UNIVERSITY OF NEWCASTLE

CARTILAGE

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ETHICAL APPROVAL IS IN PLACE

Newcastle University

Research Protocol for REC 09/H0906/72

"Biological studies of the Mechanisms of Joint Destruction in Musculoskeletal Diseases"

using samples from Newcastle NHS Foundation Trust Hospitals

version 6 - 12th September 2012

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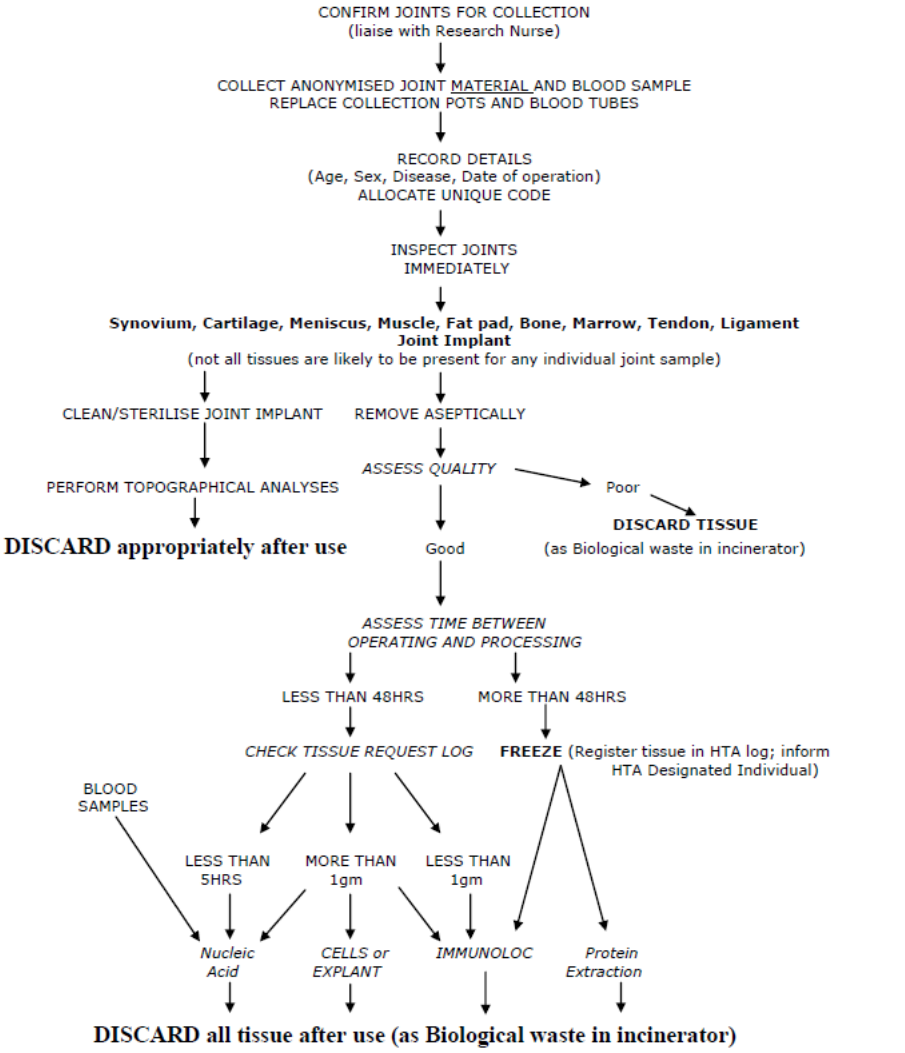
APPENDIX 5: FLOWCHART FOR SAMPLE COLLECTION AND USE within the Musculoskeletal Research Group (MRG), Newcastle University, under Ethical approval (Newcastle and North Tyneside Research Ethics Committee 1; REC 09/H0906/72) and approval by the Newcastle upon Tyne Hospitals NHS Trust Research and Development Department ("Mechanisms of Joint Destruction in Musculoskeletal Diseases")

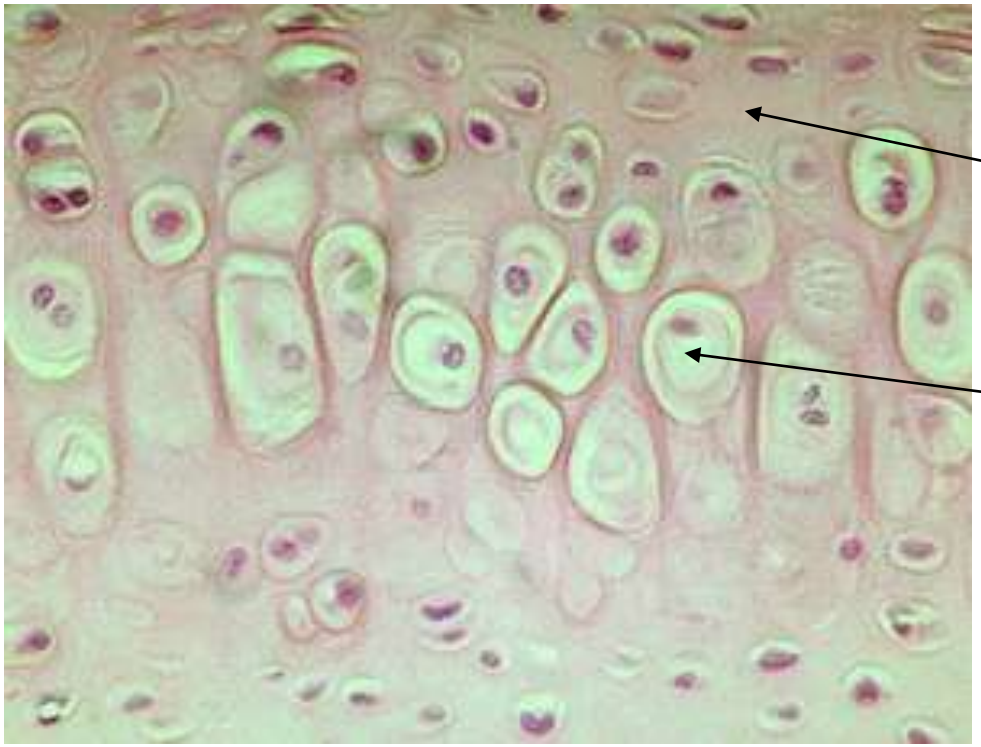


Today’s talk

Cartilage

- What it is and what it does
- What goes wrong in OA
-What we know about the process(es)
- What research offers (an example or two)

Cartilage = extracellular matrix (ECM)



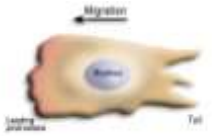
Complex network of proteins and carbohydrates surrounding cells

What does ECM do?

Support and protection

- Musculoskeletal strength
- Skin

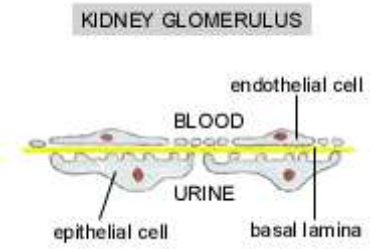
Cell migration, polarity and shape



- Wound repair
- Embryo development
- Tumour development

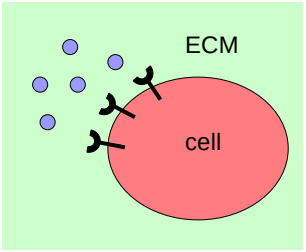
Transport of nutrients and waste products

