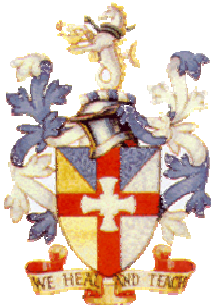
UNIVERSITY OF NEWCASTLE

CARTILAGE



Drew Rowan

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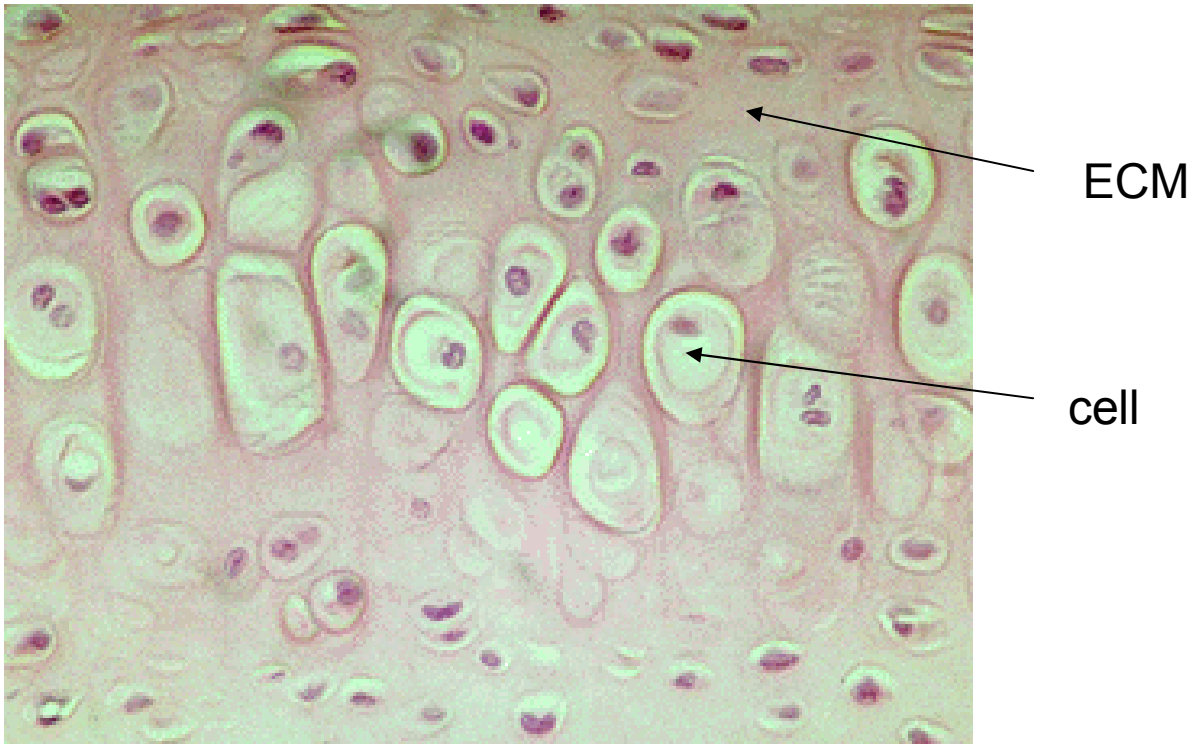
Musculoskeletal Research Group
Institute of Cellular Medicine
Newcastle University

Today's talk

Cartilage

- What it is and what it does
- What goes wrong in disease (RA and OA)
- Treatment options
-What we know about the process(es)
- What research offers

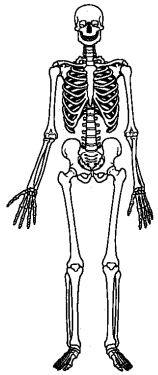
What is extracellular matrix (ECM)?



Complex network of proteins and carbohydrates surrounding cells

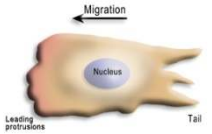
What do ECMs do?

Support and protection



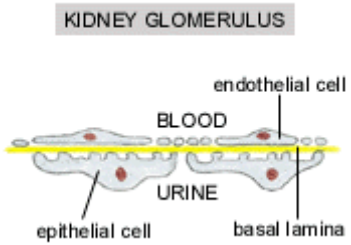
- Musculoskeletal strength
- Skin

Cell migration, polarity and shape



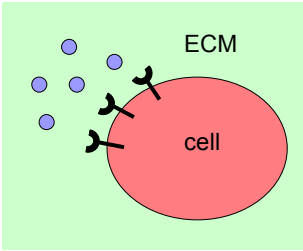
- Wound repair
- Embryo development
- Tumour development

Transport of nutrients and waste products



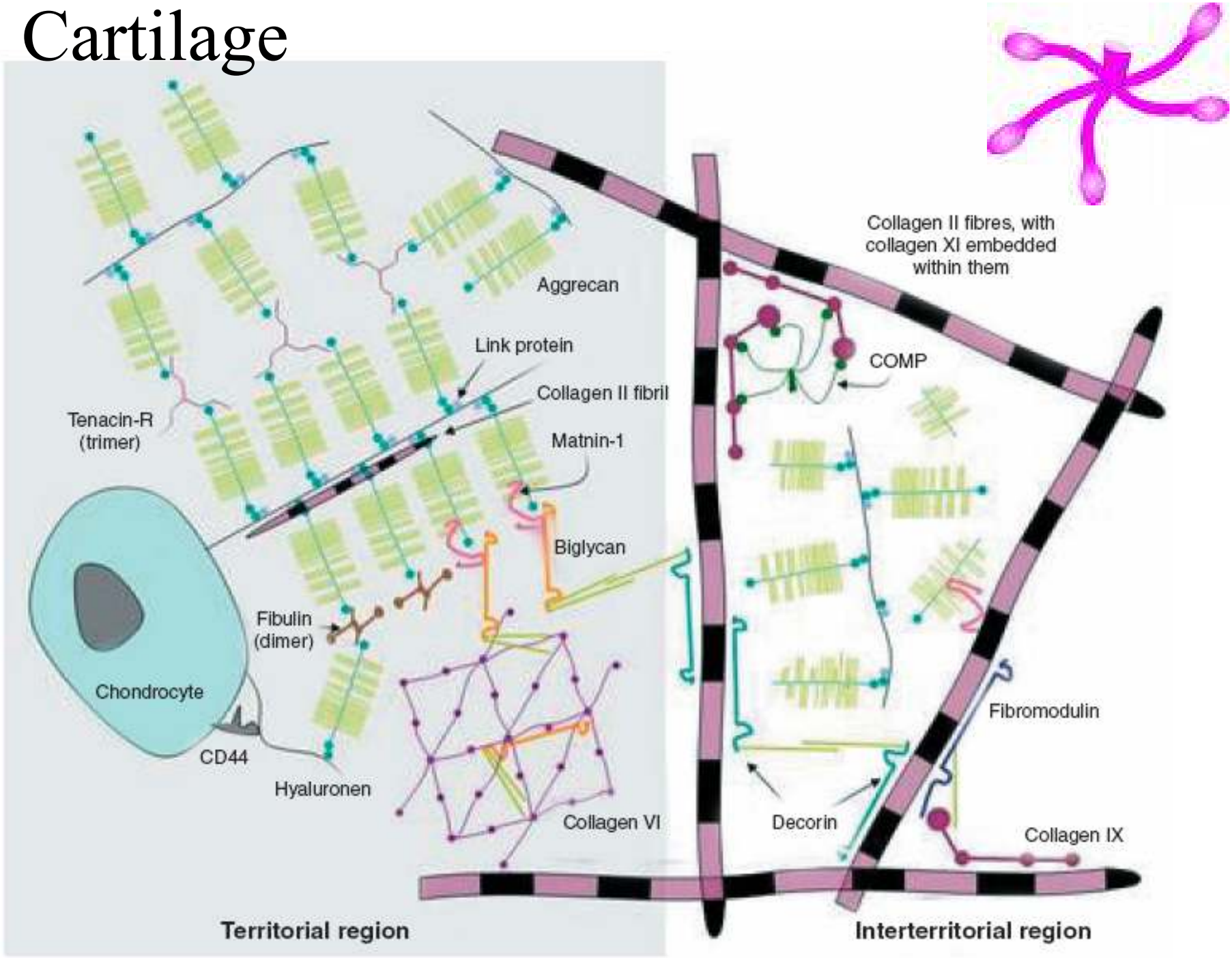
- Kidney
- Lungs
- Retina
- Pharmaceuticals

Intercellular communication

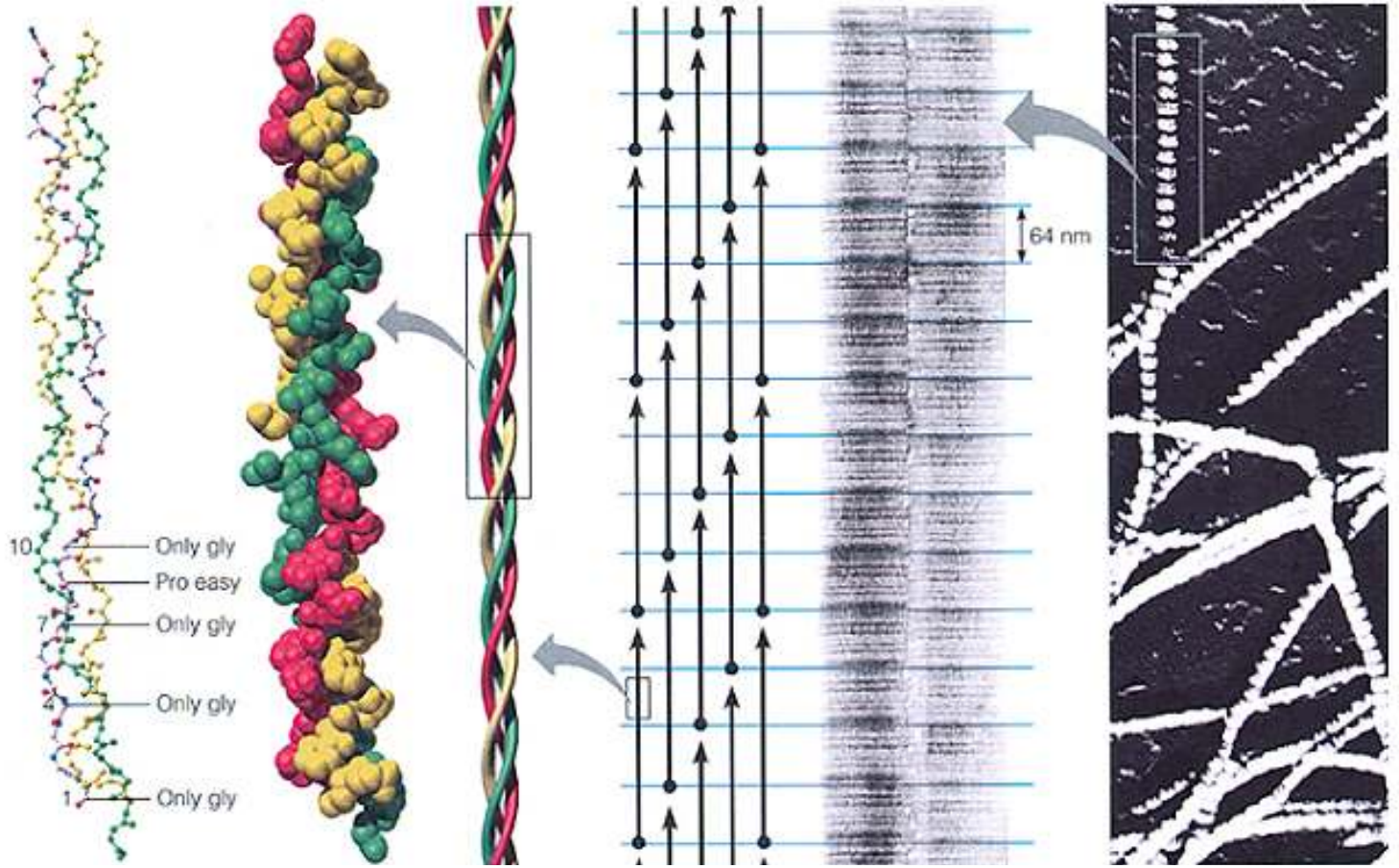


- Hormones
- Growth factors
- Cytokines

Cartilage


Aggrecan
Link protein
Collagen II fibril
Tenacin-R
(trimer)
Matnin-1
Biglycan
Fibulin
(dimer)
Chondrocyte
CD44
Hyaluronen
Collagen VI
Collagen II fibres, with
collagen XI embedded
within them
COMP
Fibromodulin
Decorin
Collagen IX
Territorial region
Interterritorial region

Collagen fibres

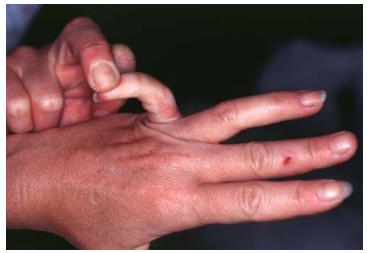


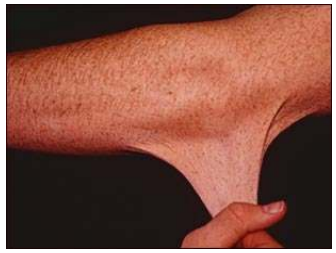
Collagen fibres provide resistance to stretching forces

