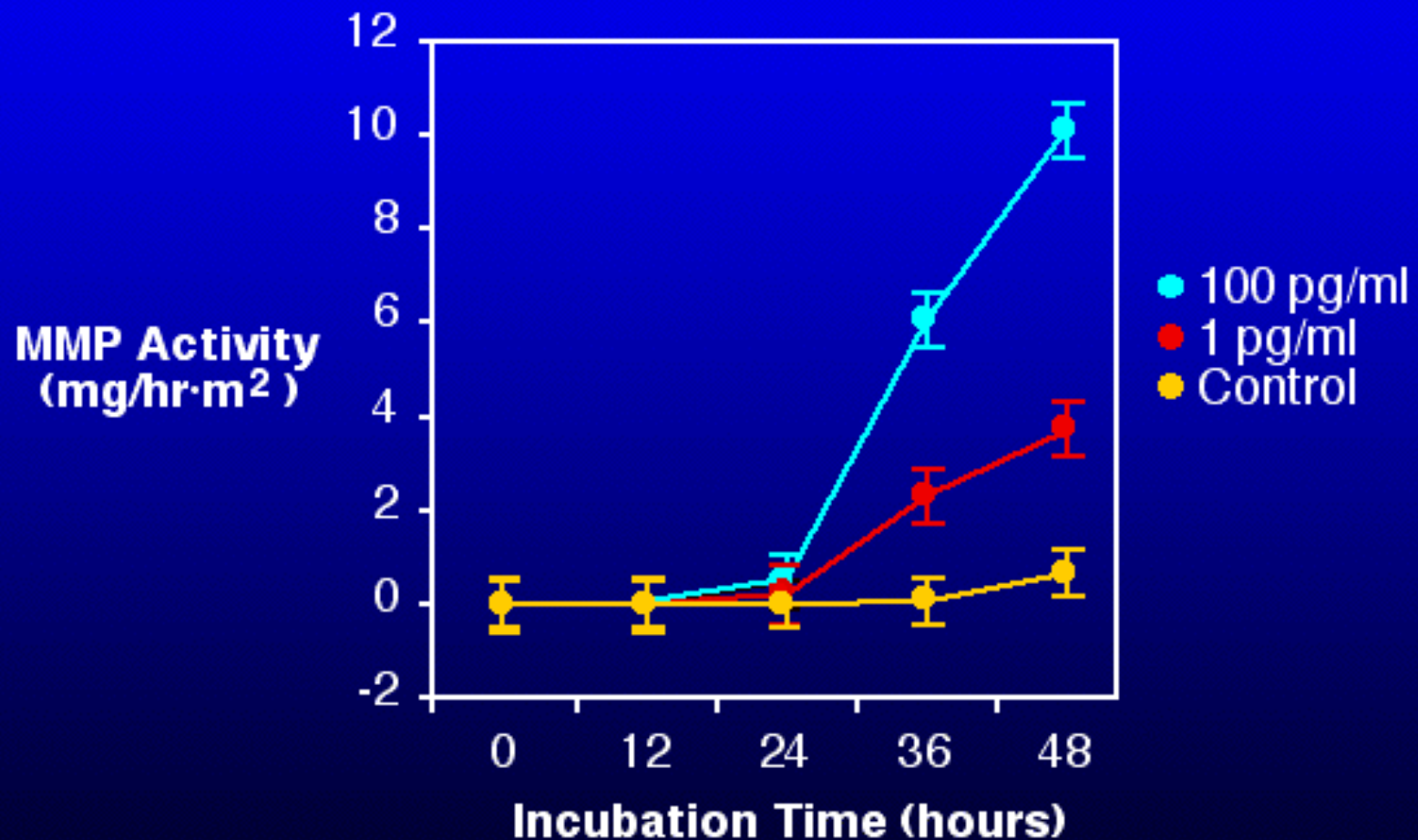
Metalloproteinase Assay



