STEM CELLS IN GLOMERULAR REPAIR

PRESENT STATUS, CHALLENGES AND FUTURE EXPECTATIONS

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GENERIC GLOMERULAR DAMAGE VIEWED THROUGH A UNIQUE EXPERIENTAL MODEL:

UNDERSTANDING THE REPAIR THAT NEEDS TO BE ACCOMPLISHED:

what WE KNOW AND

WHAT WE NEED TO FIND OUT

Paola Romagnani
Department of Biochemical, Experimental and Clinical Sciences
Excellence Centre for Research, Transfer and High Education for the development of DE-NOVO therapies
Florence, Italy

The renal parenchymal niches of progenitor cells and glomerular repair

Jiamin Teng
Louisiana State University Health Sciences Center, Shreveport, USA

EXPERIMENTAL PLATFORMS: WHAT HAS BEEN ACCOMPLISHED FROM IN-VITRO TO IN-VIVO

Elba A Turbat-Herrera
Louisiana State University Health Sciences Center, Shreveport, LA, USA

INDUCED PLURIPOTENT (iPSC) AND MESENCHYMAL STEM CELLS: POTENTIAL THERAPEUTIC ROLE IN GLOMERULAR REPAIR: WHAT WE KNOW AND WHAT NEEDS TO BE FOUND OUT

GENERIC GLOMERULAR DAMAGE VIEWED THROUGH A UNIQUE EXPERIMENTAL MODEL: UNDERSTANDING THE REPAIR THAT NEEDS TO BE ACCOMPLISHED.

WHAT WE KNOW AND WHAT WE NEED TO FIND OUT

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THE PROBLEM

- Chronic renal disease continues increasing at a rate of 6-8% per annum- most due to glomerular diseases
- It is believed that 10-20% of the total adult population of the world has some degree of chronic renal failure
- Only options available are dialysis and transplantation
- About 0.1% of the adult population on dialysis

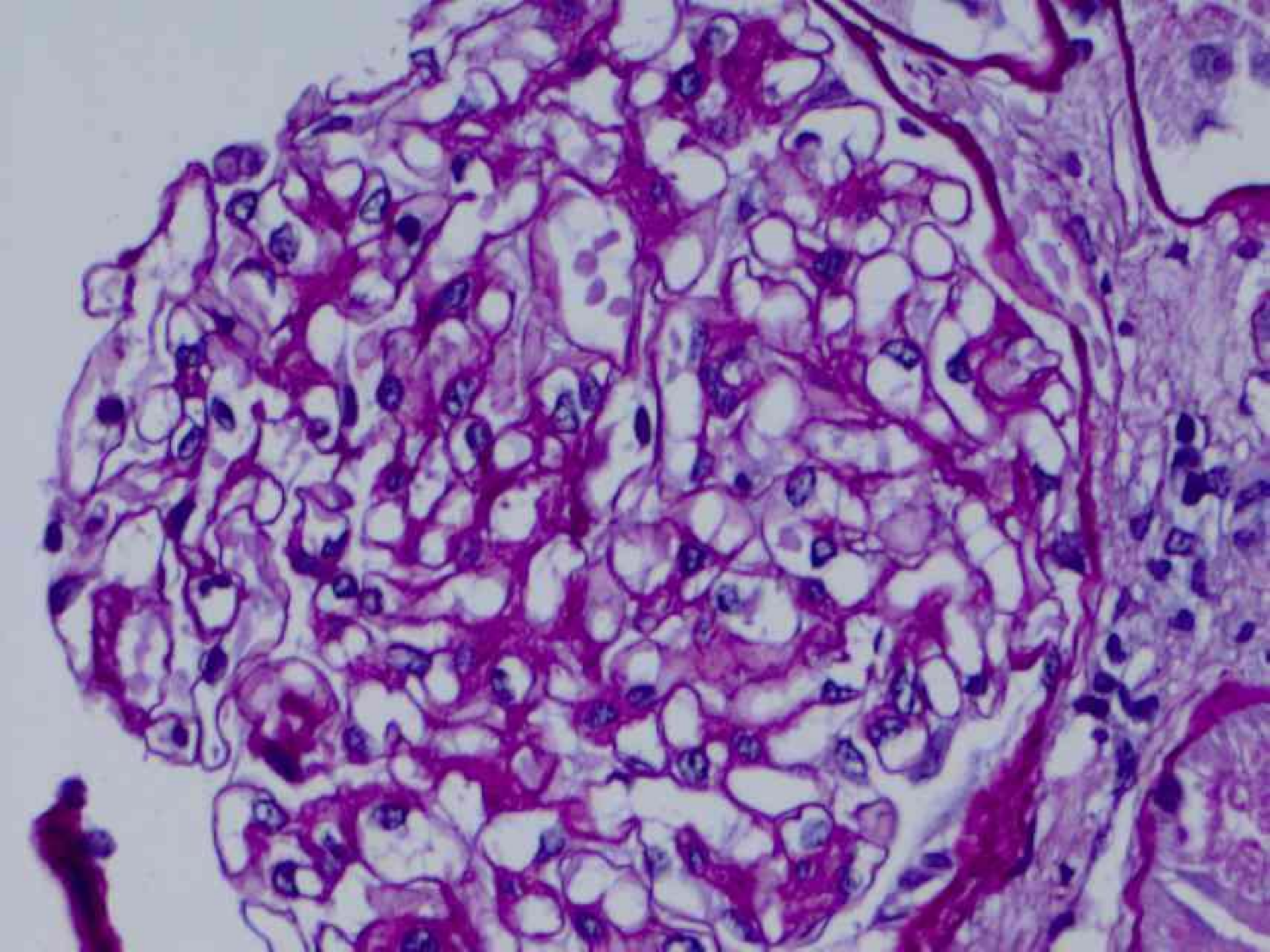
AND THIS CONSUMES ABOUT 2% of the total health expenditure in developed countries

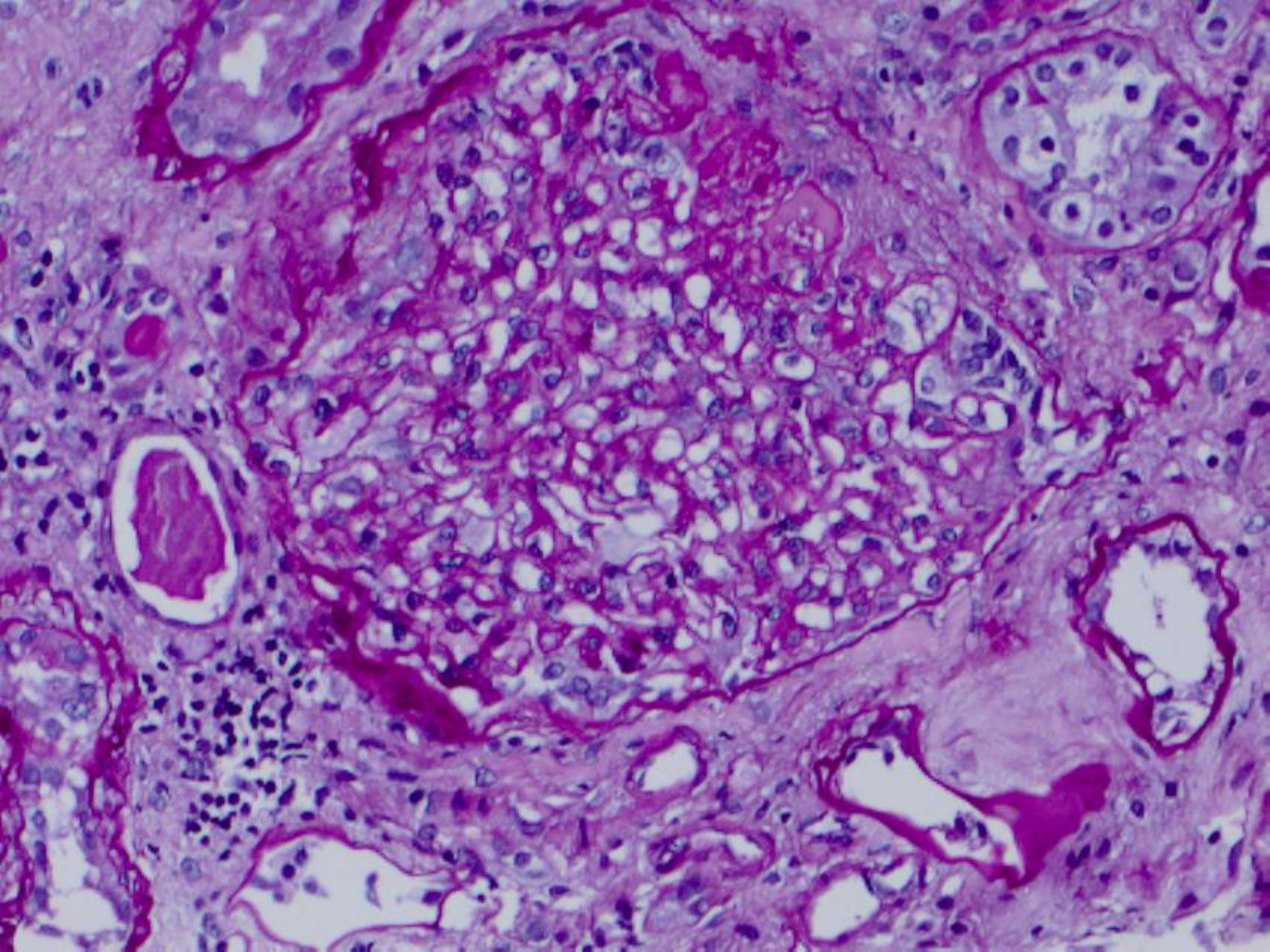
PATTERNS OF GLOMERULAR INJURY

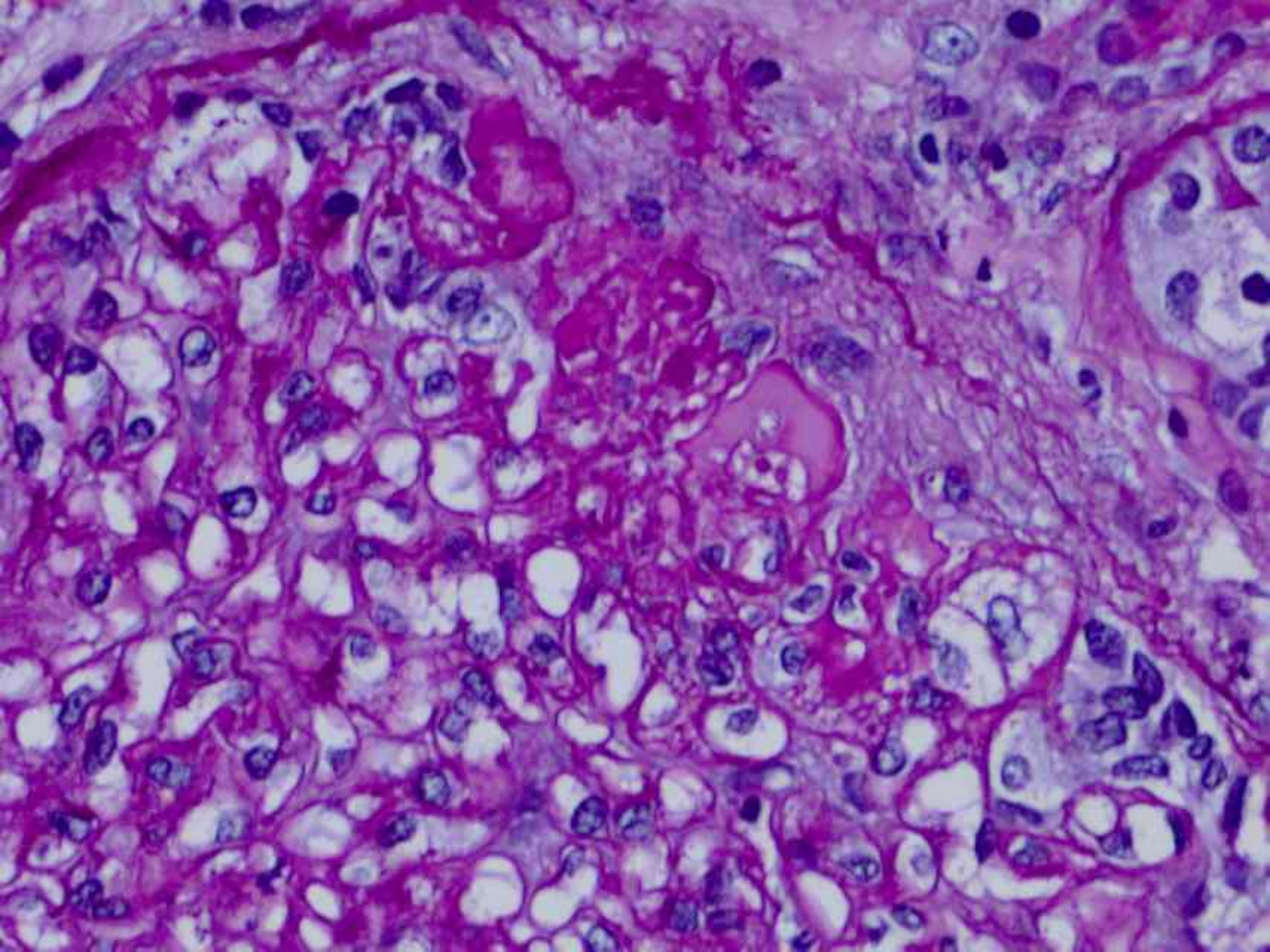
- GLOBAL
- SEGMENTAL
- MESANGIAL *****
- PERIPHERAL CAPILLARY WALL
- COMBINATIONS OF ABOVE

FACTORS THAT MAY AFFECT GLOMERULAR HEALING

- If only mesangial damage, reversibility is POSSIBLE
- When capillary spaces are compromised, they need to be open again and made functional- DIFFICULT TALK
- New capillary sapces and walls must be created
- Interactions between podocytes, endothelial and mesangial cells become essential for repair to occur- “cocktail” required