Loss of collagen integrity leads to loss of strength of the cartilage matrix

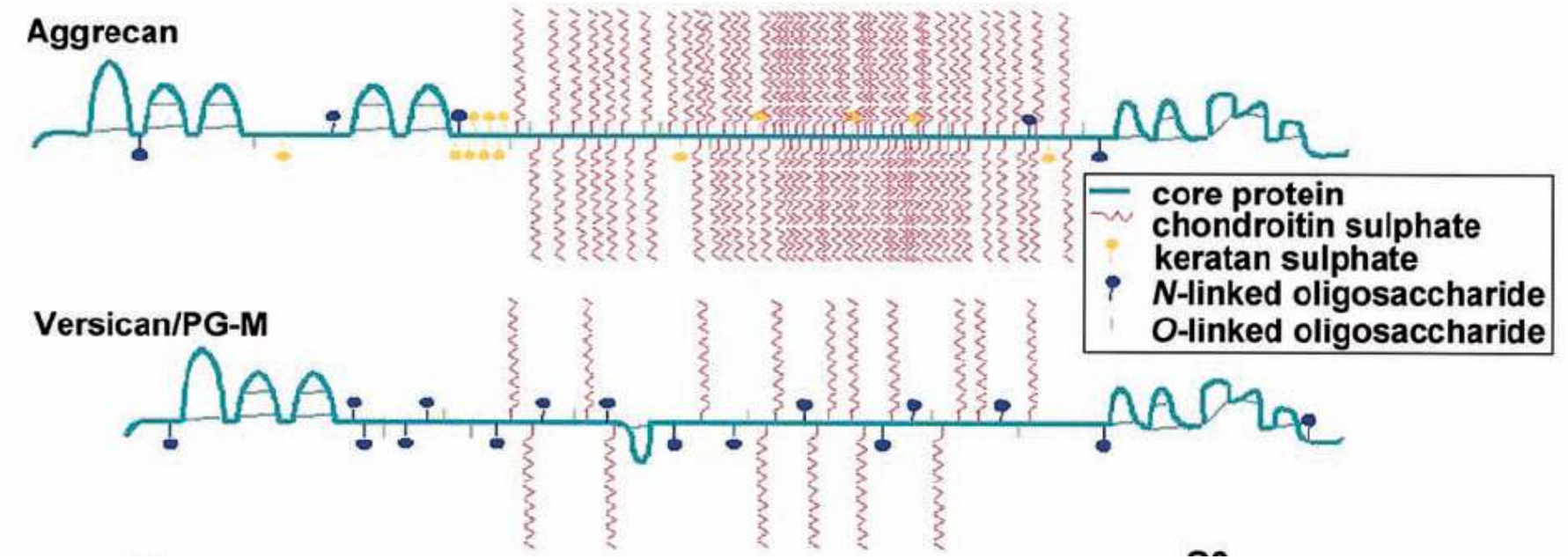
Diseases caused by mutations in collagen genes or collagen processing enzymes

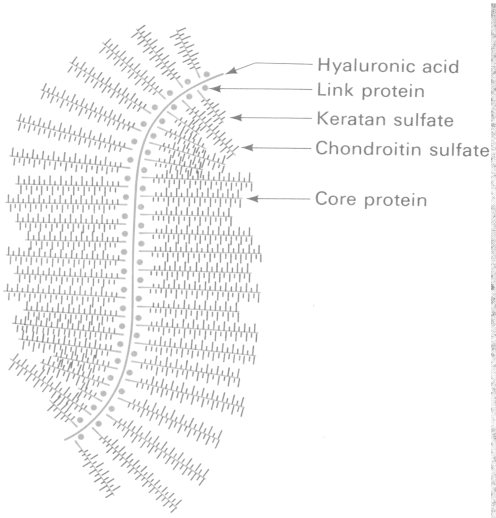
COL Gene	Disease
A1;COL1A2	OI, EDS types I, II, VIIA and VIIB, osteoporosis
2A1	**Several chondrodysplasias**
3A1	EDS type IV, arterial aneurysms
4A3; 4A4; 4A5	Alport syndrome
4A5; 4A6	Alport syndrome with diffuse oesophageal leiomyomatosis
5A1;5A2	Ehlers Danlos Syndrome (EDS) types I and II
6A1;6A2;6A3	Bethlem myopathy
7A1	EB, dystrophic forms
8A2	Some forms of corneal endothelial dystrophy
9A1;9A2;9A3	Multiple epiphyseal dysplasia, intervertebral disc disease, OA
10A1	Schmid metaphyseal dysplasia
11A1;11A2	Several mild chondrodysplasias §, non-syndromic hearing loss, OA
17A1	Generalized atrophic benign EB
18A1	Knobloch syndrome
Lysyl hydroxylase 1	EDS type VI
Procollagen N-proteinase	EDS type VIIC

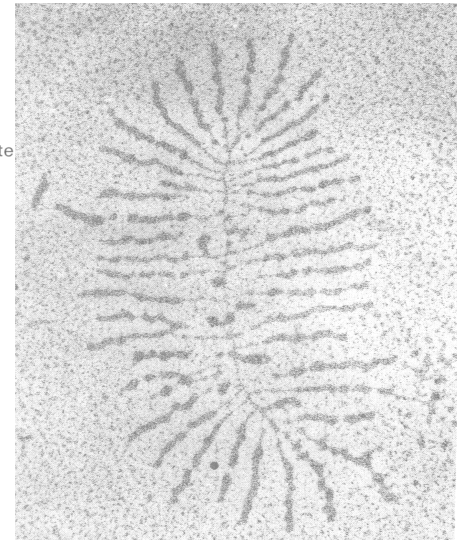




Proteoglycans



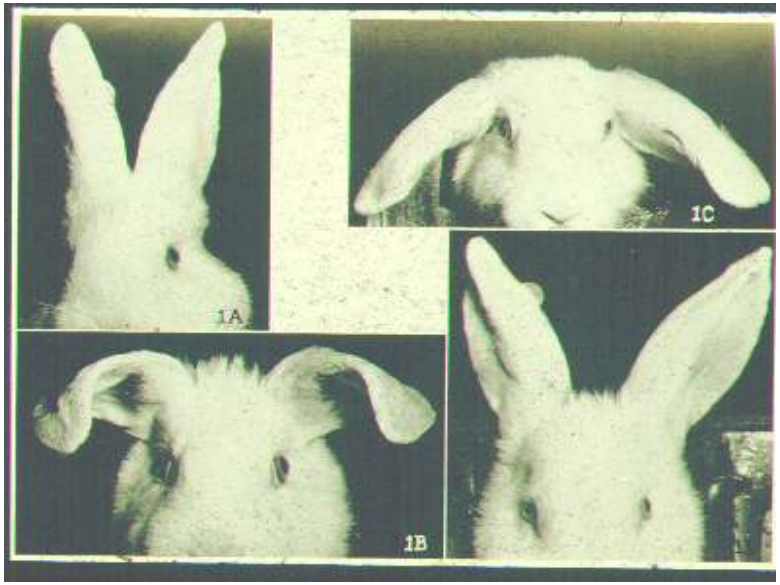




- Proteoglycans attract water, forming an hydrated gel
- results in a swelling pressure (turgor)

resistance to compression

strength and support

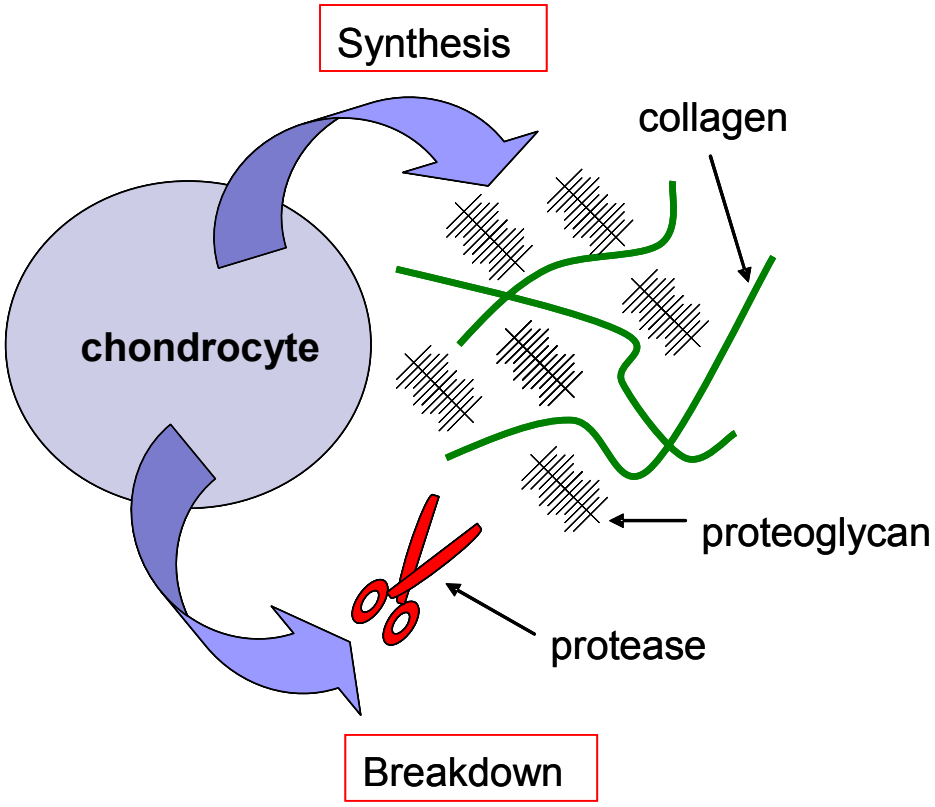


How is a healthy ECM maintained?

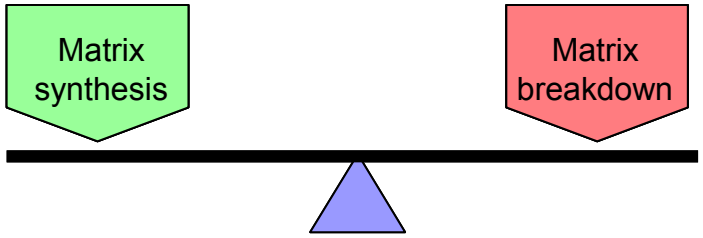
- Cells within matrix (or in contact with matrix) secrete the ECM molecules
- The same cells also secrete enzymes which are able to digest the matrix

- to remove damaged matrix

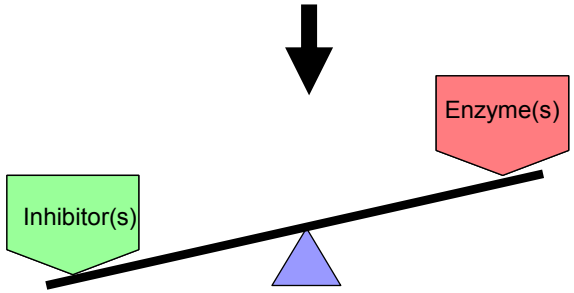
- to remodel matrix



Imbalance in **proteolytic threshold** leads to ECM destruction



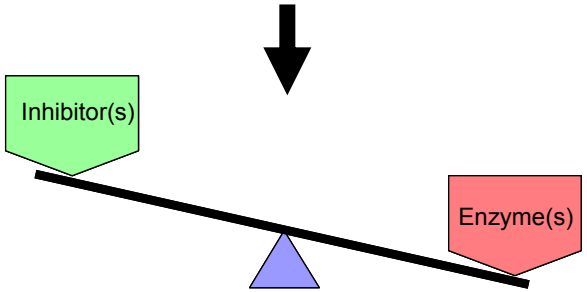
Excess proteinase(s)



Matrix destruction

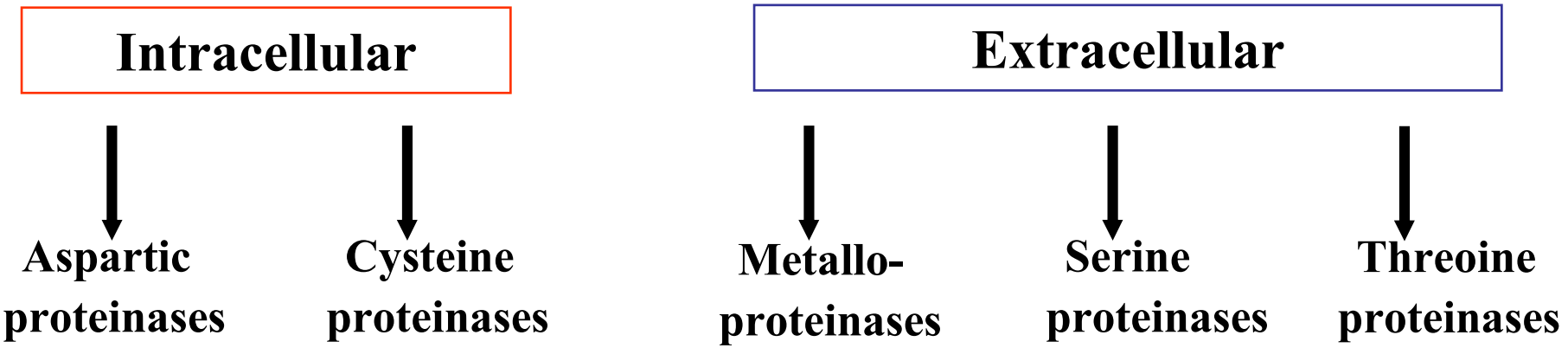
= DISEASE =

Insufficient proteinase(s)