- Kidney
- Lungs
- Retina
- Pharmaceuticals

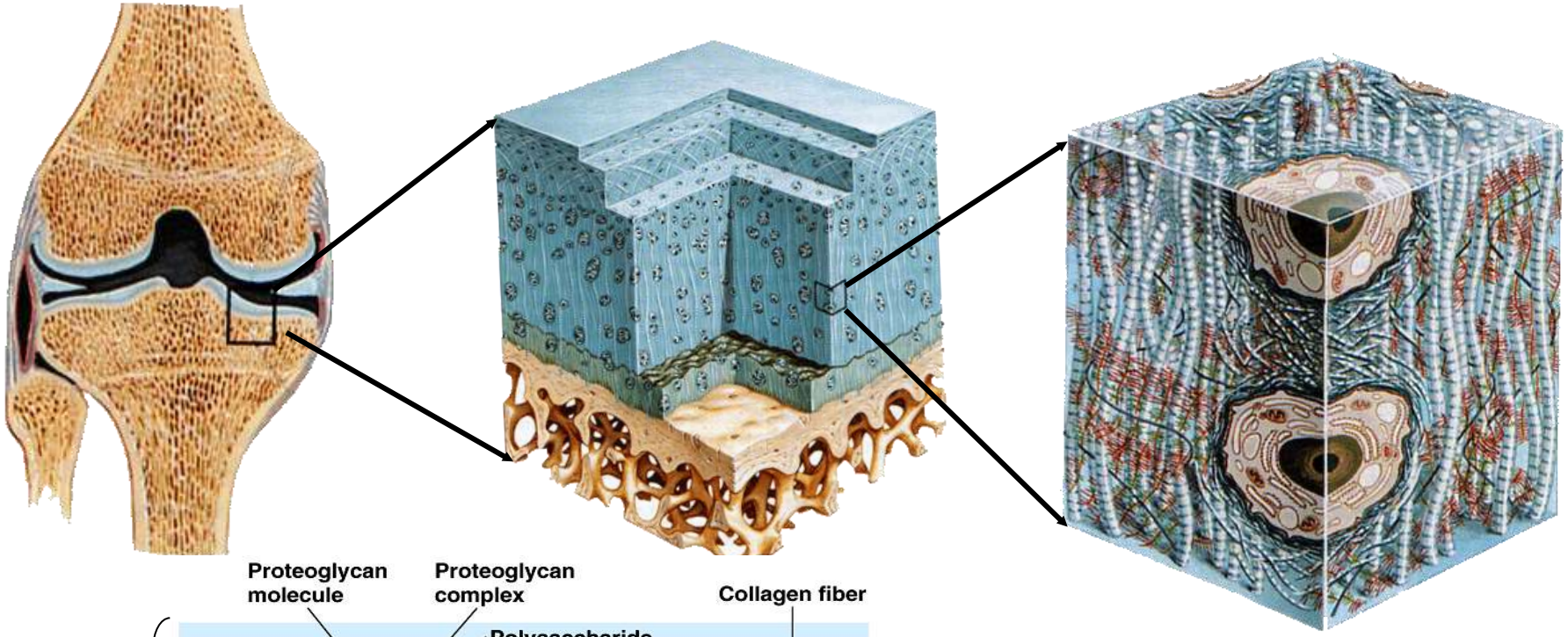


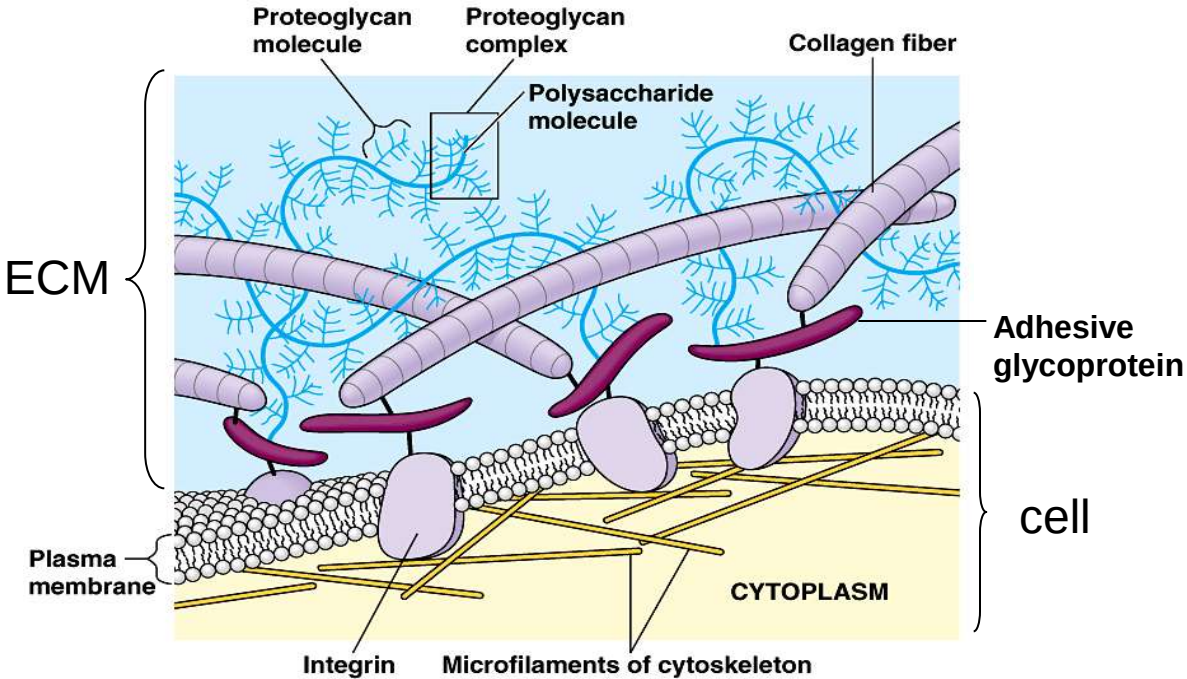
Intercellular communication

- Hormones
- Growth factors
- Cytokines

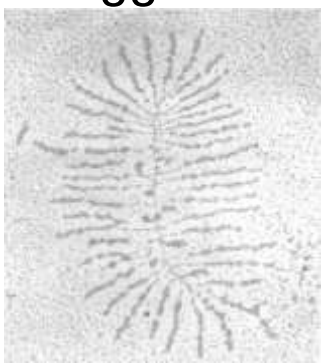


Cartilage = extracellular matrix

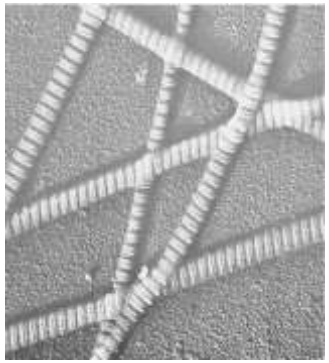




Aggrecan



Collagen



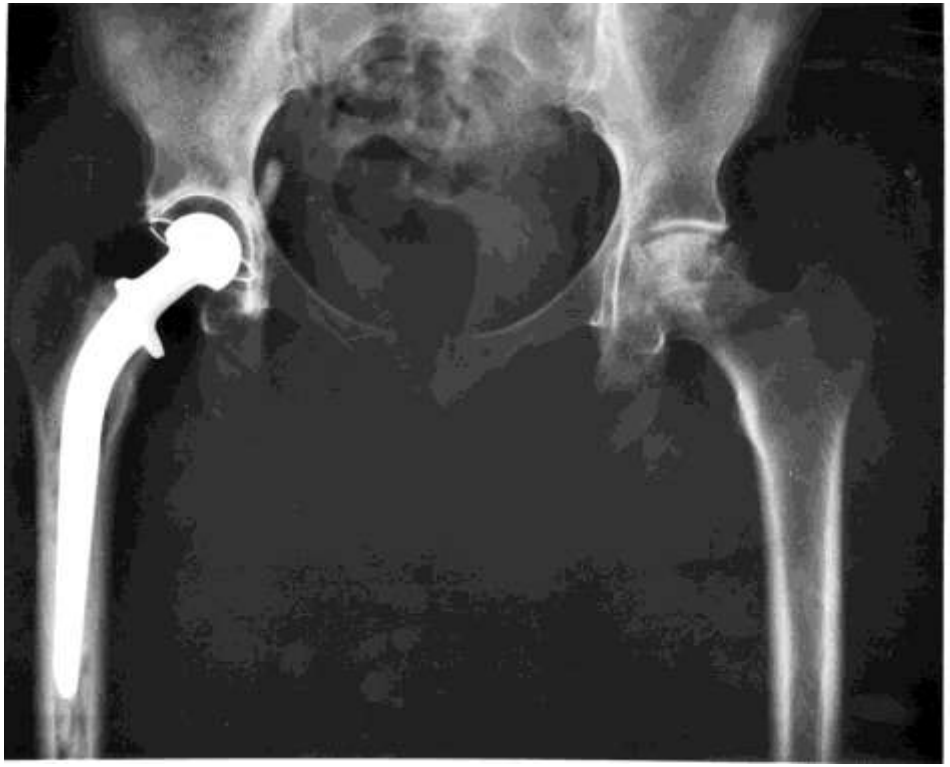
The Clinical Need

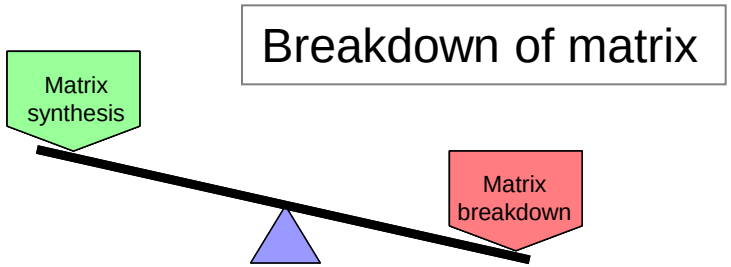
Cartilage collagen loss is essentially irreversible and therefore a critical step for therapeutic intervention

Loss of collagen = loss of joint function

Current Treatments

- **Analgesics and total joint replacement are only current treatments**





Proteinases

Intracellular

Extracellular

Aspartic proteinases

Cysteine proteinases

Threonine proteinases

Serine proteinases

Metallo-proteinases

Low pH → **Neutral pH**

Cathepsin D

Cathepsin B
Cathepsin K
Cathepsin L
Cathepsin S

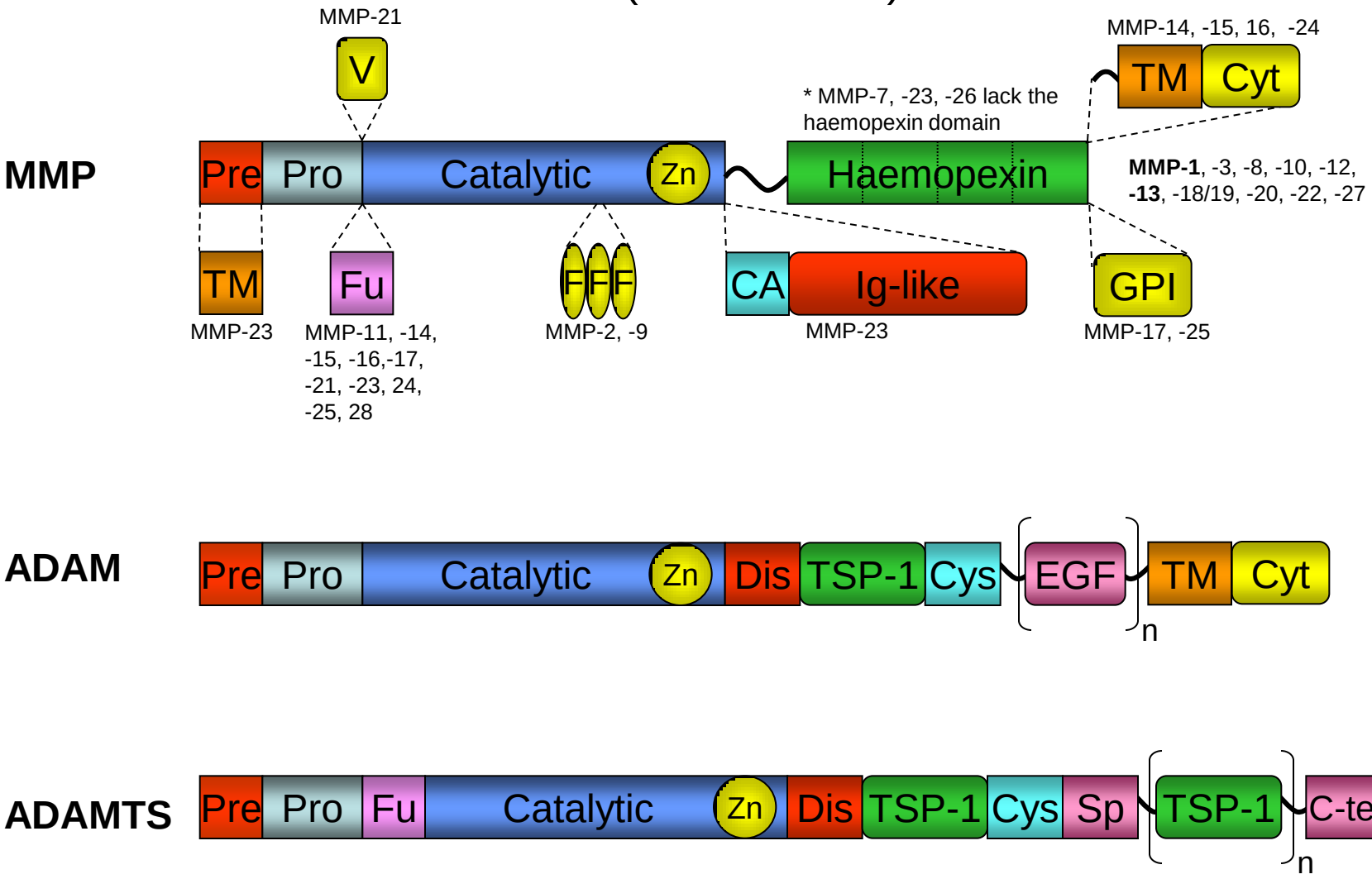
Proteosome

Elastase
Cathepsin G
Plasmin
PAs
Furin

MMP-1, -8, -13
MMP-3, -10, -11
MMP-2, -9
MT1-6 MMP
ADAMs
ADAMTSs

Metalloproteinases family

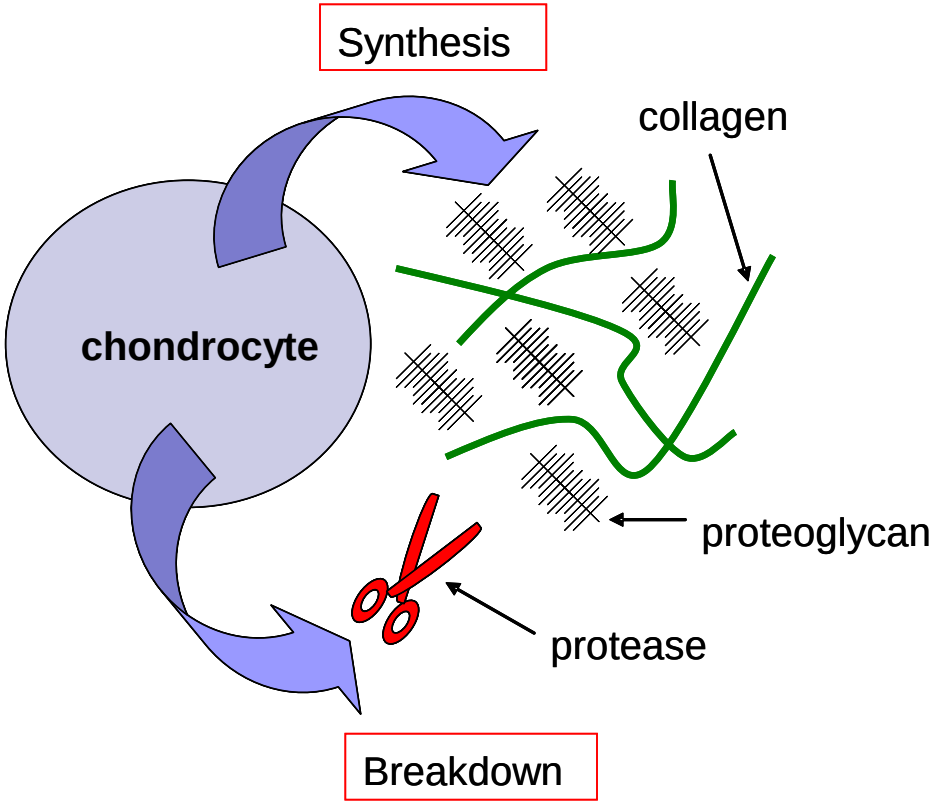
(~75 member)



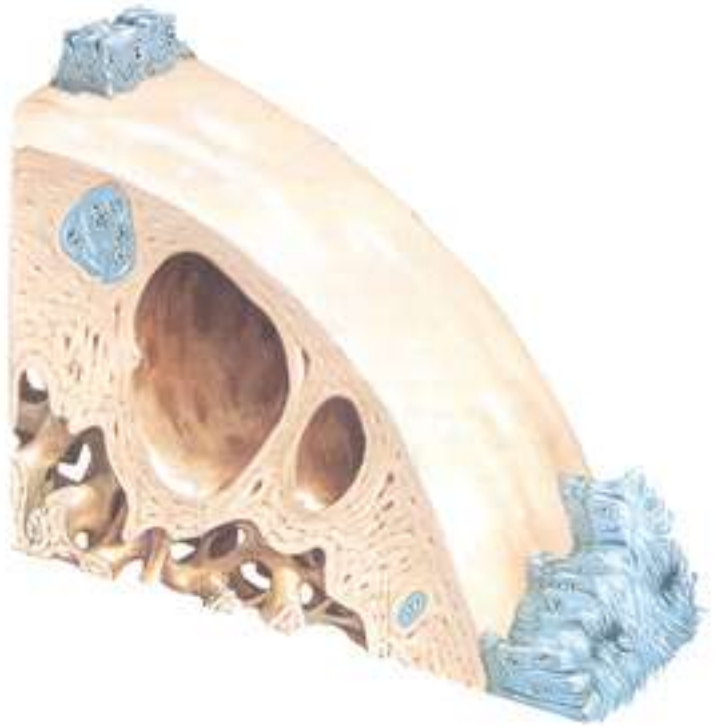
How is a healthy ECM maintained?