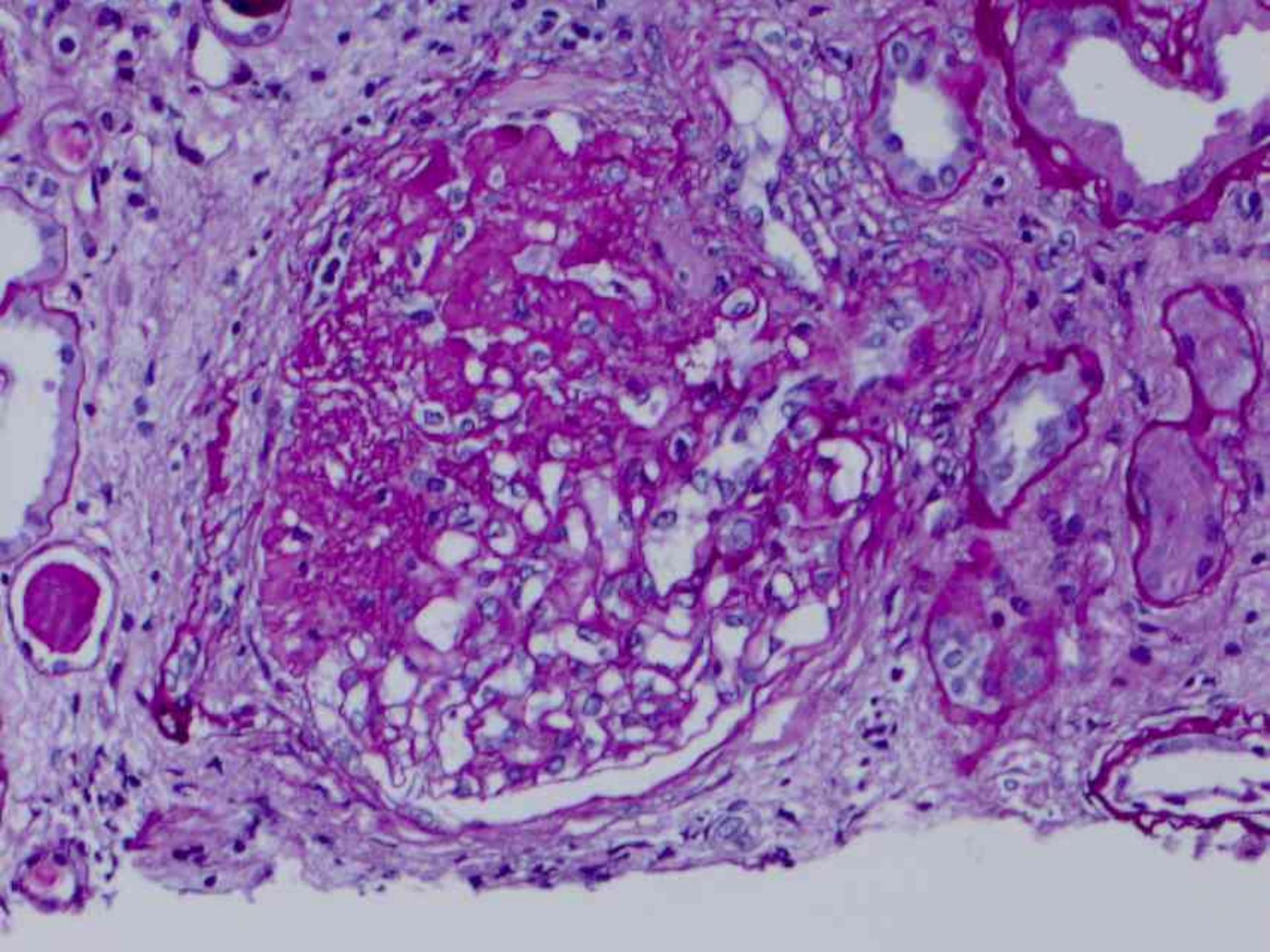




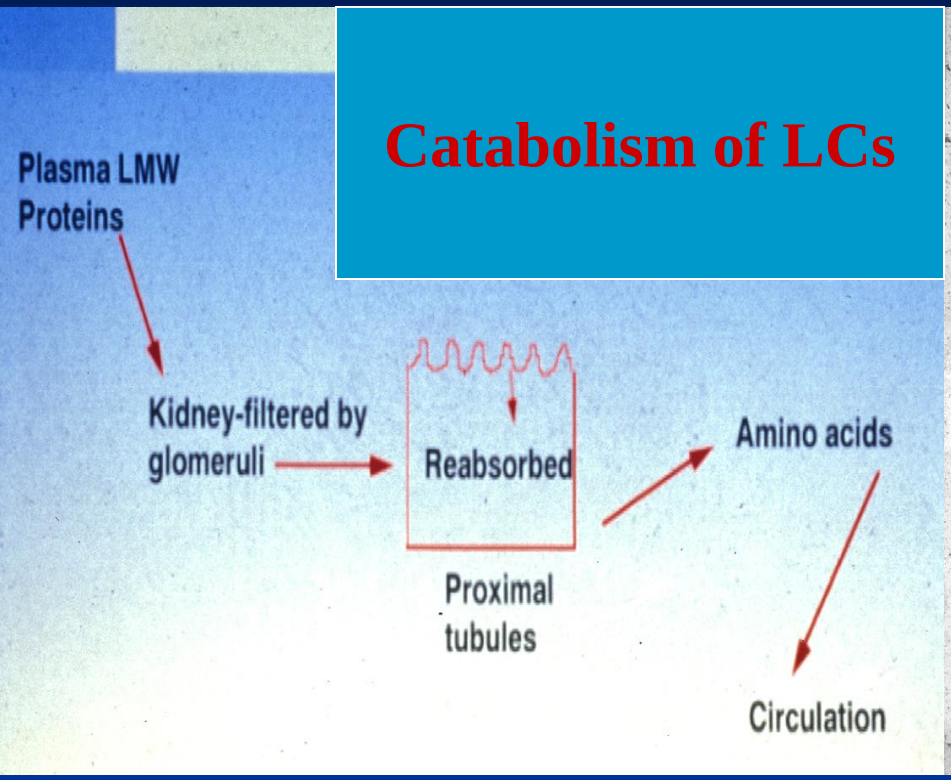


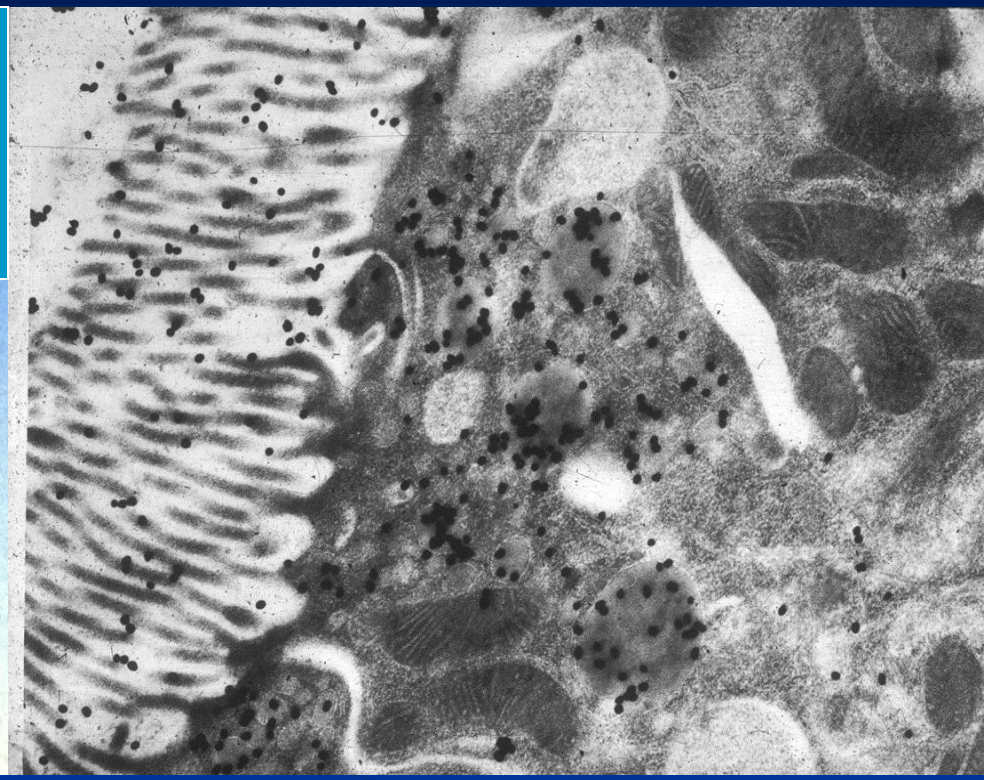
GLOMERULAR INJURY IRREVERSIBILITY

- If more than 50% of a glomerulus becomes sclerosed, reversibility is markedly affected and repair of that glomerulus may no longer be possible

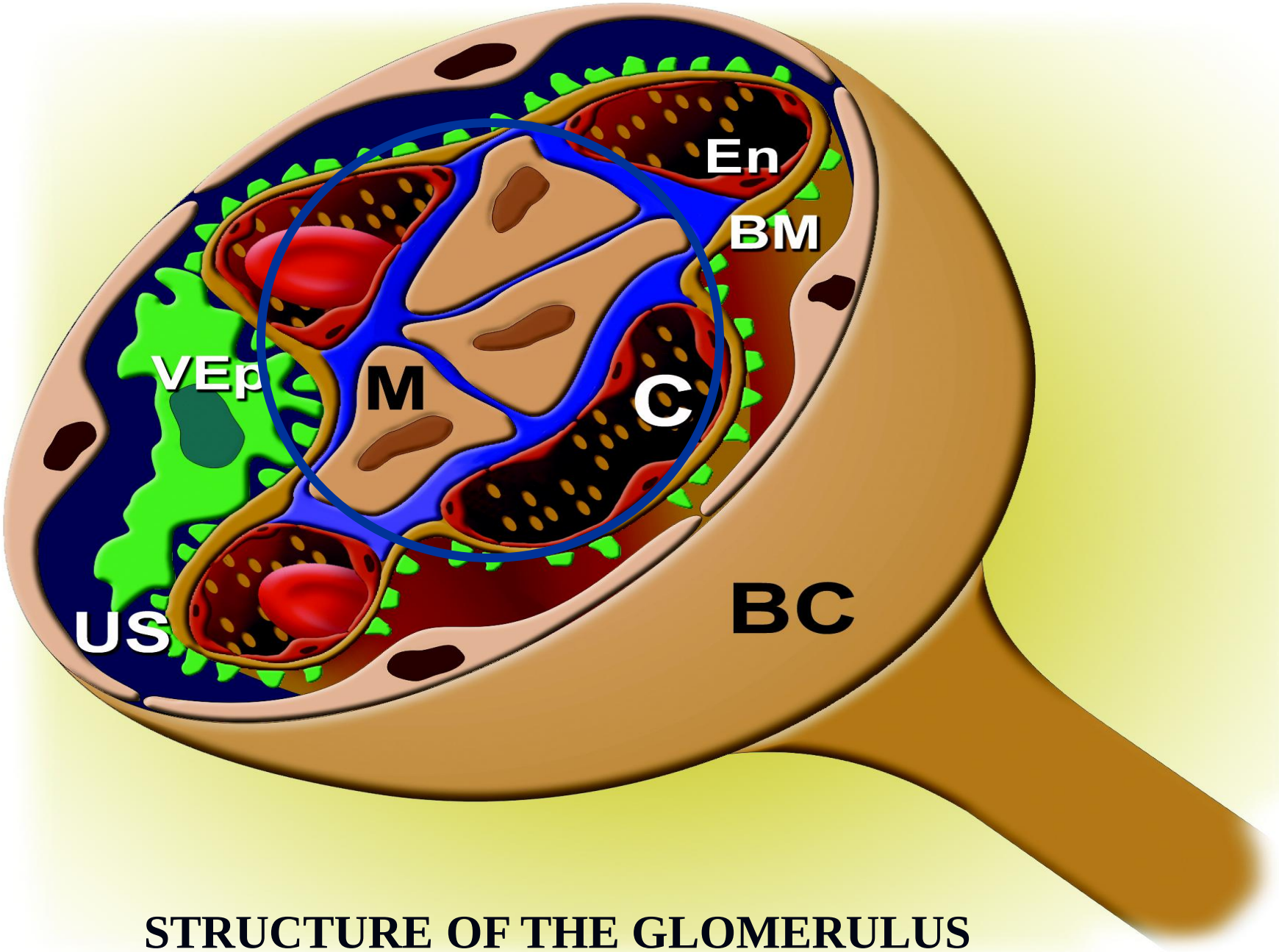


Catabolism of LCs





NORMAL LIGHT CHAINS DO NOT INTERACT WITH GLOMERULAR COMPARTMENT



STRUCTURE OF THE GLOMERULUS

PDGF-B
Platelet derived growth factor-beta

TGF-B1
Transforming growth factor-beta

Ras/MEK/ERK kinase pathways

cTGF

Metalloproteinases
MMPs

TIMPs
Tissue inhibitors of MMPs

Plasminogen Activator Inhibitor PAI-1

Alpha 2-macroglobulin

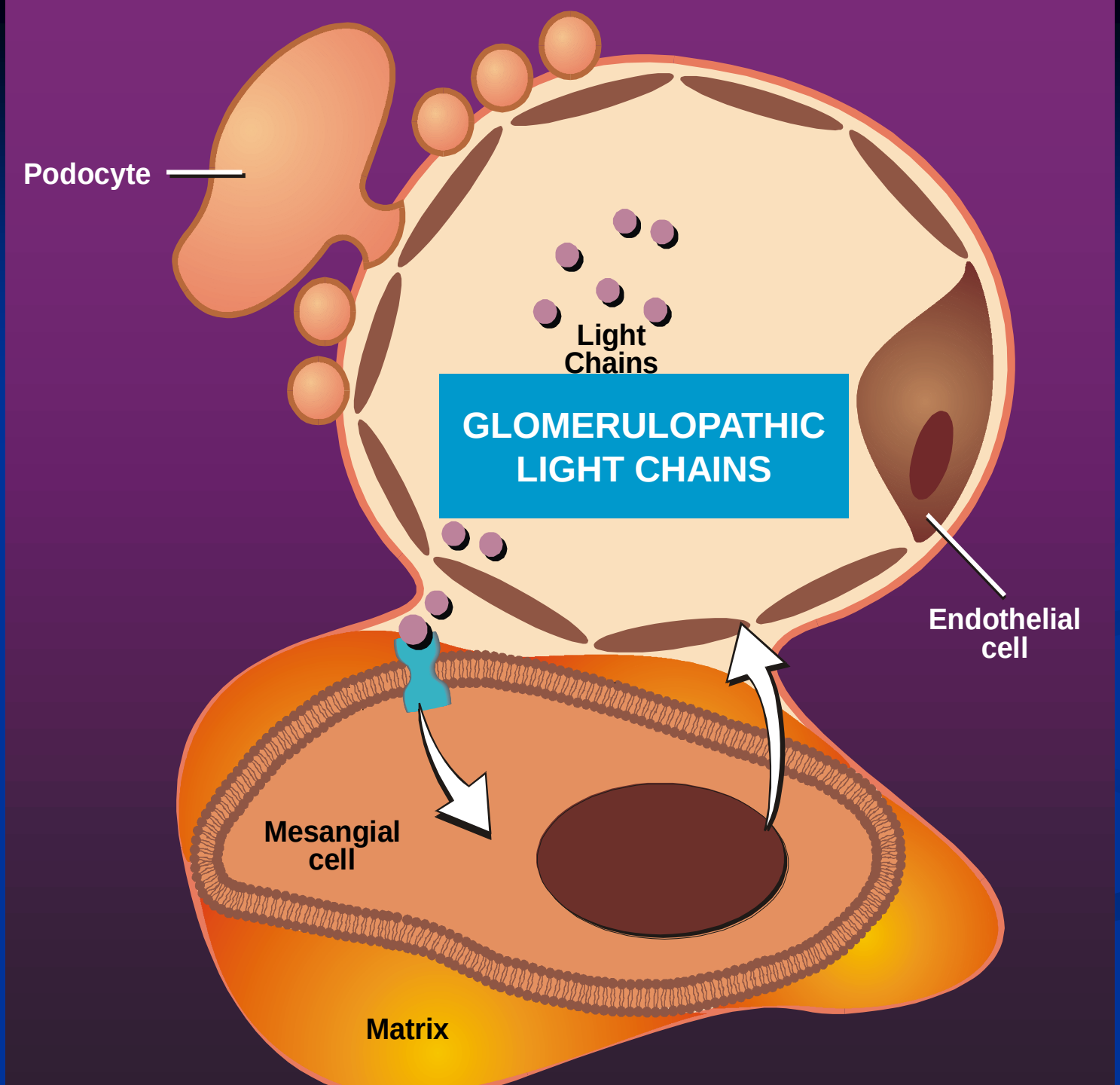
MESANGIAL HOMEOSTASIS

PROLIFERATION

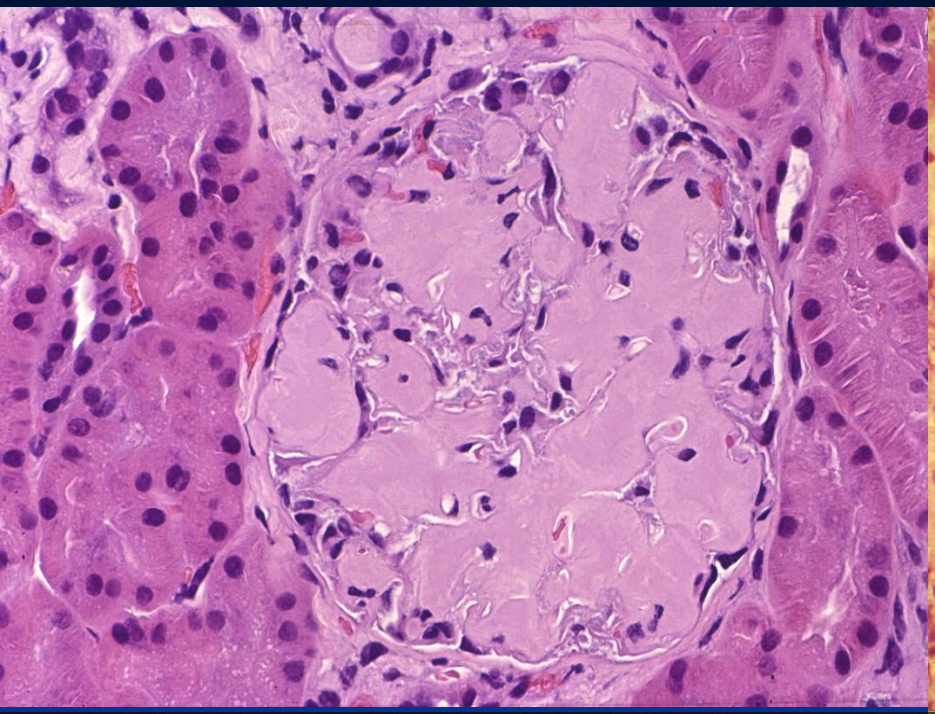
ECM FORMATION

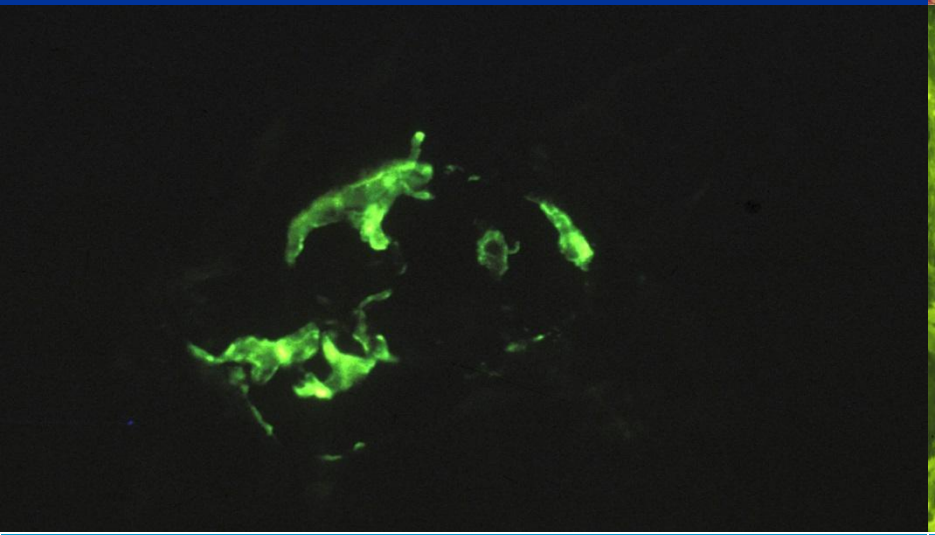
ECM DESTRUCTION

MMP MODULATORS


Podocyte
Light Chains
GLOMERULOPATHIC LIGHT CHAINS
Endothelial cell
Mesangial cell
Matrix