Tissue fibrosis

Proteolysis

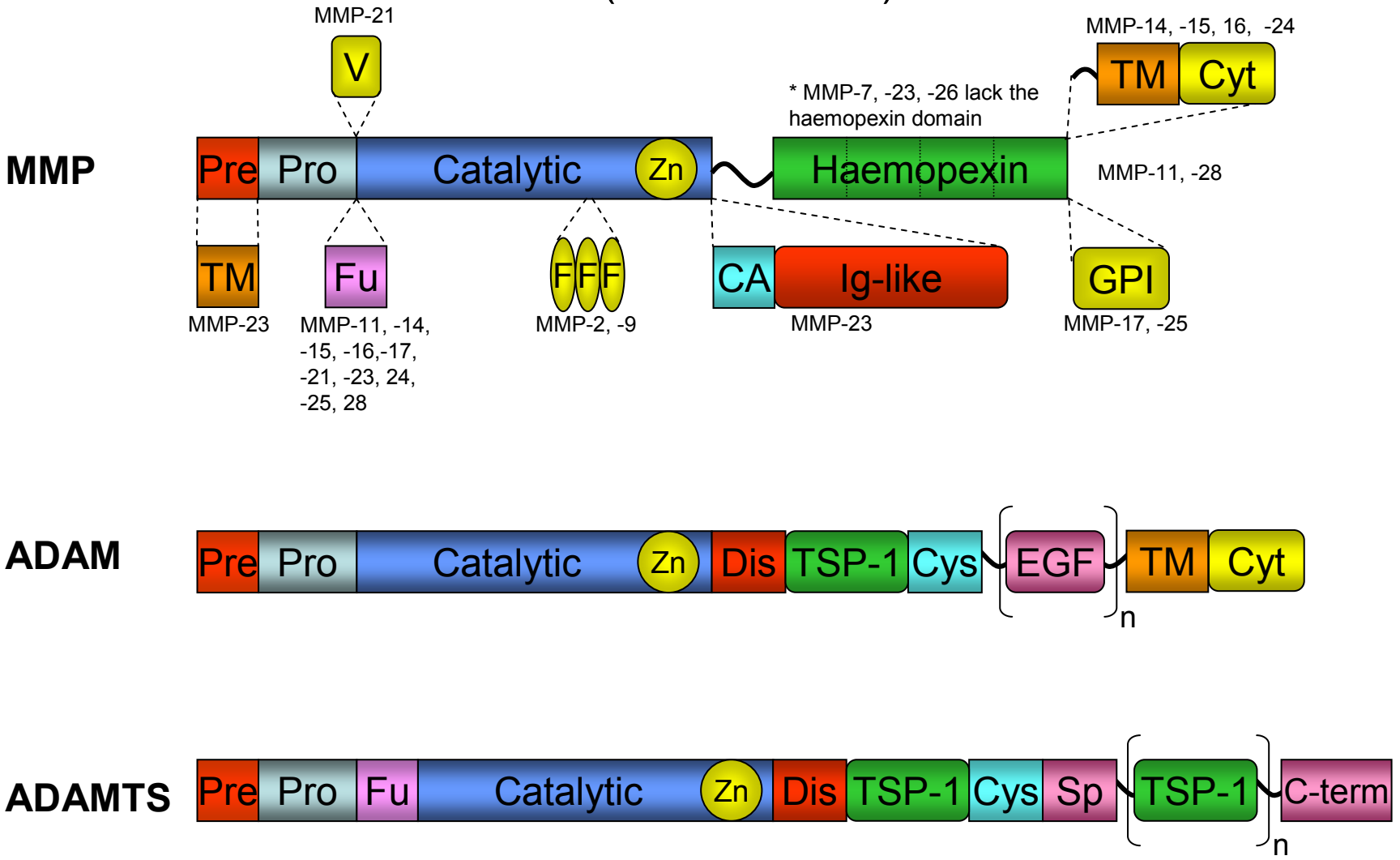


Low pH → Neutral pH

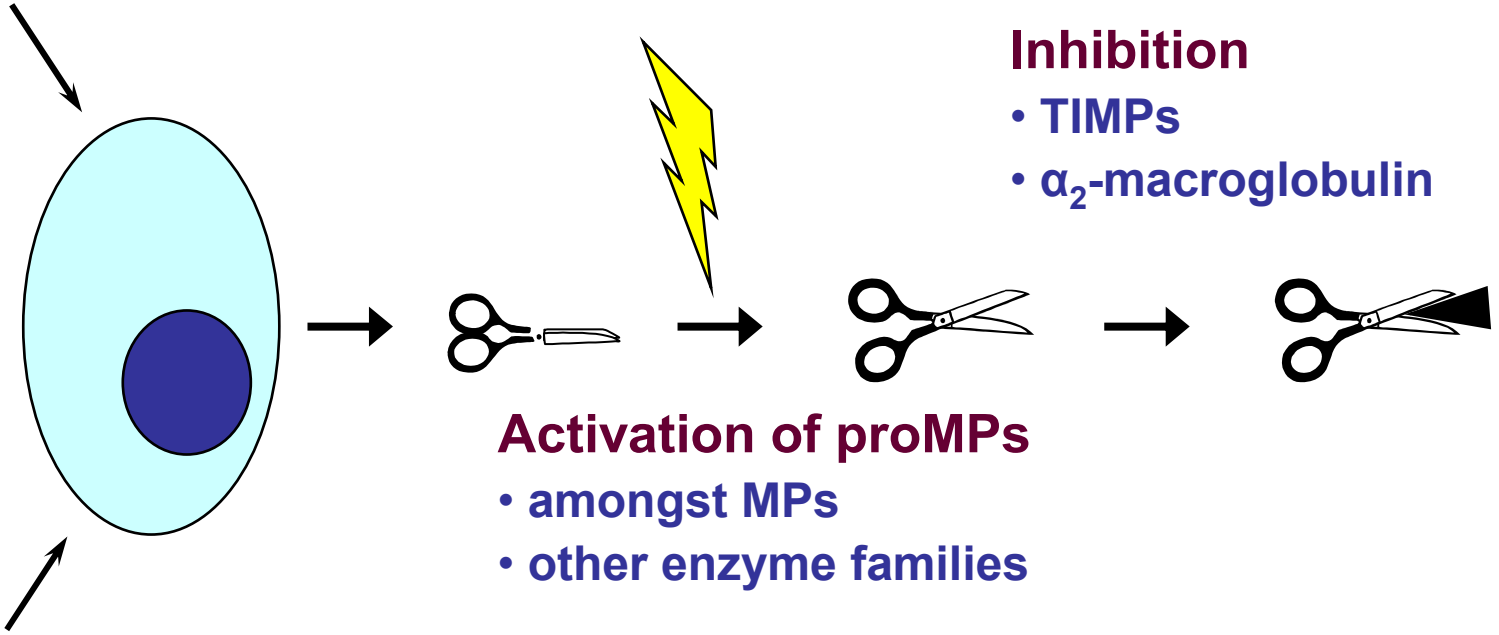
Cathepsin D	Cathepsin B	MMP-1, -8, -13	Elastase	Proteosome
	Cathepsin H	MMP-3, -10, -11	Cathepsin G	
	Cathepsin K	MMP-2, -9	Plasmin	
	Cathepsin L	MT1-6 MMP	PAs	
	Cathepsin S	ADAM/ADAMTS	Furin	

Metalloproteinases family

(~75 members)



Control of metalloproteinase activity



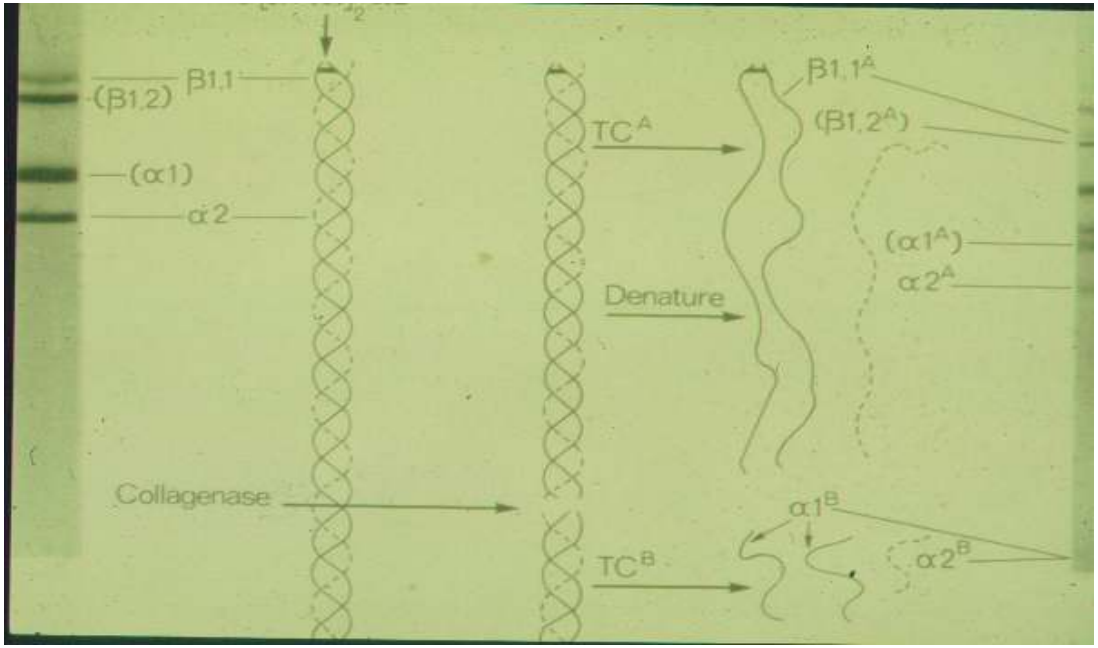
Inhibition
- TIMPs
- α_2-macroglobulin

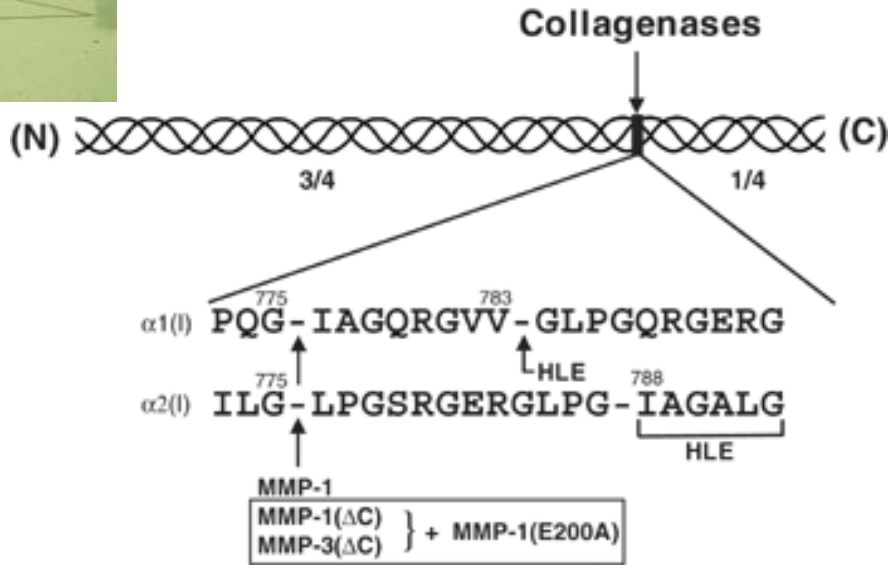
Activation of proMPs
- amongst MPs
- other enzyme families

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

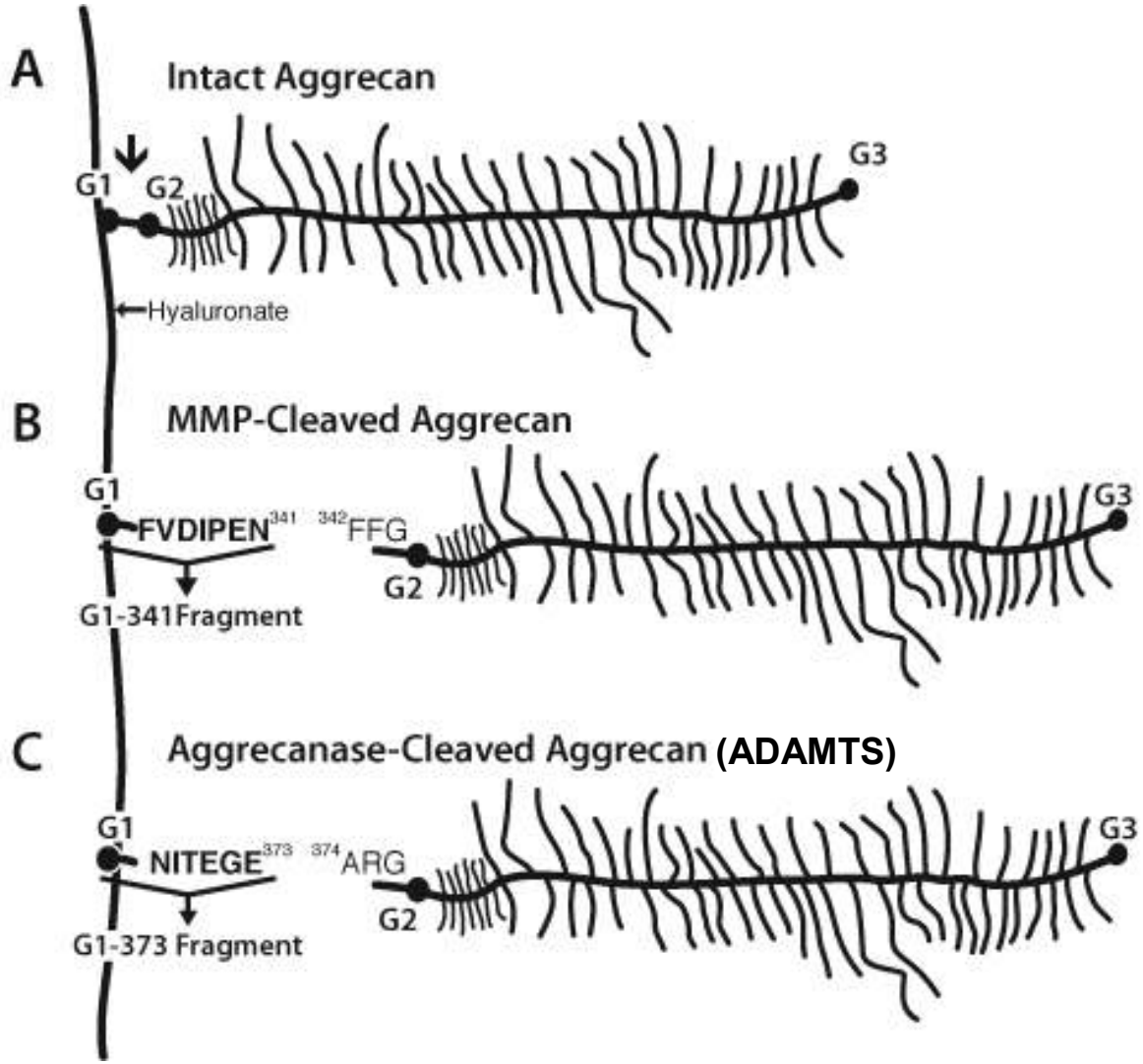
The first collagenase was discovered in 1962

Collagenases cleave collagen at a single and unique site



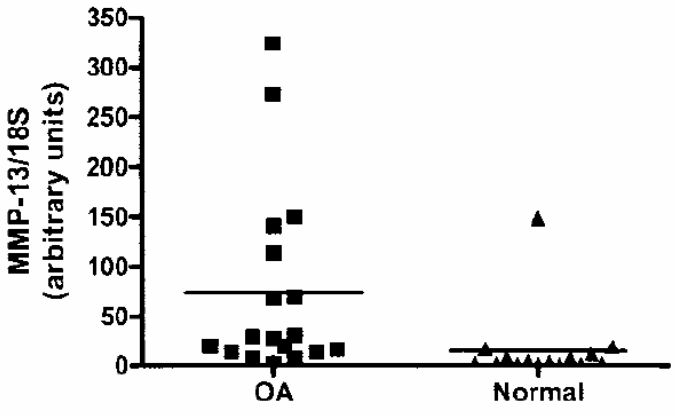


Aggrecan cleavage

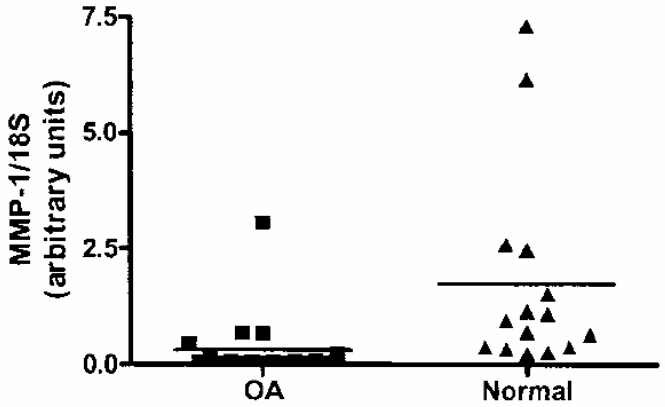


MMP expression changes

MMP-13



MMP-1



RA and OA are both characterized by proteolytic degradation of cartilage collagen

Jubb & Fell (1980)
J Pathol 130: 159-167

"The preservation of the collagen network is crucial for the survival of cartilage; there is evidence from organ culture experiments that when the collagen is lost, matrix is not regenerated whereas if the proteoglycan alone is removed, it is rapidly replaced."