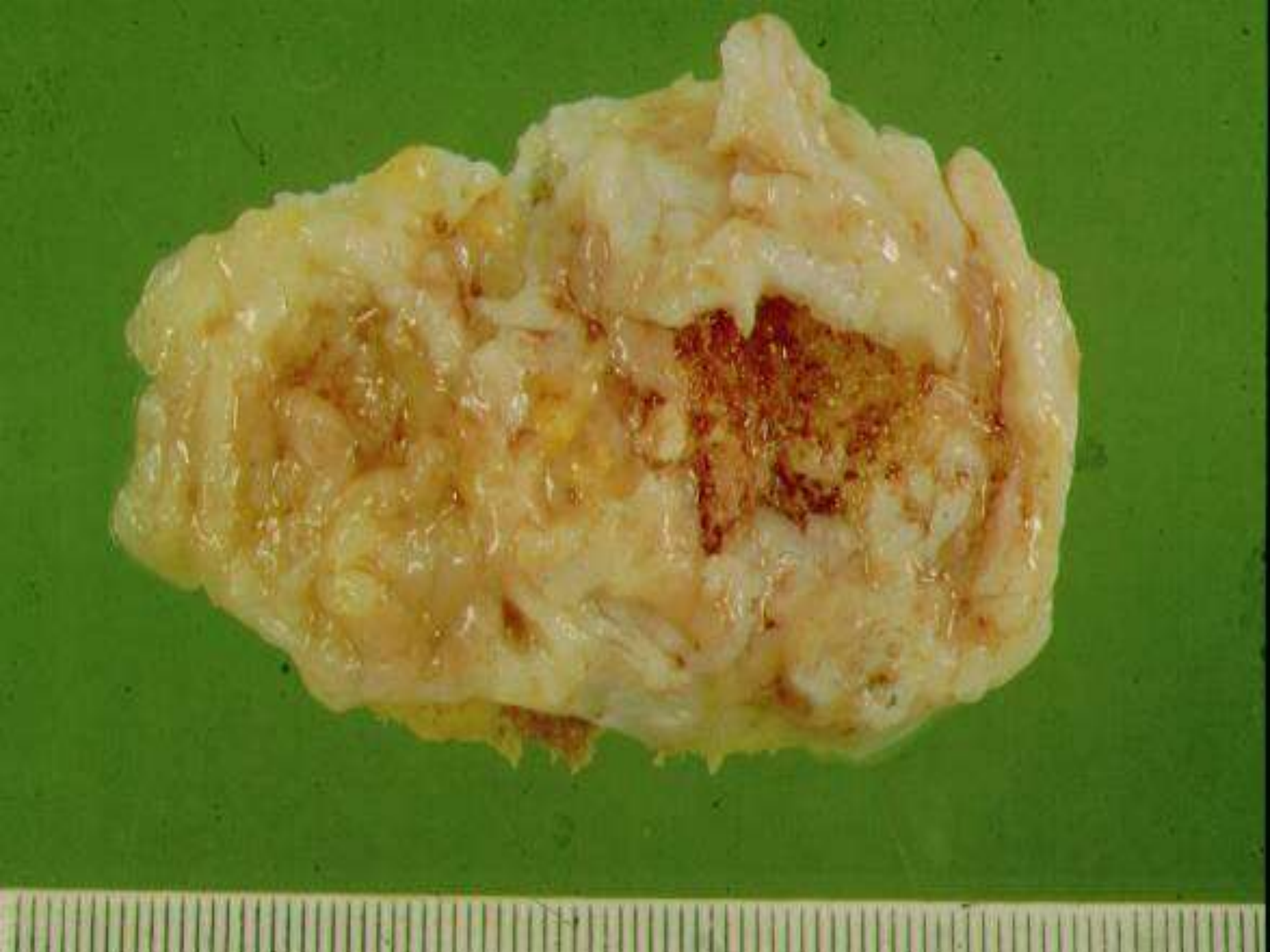
- Cells within matrix (or in contact with matrix) secrete the ECM molecules
- The same cells also secrete enzymes which are able to digest the matrix

- to remove damaged matrix
- to remodel matrix



OSTEOARTHRITIS: ends in cartilage degradation





Disease mechanisms

- RA

  - autoimmune; genetic; inflammatory; synovium-driven

- OA

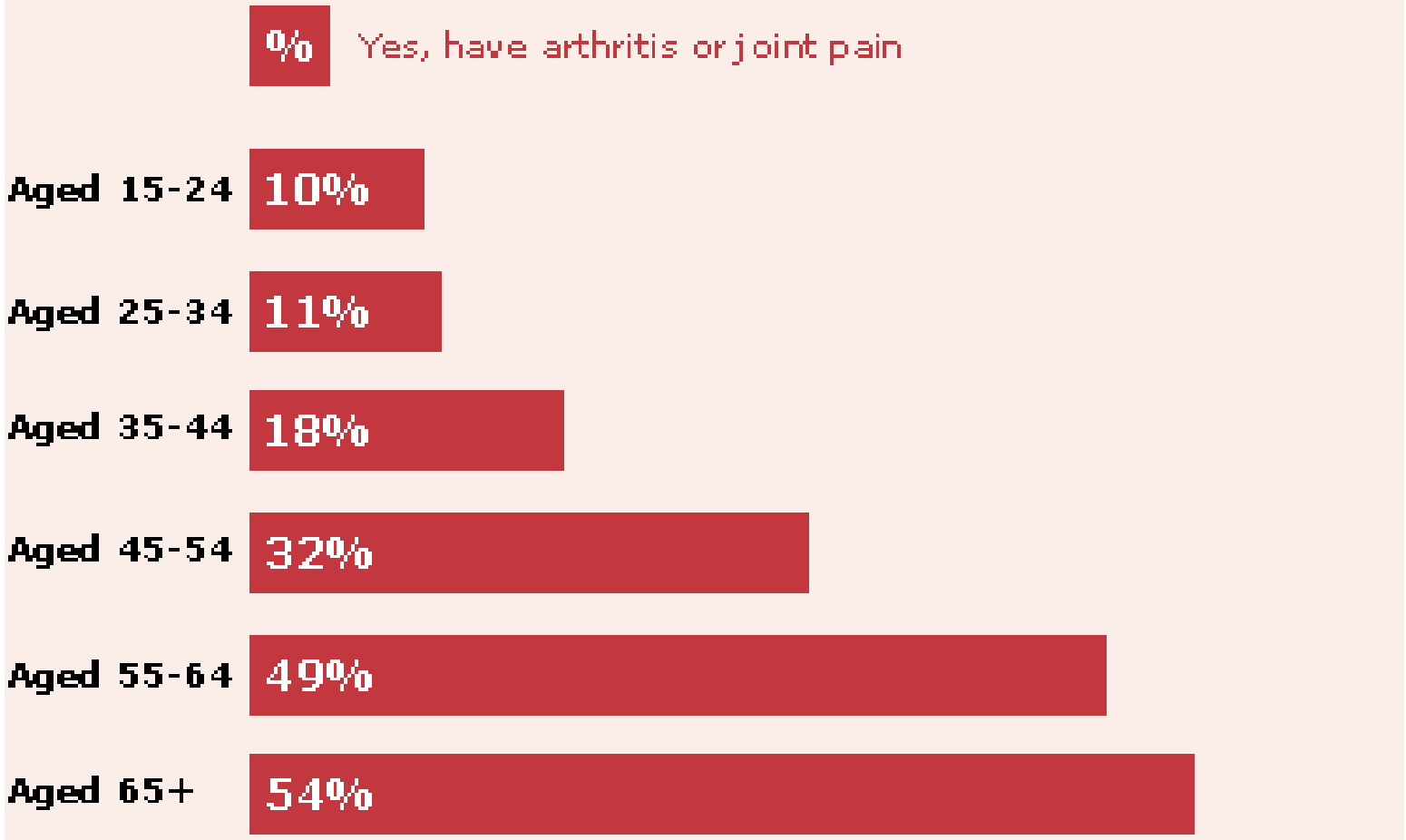
  - mechanical or trauma; non-inflammatory; cartilage-driven

- Treatments

  - most modulate pain and inflammation, **NOT** destruction

Incidence of arthritis and joint pain

Do you have or have you ever had arthritis or joint pain?



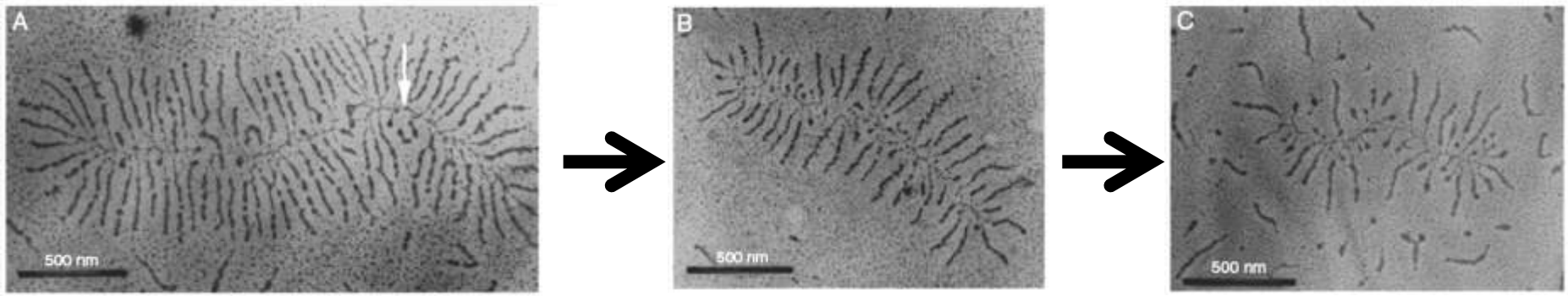
Base: Adults aged 15+ in Great Britain (2,031) Source: MORI

Age-related cartilage changes

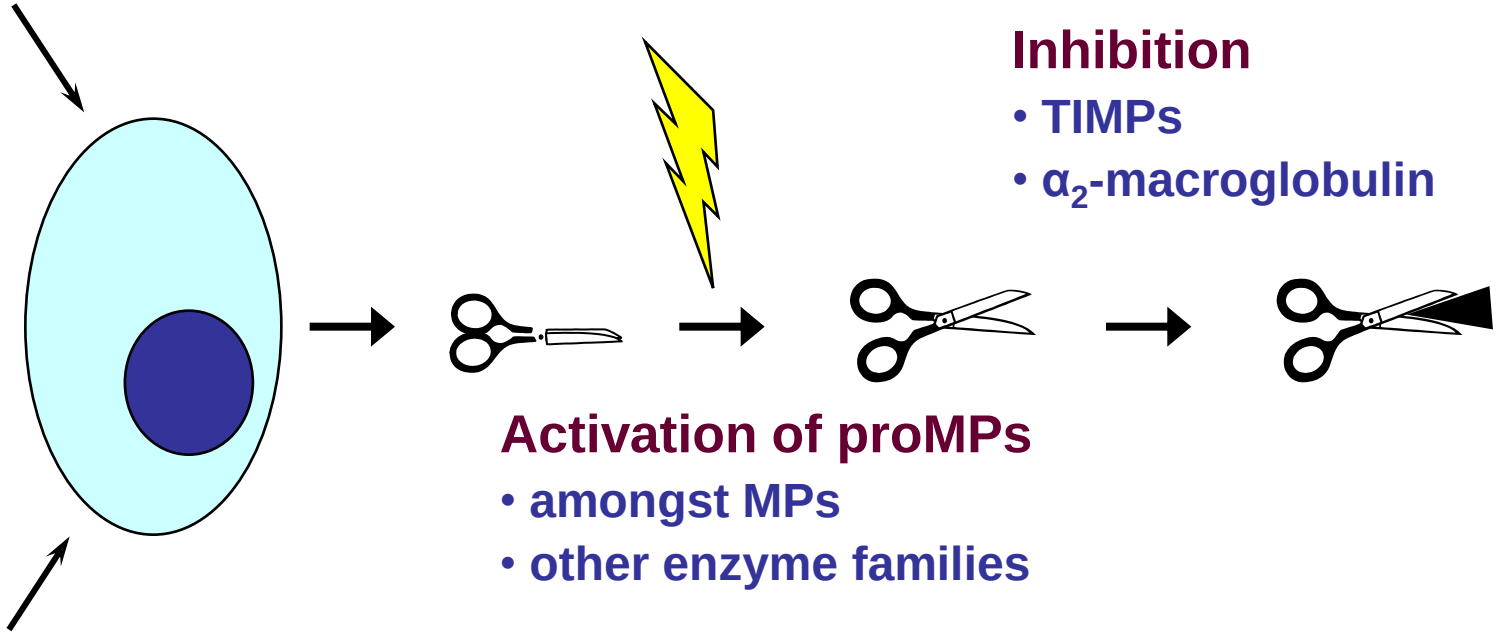
Glycation endproducts
Senescence
Oxidative stress
Apoptosis
Protein mis-folding
Epigenetics

??? A little of everything ???