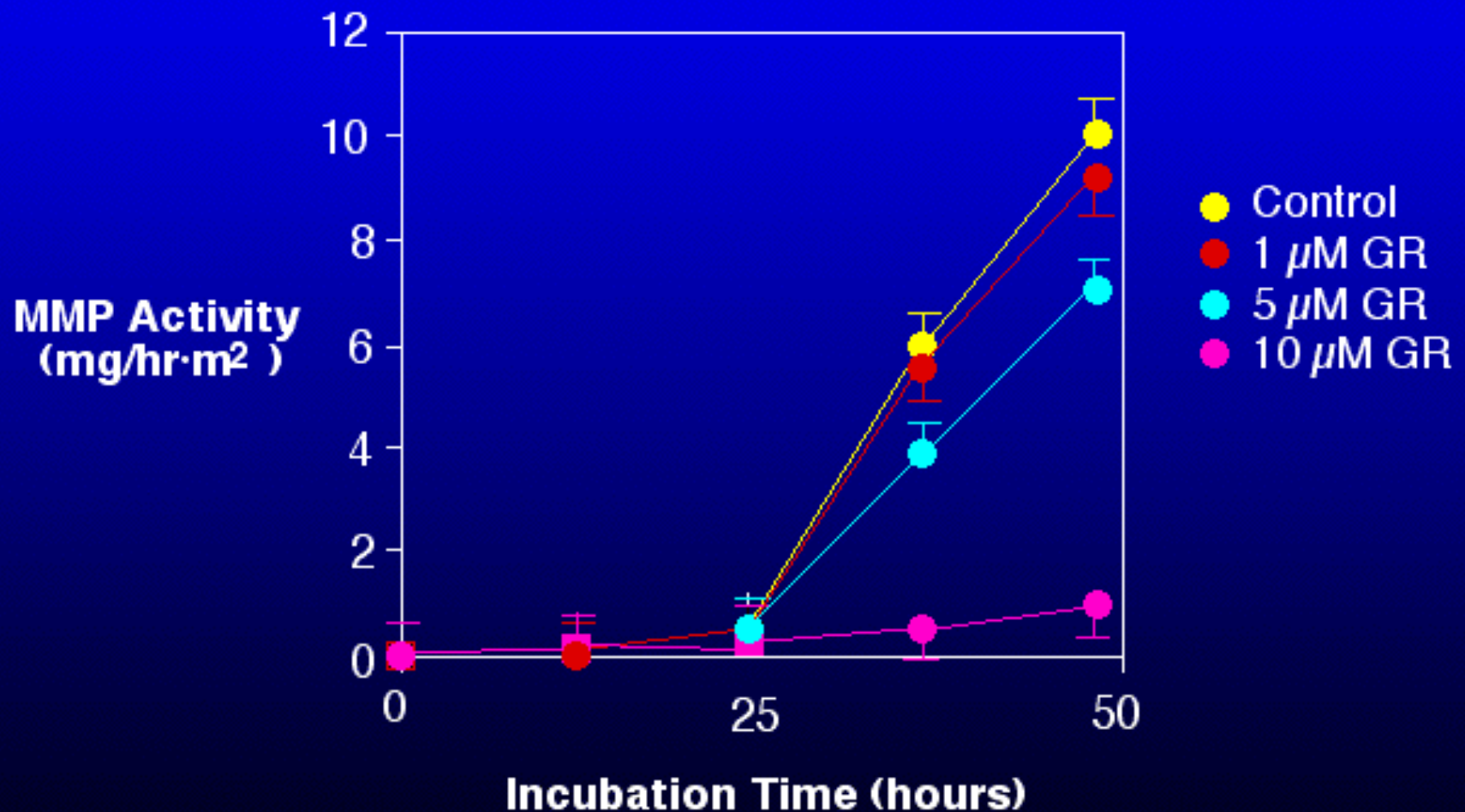
Protease Activity of IL-1 β Treated Synovial Cells