Collagen loss is a key therapeutic target

Biologics as therapeutics (in RA)

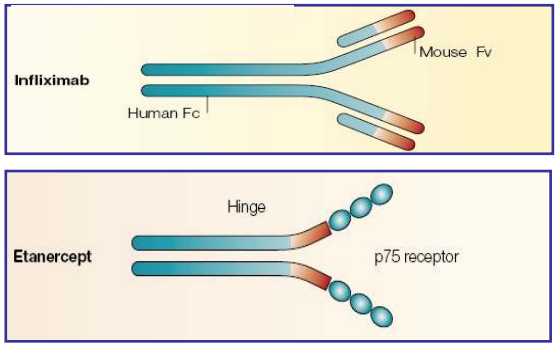
Anakinra = Anti-IL-1

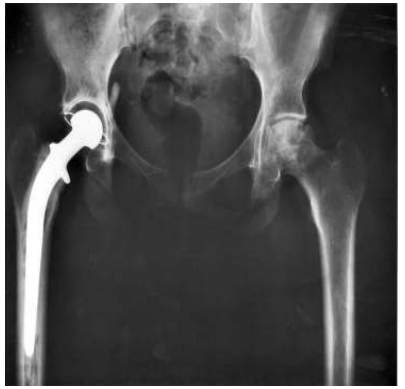
Atlizumab = Anti-IL-6

Etanercept = Anti-TNF

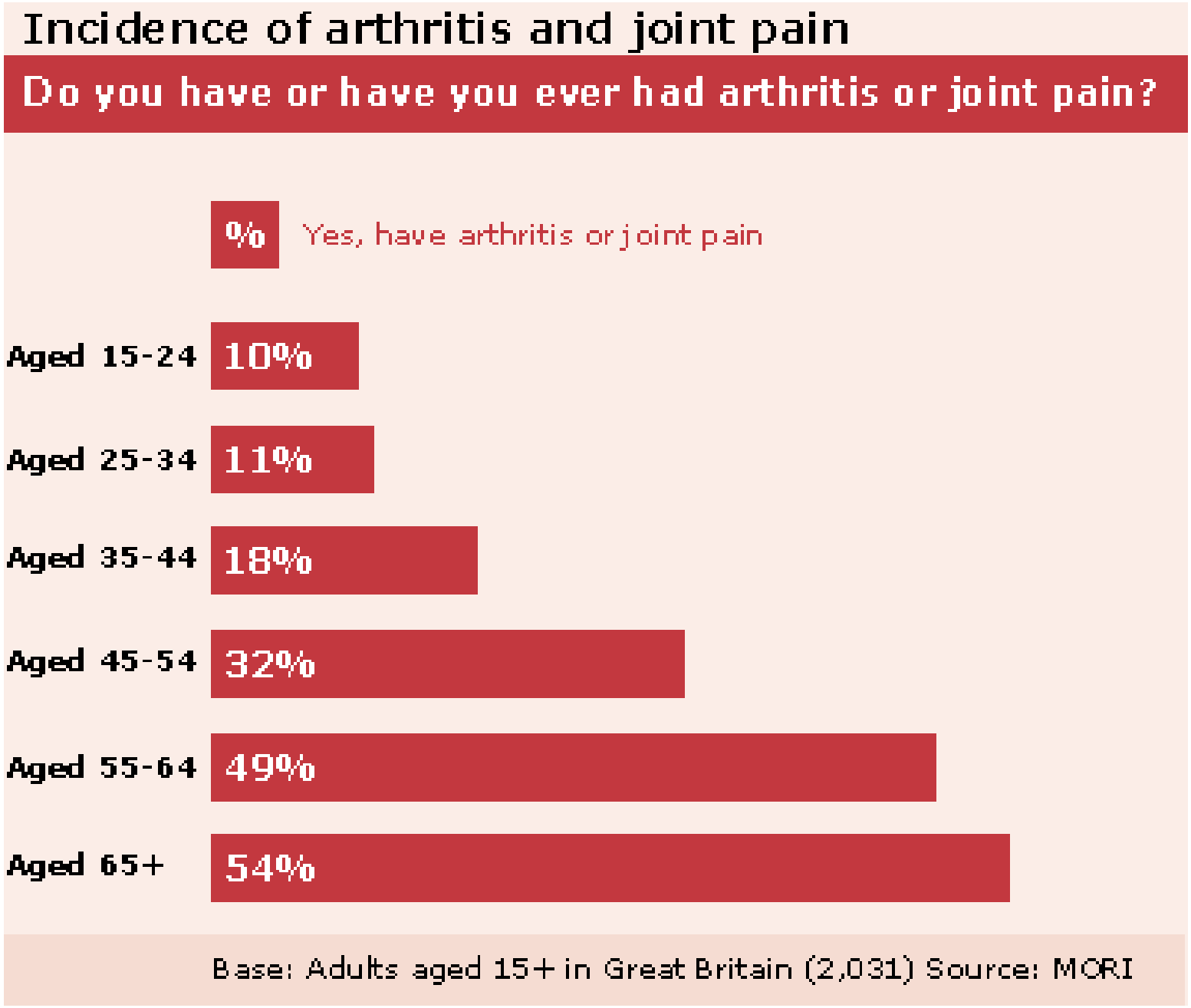
Infliximab = Anti-TNF

Retuximab = anti-CD20

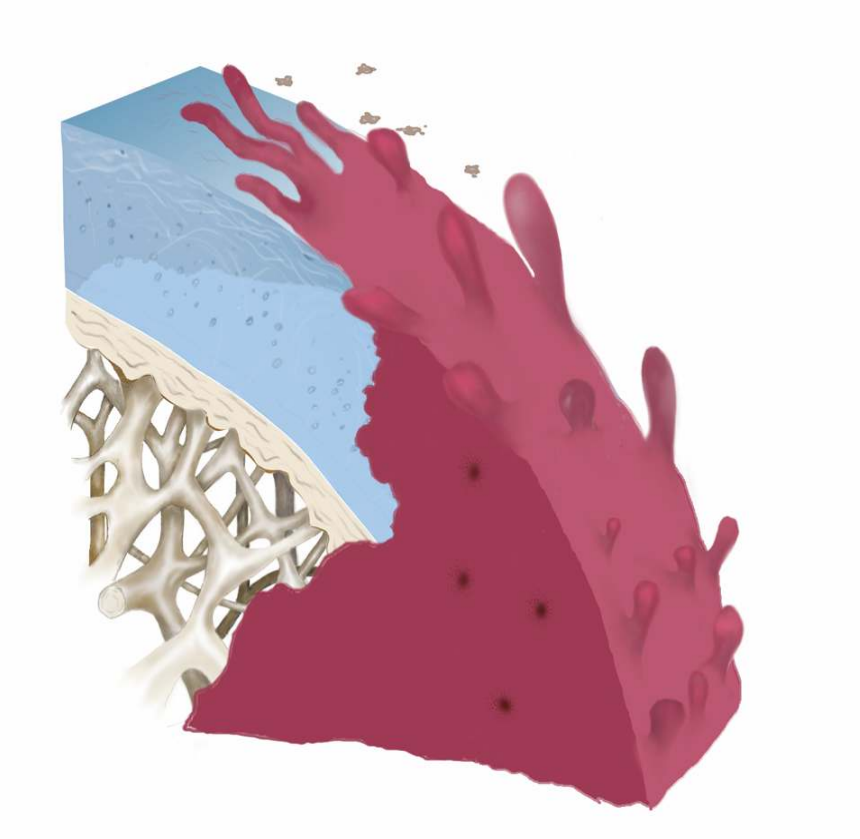




OA therapeutics

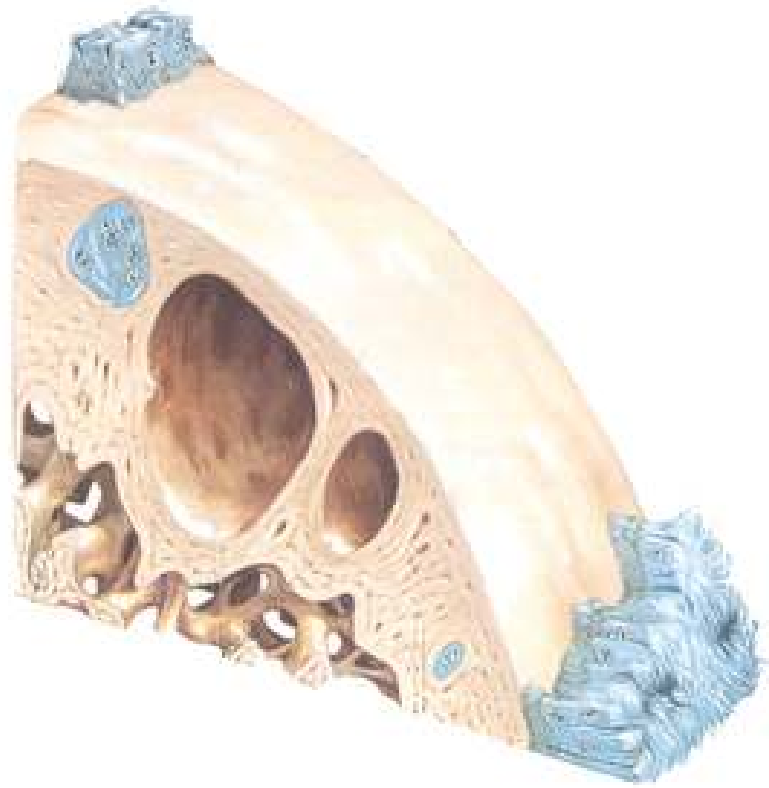

Incidence of arthritis and joint pain
Do you have or have you ever had arthritis or joint pain?
%
Yes, have arthritis or joint pain
Aged 15-24
10%
Aged 25-34
11%
Aged 35-44
18%
Aged 45-54
32%
Aged 55-64
49%
Aged 65+
54%
Base: Adults aged 15+ in Great Britain (2,031) Source: MORI

RA Progression



Often a rapid process

OA Progression



Typically a slow process

Disease mechanisms

- RA

  - autoimmune; genetic; inflammatory; synovium-driven

- OA

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

- Treatments

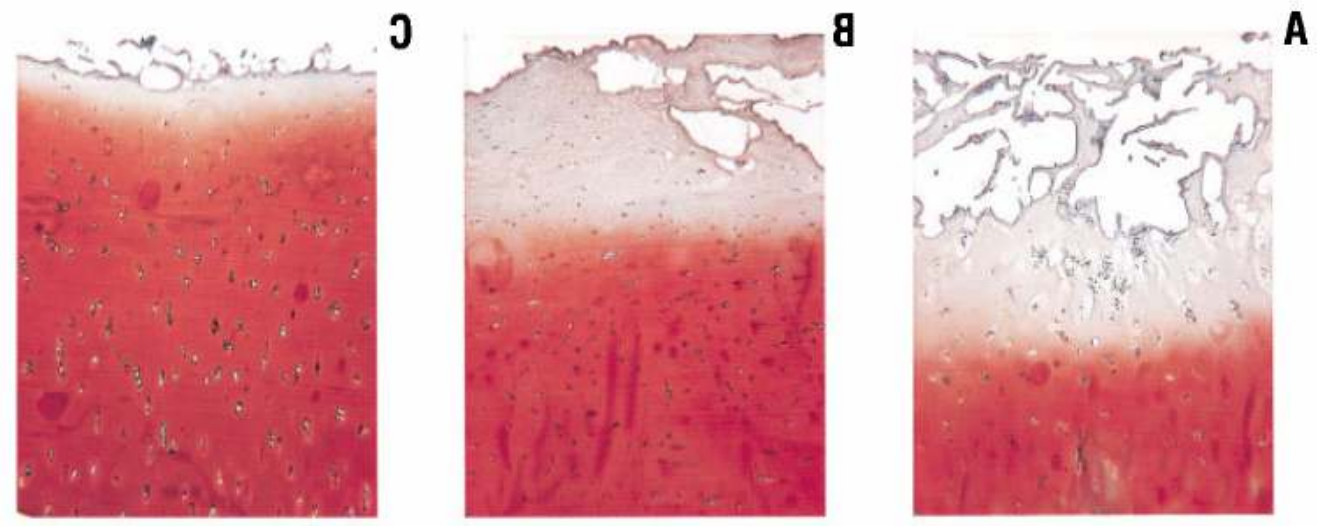
  - most modulate pain and inflammation, **NOT** destruction

Current anti-inflammatory treatments (eg. NSAIDs) effectively reduce synovial inflammation, but do not prevent joint destruction