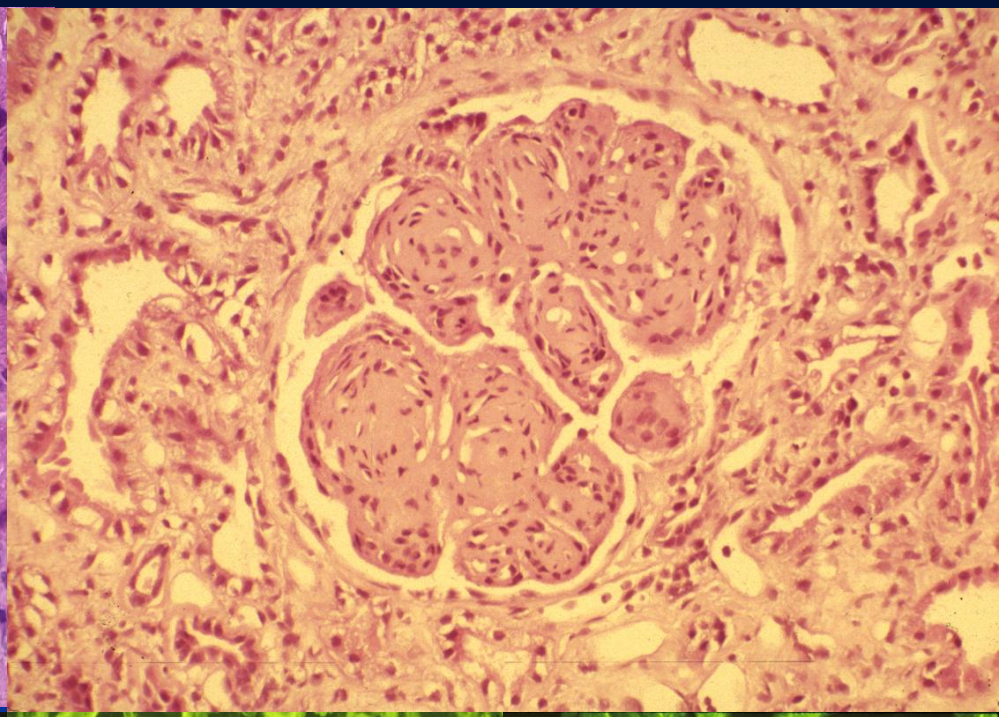
AL-AMYLOIDOSIS

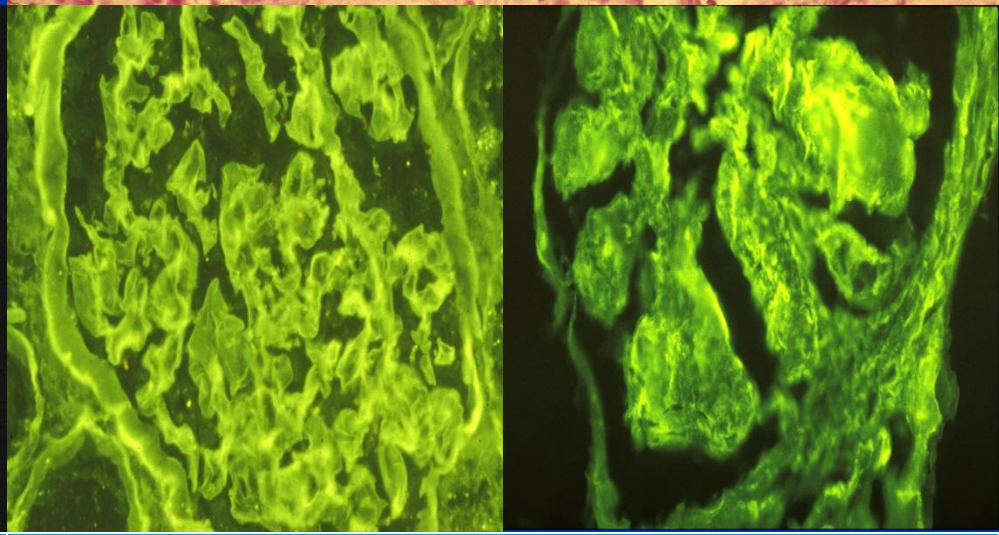




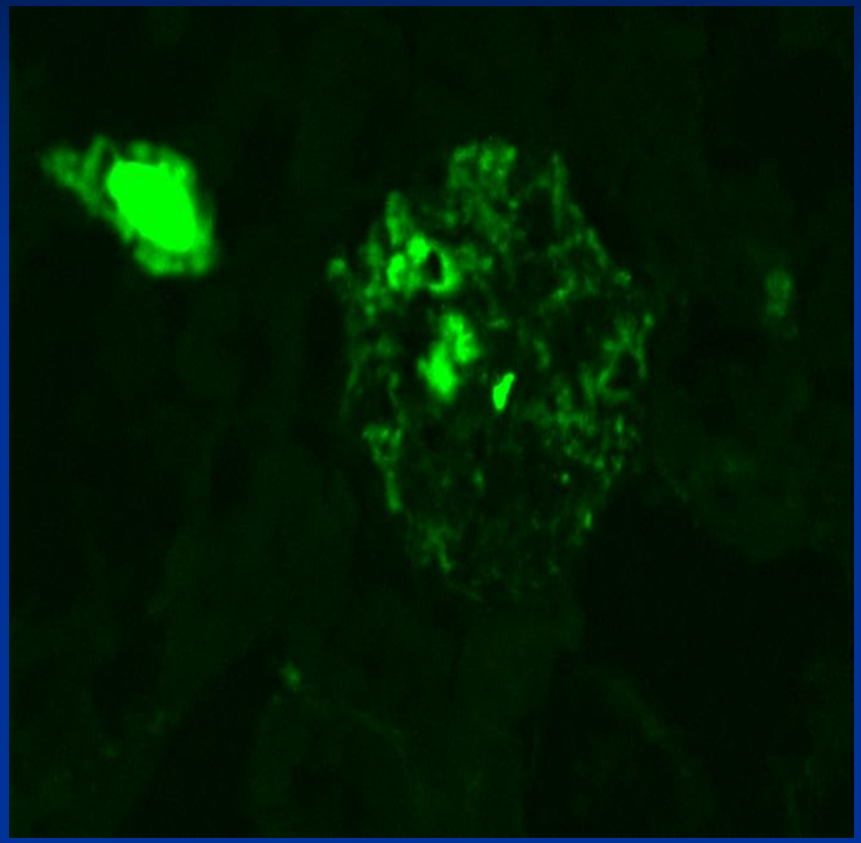
TYPICAL IF, λ LIGHT CHAINS λ6

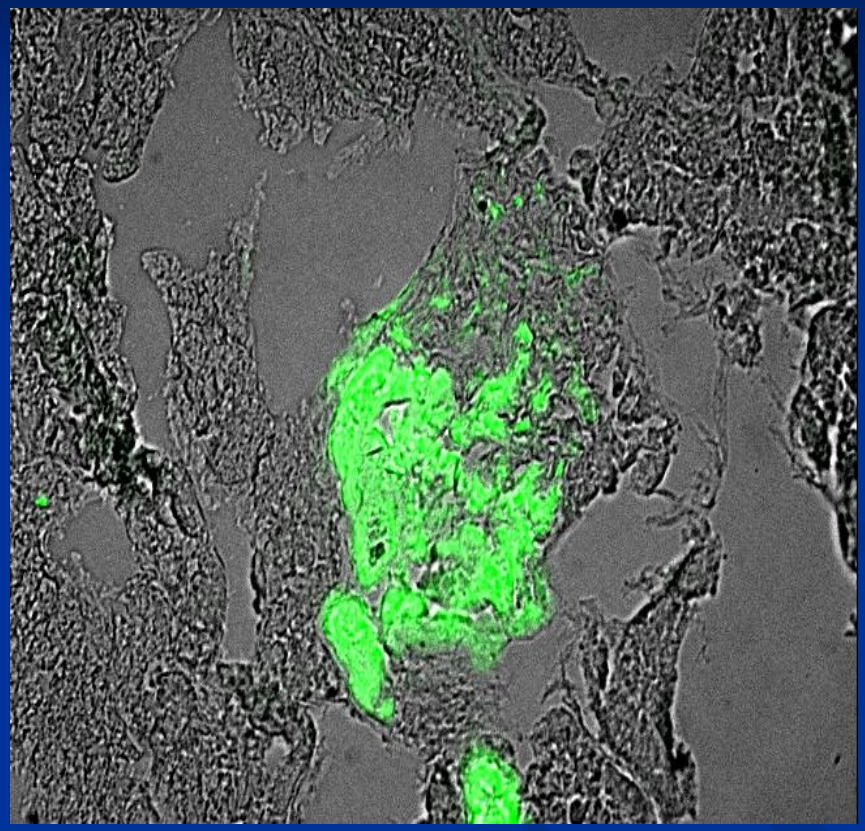
LIGHT CHAIN DEPOSITION DISEASE



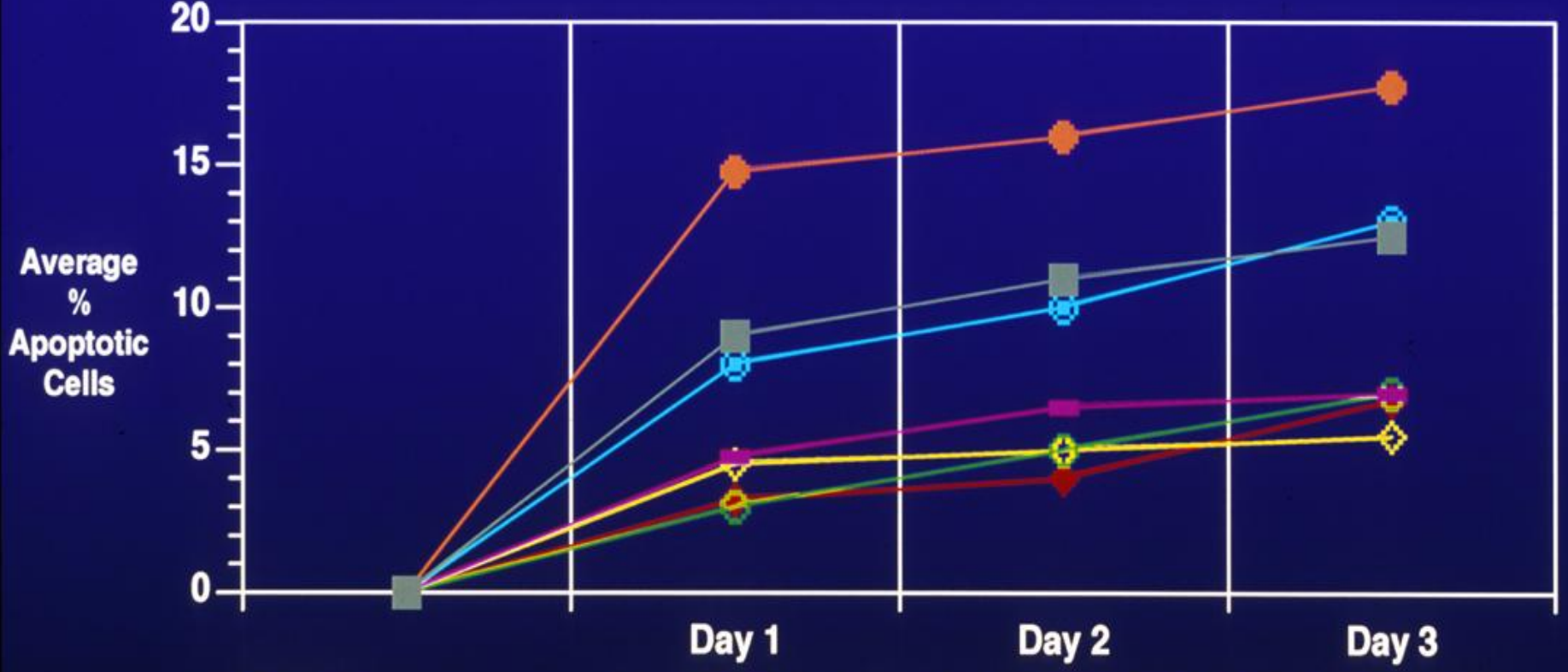


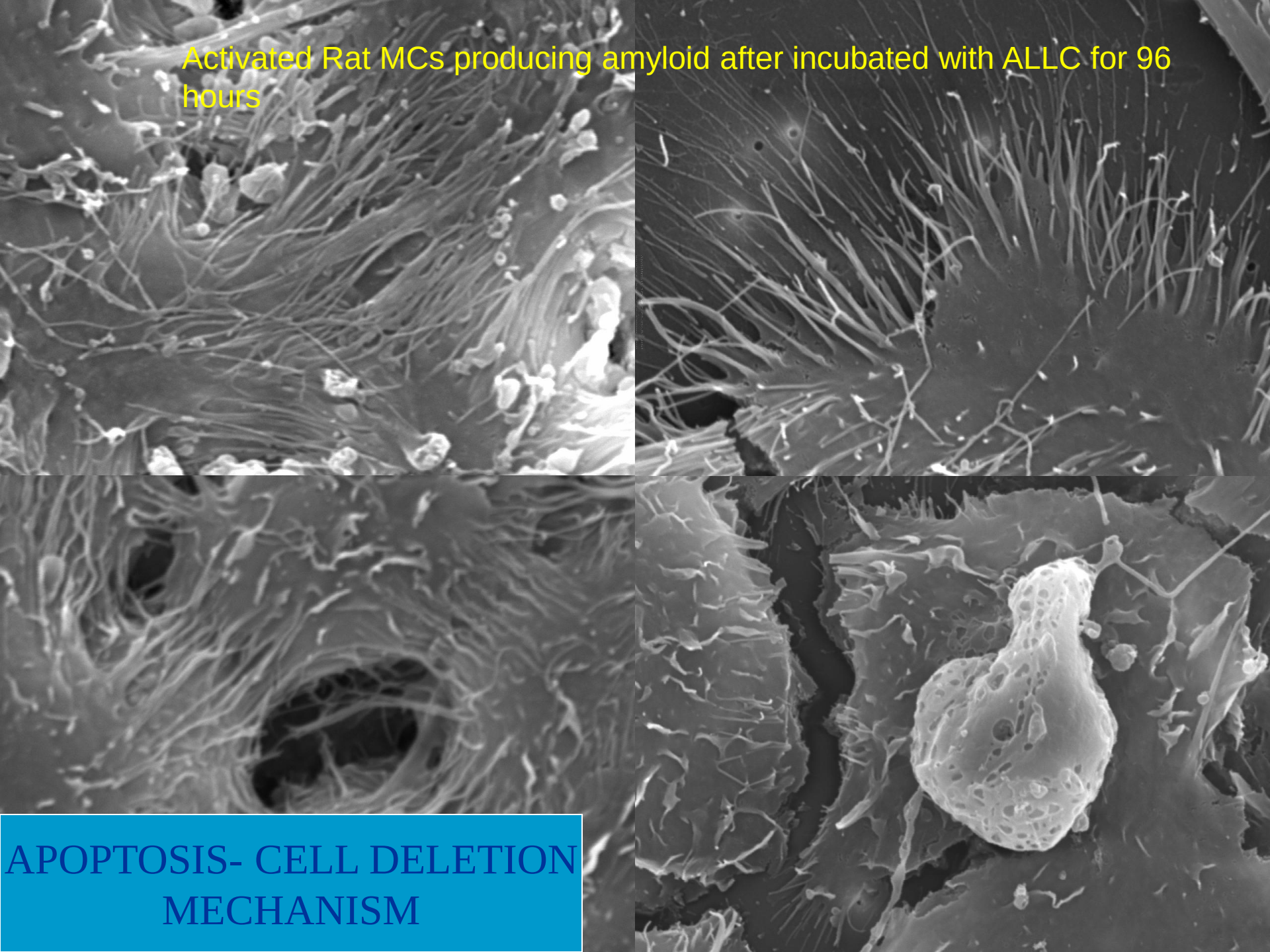
TYPICAL IF, κ LIGHT CHAINS κI and IV





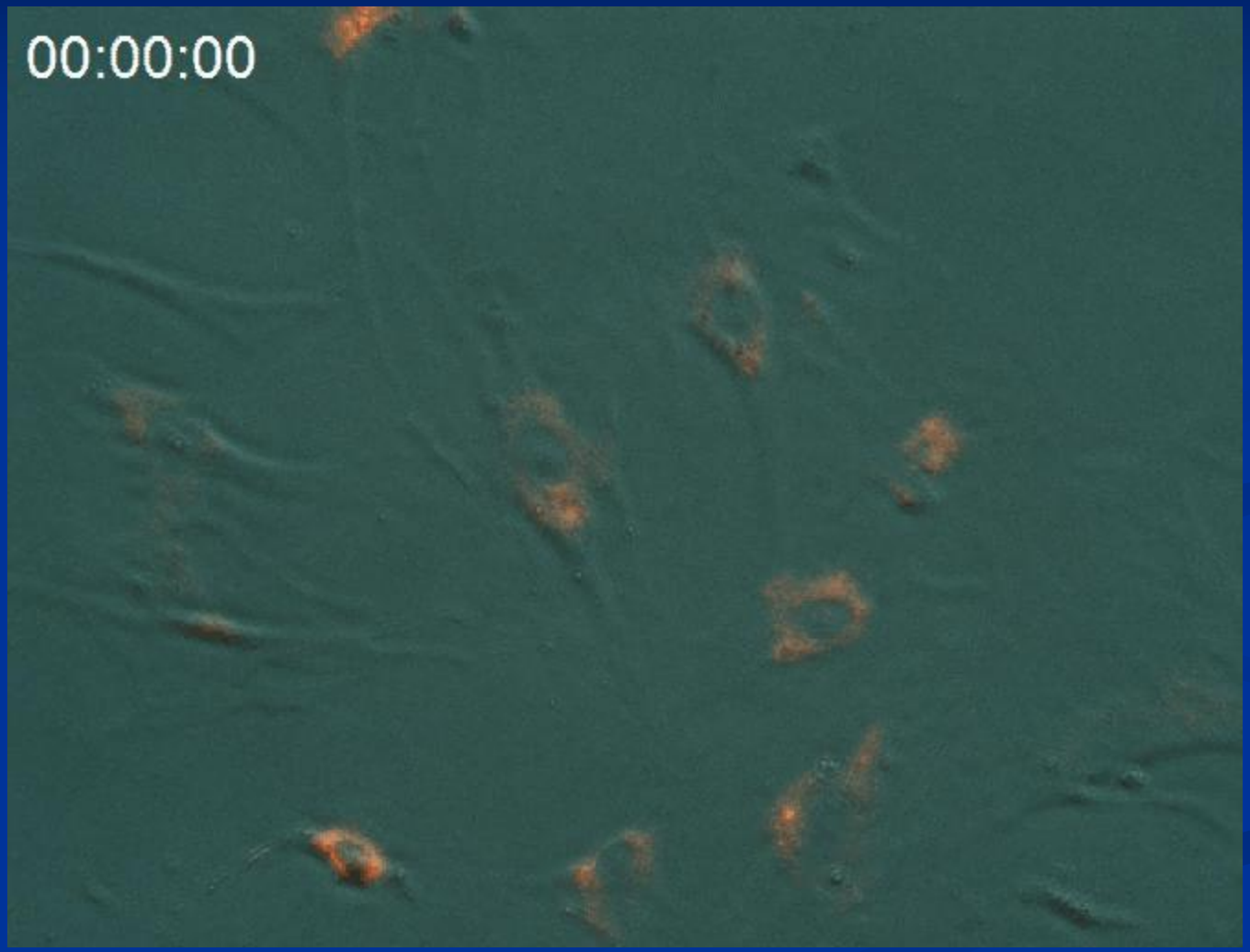
Rat Mesangial Cells in Culture
Monoclonal Light Chains and Apoptosis