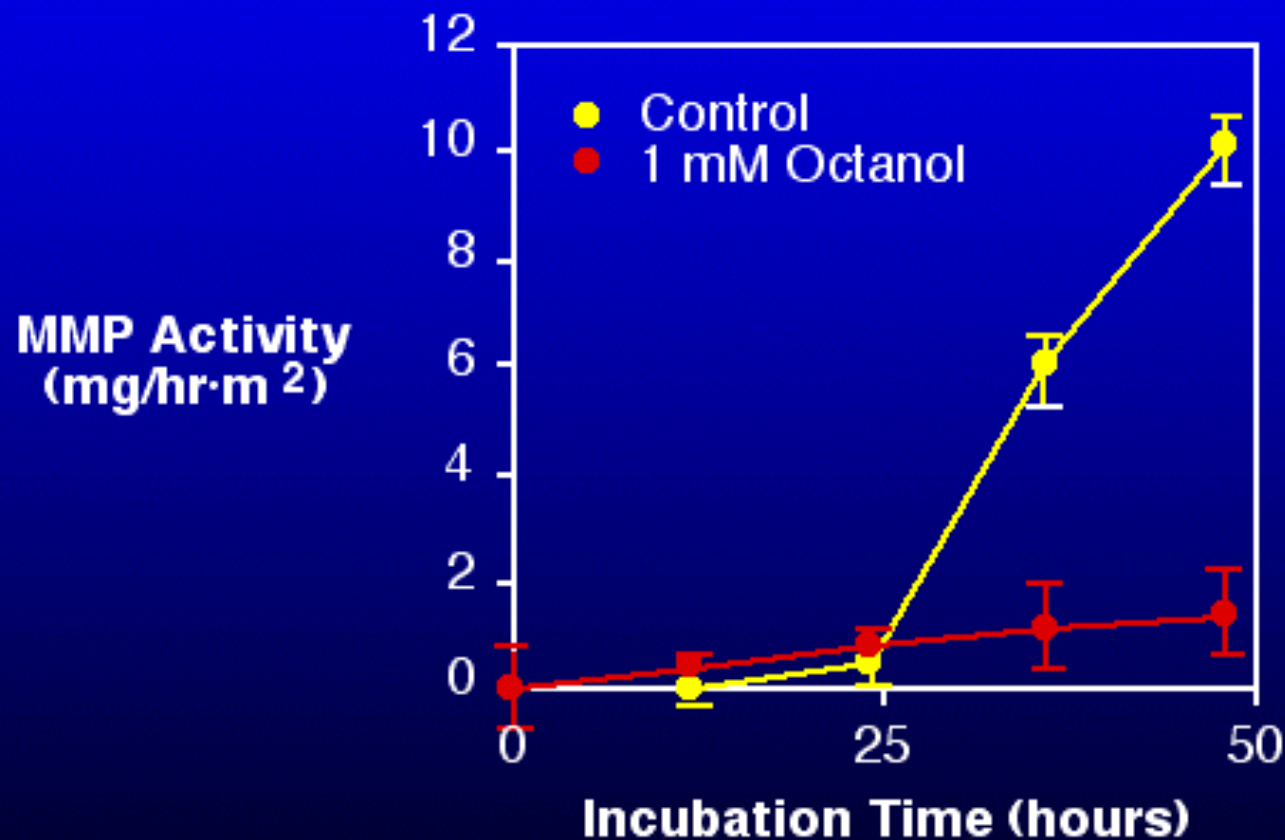
Effect of IL-1 β on Protease Activity



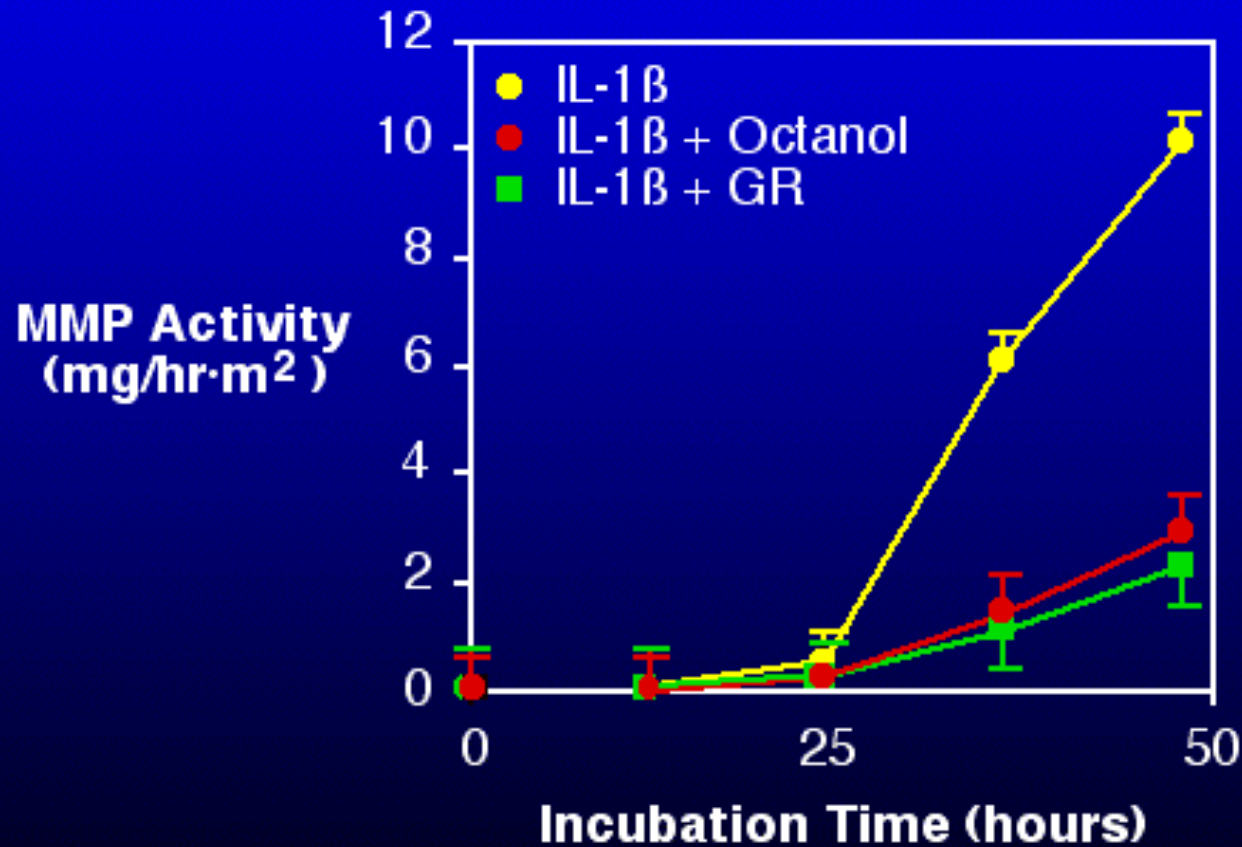
Effect of 18 α -glycyrrhetic acid (GR) on Protease Production