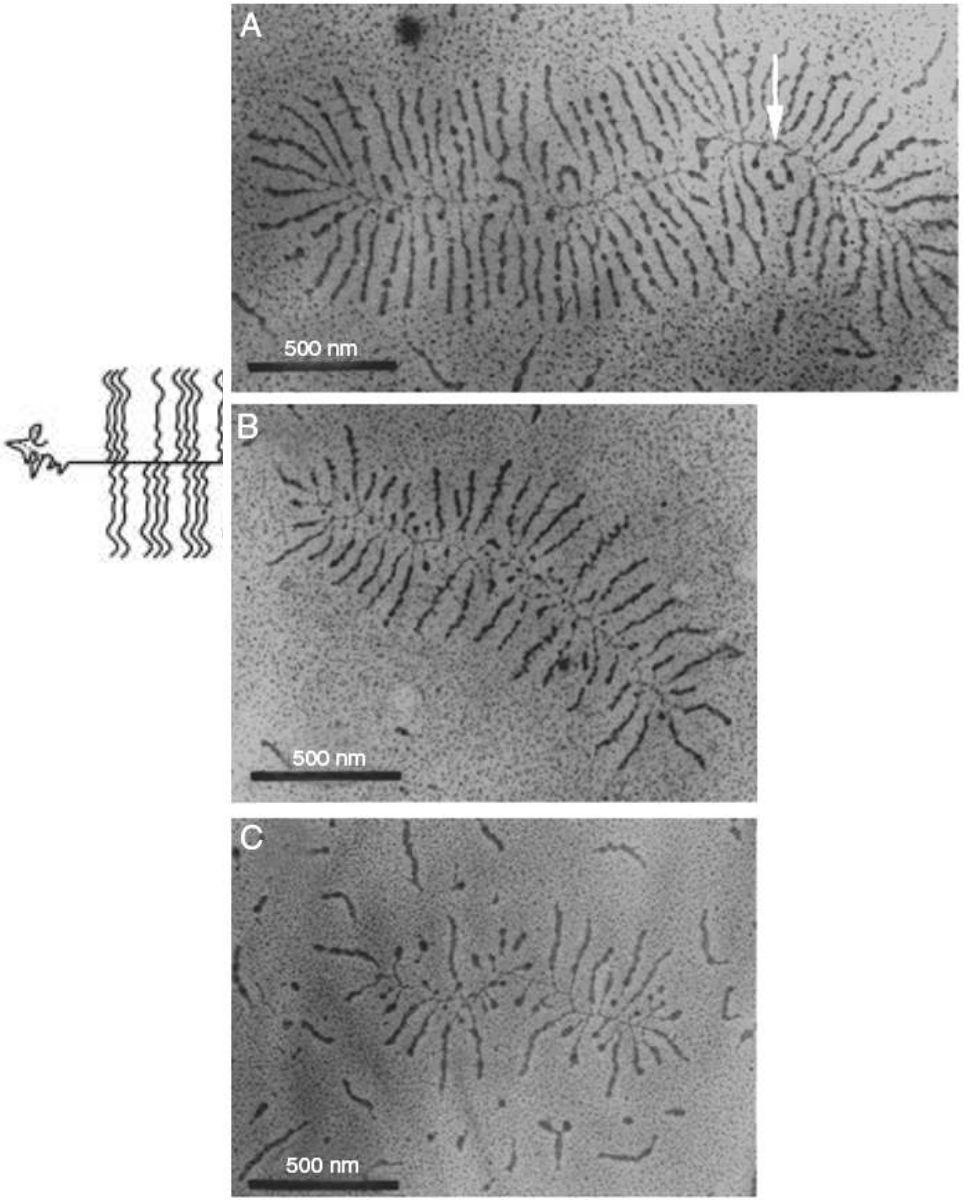
In RA, cartilage destruction clearly involves more than just the synovium

Example of a very ‘florid’ RA synovial sample typically obtained at time of synovectomy in the 1980s

Phenotypic changes in cartilage



Age-related changes to aggrecan



G3-domain & link protein cleavage

IGD & CS cleavage

Further G1, link & HA cleavage

Collagen – Advanced glycation endproducts

- Sugar molecules bond to proteins & DNA which overtime are modified into AGEs
- Some AGEs form covalent cross-links – stiffening tissues
- The ½ life of cartilage collagen >100yrs
- AGE levels in cartilage collagen increase >50-fold

AGE consequences for cartilage

- AGE increases in cartilage cause
  - > tissue stiffness
  - > brittleness (mechanical damage)
  - < cellular adhesion to ECM
  - < proliferation
  - Altered gene expression
  - < MMP-mediated collagen cleavage

- >AGE levels in cartilage result in more severe OA in an animal model (DeGroot et al A&R, 2004, 50, 1207-1215)

Age-related cartilage changes

Glycation endproducts
Senescence
Oxidative stress
Apoptosis
Protein mis-folding
Epigenetics

??? A little of everything ???

What do we know about the factors that promote cartilage destruction?

Inflammation in arthritis is perpetuated by cytokines

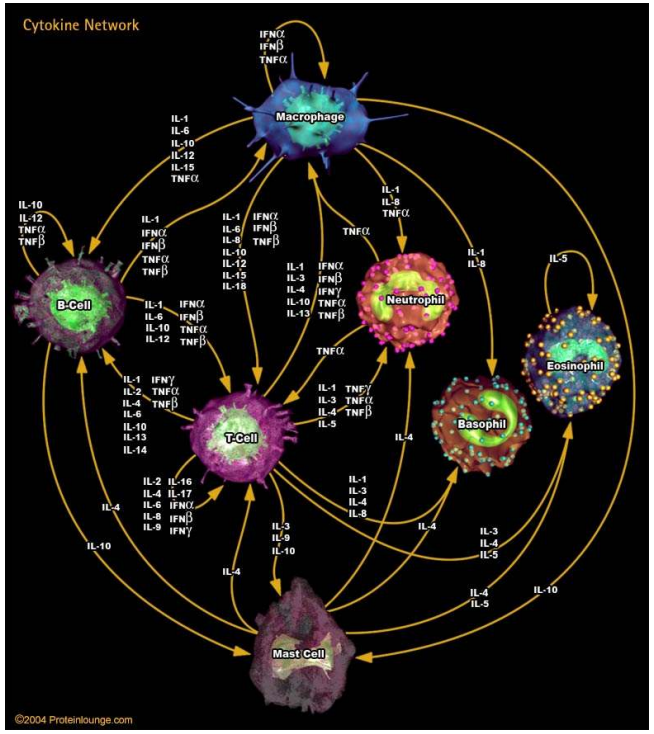


TABLE 77.3 CELLULAR SOURCES OF SYNOVIAL CYTOKINES IN RA

Cytokines	Detected in RA synovium/synovial fluid
Products of T cells	
IL-2	–
IL-3	–
IL-4	–
IL-6	±
IL-17	±
IFN-γ	–
TNF-α	–
TNF-β	–
GM-CSF	–
Products of macrophages/ type A synoviocytes	
IL-1	+++
IL-6	+
IL-8	+++
TNF-α	++
M-CSF (CSF-1)	+
GM-CSF	+
TGF-β	++
MCP-1	++
Products of fibroblasts/ type B synoviocytes	
IL-6	+++
GM-CSF	+
MCP-1	+

Comparision of cytokines produced by T cells and macrophages/fibroblasts in the RA synovium. A dash in the right-hand column indicates negligible or no detection. MCP-1, monocyte chemoattractant protein-1

© www.rheumtext.com - Hochberg et al (eds)

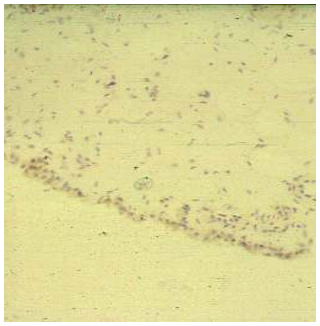
Key cytokines in arthritis:

Tumour necrosis factor α (TNFα)
Interleukin-1 (IL-1)

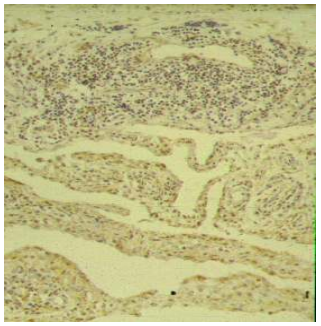
Oncostatin M (OSM) in arthritis

- interleukin-6-type cytokine
- family share common surface receptor (gp130)
- produced by T-cells and monocytic cells
- increases acute-phase response in the liver
- induces TIMP-1 production in chondrocytes
- was thought to be anti-inflammatory