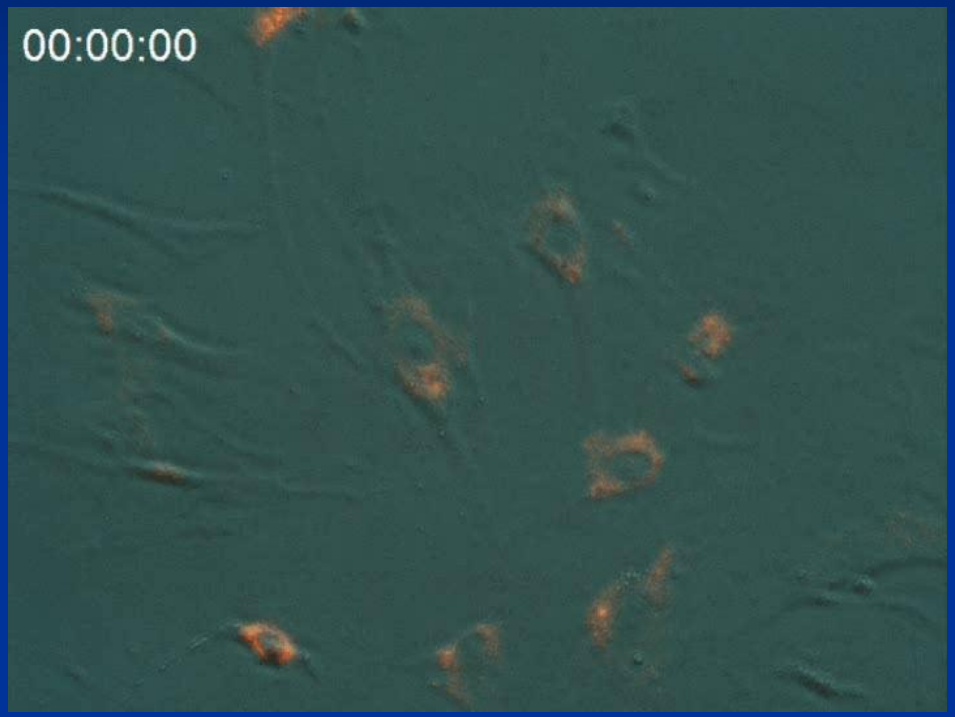


APOPTOSIS- CELL DELETION MECHANISM

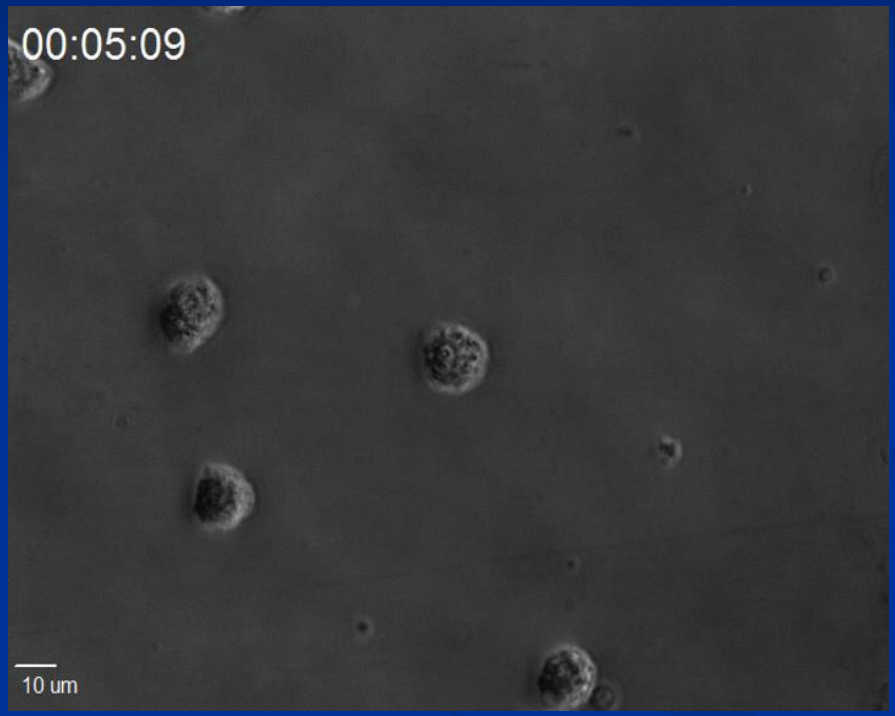
ALLC induce apoptosis in MC



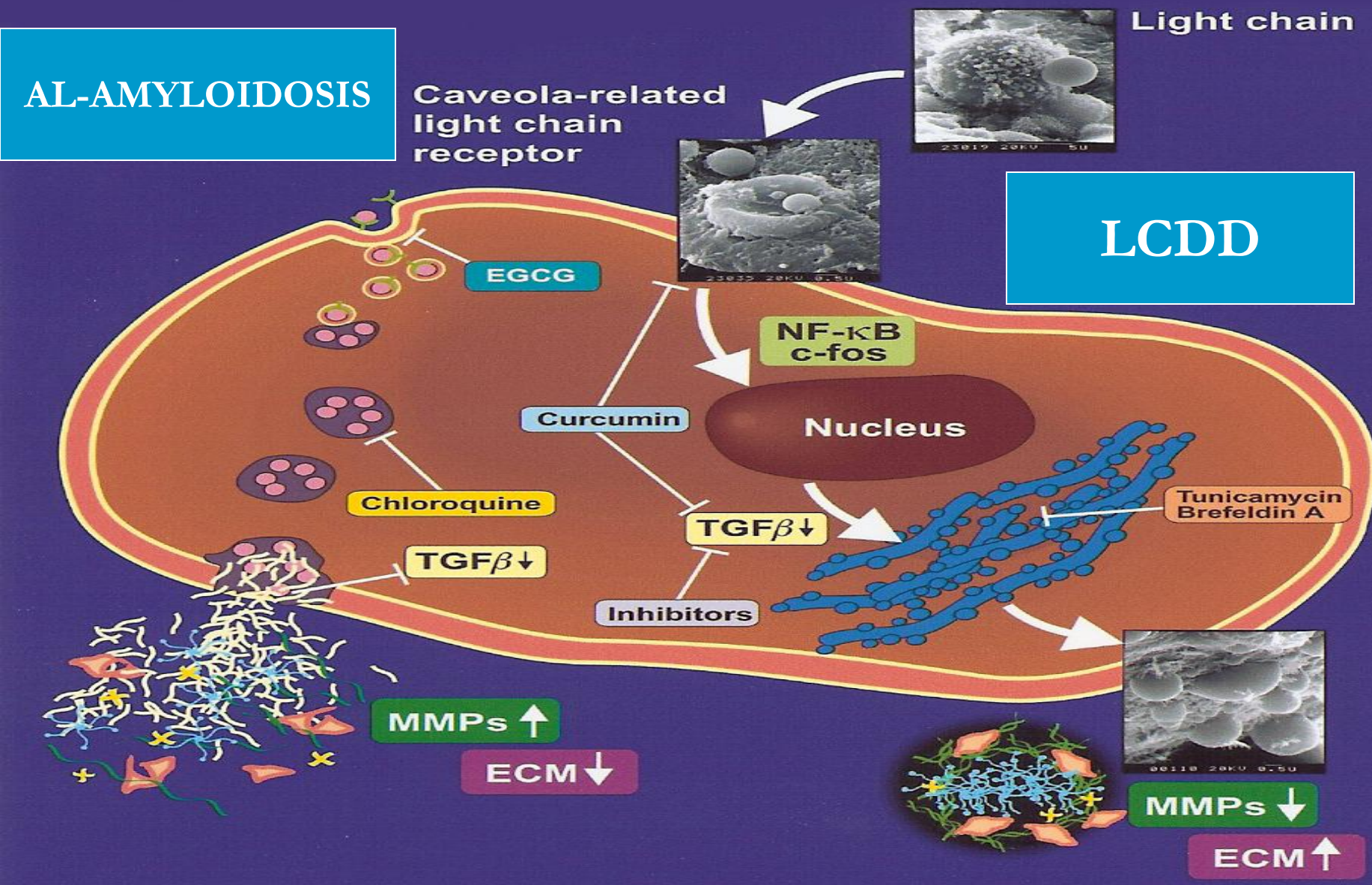
LCDD-LC induces RMC (Caspase-3) apoptosis



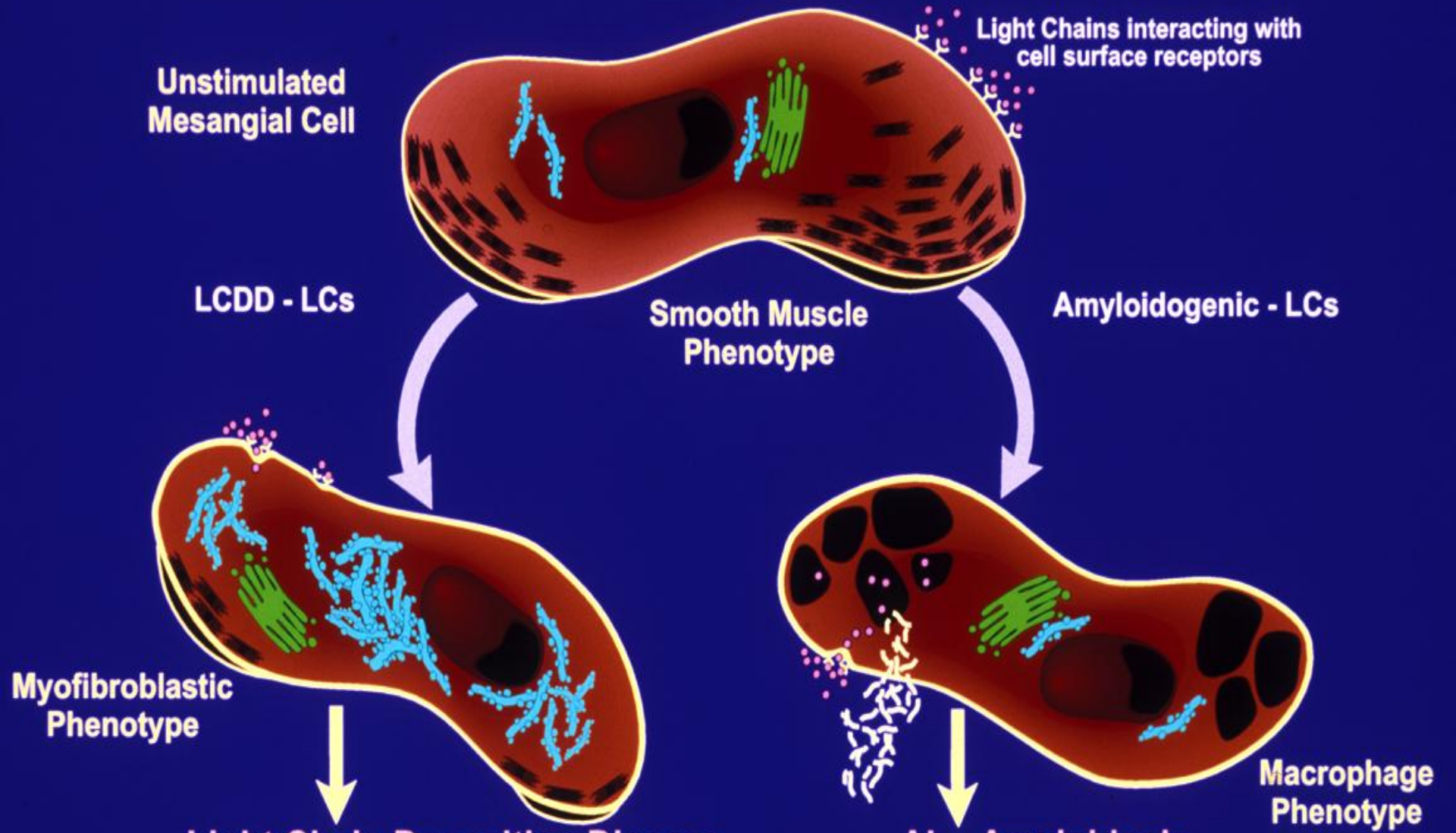
RMSC phagocytosis of LCDD-LC-induced apoptotic bodies / cells



G-LCs and glomerulopathies



Phenotypic Transformation of Mesangial Cells

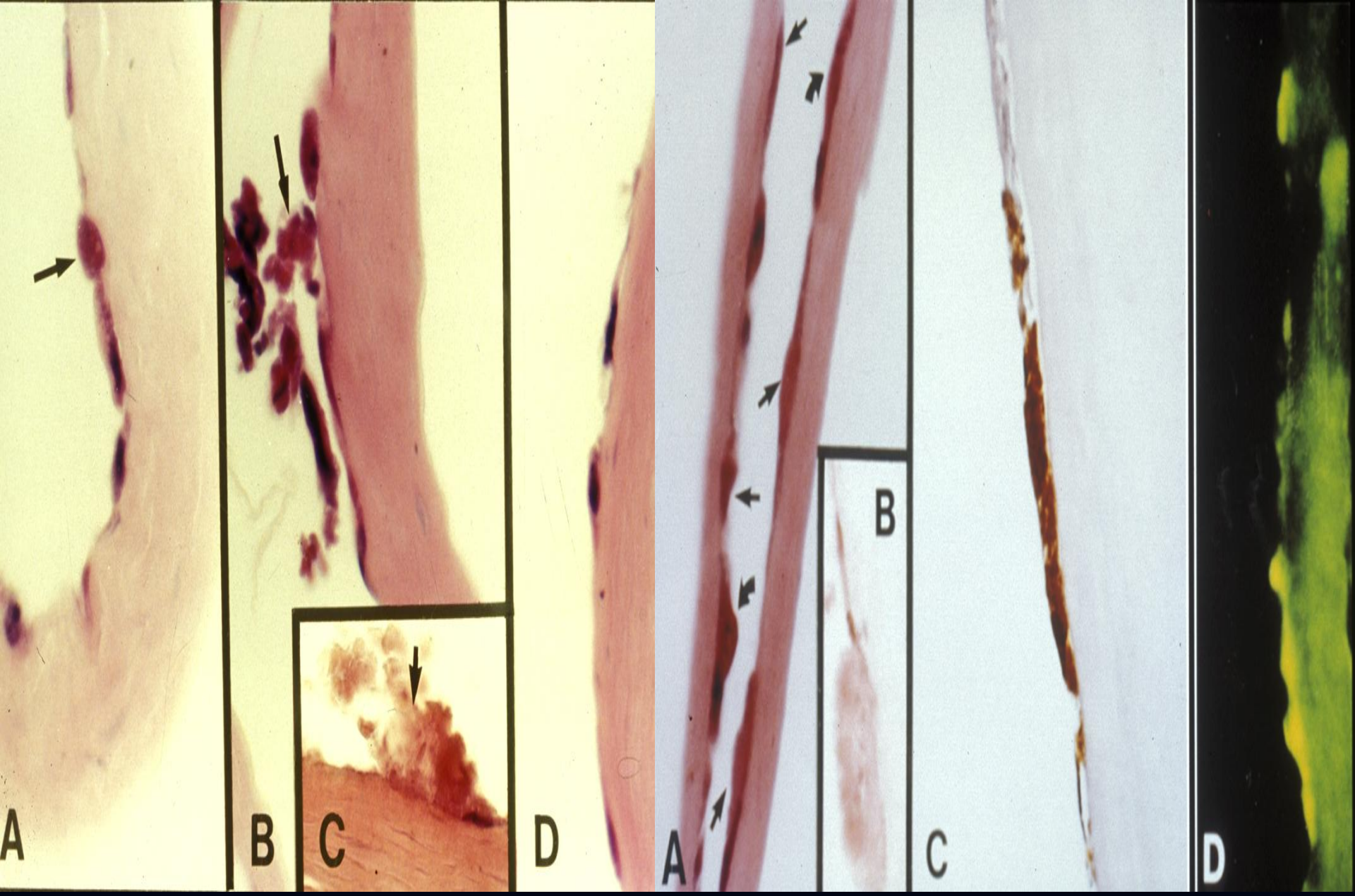


Light Chain Deposition Disease

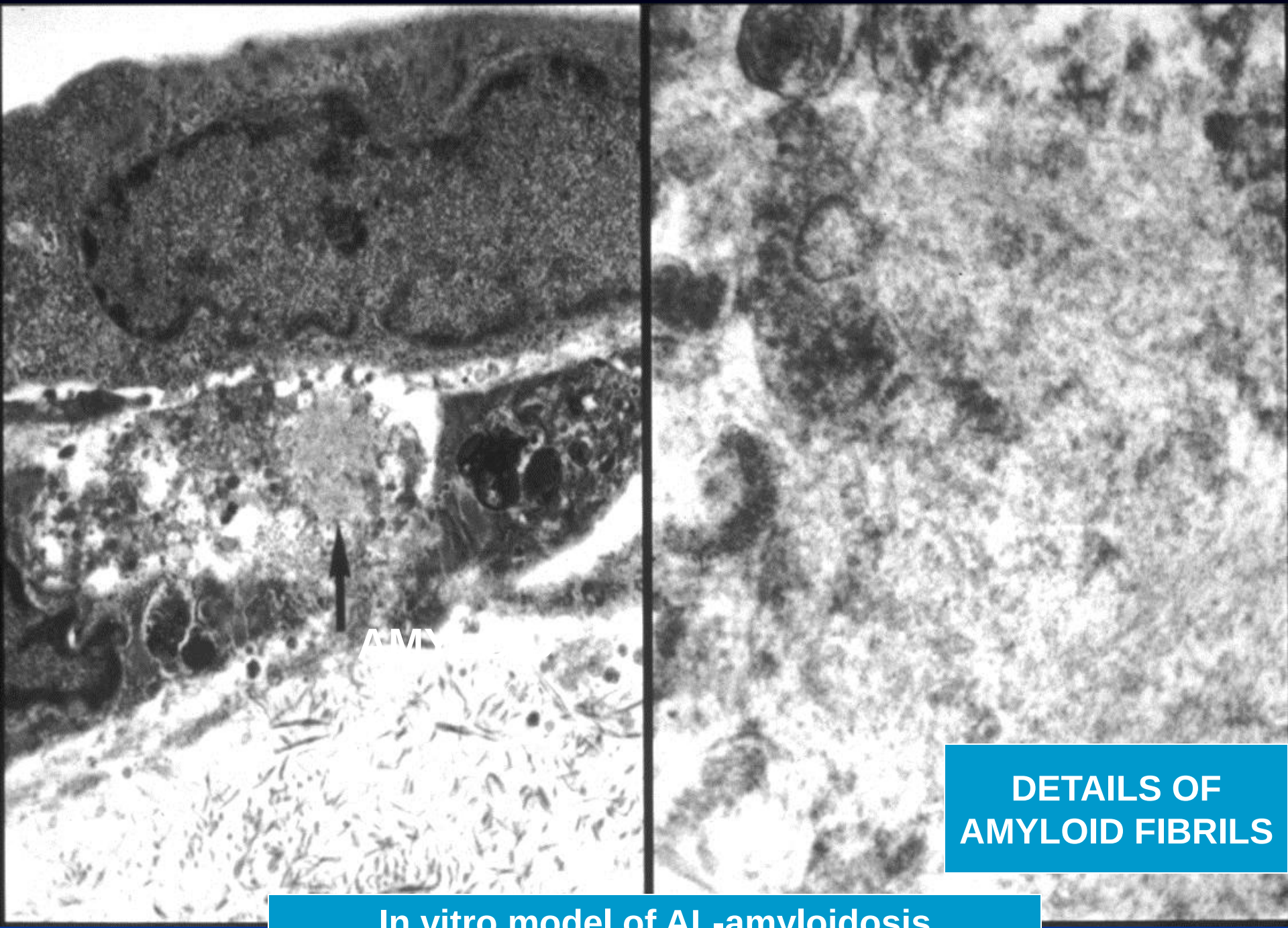
- Abundant RER
- Activation of protein synthesis
- Decreased MMP activity