by Synovial Cells in the Presence of IL-1 β



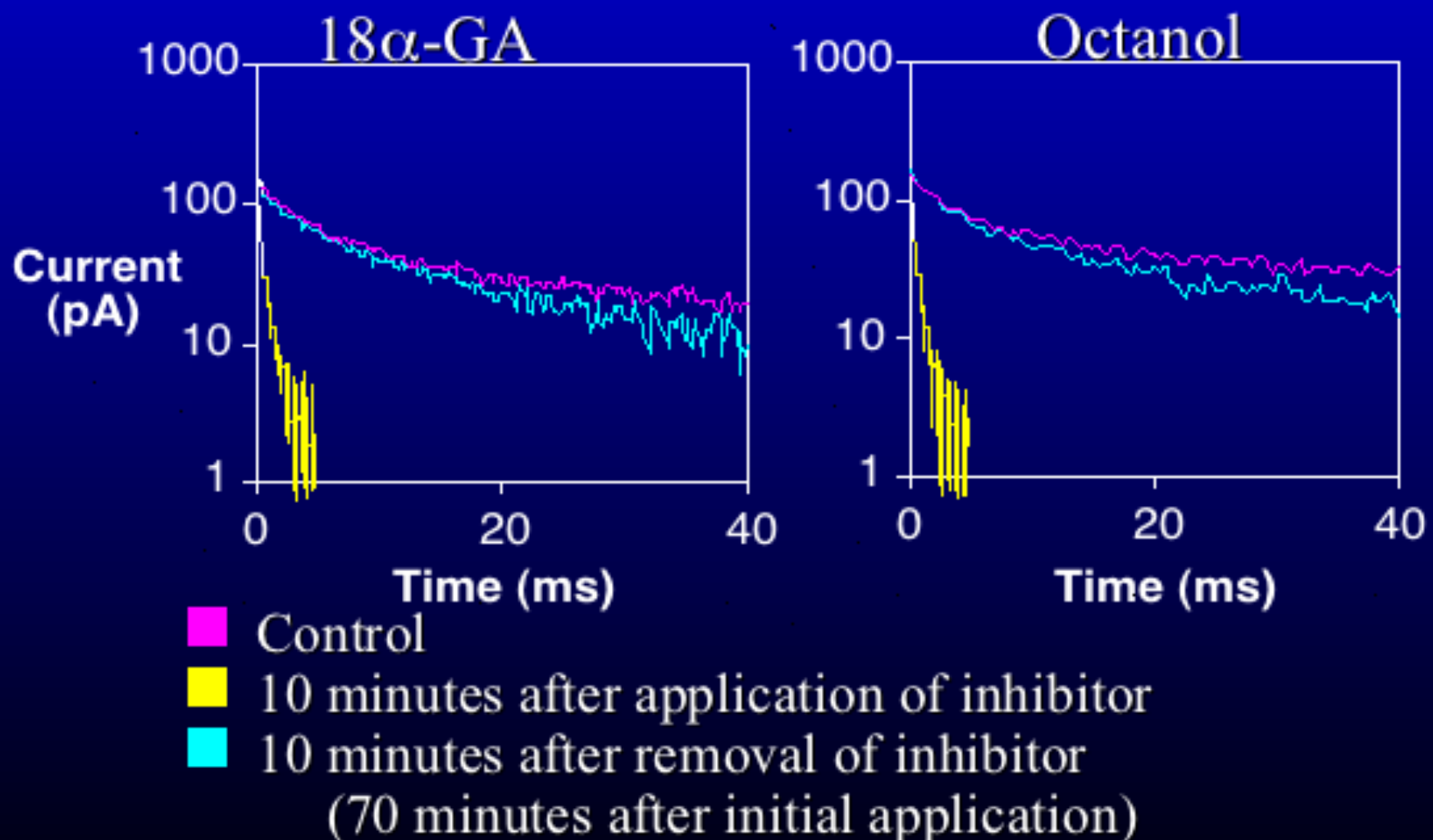
Effect of Octanol on Protease Production by Synovial Cells in the Presence of IL-1 β