Phenotypic changes in cartilage



Control of metalloproteinase activity



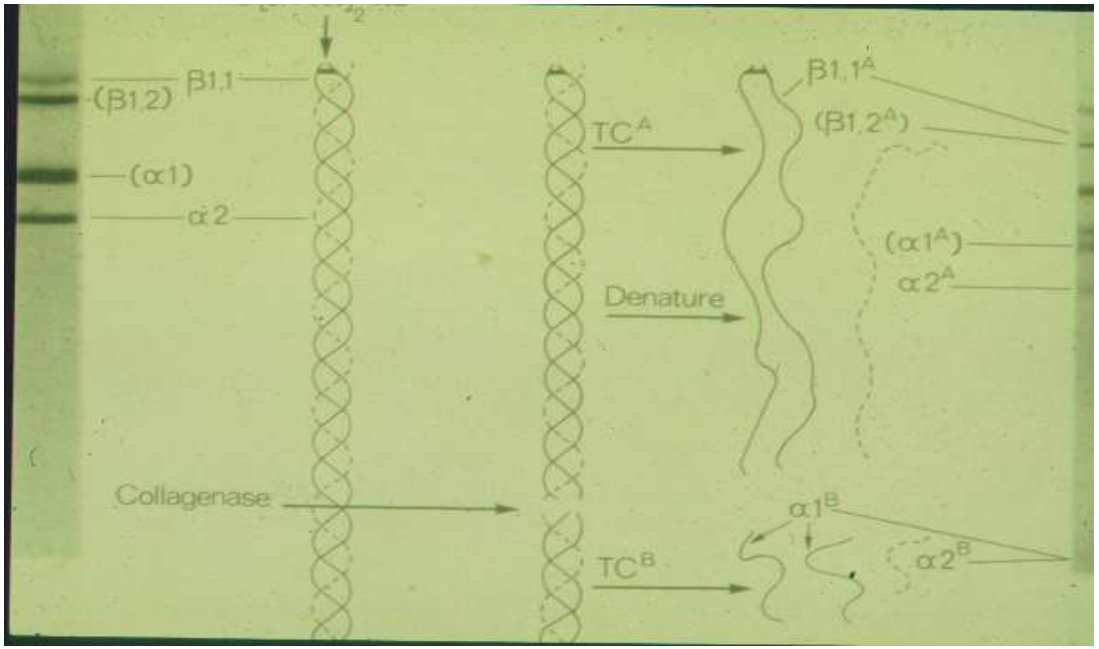
Inhibition
- **TIMPs**
- **α_2-macroglobulin**

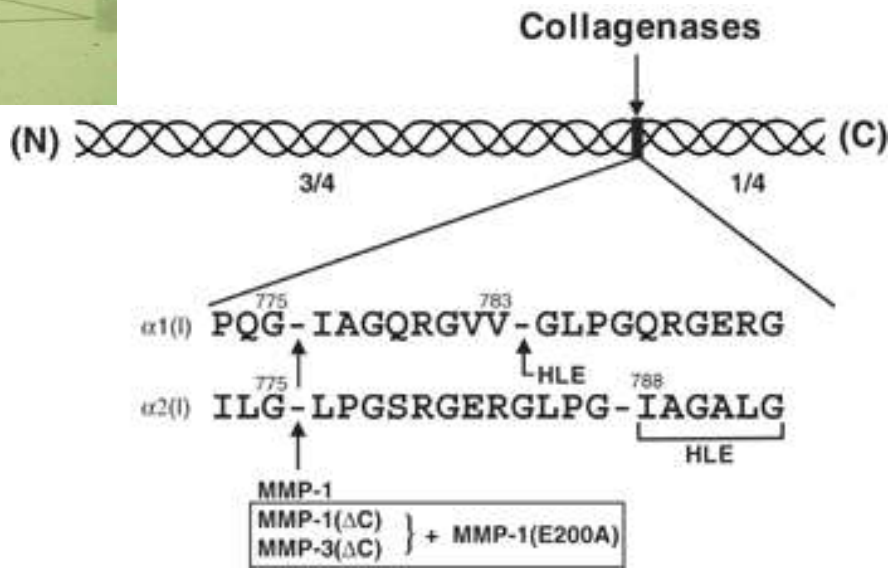
Activation of proMPs
- **amongst MPs**
- **other enzyme families**

Transcriptional control of MPs and TIMPs
- **cytokines/growth factors**
- **cell-matrix interaction**

The first collagenase was discovered in 1962

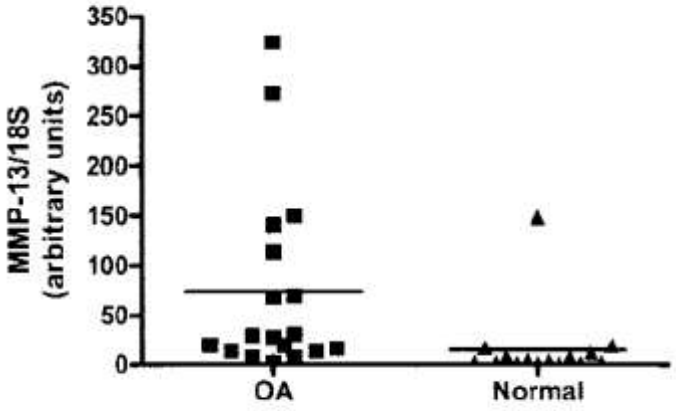
Collagenases cleave collagen at a single and unique site



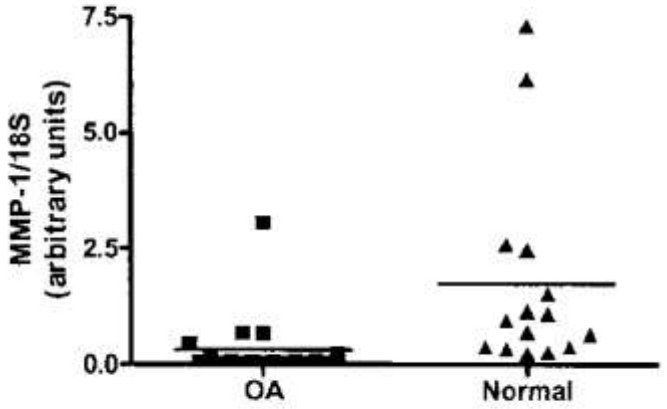


MMP expression changes

MMP-13



MMP-1

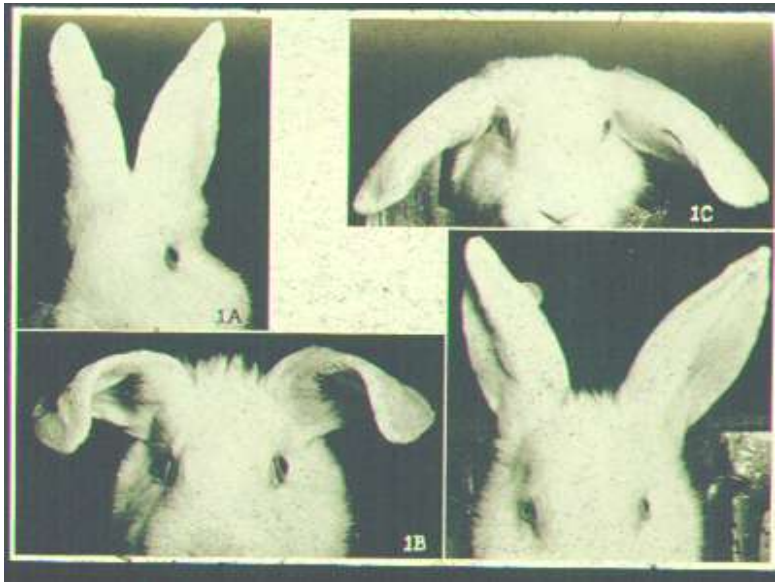


Aggrecan cleavage

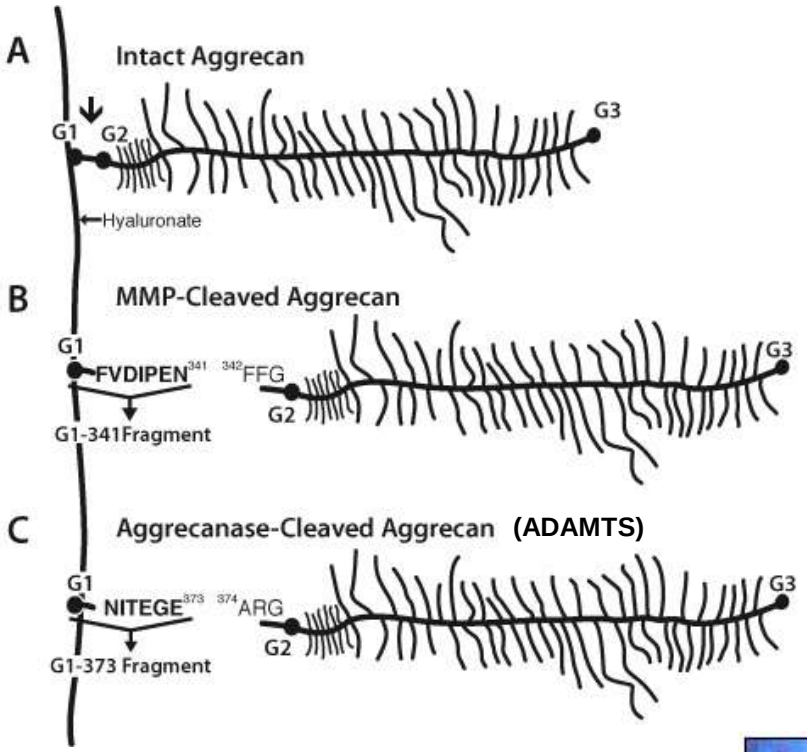
- Proteoglycans attract water, forming an hydrated gel
- results in a swelling pressure (turgor)

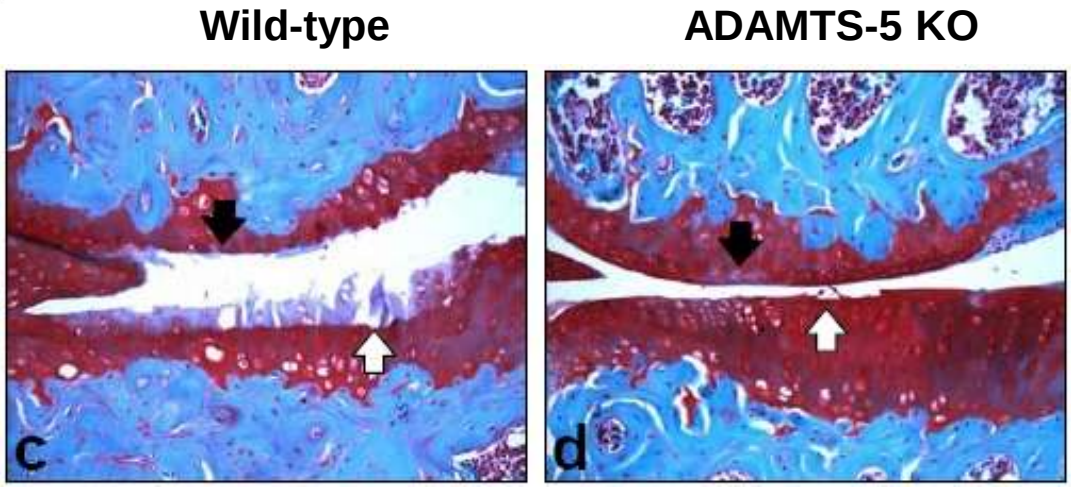
resistance to compression

strength and support



Aggrecan cleavage





Pro-inflammatory cytokines

Interleukin-1 (IL-1)