AL - Amyloidosis

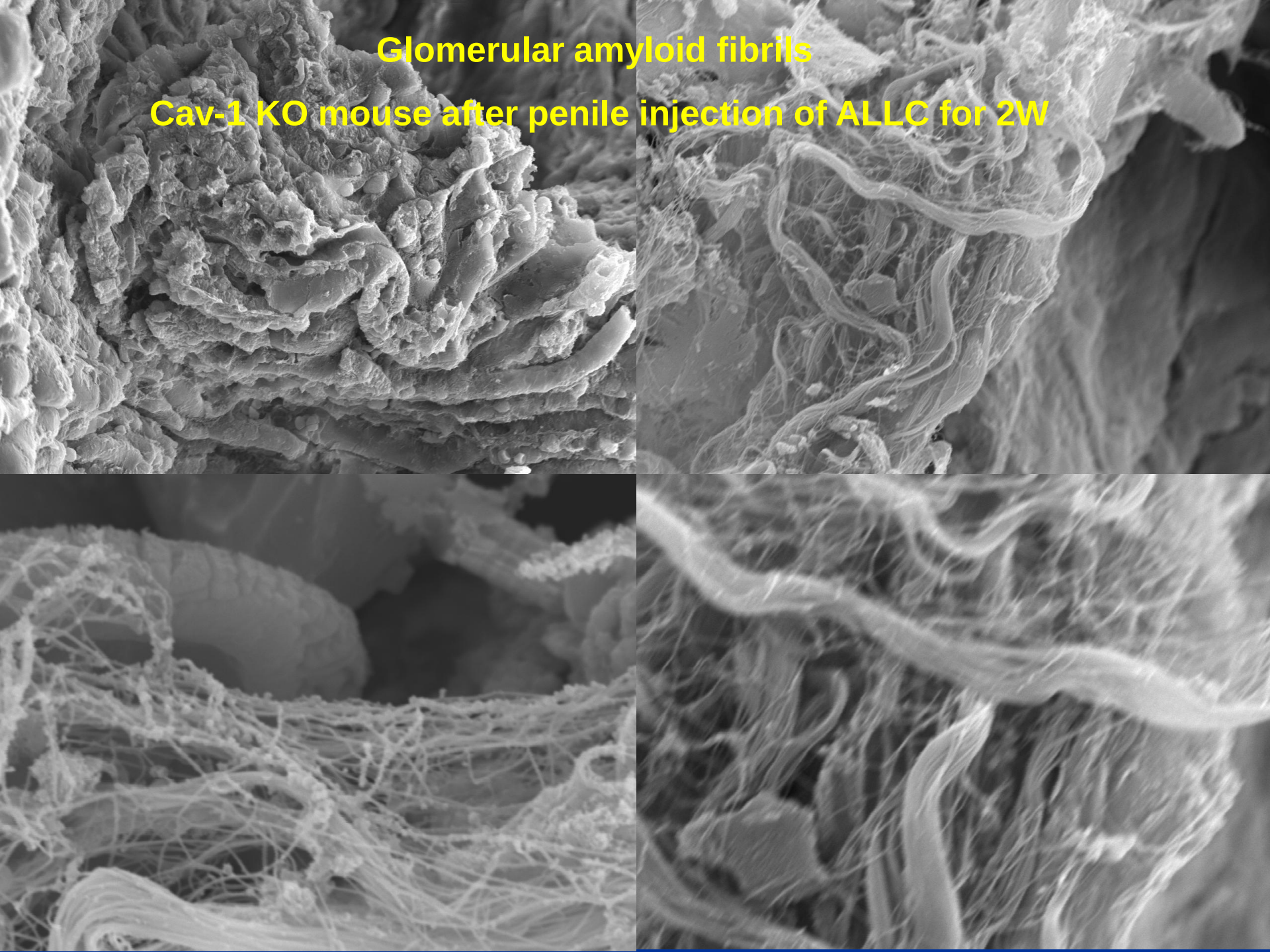
- Abundant lysosomes
- Amyloid formation and secretion
- Increased MMP activity

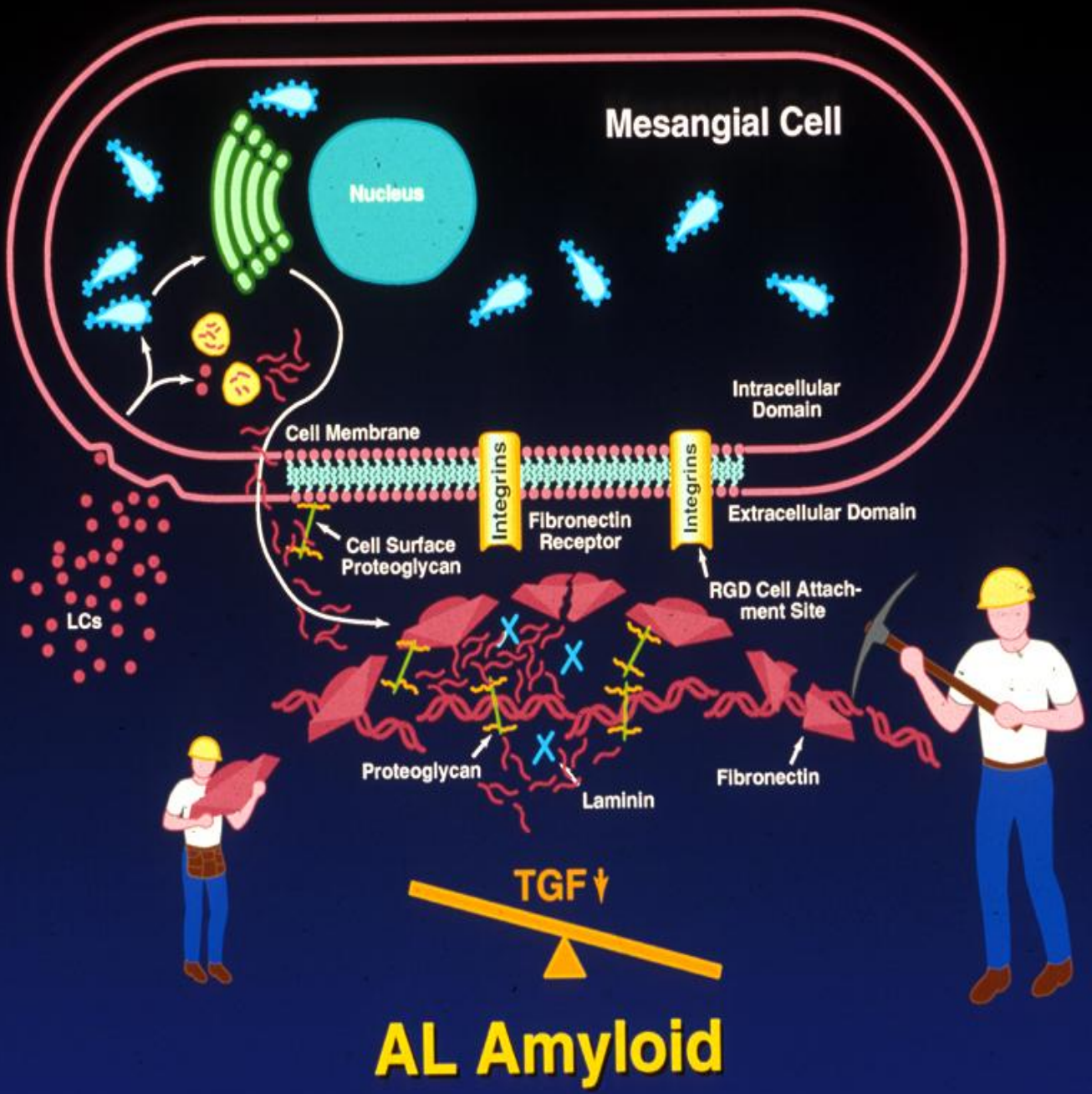


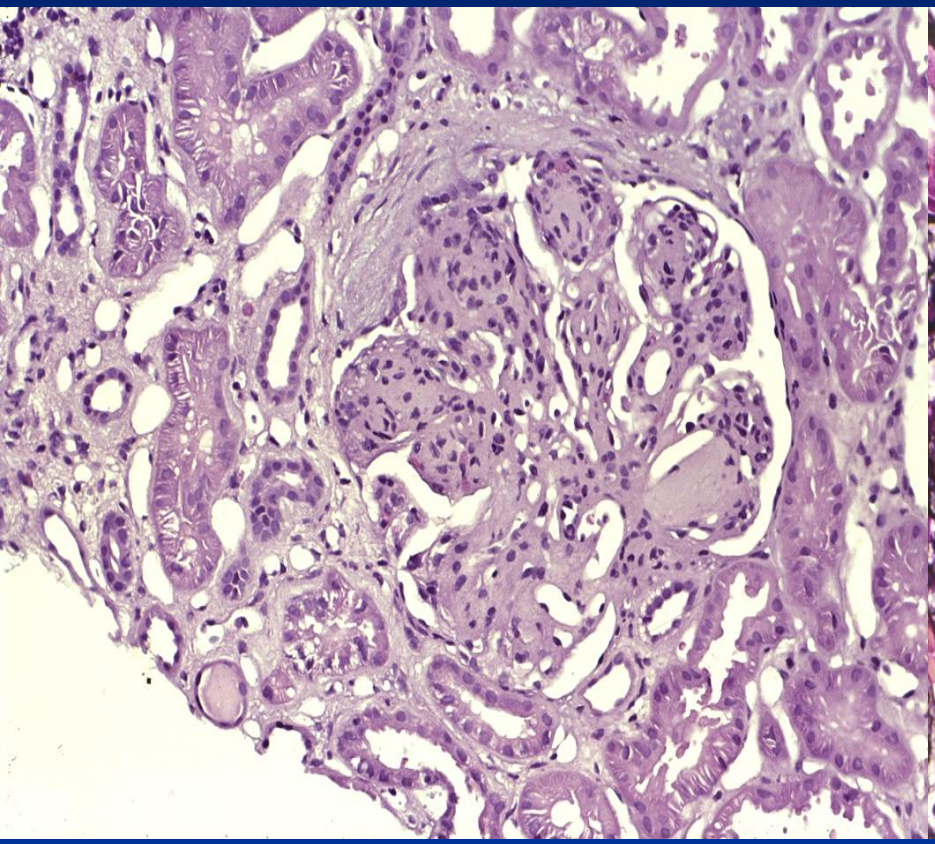
(Left) A+B- Early amyloid formation D-Control TLC (Right) A+B-More advanced amyloid formation C-Lambda LC IH & D- Thioflavin T stain

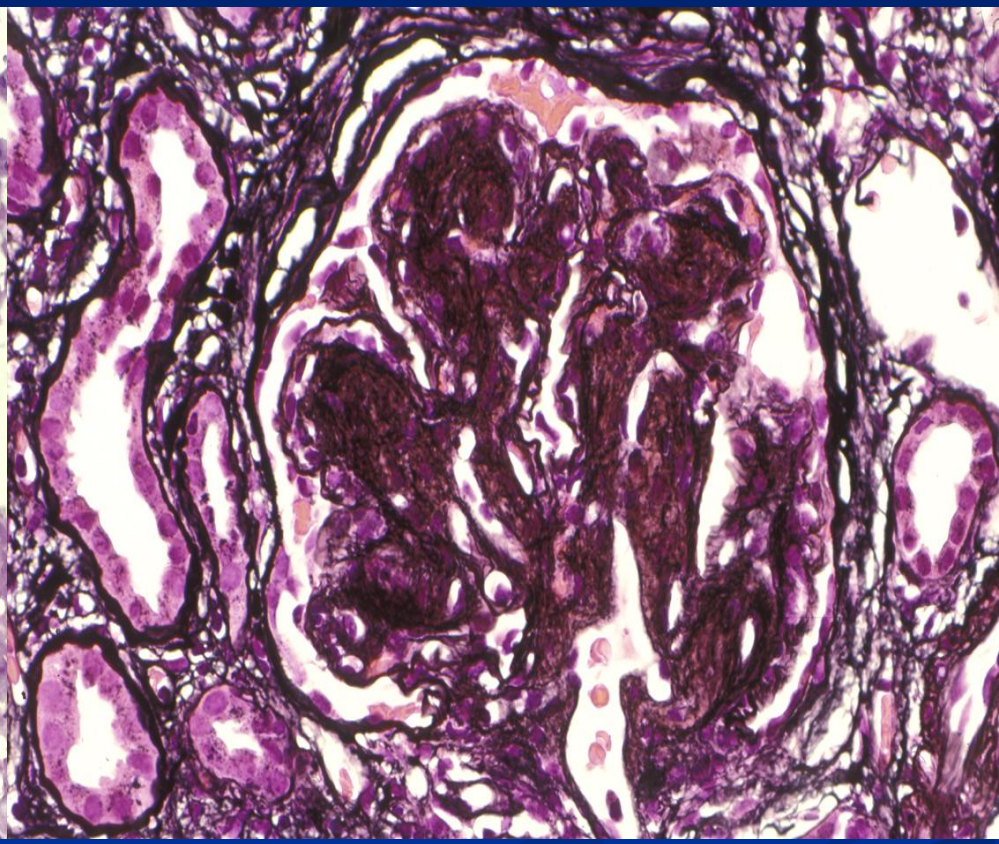


In vitro model of AL-amyloidosis

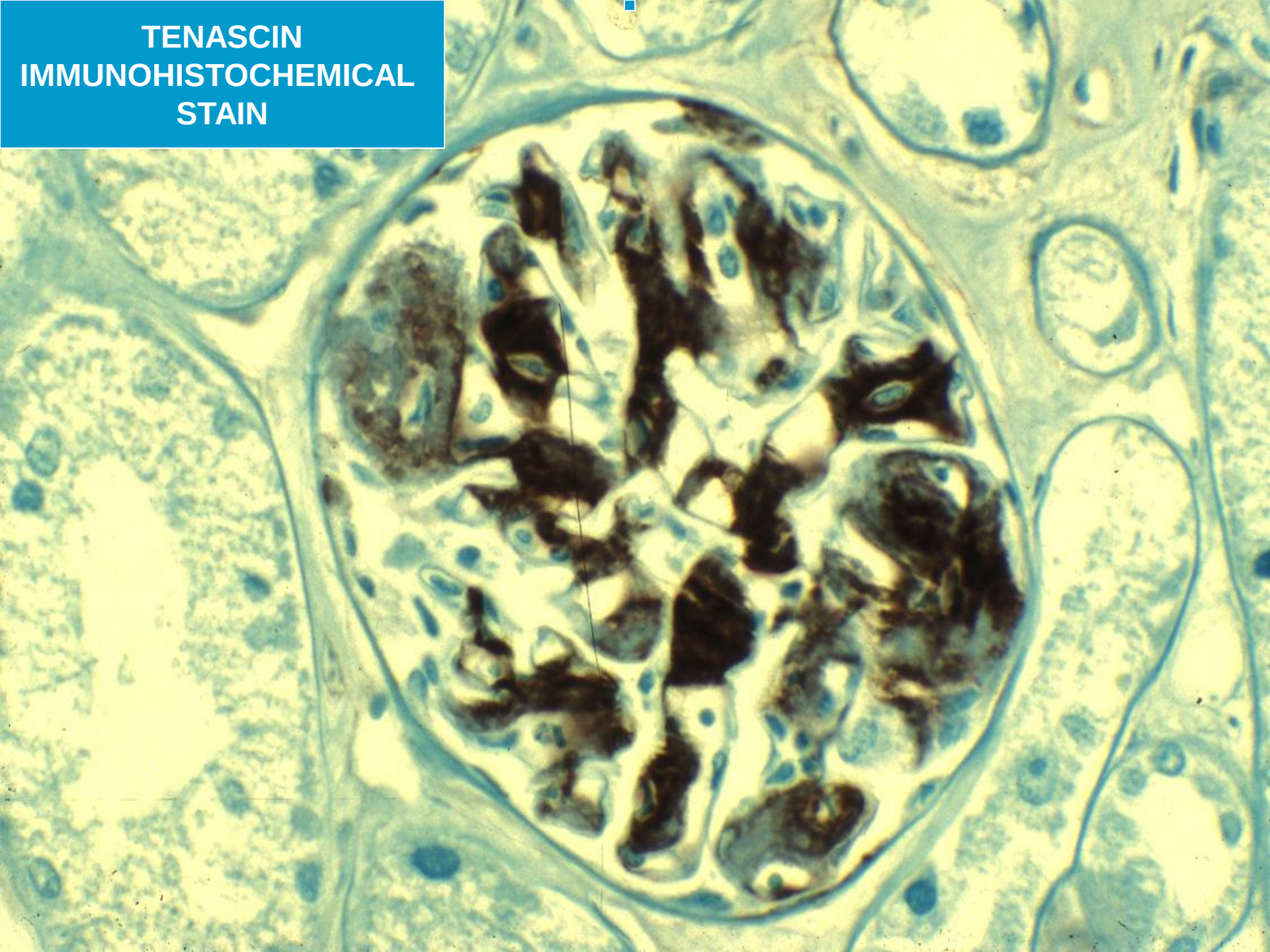

Glomerular amyloid fibrils
Cav-1 KO mouse after penile injection of ALLC for 2W


Mesangial Cell
Nucleus
Intracellular Domain
Cell Membrane
Integrins
Integrins
Extracellular Domain
Fibronectin Receptor
Cell Surface Proteoglycan
RGD Cell Attach-ment Site
LCs
Proteoglycan
Laminin
Fibronectin
TGF
AL Amyloid