Effect of Brief Application of Gap Junction Inhibitors on Protease Production



Effect of Gap Junction Inhibitors on Gap Junction Intercellular Communication



Summary (Know)



Rabbit Cells



**Rabbit Cells
Human Cells**



$$N_{\text{ARTHRITIS}} > N_{\text{NORMAL}}$$

**Rabbit Cells
Human Cells
Tissue**

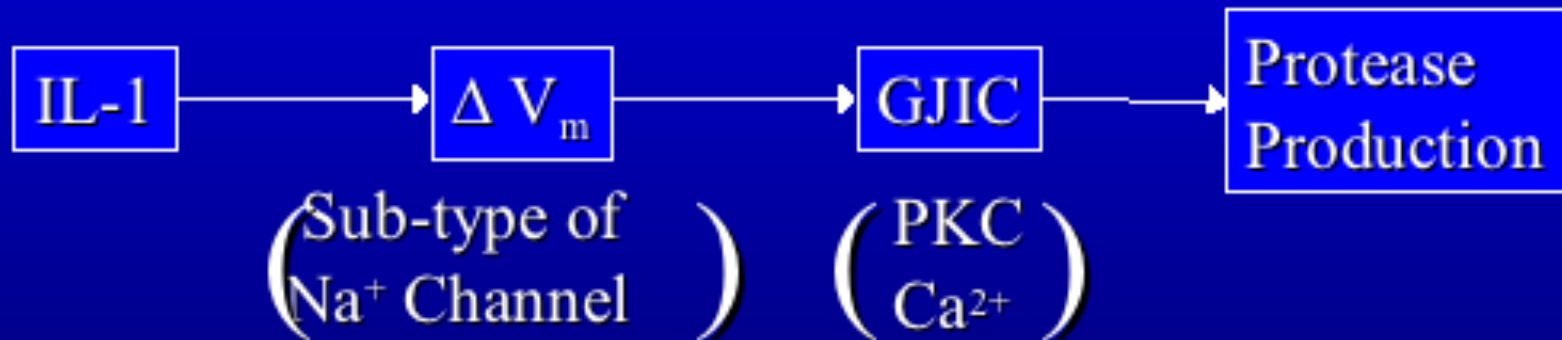
 **Cx43**

**Human Cells
Synovial Tissue**



Rabbit Cells

Summary (Think)



Acknowledgements

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