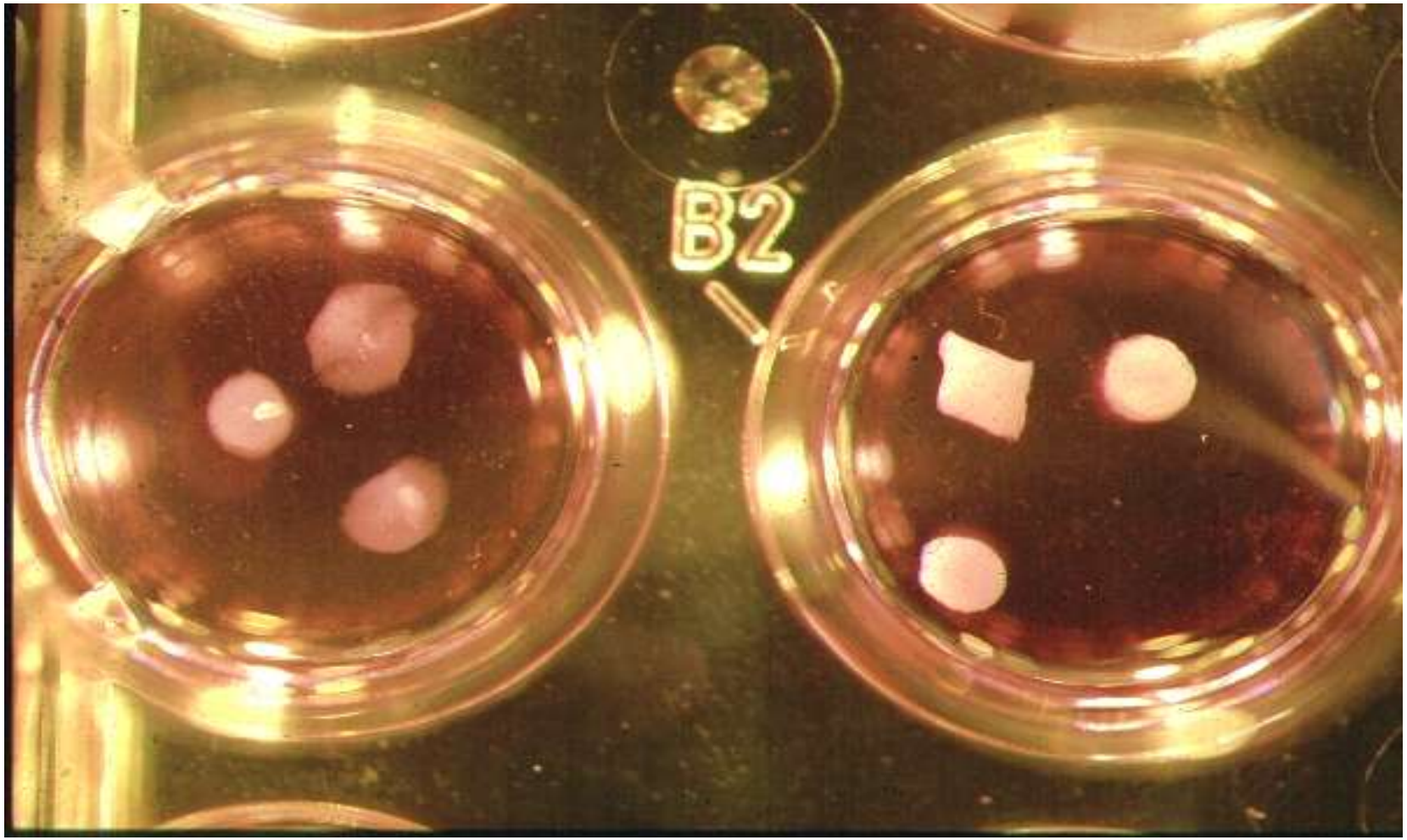
Interleukin-6 (IL-6)

Interleukin-17 (IL-17)

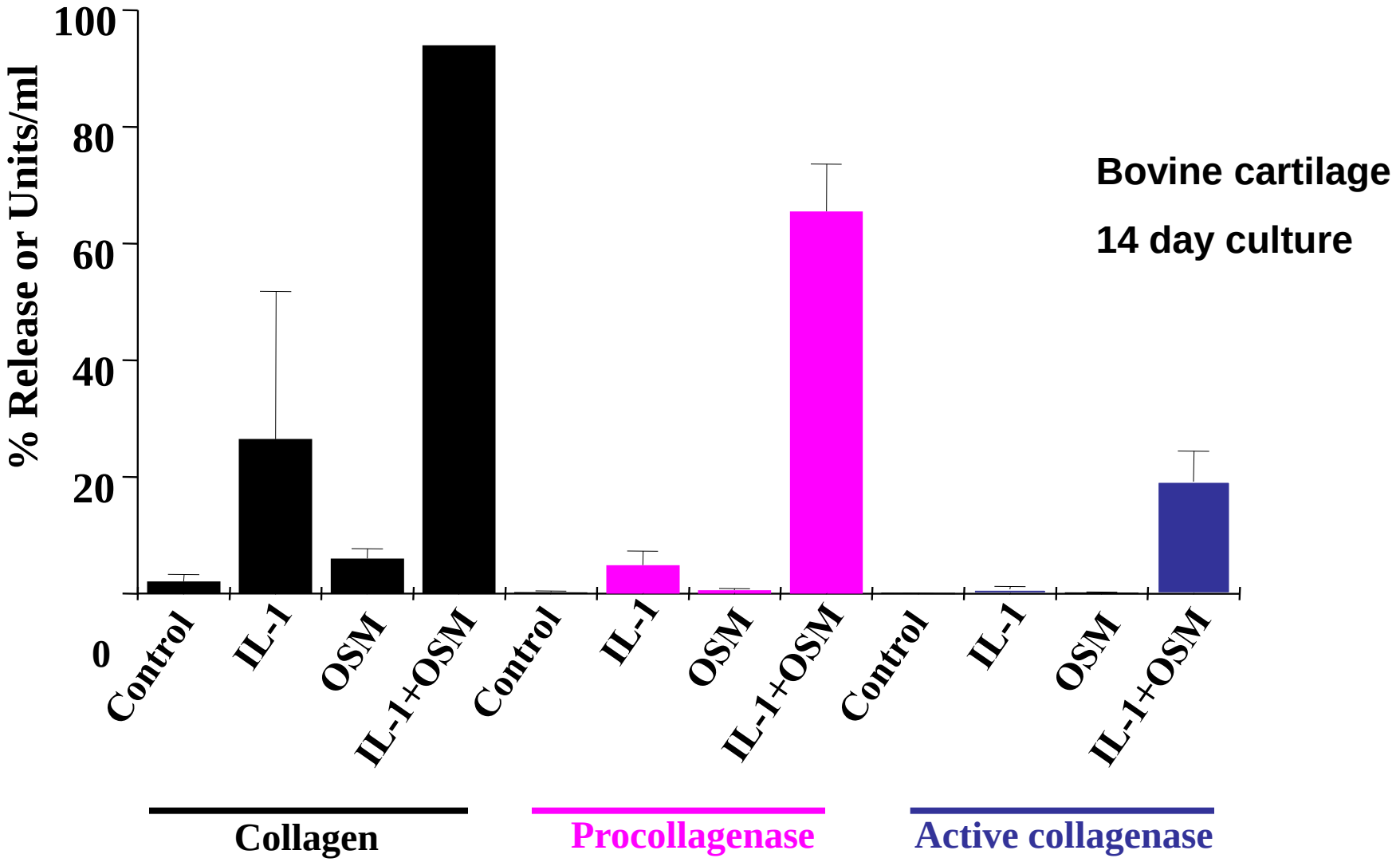
Tumour necrosis factor α (TNFα)

Oncostatin M (OSM)

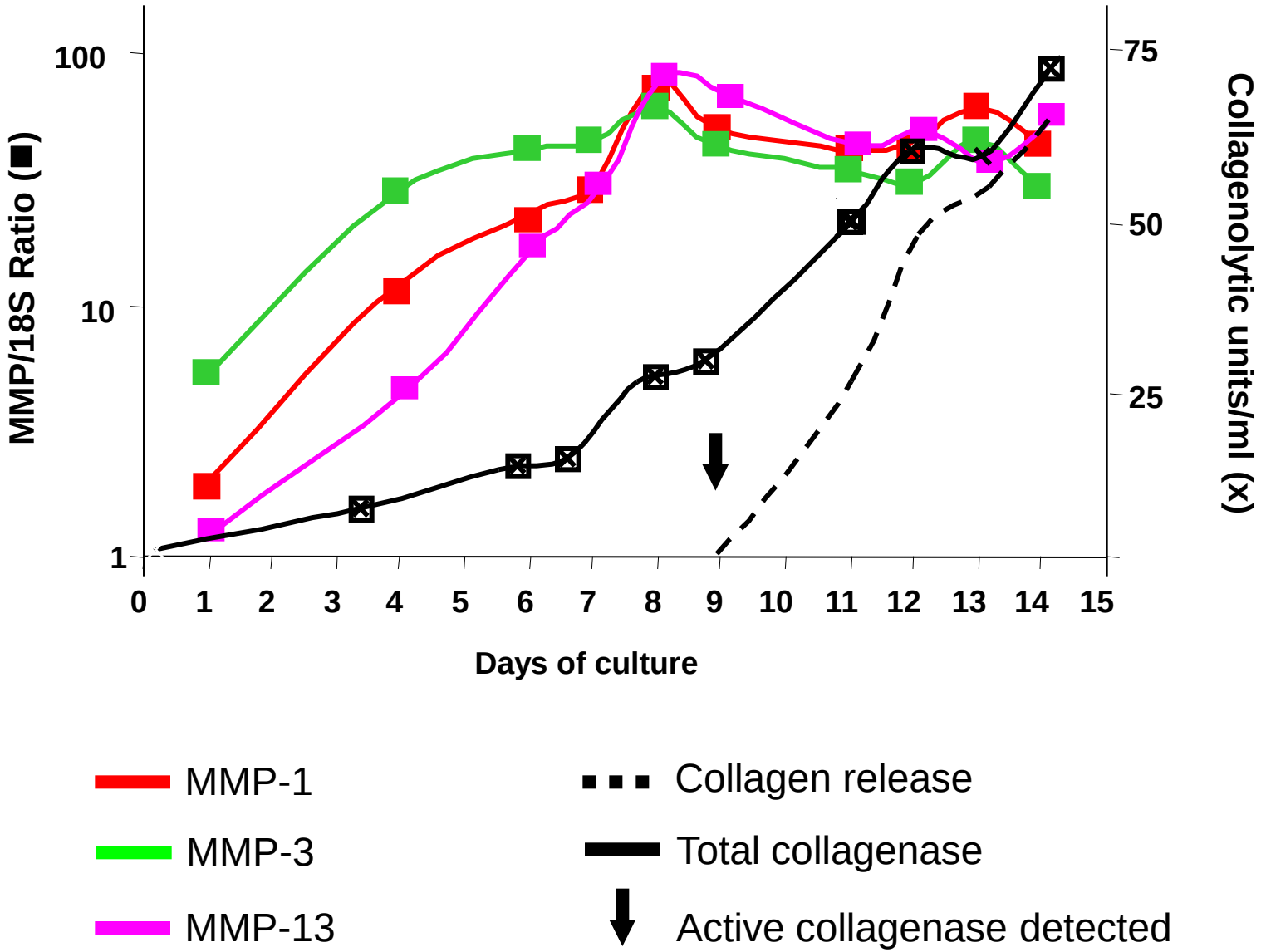
Cartilage collagenolysis model



IL-1+OSM-induced cartilage collagenolysis

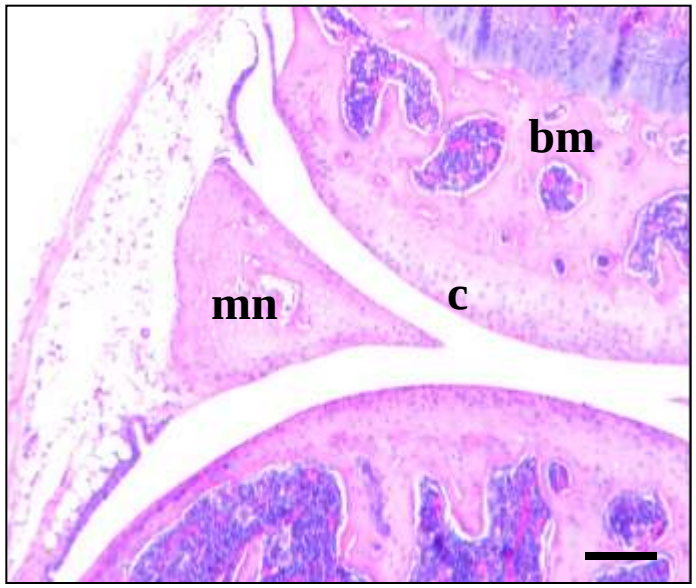


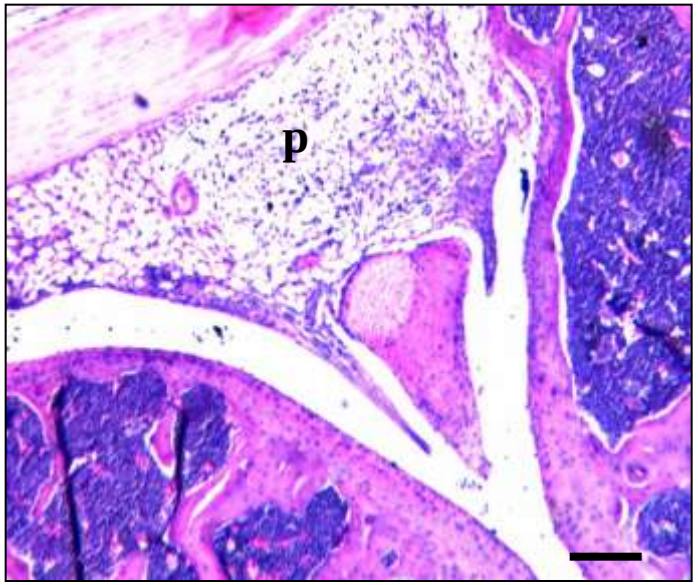
MMP expression profiling in resorbing cartilage