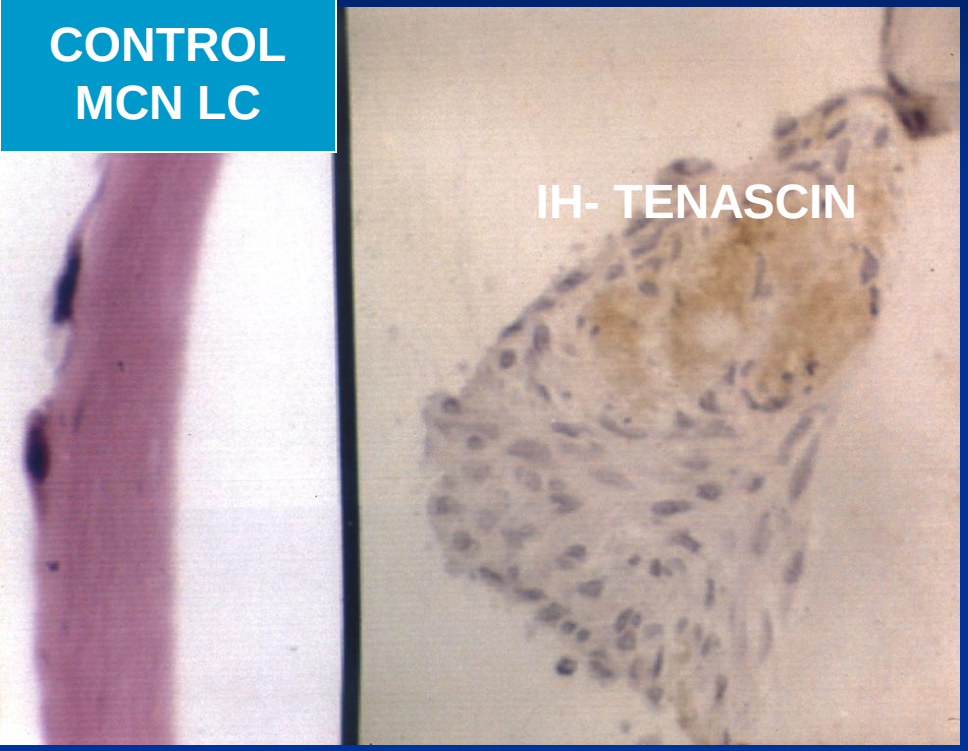




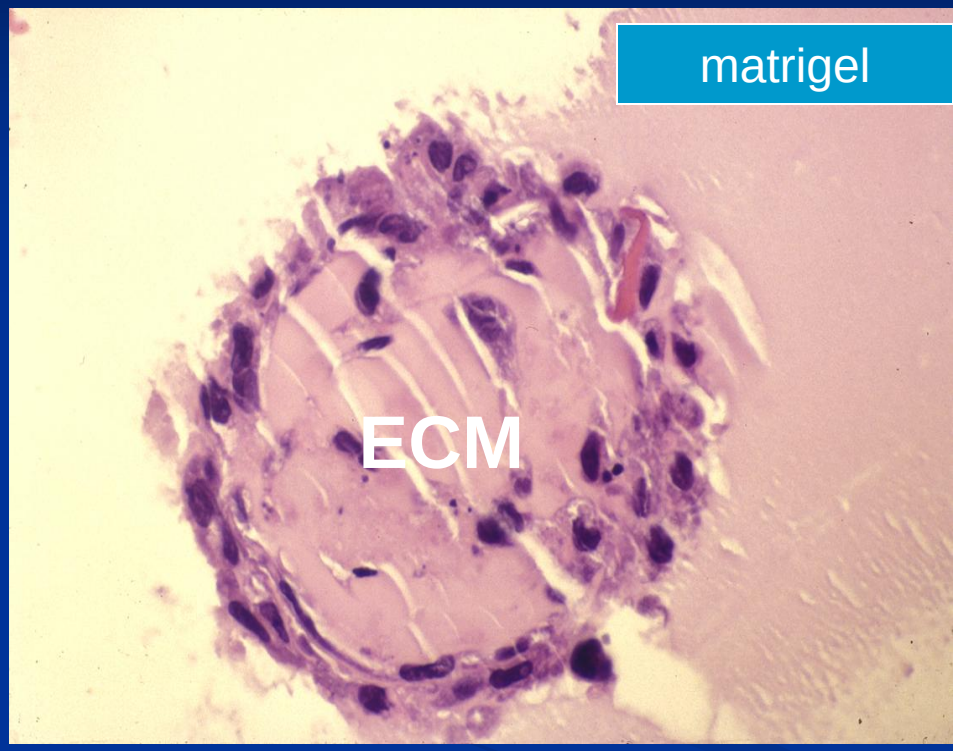
TENASCIN IMMUNOHISTOCHEMICAL STAIN



CONTROL
MCN LC



IH- TENASCIN

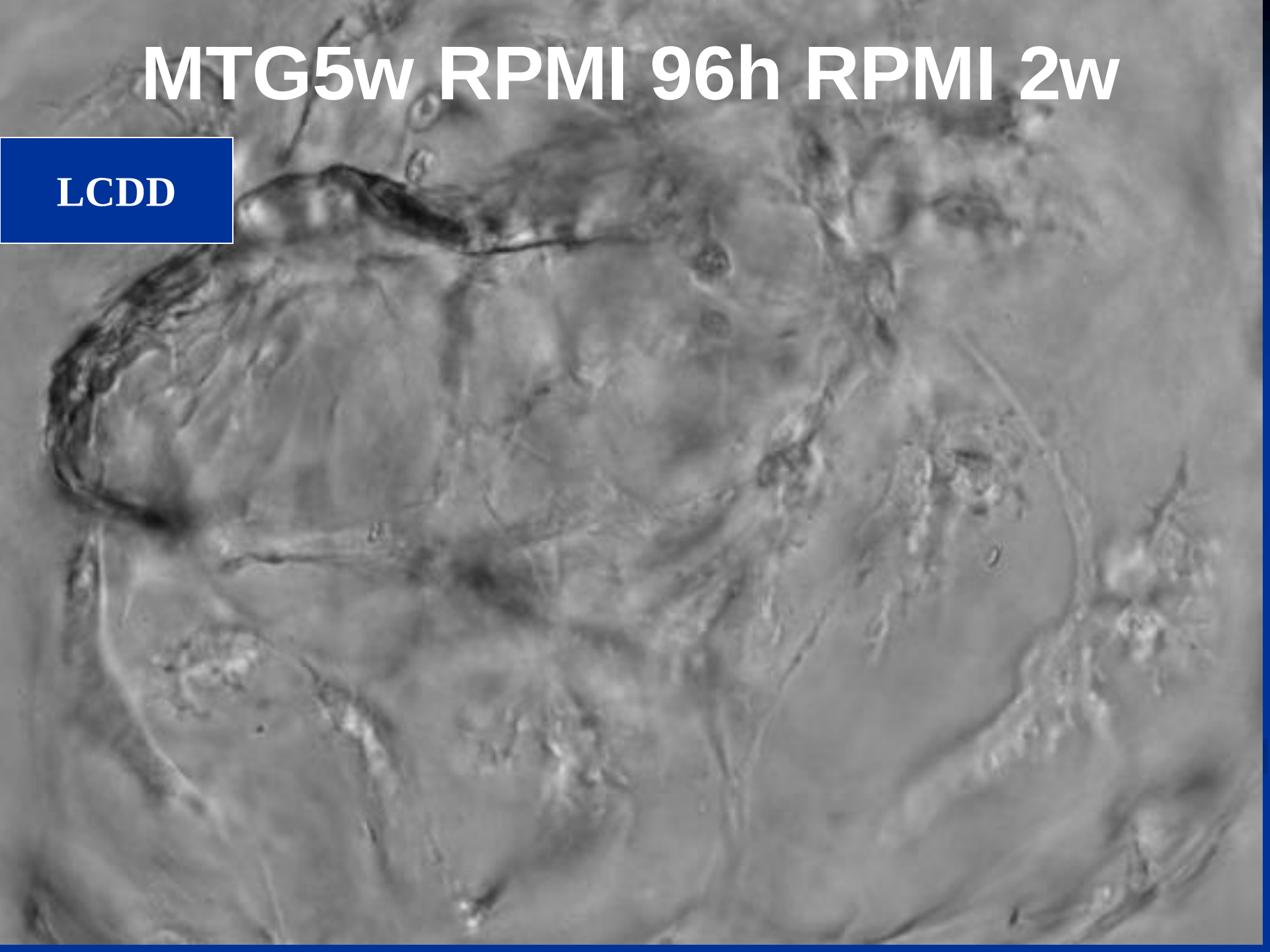


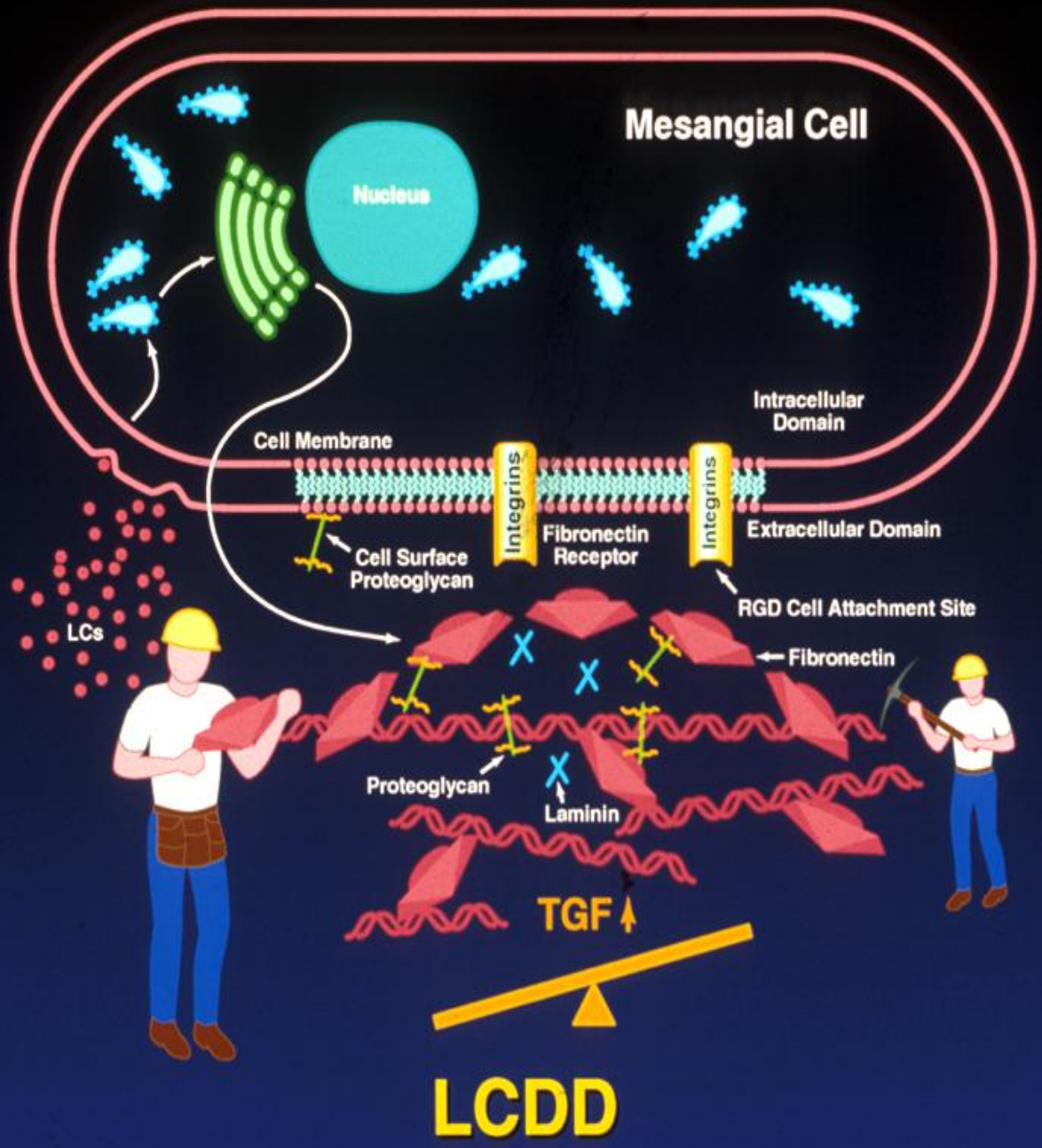
matrigel

ECM

MTG5w RPMI 96h RPMI 2w

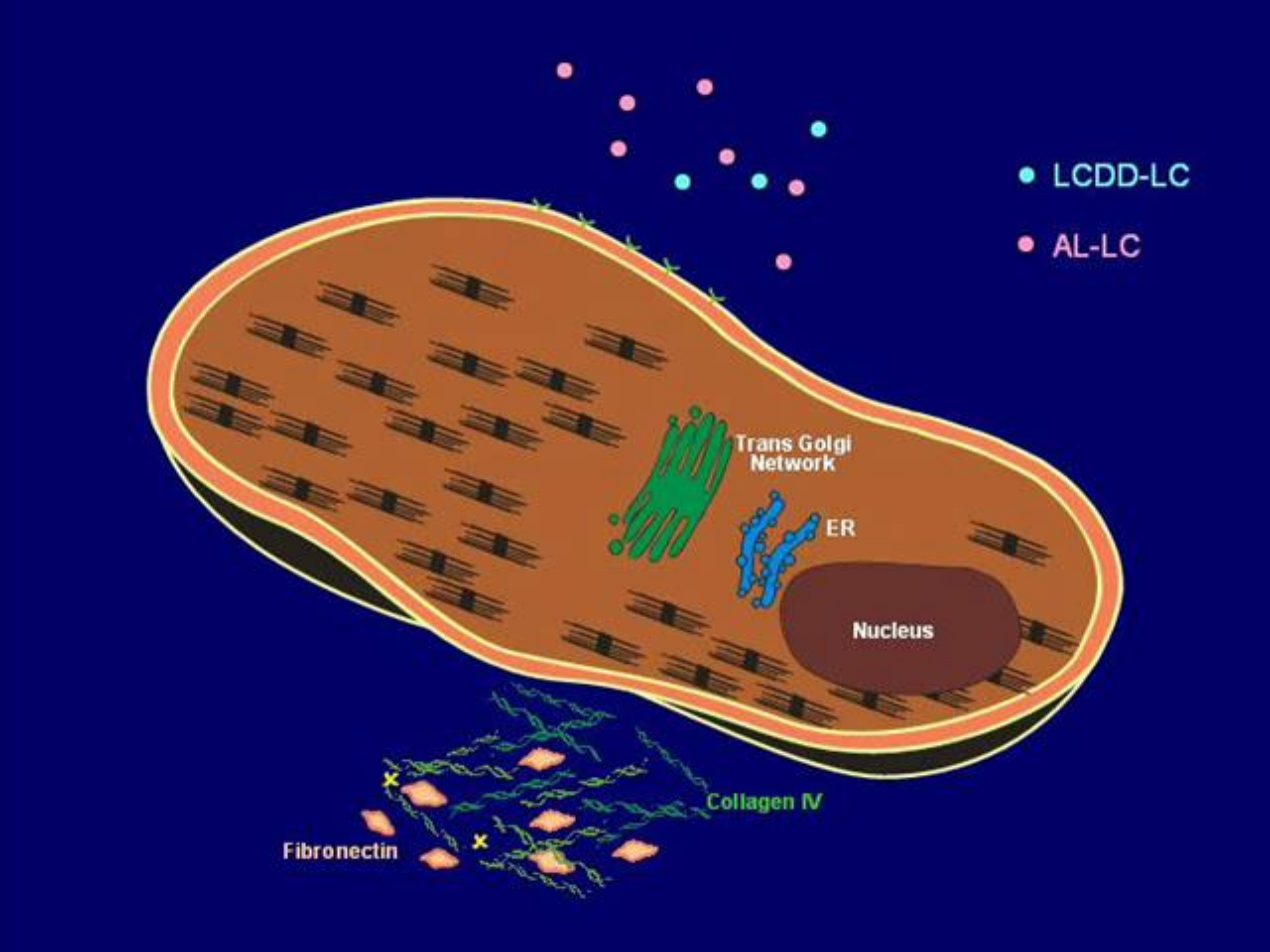
LCDD




Mesangial Cell
Nucleus
Intracellular Domain
Cell Membrane
Integrins
Integrins
Fibronectin Receptor
Extracellular Domain
Cell Surface Proteoglycan
RGD Cell Attachment Site
LCs
Fibronectin
Proteoglycan
Laminin
TGF
LCDD