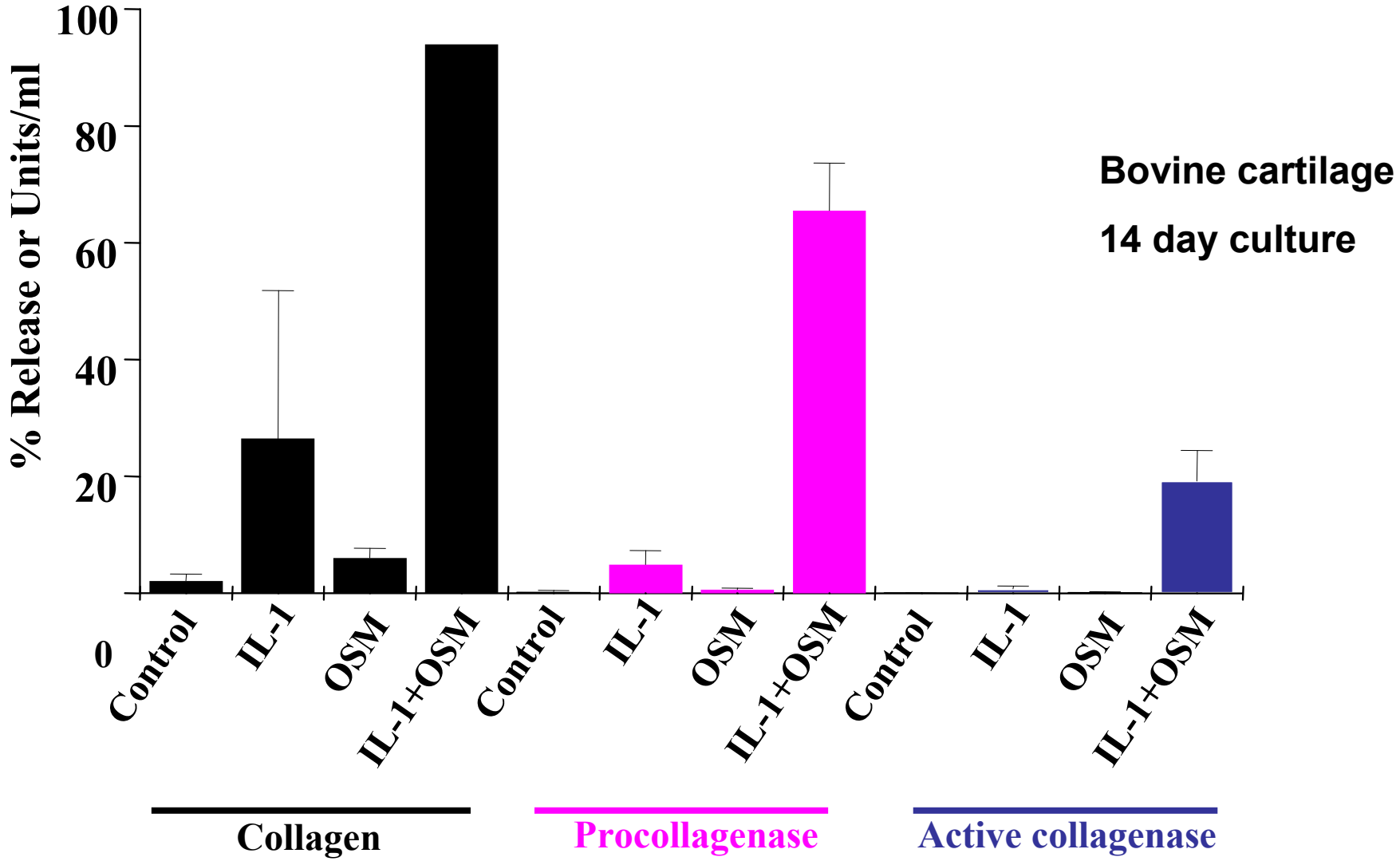
normal



rheumatoid

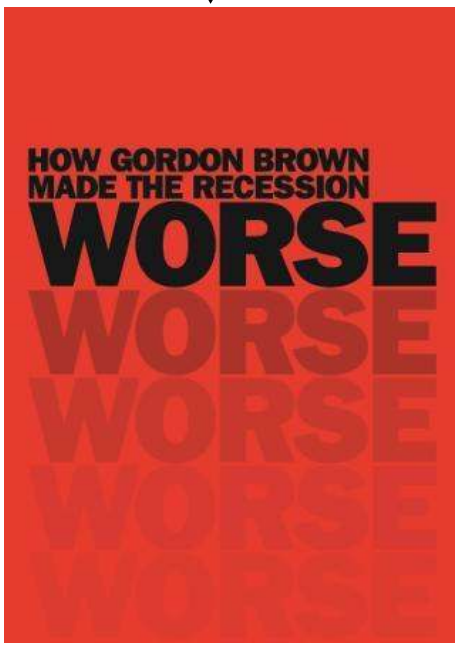


IL-1+OSM-induced cartilage collagenolysis



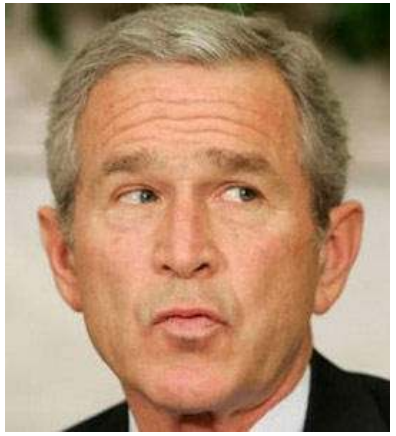
Hierarchy

eg. TNFα



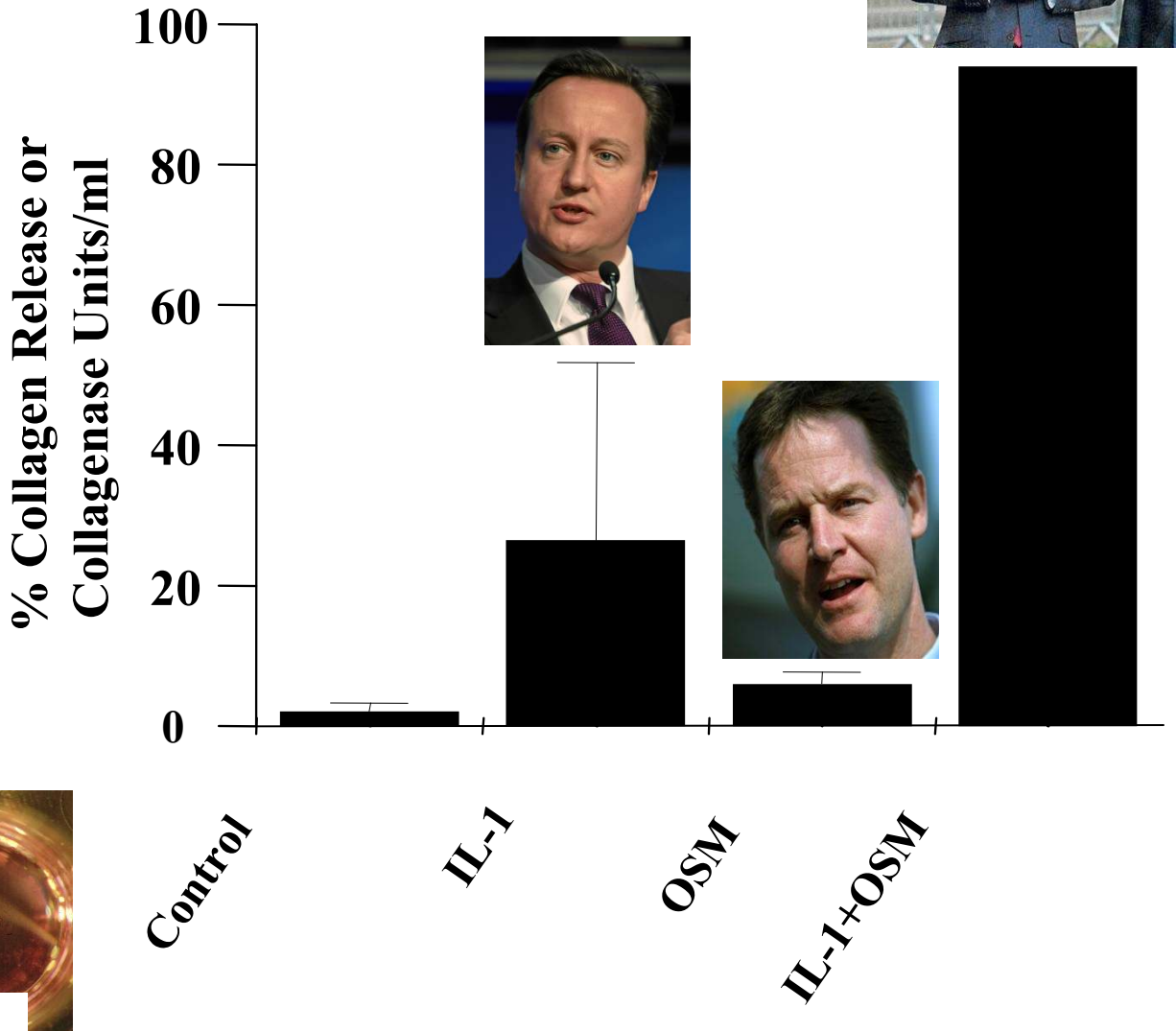
Co-operation

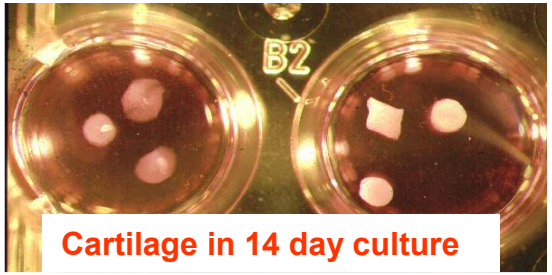
eg. IL-1



eg. OSM

CYTOKINE SYNERGY – a little can have a big impact!



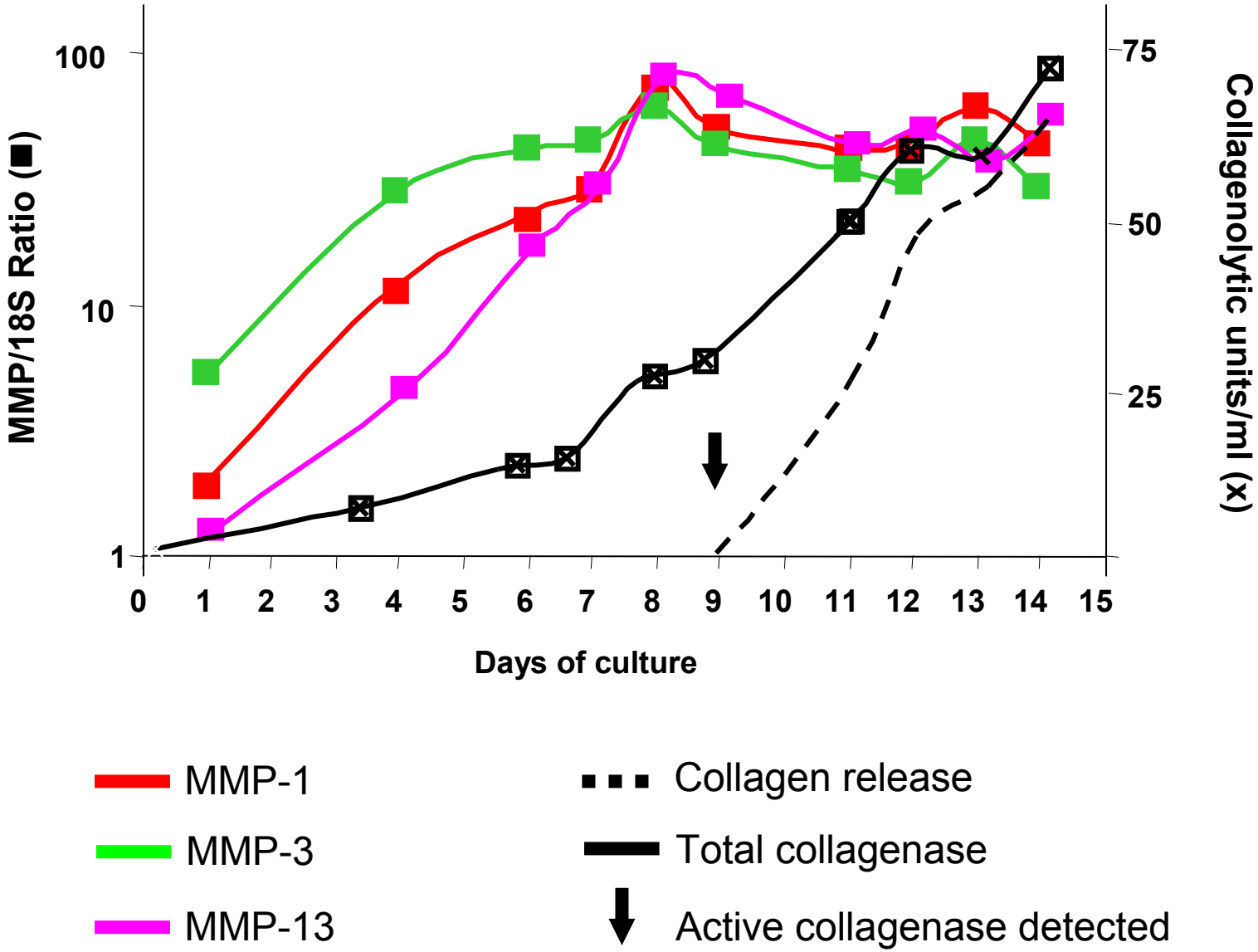


Cytokine combinations that work and are present in inflammatory arthritis

- **IL-1 (Cawston *et al*., 1995, 1998)**
- **IL-17 (Koshy *et al*., 2002a)**
- **TNFα (Hui *et al*., 2003a,b)**
- **IL-6 (+sIL-6R) also identified (Rowan *et al*., 2001)**

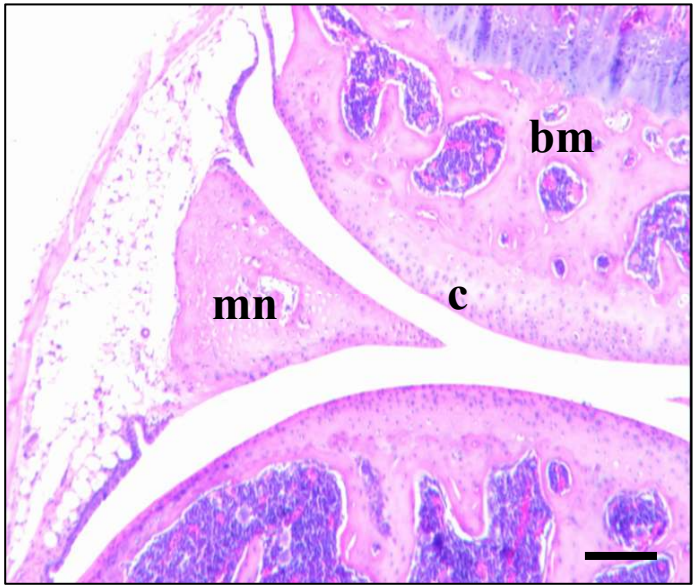
We now use IL-1+OSM as a potent, model stimulus of cartilage breakdown and MMP expression within the context of inflammatory arthritis

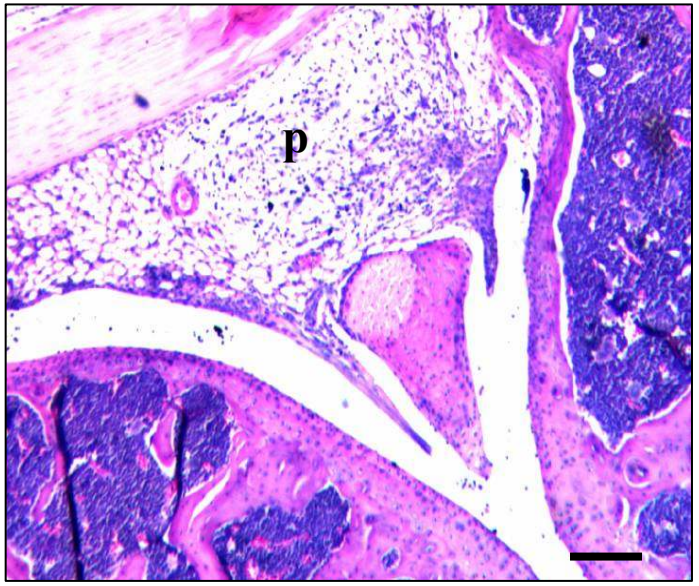
MMP expression profiling in resorbing cartilage