Adenoviral delivery of IL-1+OSM in murine joints

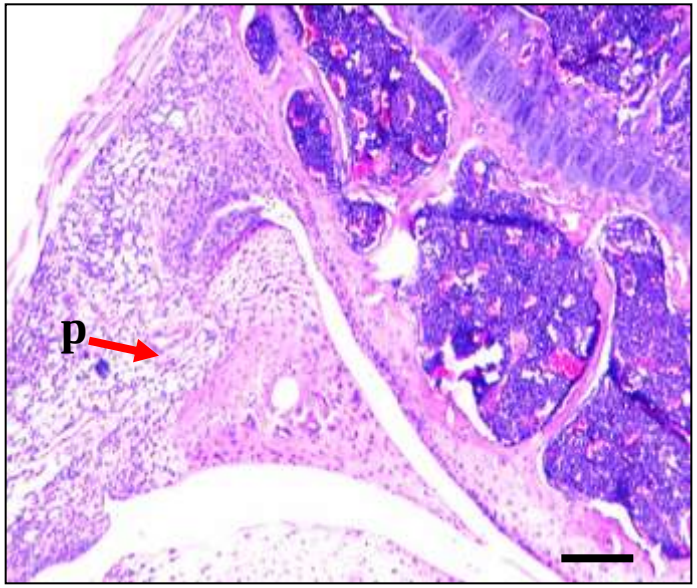
Control

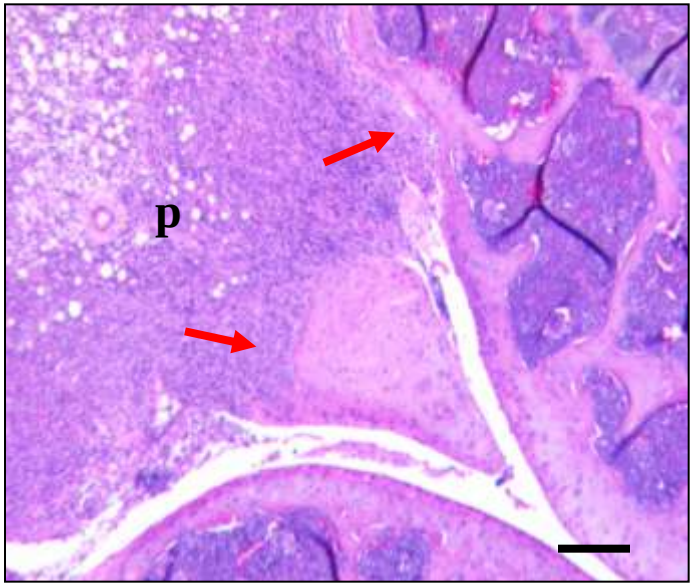




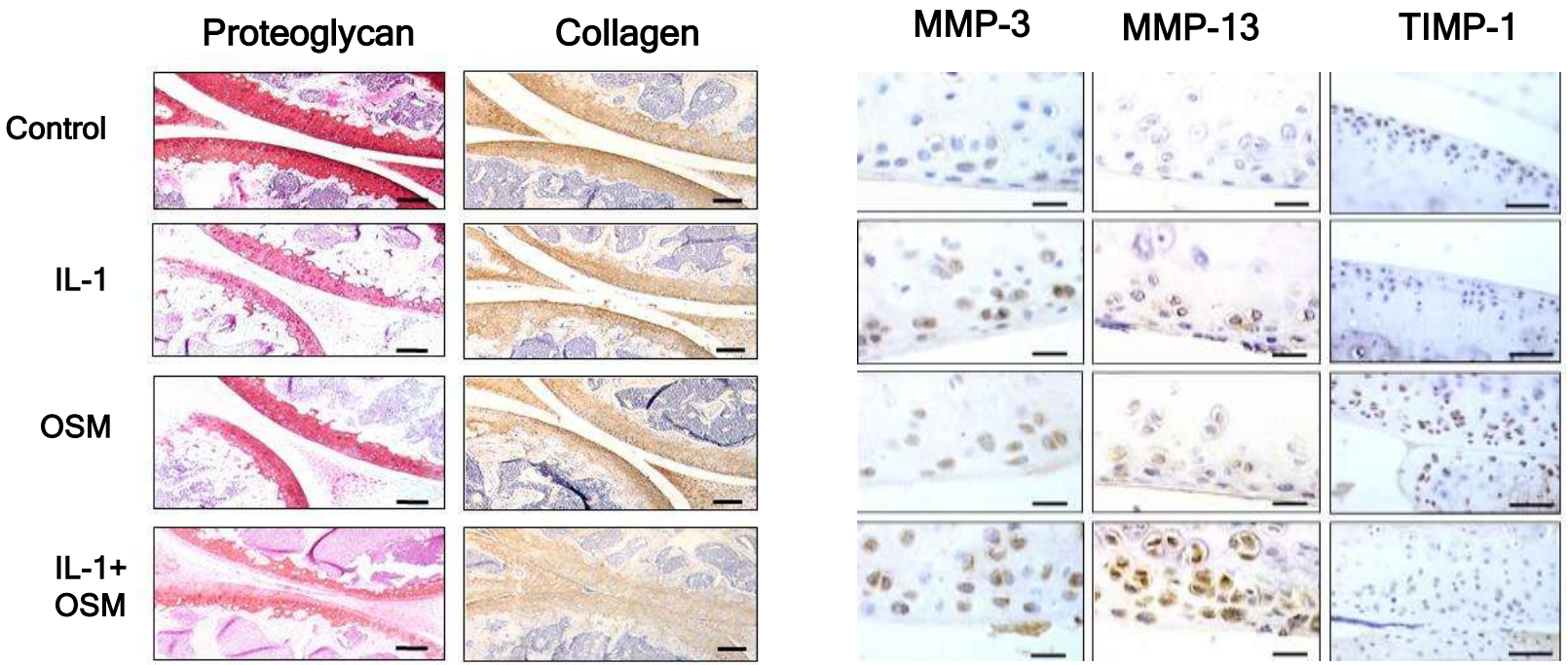
IL-1

OSM





IL-1+
OSM

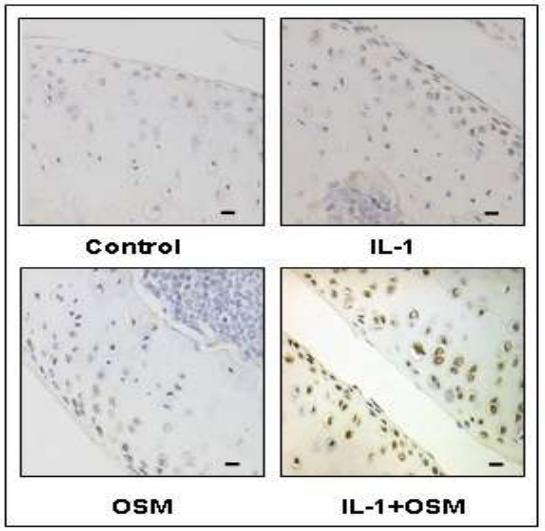


Rowan *et al*., 2003

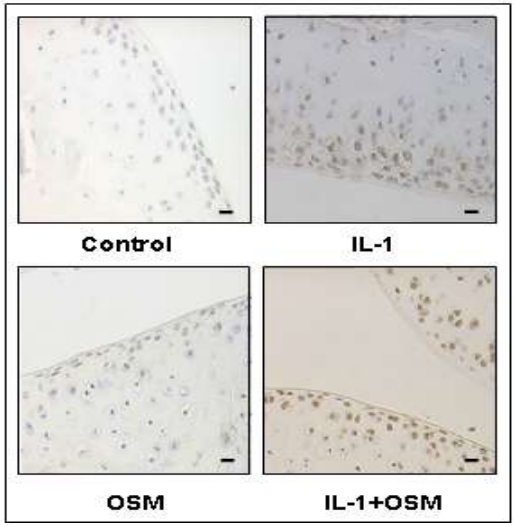
Gene profiling – “transcriptomics”

Gene	Signal				Signal log ratio vs. control			Fold change vs. control		
	Control	IL-1	OSM	IL-1+ OSM	IL-1	OSM	IL-1+ OSM	IL-1	OSM	IL-1+ OSM
Proteases and inhibitors										
MMP-1	22A	1875P	28P	4217P	6.1	0.3	7.3	67.6	1.3	157.6*
MMP-3	376P	5862P	318P	8910P	3.4	0.2	4.4	10.2	1.1	21.1*
MMP-10	30A	99P	14A	177A	1.2	-0.6	2.3	2.3	-0.7	4.8*
MMP-12	57A	1015P	37A	2447P	3.6	-0.3	4.9	12.5	-0.8	30.7*
MMP-13	3A	280P	18P	1548P	5.9	2.0	8.4	58.9	4.1	342.5*
MMP-14	22A	28A	97A	248P	0.0	1.8	3.2	1.0	3.5	8.9
Antileukopeptidase	112P	175P	68A	400P	0.4	-0.7	2.4	1.1	-0.6	6.9
SCCA 2	4A	20A	525P	1125P	2.2	5.8	7.2	4.4	55.7	147.0
C1r	274P	1217P	509P	1912P	2.2	1.2	2.9	4.6	2.3	7.5
Chemokines, cytokines, receptors and signal transduction										
IL-8	2A	896P	2A	1972P	7.7	-0.2	9.0	207.9	-0.9	512.0*
IL-1β	24A	150A	48A	560P	2.9	0.8	4.2	7.2	1.7	18.4
MCP-1	3A	175P	43A	285P	5.0	3.9	6.3	32.0	14.4	78.8*
MCP-3	43A	369P	89P	734P	3.8	1.6	7.3	13.9	3.0	28.8*
IL-6	53A	251P	43A	1486P	1.7	-0.1	4.3	3.3	-0.9	20.4*
LIF	12A	125M	9A	226P	3.1	-0.1	4.0	4.3	-0.9	10.2*
OSMβR	25P	71P	155P	253P	1.2	2.7	3.3	2.4	6.5	9.2
ENA-78	9A	10A	6A	217P	0.6	-0.2	4.4	1.5	-0.3	20.4*
PBEF	305P	928P	604P	2303P	1.6	1.0	3.0	3.0	2.0	8.1*
Activin A	58A	90P	17A	125A	0.7	-2.3	1.5	1.8	-0.7	2.3*
Jak 2 kinase	28A	88P	98P	371P	1.4	1.5	3.2	2.7	2.8	9.1
Extracellular proteins										
Decorin variant A	14P	64P	23P	126P	1.8	0.1	3.3	2.3	1.2	18.4*
Decorin variant C	98P	255P	99P	826P	1.7	0.0	3.5	3.3	1.0	11.3
Fibronectin	130P	140P	397P	709P	0.11	1.6	2.7	1.0	2.9	6.3
Serum amyloid A2	5A	289P	9A	2288P	5.15	0.9	8.9	85.0	1.1	362.0
Calcium binding protein A9	7A	45A	18A	339P	1.51	1.4	338.9	2.8	2.6	45.3
Calcium binding protein A8	33A	63A	19A	604P	0.91	-0.2	4.0	1.9	1.7	16.0
PTX 3	18A	123P	24P	407P	2.59	0.7	5.1	6.0	1.6	33.6*
Chitinase-3-like-2	79A	215P	71A	1750P	1.56	-0.3	3.9	2.9	1.0	14.9
Chitinase-3-like-1	243P	579P	1666P	2320P	1.21	2.7	3.2	2.4	6.4	9.2
SOD 3	24P	232P	25A	404P	3.45	-0.2	5.7	24.8	1.5	41.6