LIGHT CHAIN DEPOSITION DISEASE
"healing" the damaged mesangium

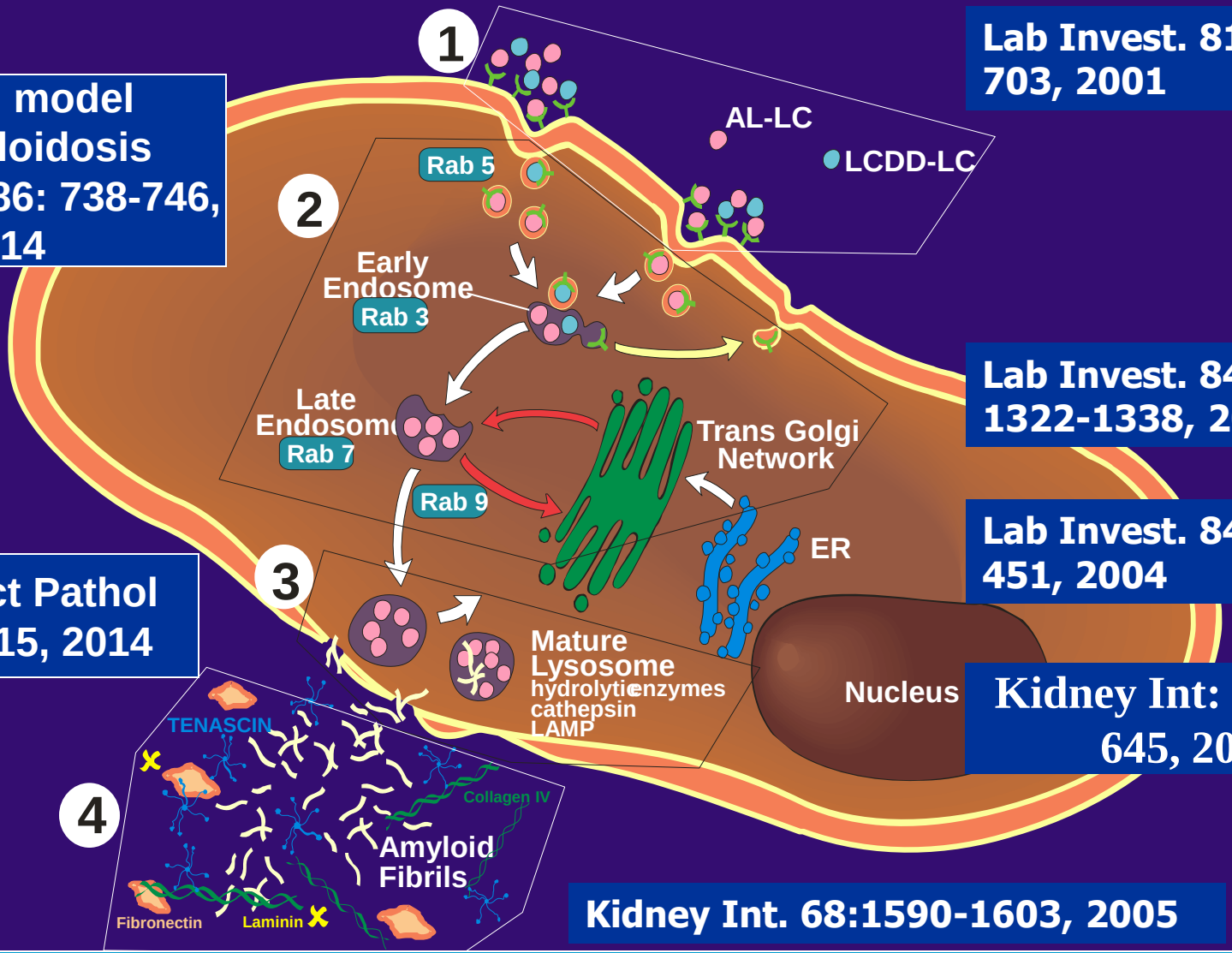
- **Few case reports in the literature of patients who have had their an initial renal biopsy with nodular GS / inciting factor controlled / repeat biopsy showing disappearance of mesangial nodularity**
- **NEED TO DESTROY MATRIX THAT HAS BEEN DEPOSITED BEFORE REPAIR CAN TAKE PLACE**


LCDD-LC
AL-LC
Trans Golgi Network
ER
Nucleus
Collagen IV
Fibronectin

Renal lesions associated with plasma cell dyscrasias: practical approach to diagnosis, new concepts, and challenges. Arch Pathol Lab Med; 133: 249-267, 2009.

Glomerulopathic Light Chain - Mesangial Cell Interactions

Animal model AL-amyloidosis Kidney Int 86: 738-746, 2014



Lab Invest. 81: 869-703, 2001

Lab Invest. 84: 1322-1338, 2004

Lab Invest. 84: 440-451, 2004

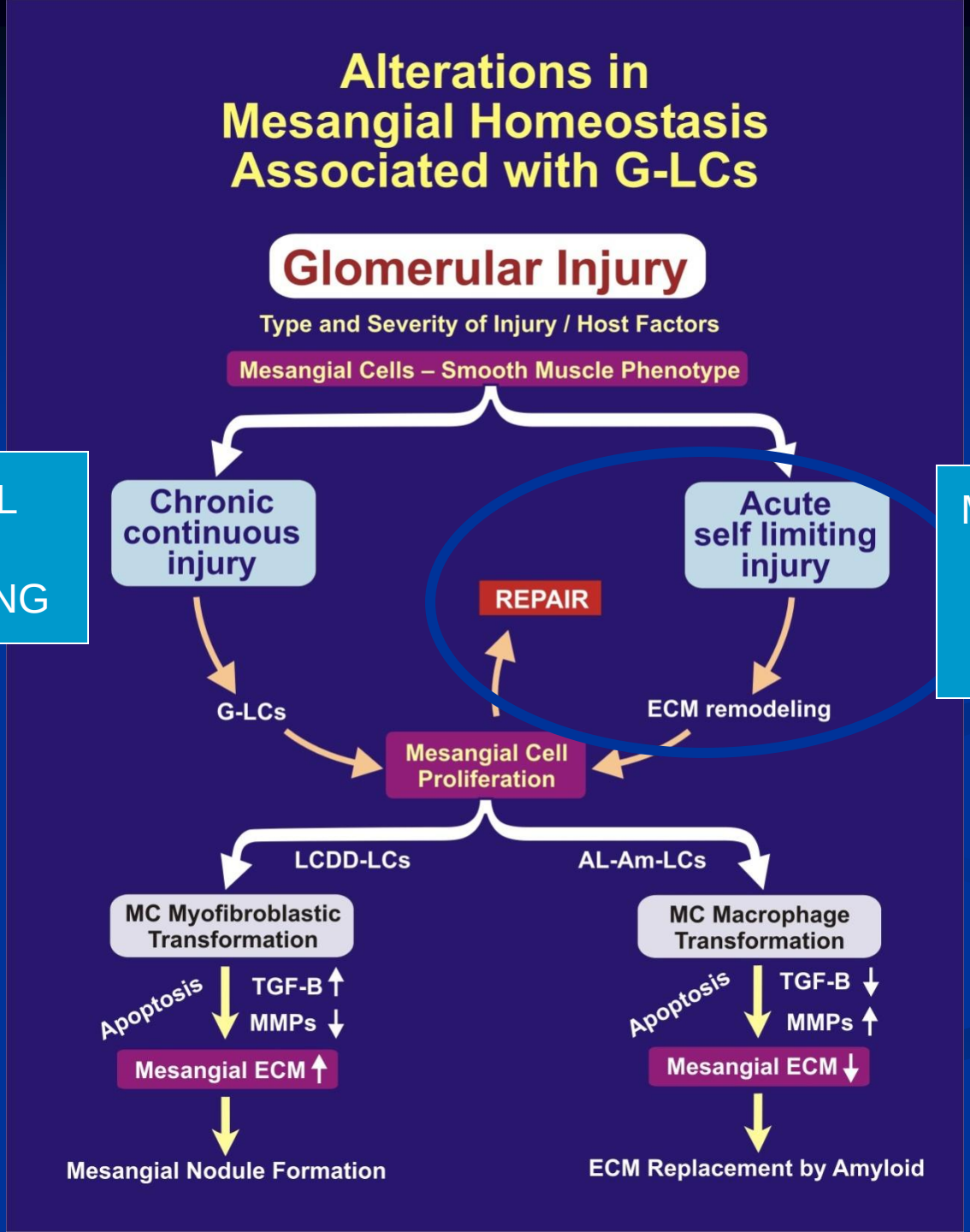
Ultrastruct Pathol 38: 104-115, 2014

Kidney Int: 75: 634-645, 2009

Kidney Int. 68:1590-1603, 2005

EXPERIMENTAL MODELS FOR RENAL DISEASES: Pathogenesis and diagnosis. Contributions to Nephrology series. Karger Pub. 2011

MESANGIAL REPAIR IS CHALLENGING

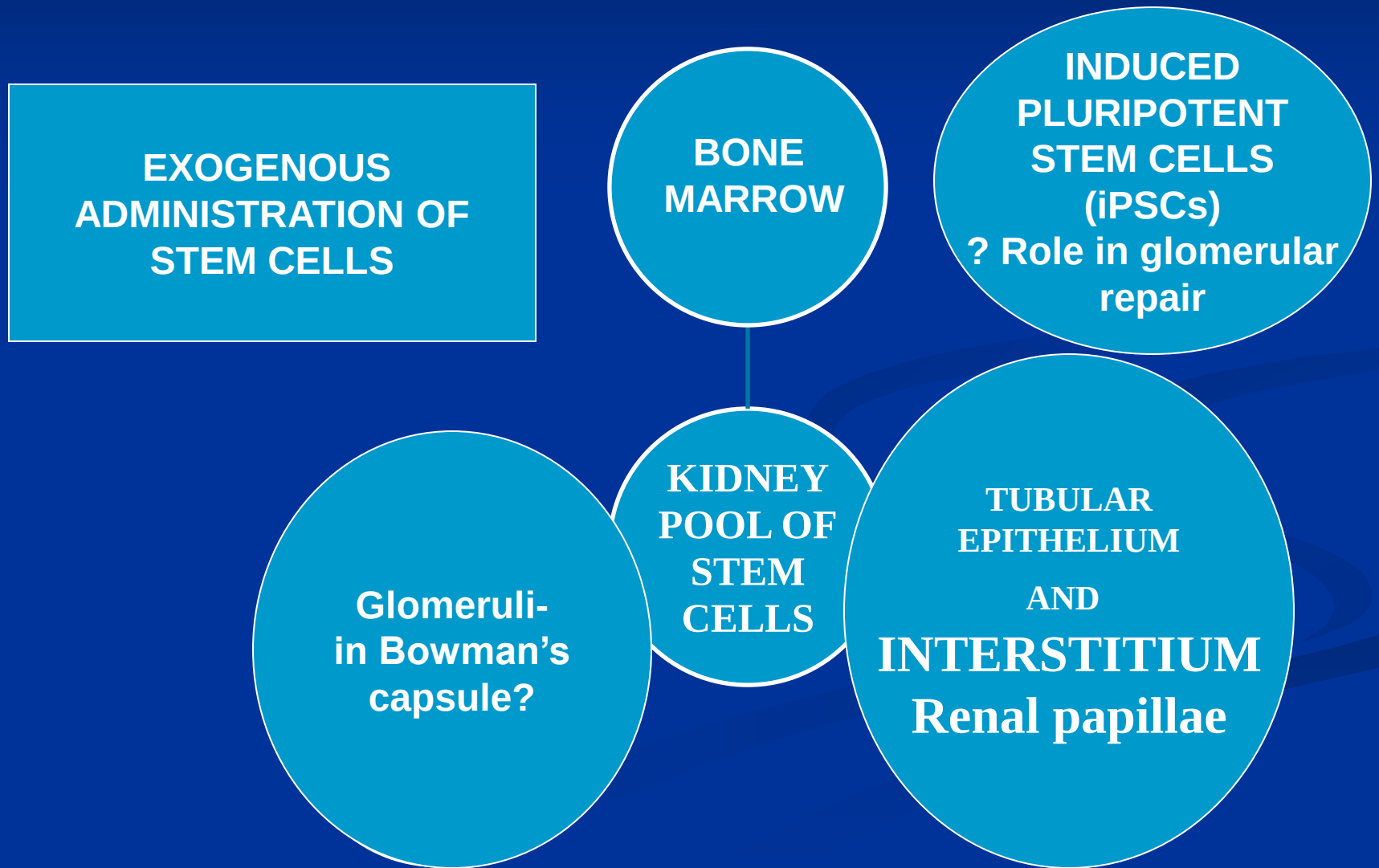


MESANGIAL REPAIR IS VERY LIKELY

WHAT HAPPENS IN THE INJURED MESANGIUM

- DECREASED NUMBERS OF CELLS CAPABLE OF REBUILDING IT
- ACCUMULATION OF MATRIX DIFFICULT TO DISPOSE OF
- ACCUMULATION OF "FOREIGN" MATERIAL AND MATRIX DEBRIS THAT REQUIRES REMOVAL
- ALTERATIONS OF THE MATRIX- ABNORMAL MATRIX
- PRESENCE OF SUBSTANCES THAT INHIBIT REPAIR THAT ALSO REQUIRE REMOVAL

WHERE DO NEW MESANGIAL CELLS MAY COME FROM TO REBUILD DAMAGED MESANGIUM?

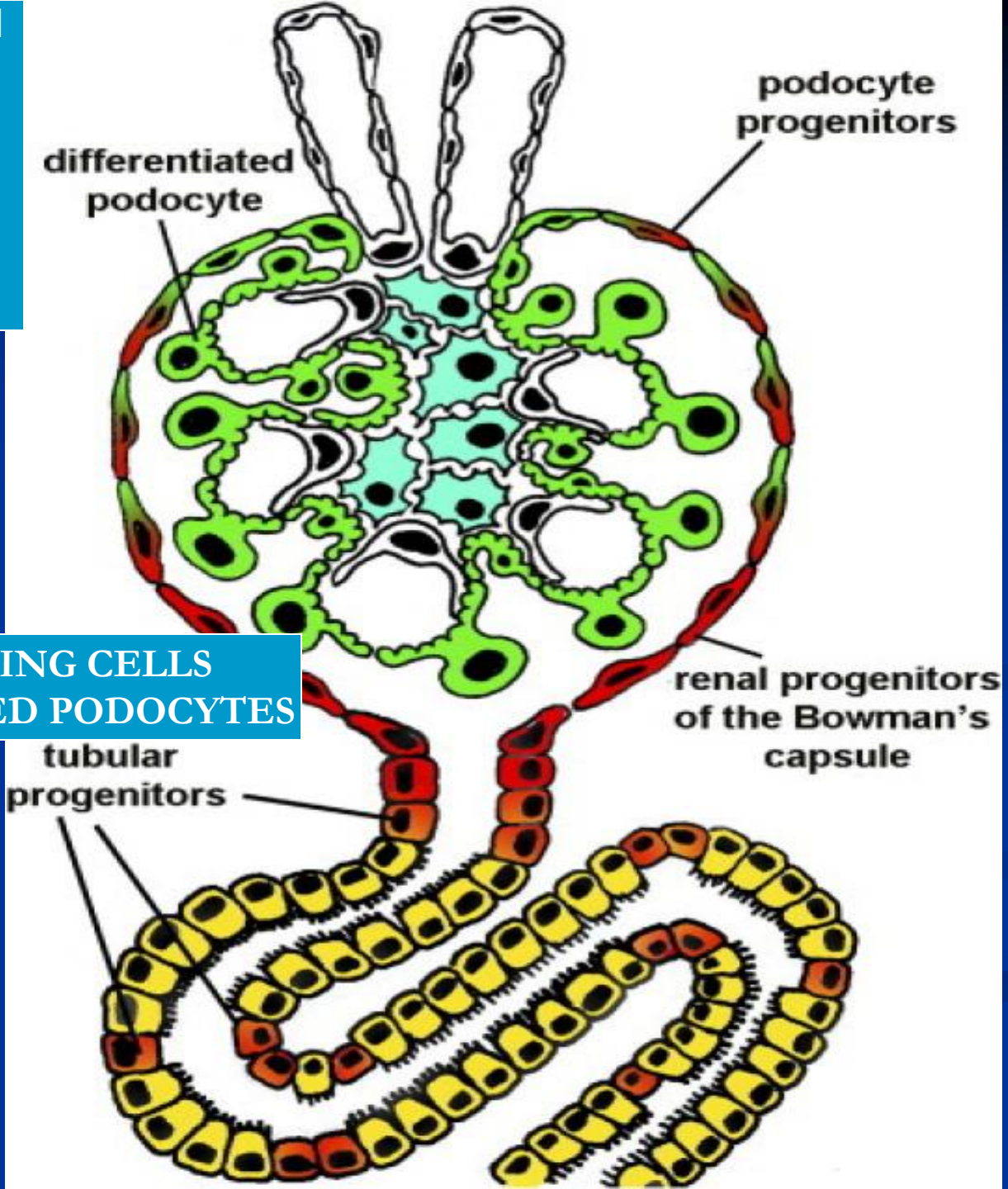


WHERE ARE THE POSSIBLE STEM CELLS IN KIDNEY?

- IN RENAL PAPILLAE- UPPER PAPILLAE- only solid evidence of role in tubular repair
- IN TUBULAR EPITHELIUM AND INTERSTITIUM- only role in tubular healing
- PARIETAL EPITHELIAL CELLS-
- CELLS AROUND MACULA DENSA- cells of renin lineage

? GLOMERULAR HEALING

Romagnani, P: Renal Progenitor of Bowman's capsule and tubular brothers.
Am J Pathol
178: 490-493, 2011.