Adenoviral delivery of IL-1+OSM in murine joints

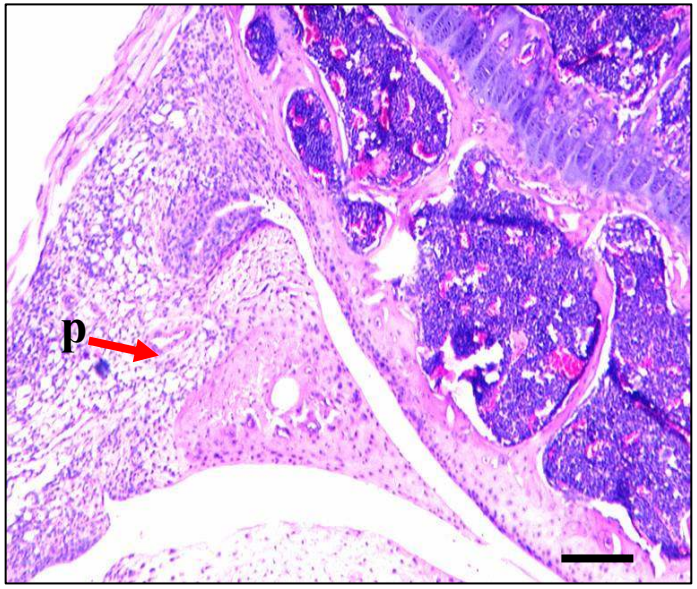
Control

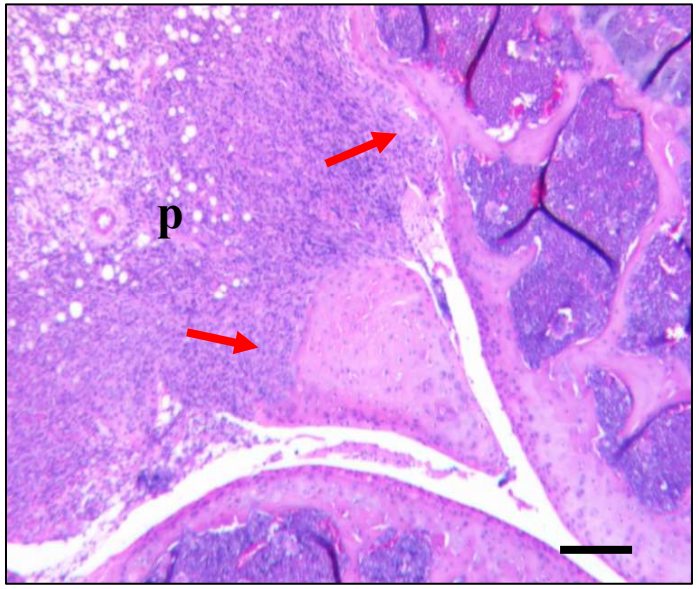




IL-1

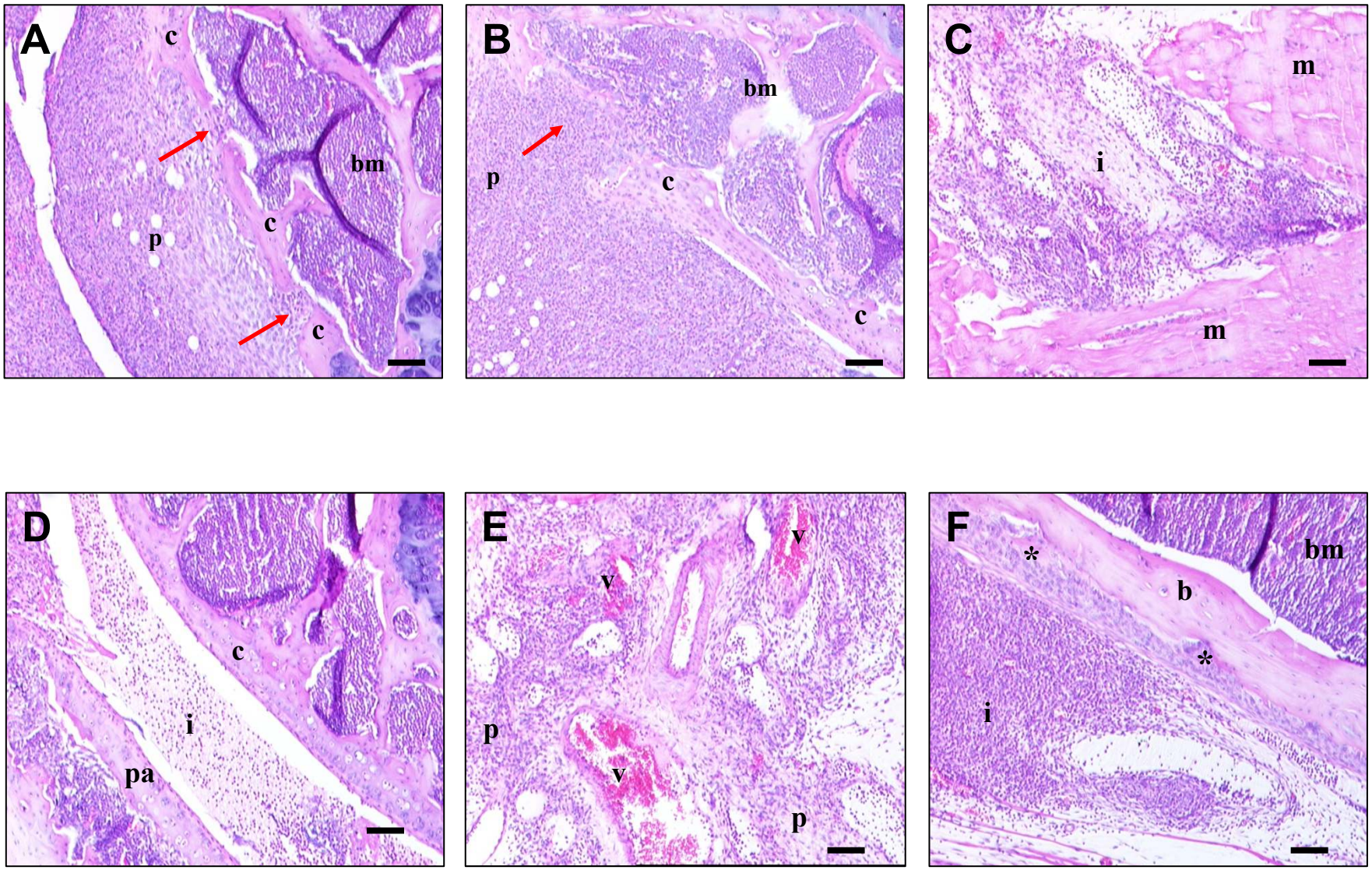
OSM

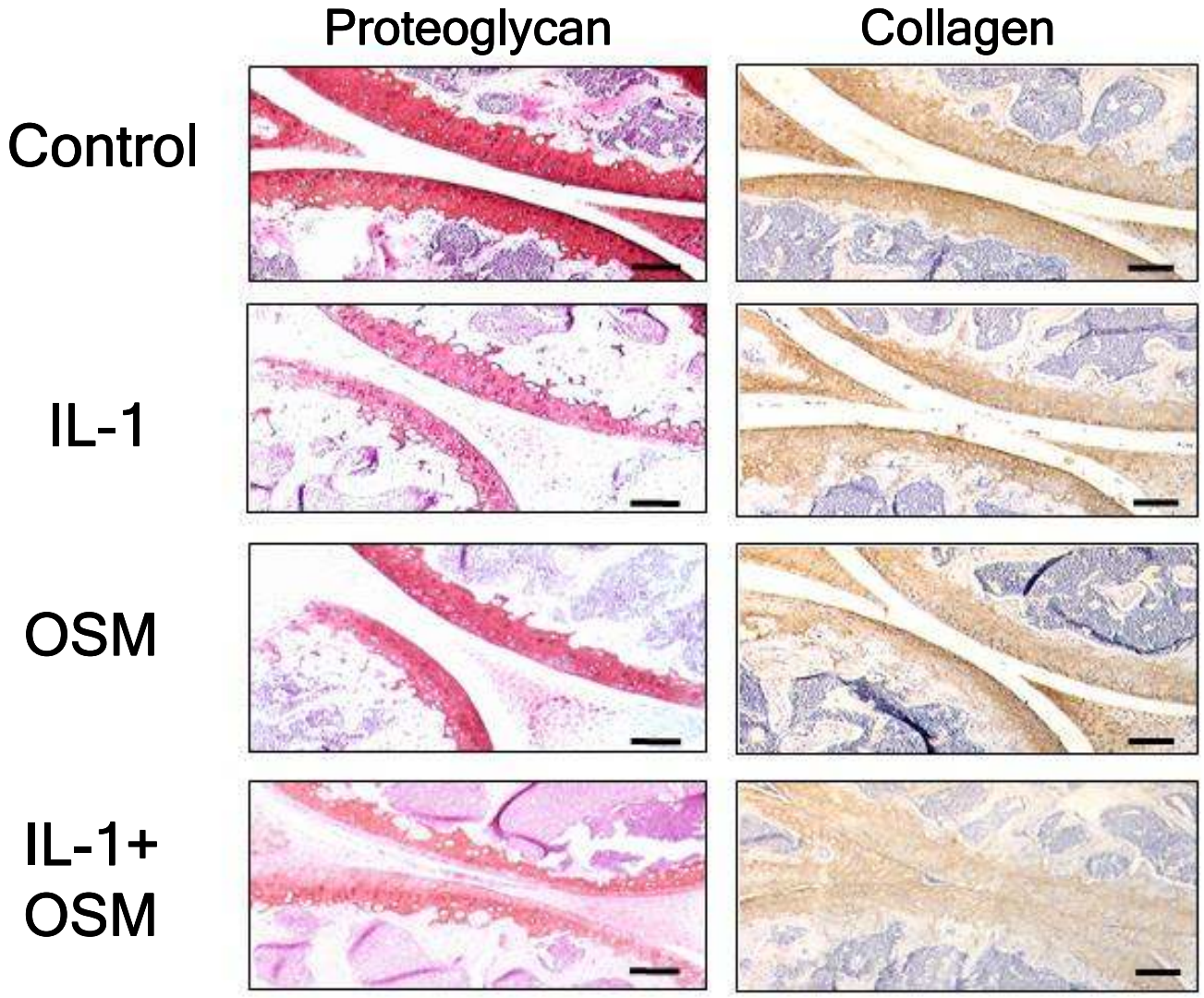




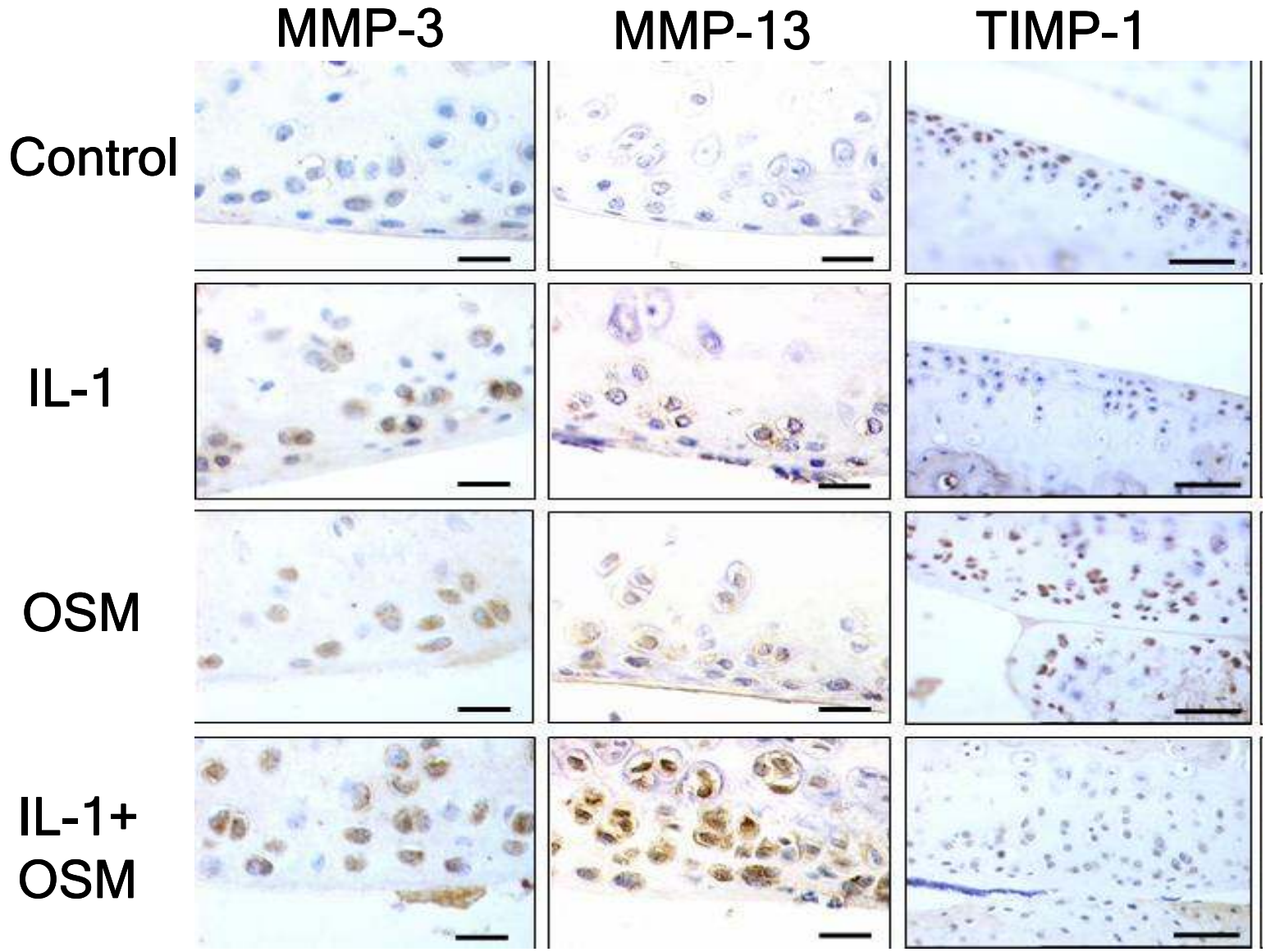
IL-1+
OSM

Adenoviral delivery of IL-1+OSM in murine joints




Proteoglycan
Collagen
Control
IL-1
OSM
IL-1+
OSM

Rowan *et al.*, 2003



Gene profiling – "transcriptomics"

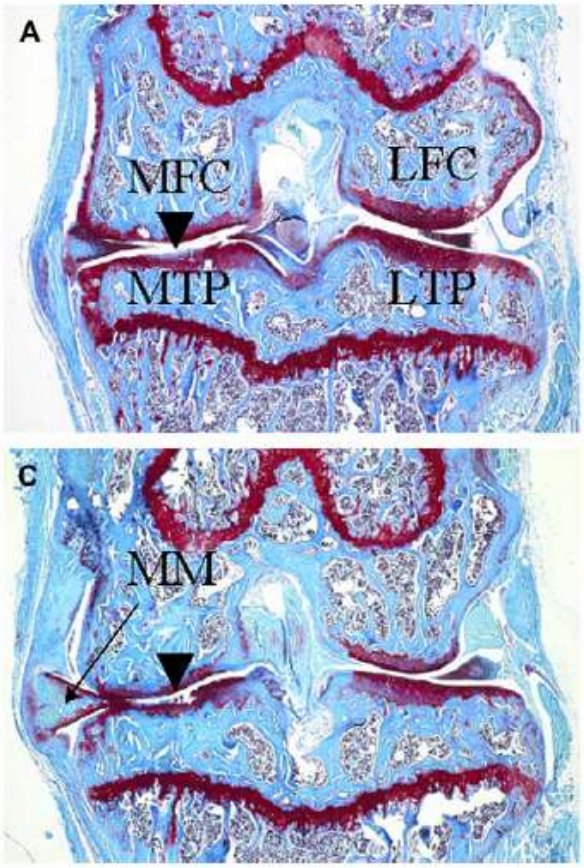
	Signal				Signal log ratio vs. control			Fold change vs. control		
Gene	**Control**	**IL-1**	**OSM**	**IL-1+ OSM**	**IL-1**	**OSM**	**IL-1+ OSM**	**IL-1**	**OSM**	**IL-1+ OSM**
Proteases and inhibitors										
MMP-1	22A	1875P	28P	4217P	6.1	0.3	7.3	67.6	1.3	157.6*
MMP-3	376P	5862P	318P	8910P	3.4	0.2	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-3-1ike-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

When studying OA, what is an appropriate control tissue????

Cartilage obtained from OA patients undergoing joint replacement is END-STAGE disease

Animal model of OA

Surgical destabilisation of the medial meniscus (DMM)