Pentraxin-3

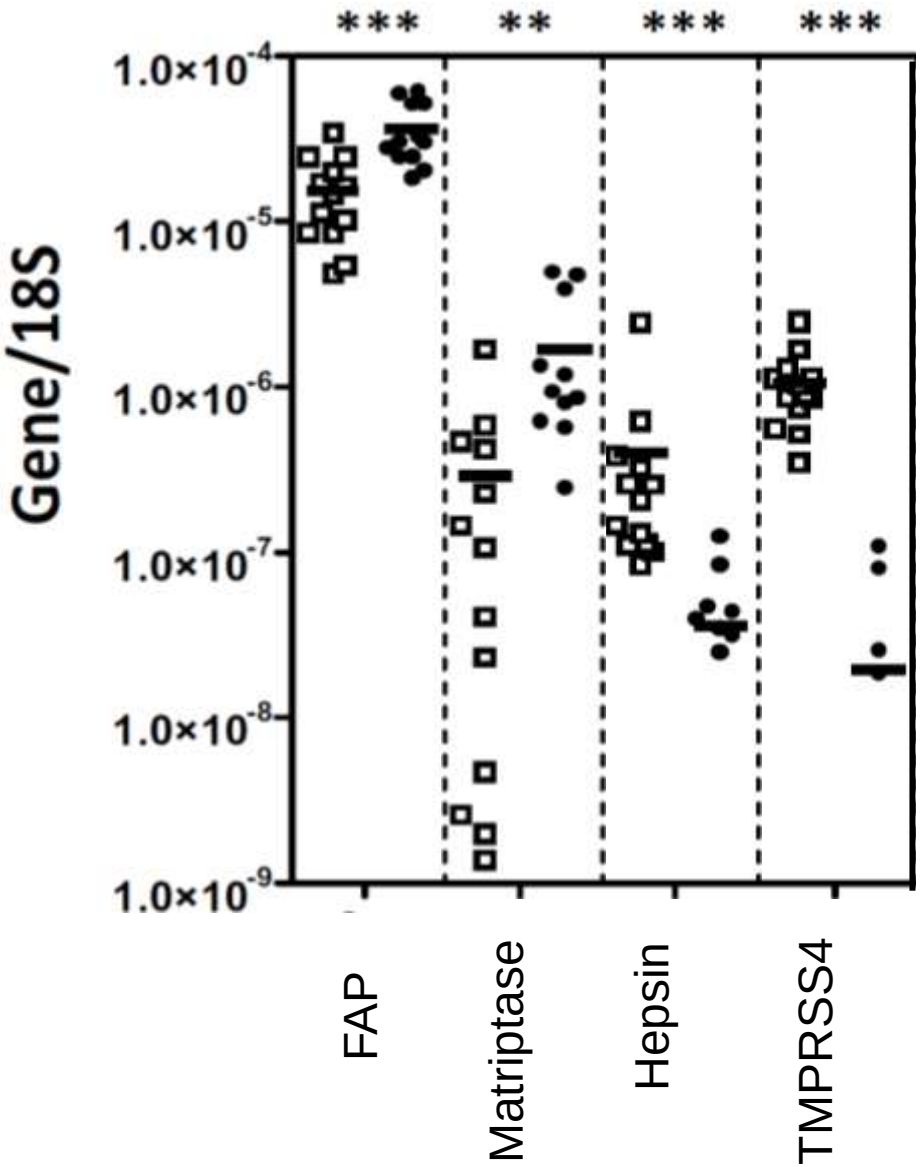


Activin A



Barksby *et al.*, 2006 (A&R)