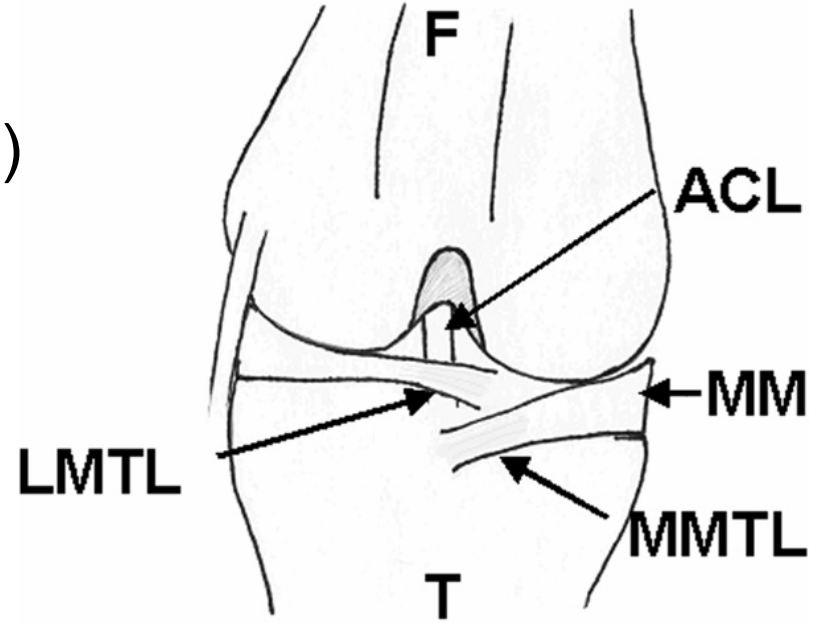
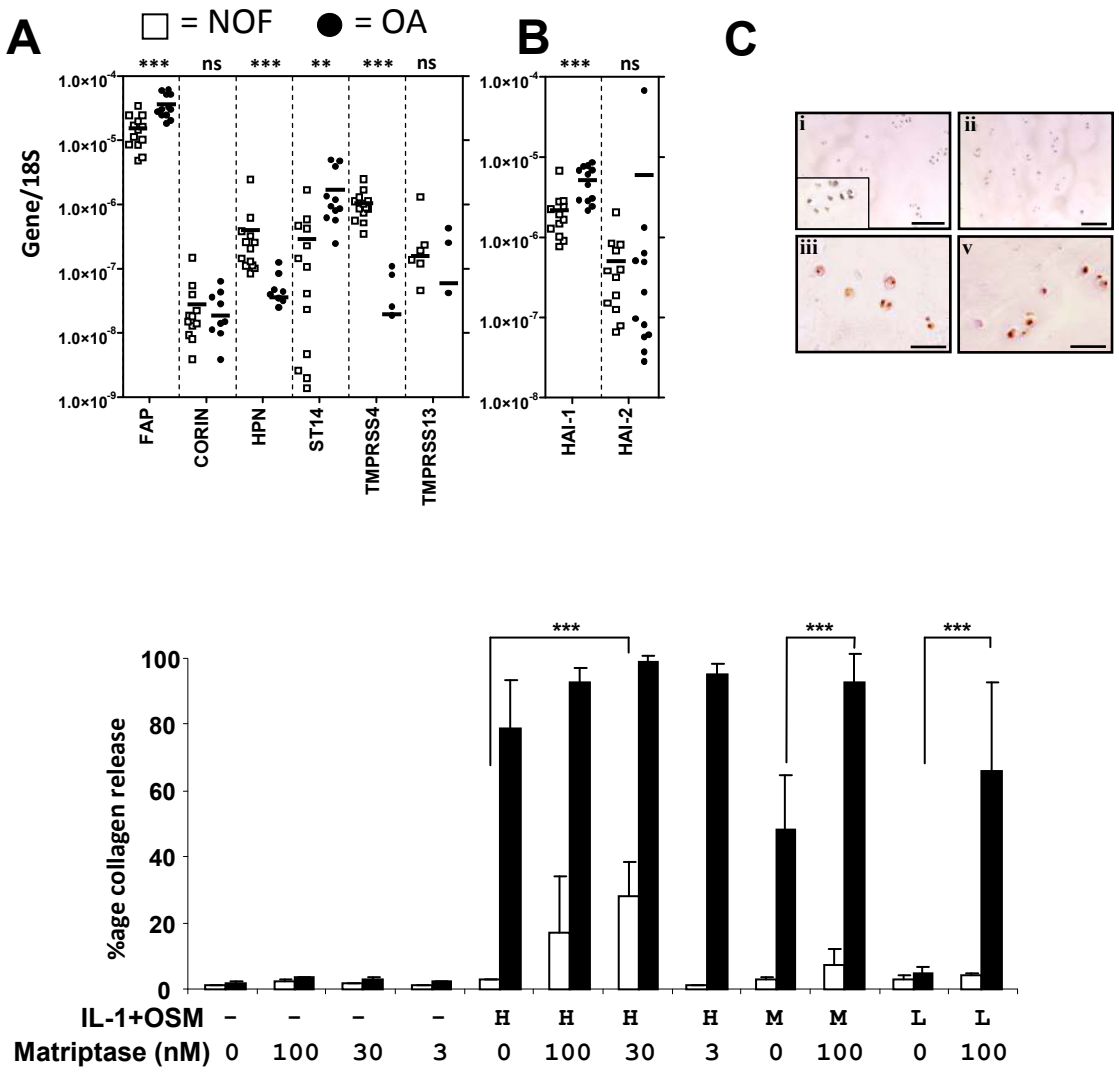
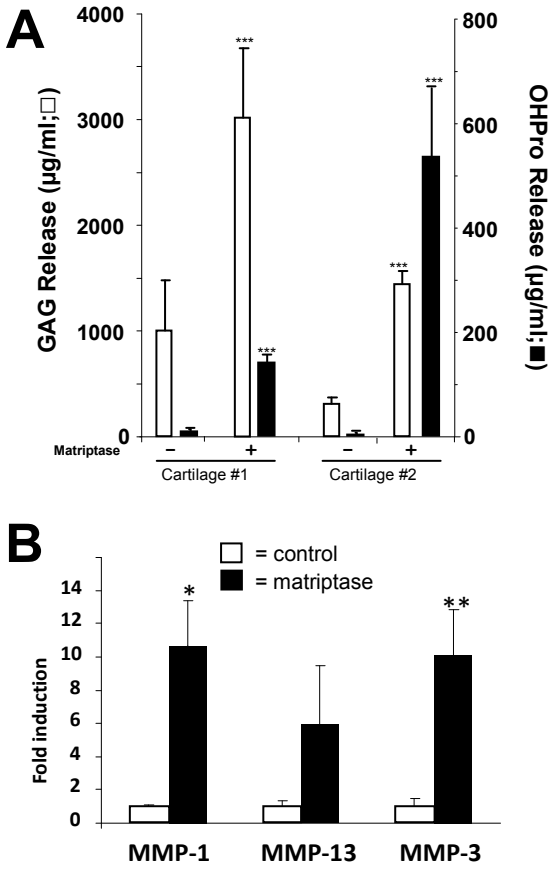
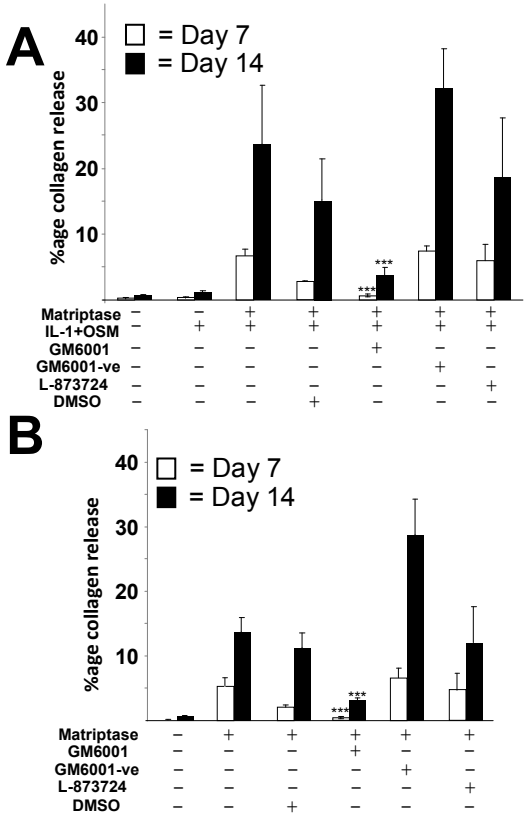
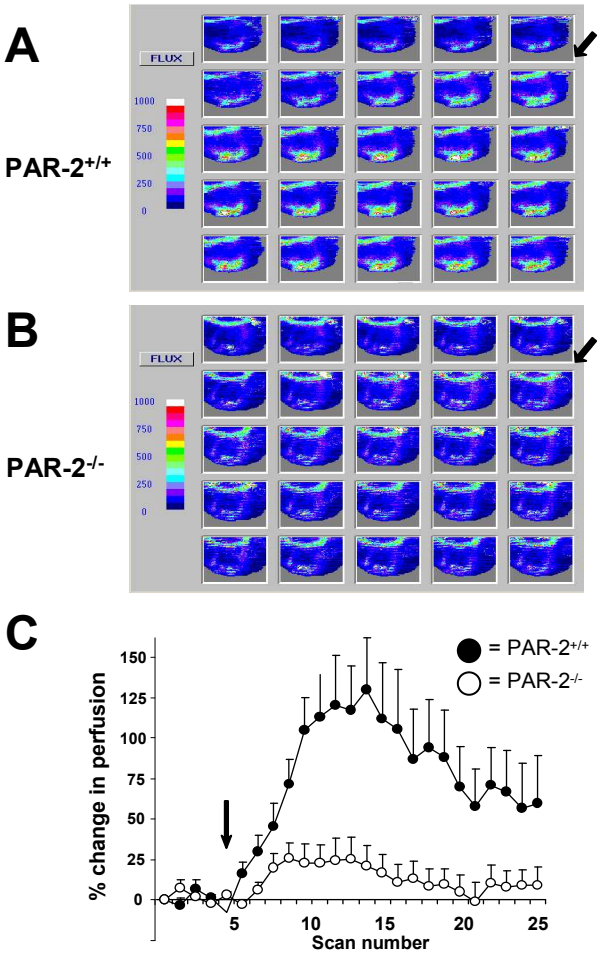


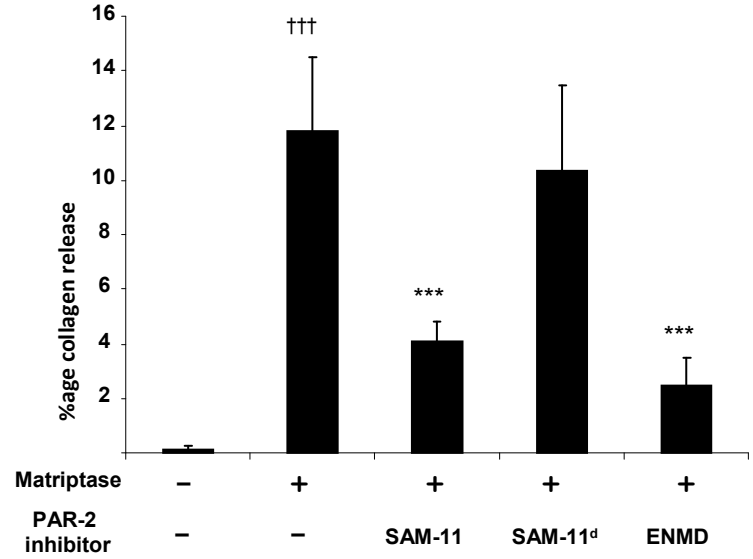
Fig. 1. Diagram of the right knee joint of the mouse. F = femur; T = tibia; MM = medial meniscus; ACL = anterior (cranial) cruciate ligament; MMTL = medial meniscotibial ligament; LMTL = lateral meniscotibial ligament. The MMTL is transected to generate destabilization of the medial meniscus (DMM). The ACL is transected in the ACLT model.


A
□ = NOF
● = OA
***
ns
***
**
***
ns
Gene/18S
1.0×10⁻⁴
1.0×10⁻⁵
1.0×10⁻⁶
1.0×10⁻⁷
1.0×10⁻⁸
1.0×10⁻⁹
FAP
CORIN
HPN
ST14
TMPRSS4
TMPRSS13
B
***
ns
1.0×10⁻⁴
1.0×10⁻⁵
1.0×10⁻⁶
1.0×10⁻⁷
1.0×10⁻⁸
HAI-1
HAI-2
C
i
ii
iii
v
***
***
***
%age collagen release
100
80
60
40
20
0
IL-1+OSM – – – – H H H H M M L L
Matriptase (nM) 0 100 30 3 0 100 30 3 0 100 0 100


A
4000
3000
2000
1000
0
800
600
400
200
0
GAG Release (µg/ml;□)
OHPro Release (µg/ml;■)
***
Matriptase
–
+
Cartilage #1
Cartilage #2
B
= control
= matriptase
Fold induction
14
12
10
8
6
4
2
0
*
**
MMP-1
MMP-13
MMP-3


A
= Day 7
= Day 14
%age collagen release
40
30
20
10
***
Matriptase
IL-1+OSM
GM6001
GM6001-ve
L-873724
DMSO
B
= Day 7
= Day 14
%age collagen release
40
30
20
10
***
Matriptase
GM6001
GM6001-ve
L-873724
DMSO


A
PAR-2+/+
FLUX
1000
750
500
250
0
B
PAR-2-/-
FLUX
1000
750
500
250
0
C
= PAR-2+/+
= PAR-2-/-
% change in perfusion
150
125
100
75
50
25
0
5
10
15
20
25
Scan number
D
DMM
Sham
Matriptase
PAR-2


%age collagen release
16
14
12
10
8
6
4
2
†††
***
***
Matriptase
–
+
+
+
+
PAR-2 inhibitor
–
–
SAM-11
SAM-11d
ENMD

A model for cartilage destruction in OA in the absence of inflammation

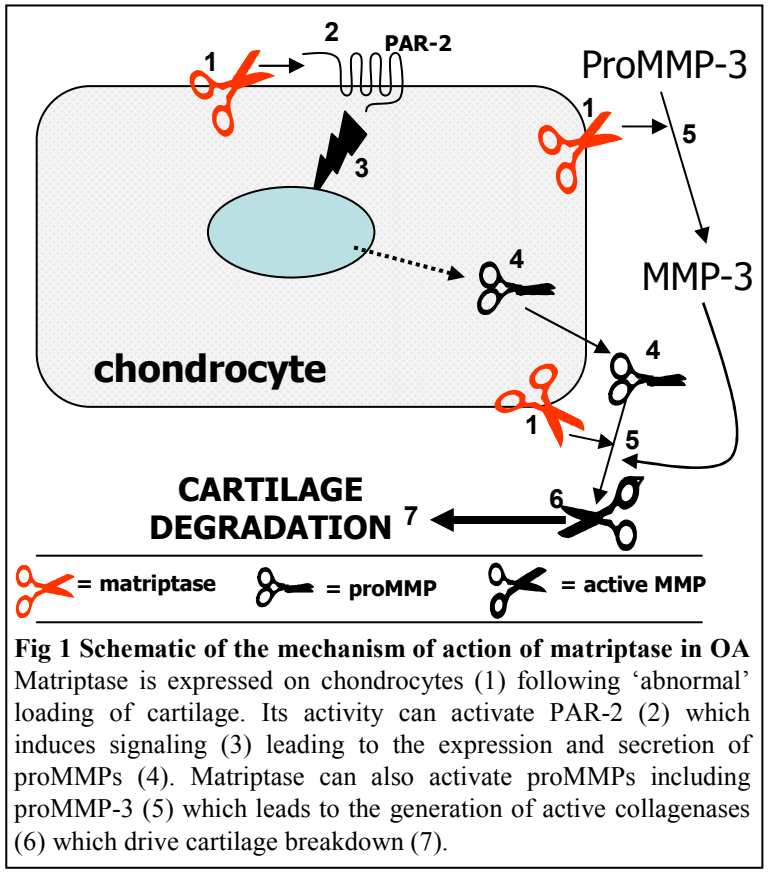


Fig 1 Schematic of the mechanism of action of matriptase in OA Matriptase is expressed on chondrocytes (1) following 'abnormal' loading of cartilage. Its activity can activate PAR-2 (2) which induces signaling (3) leading to the expression and secretion of proMMPs (4). Matriptase can also activate proMMPs including proMMP-3 (5) which leads to the generation of active collagenases (6) which drive cartilage breakdown (7).

Summary

Cartilage is a complex ECM

Chondrocytes do have a limited “repair response”

OA and RA are characterised by cartilage destruction

Current treatments are not universally effective

Metalloproteinases (MMPs) primarily mediate this destruction

Pro-inflammatory cytokines drive MMP expression

Inflammatory intracellular signaling is complex

Mechanisms that do not involve inflammation per se mediate destruction too

Need to identify the molecular mechanisms that drive cartilage destruction in order to develop therapeutics

The Key Questions

Can we cure arthritis?

Probably not!

Can we negate the need for joint replacements?

Probably not!

Can we manage arthritis better?

Most definitely!

Any Questions??