Membrane serine proteases expressed in cartilage

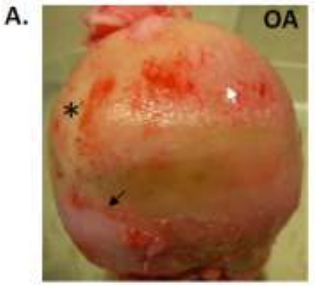


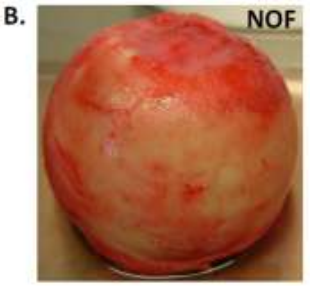
□ = NOF

● = OA

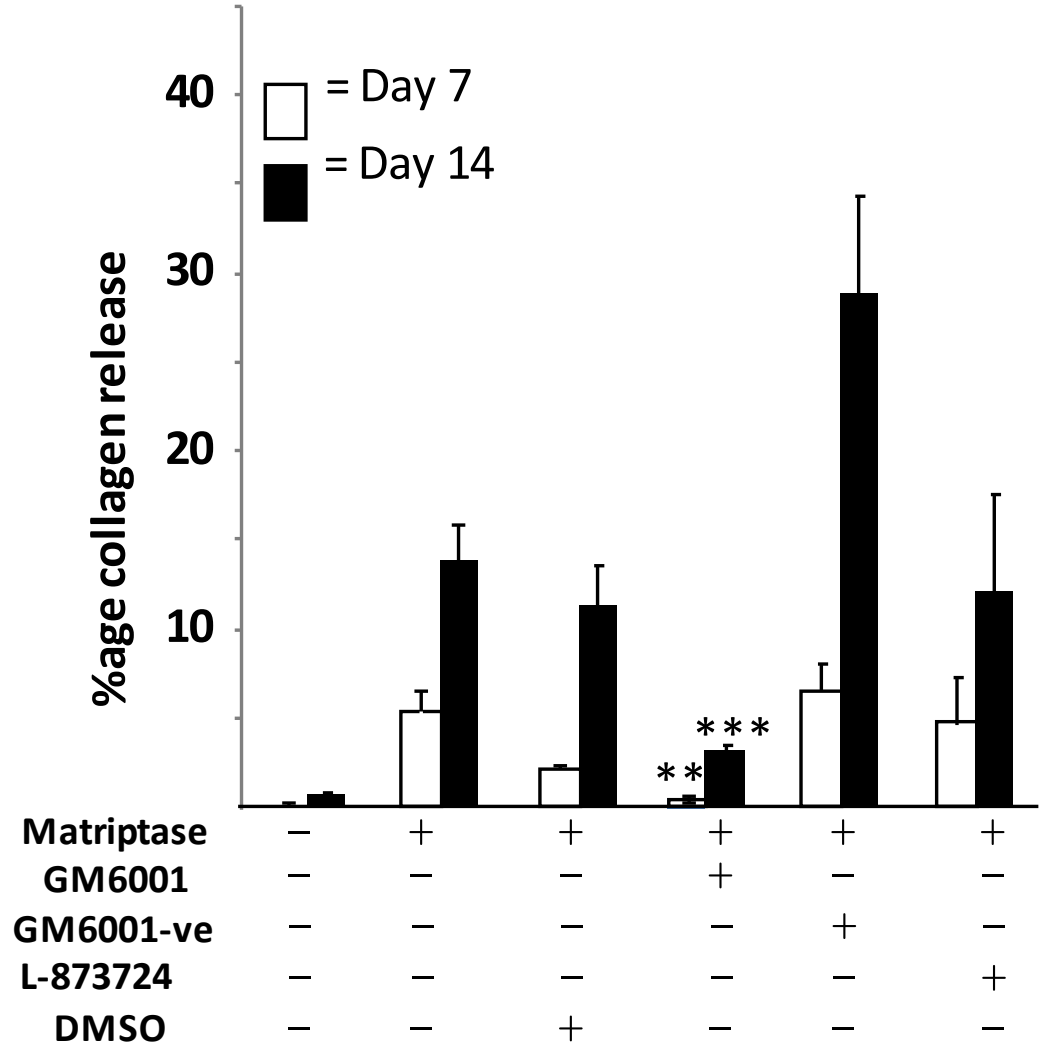
*** p<0.001

** p<0.01



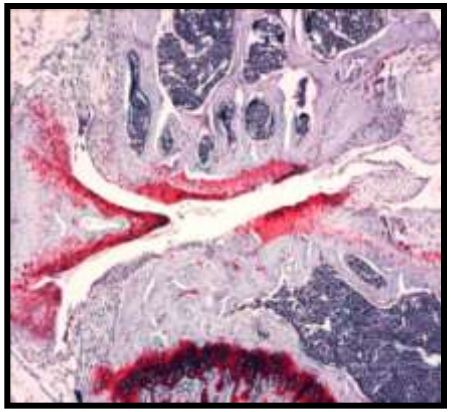


Matriptase-induced cartilage collagen release is MMP-dependent



Matriptase inhibition reduces cartilage damage

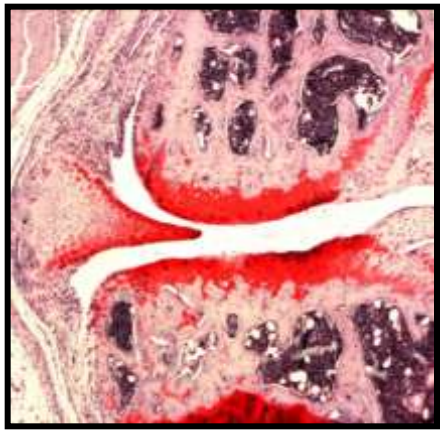
0 mg/kg/day

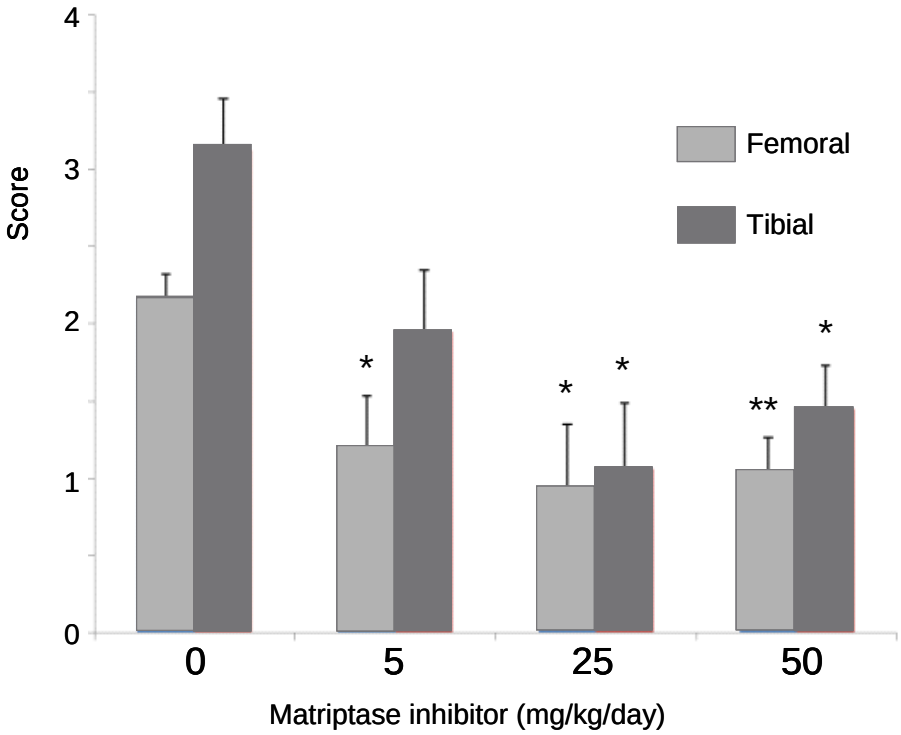


5 mg/kg/day

25 mg/kg/day

50 mg/kg/day





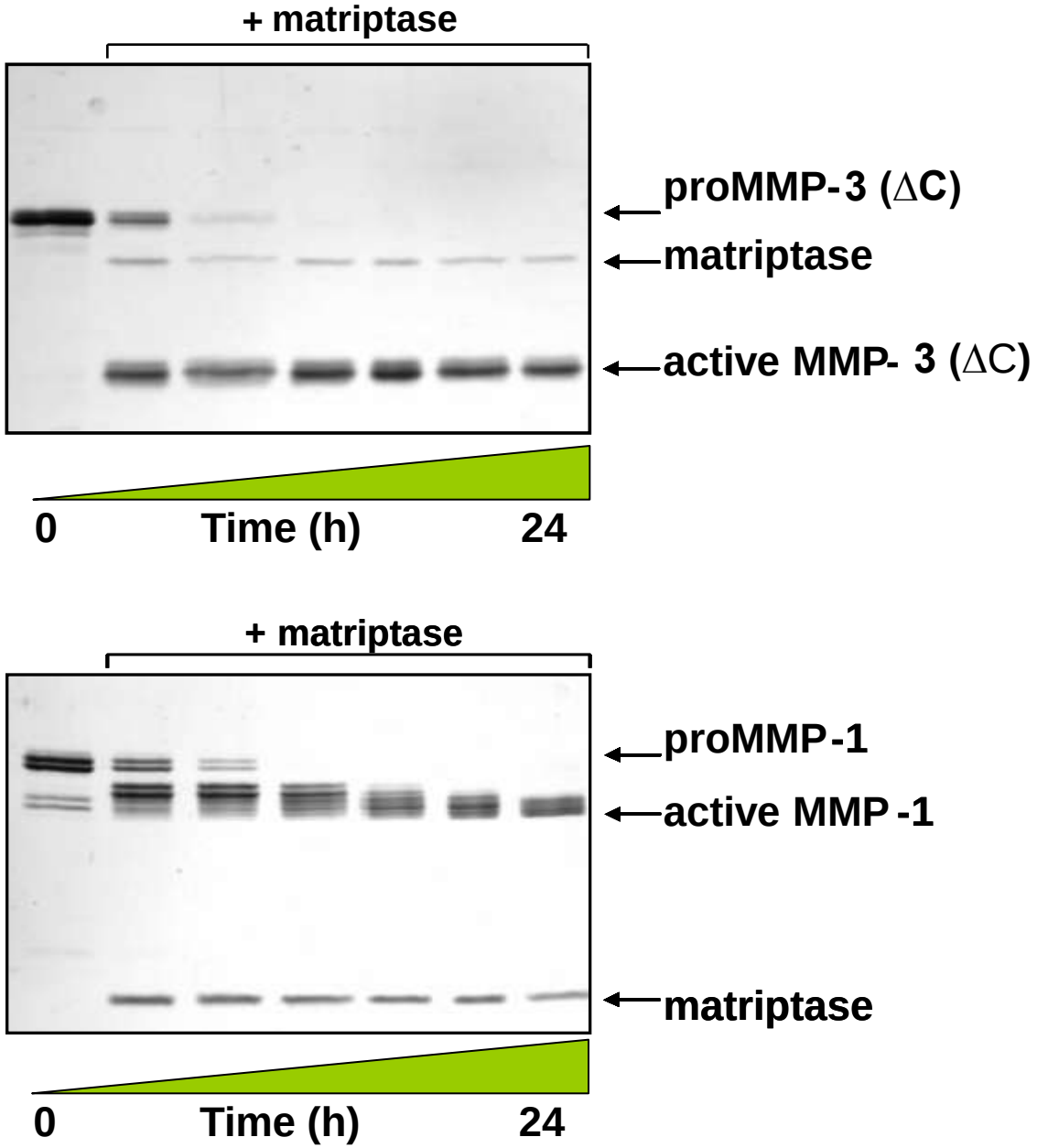
** p<0.01
* p<0.05

Matriptase activates

proMMP-3

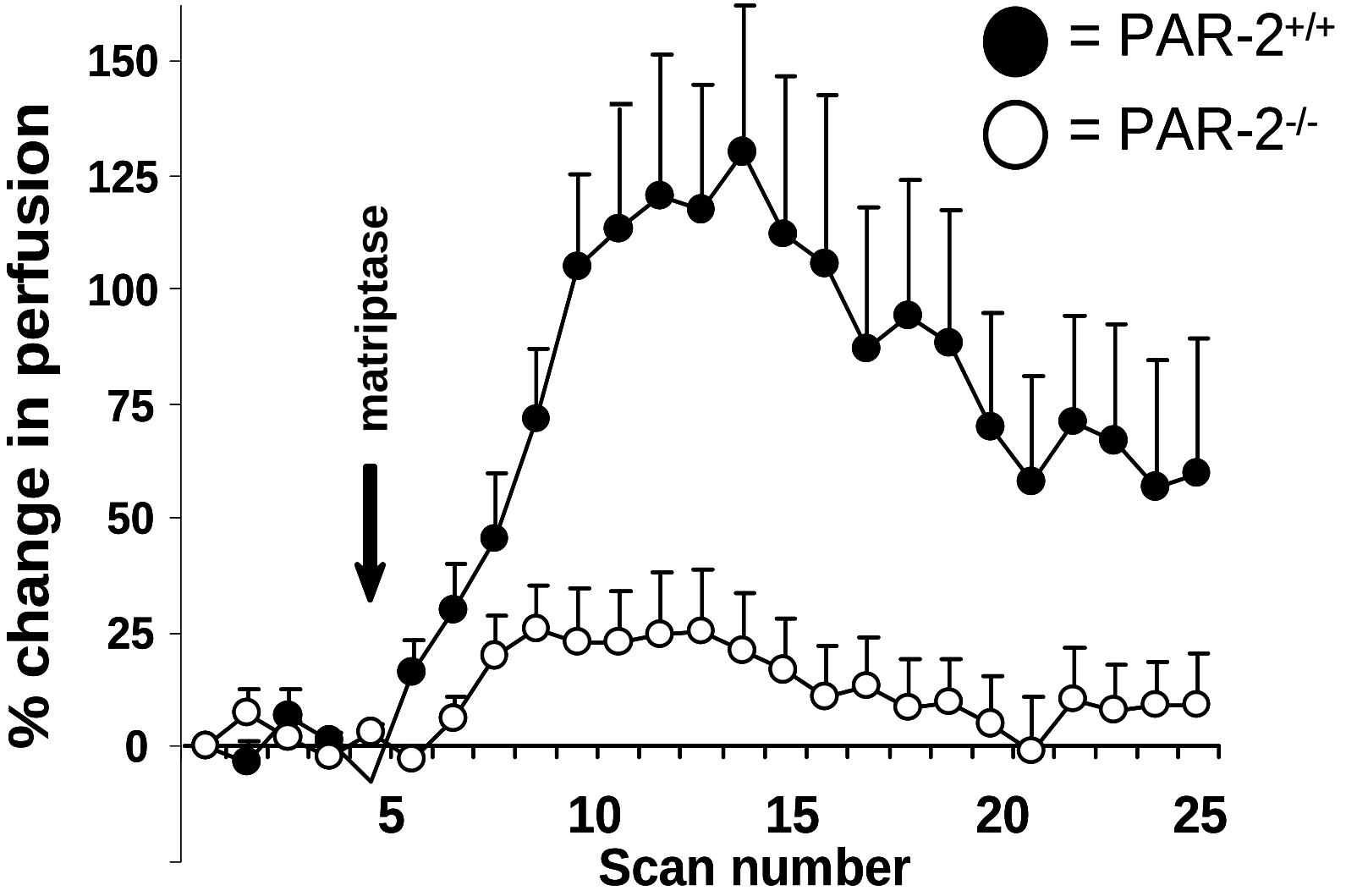
and

proMMP-1



Milner et al 2010 Arthritis Rheum

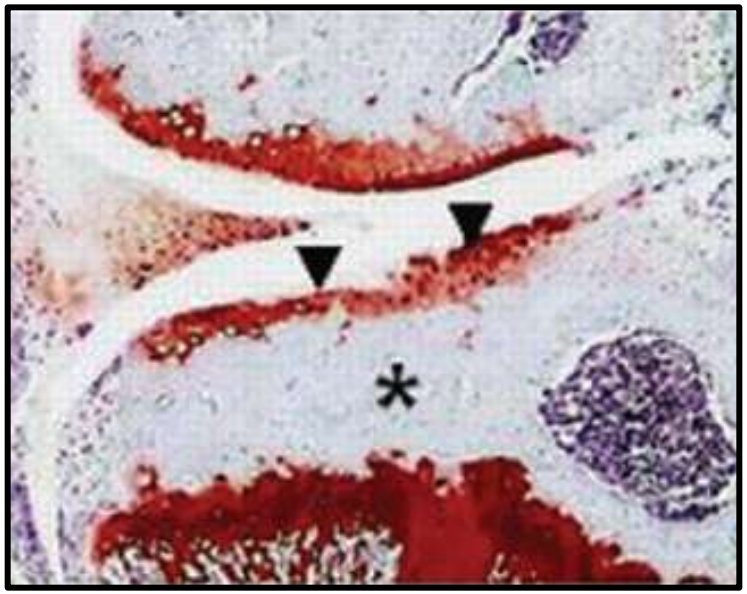
Matriptase activates PAR-2



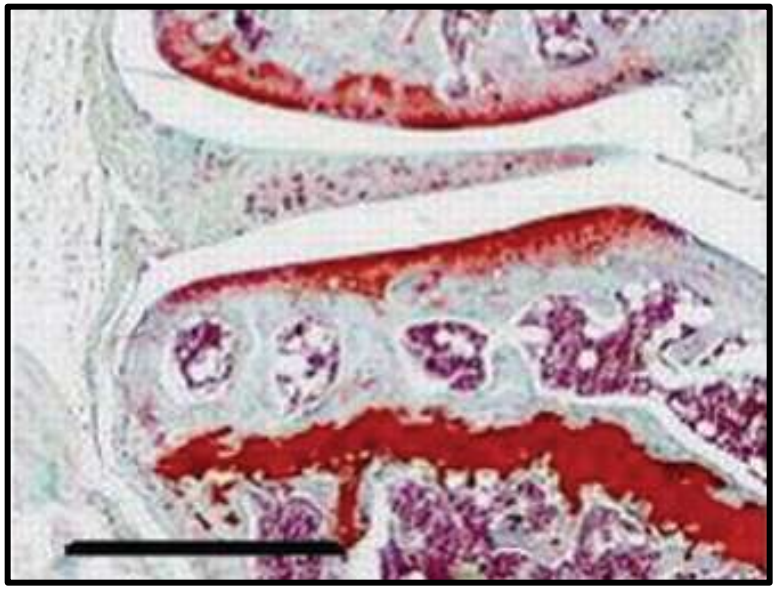
Milner et al 2010 Arthritis Rheum

PAR-2 absence prevents cartilage erosions in a murine model of OA

Wild-type



PAR-2 knockout



Ferrell W R et al. Ann Rheum Dis 2010;69:2051-2054

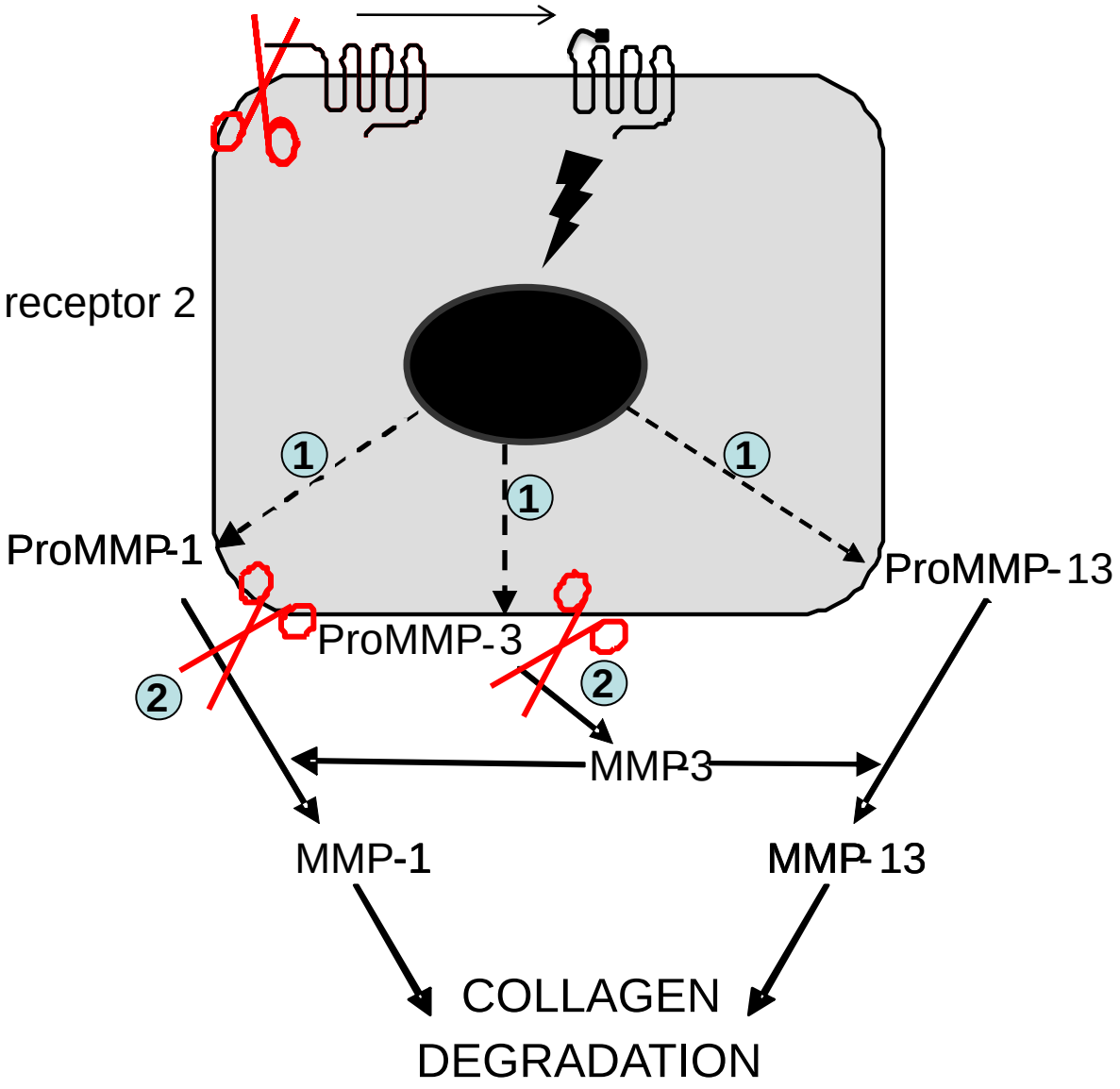
ARD

Mechanism = a ‘definable’ molecular pathway

= matriptase

= Proteinase activated receptor 2
PAR-2

= signalling



1 induction

2 activation

Milner et al 2010 Arthritis Rheum

Summary

Cartilage is a complex ECM

OA (and RA) are characterised by cartilage destruction

No Current treatments for OA

Metalloproteinases (MMPs) primarily mediate this destruction

Pro-inflammatory cytokines drive MMP expression

Chondrocytes have a limited “repair response”

Distinct molecular pathways contribute to disease

Need to identify the molecular mechanisms that drive cartilage destruction

The Key Questions

Can we cure arthritis?

Can we negate the need for joint replacements?

Can we manage arthritis better?

Any Questions??