RENIN-PRODUCING CELLS TO REPLACE INJURED PODOCYTES

Renal cell progenitors in Bowman's capsule (urinary pole)

- Characterized by expression of CD24, CD 133, CK 7 and 19, bcl 2, MYOF (regenerating promoting molecule), vimentin, OCT4 (stem cell transcription factor) and PAX 2
- Can indeed repair injured tubular cells and podocytes
- ROLE IN MESANGIAL CELL REPAIR- WORK IN PROGRESS
- Likely play an important role in cell replacement in aging / certain renal disorders with minor damage

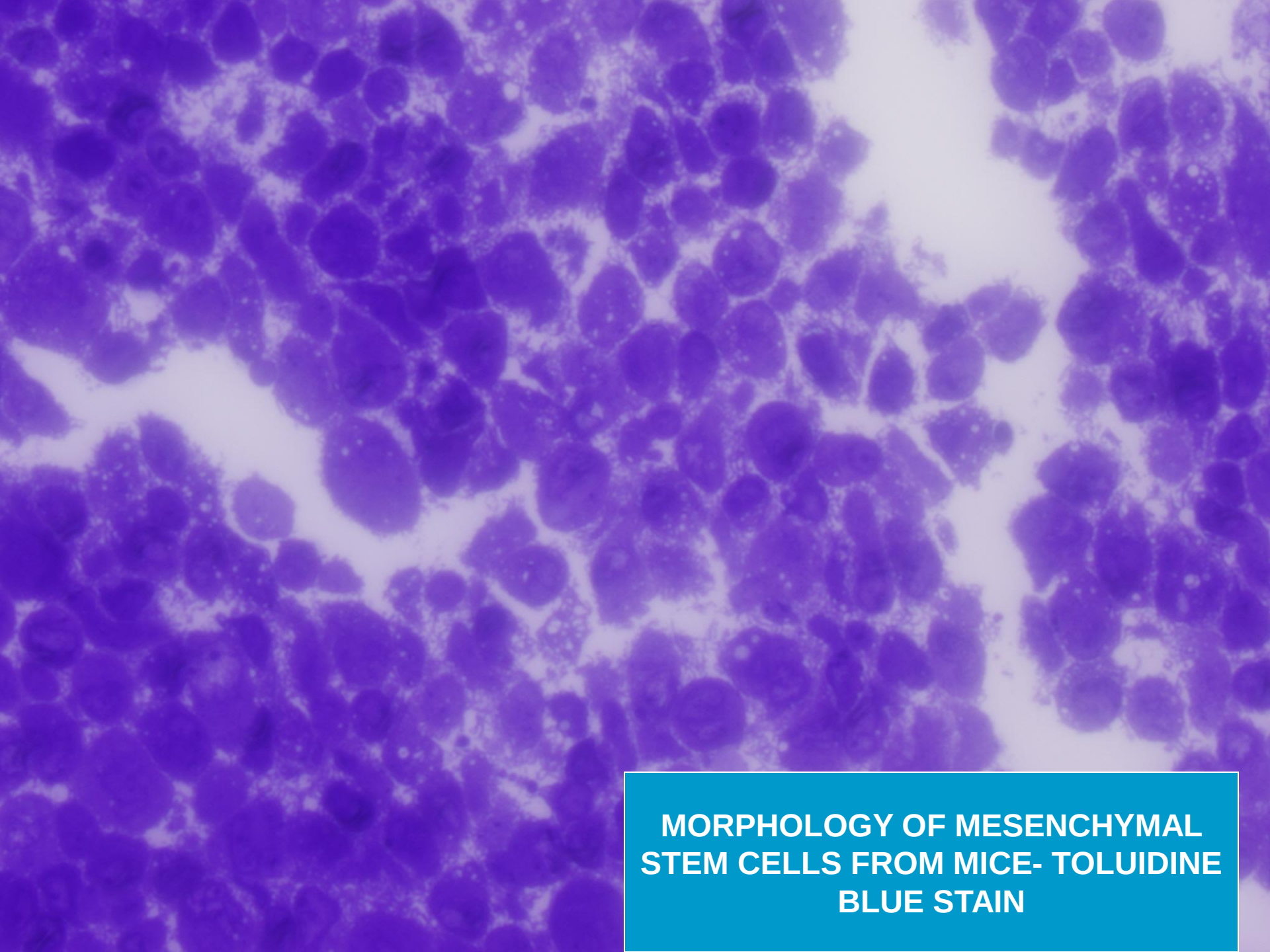
What do we know about mesenchymal stem cells?

- **Bone marrow-derived cells- Less than 0.01% of all bone marrow cells**
- 2004- Suzuki. A. et al- Bone marrow cells can differentiate into mesenchymal cells
- Can repair renal injury to proximal tubular cells (Herrera MB et al. glycerol-induced acute renal failure model, 2004; Lange et al. ischemia-reperfusion model 2005)

  ? which cells are best for MESANGIAL repair- **mesenchymal stem cells** or hematopoieitic stem cells OR resident cells in renal parenchyma

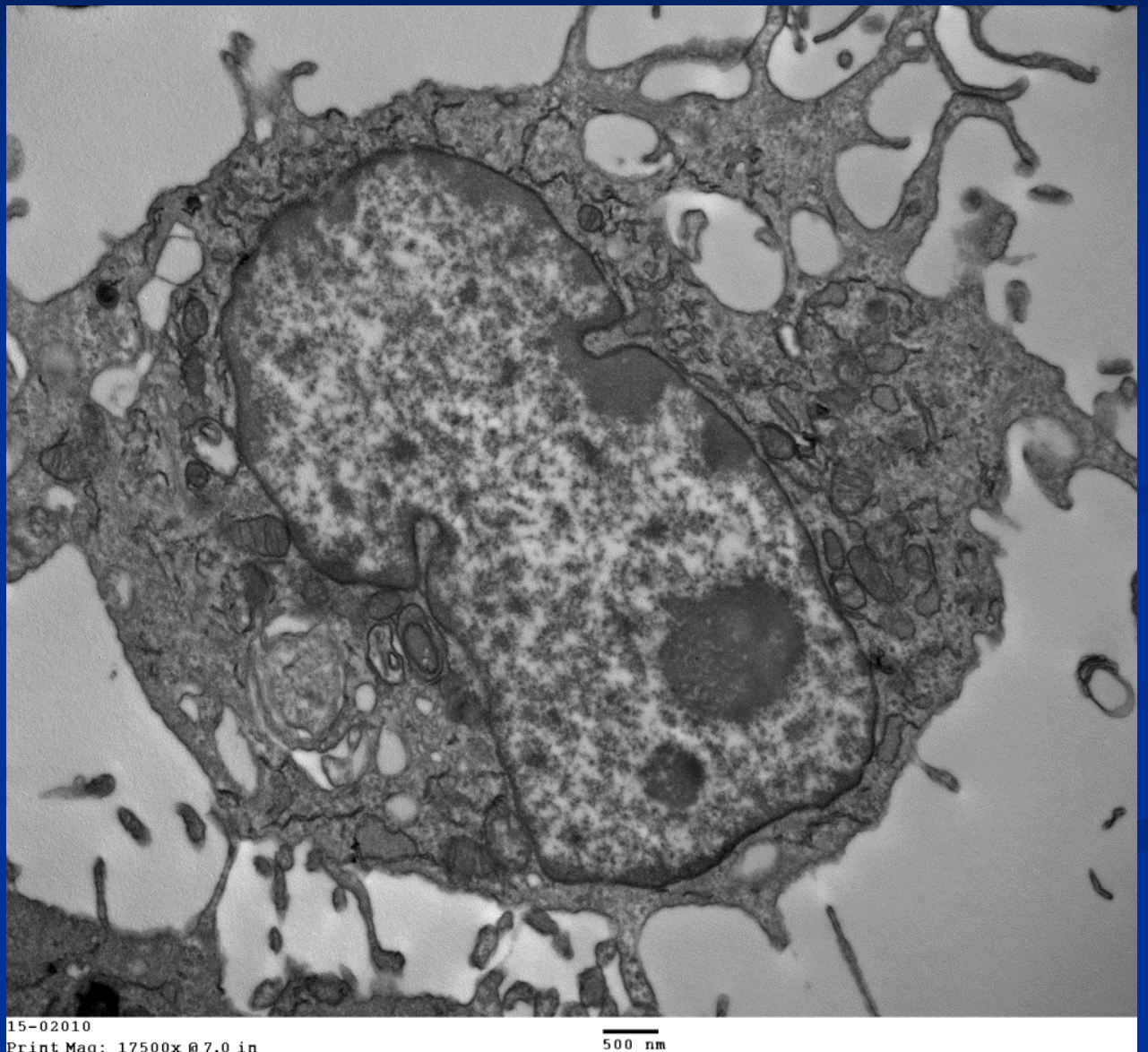
MESENCHYMAL STEM CELLS

- Multipotent bone marrow stromal cells
- Home to sites of tissue damage / inflammation
- Cell signaling transduction is required for determination of fate of MSCs
- Adult tissue stem cells **transdifferentiate** into parenchymal cells from outside of their germ cell layer of origin repairing damaged cells
- Possess immunomodulatory functions
- Do **not** express blood group antigens, MHC Class II antigens or stimulatory factors



MORPHOLOGY OF MESENCHYMAL STEM CELLS FROM MICE- TOLUIDINE BLUE STAIN

Mouse MSC



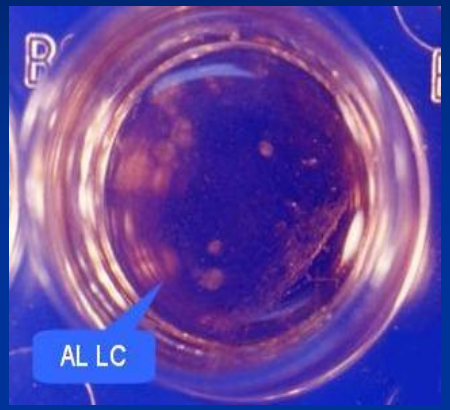
Mesenchymal stem cells
Phase I- Preliminary trials

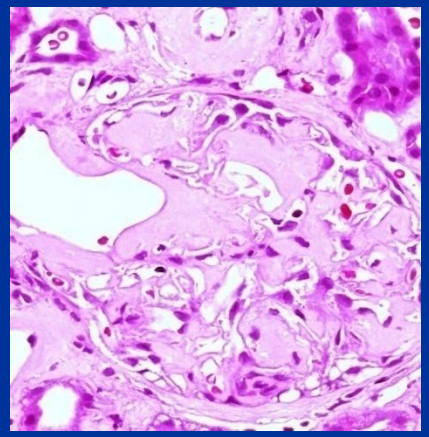
- **Have demonstrated feasibility, efficacy, and safety in animals and humans**
- Allogenic MSCs administered to animals with experimental AKI
- In patients with high risk of AKI undergoing on-pump cardiac surgery (CA bypass or valve surgery)- post-operative infusion of allogenic MSCs into the suprarenal aorta- stable renal function up to 16 months post surgery (20% control pts required dialysis)

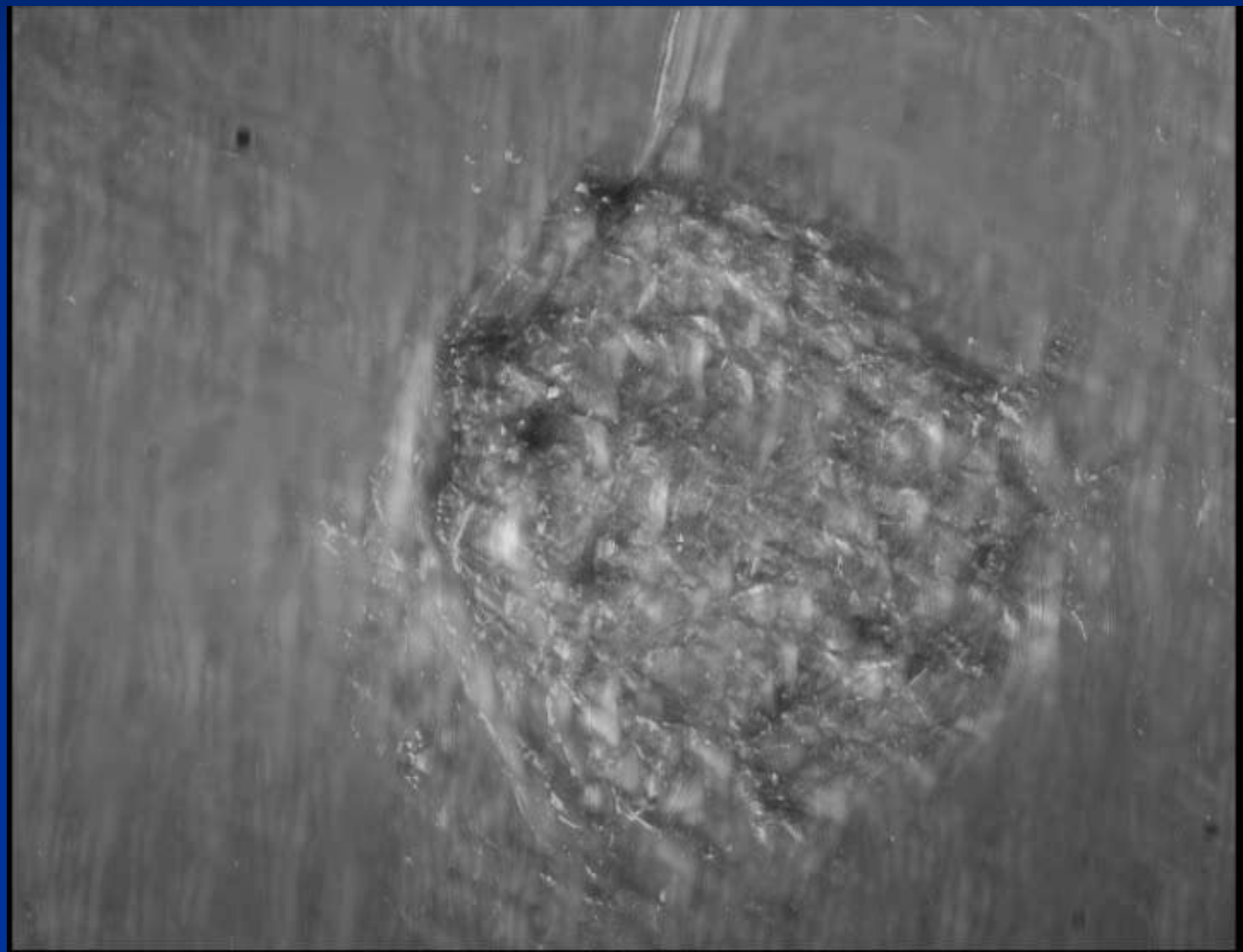
MESENCHYMAL STEM CELLS

- INTERPRET AND RESPOND TO CUES GENERATED BY INJURED RENAL CELLS
- PRODUCE GROWTH FACTORS
- CROSS TALK WITH SURROUNDING CELL TYPES

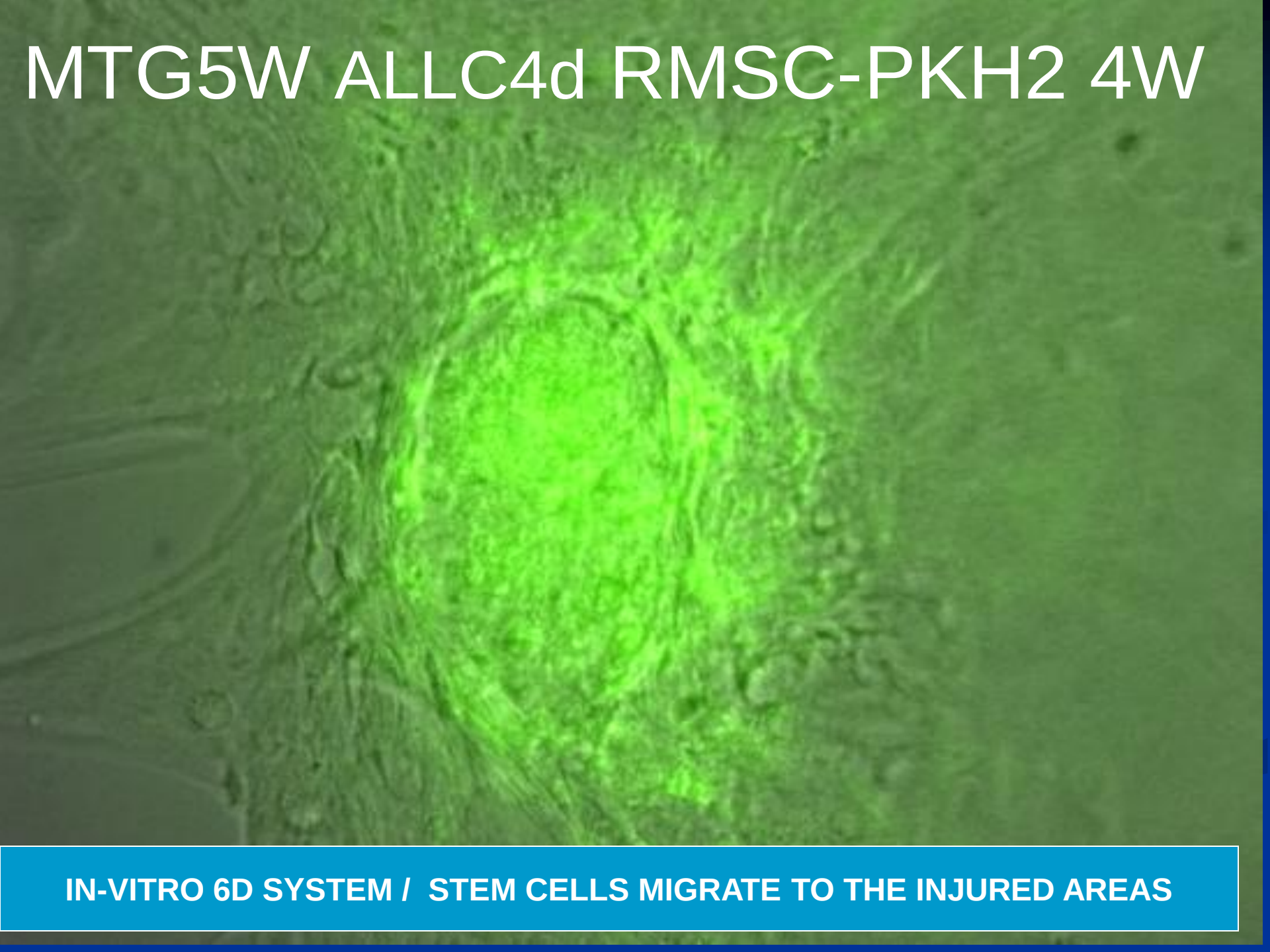
HMCs growing on Matrigel and incubated with AL-LC forming amyloid- FOR 4 days



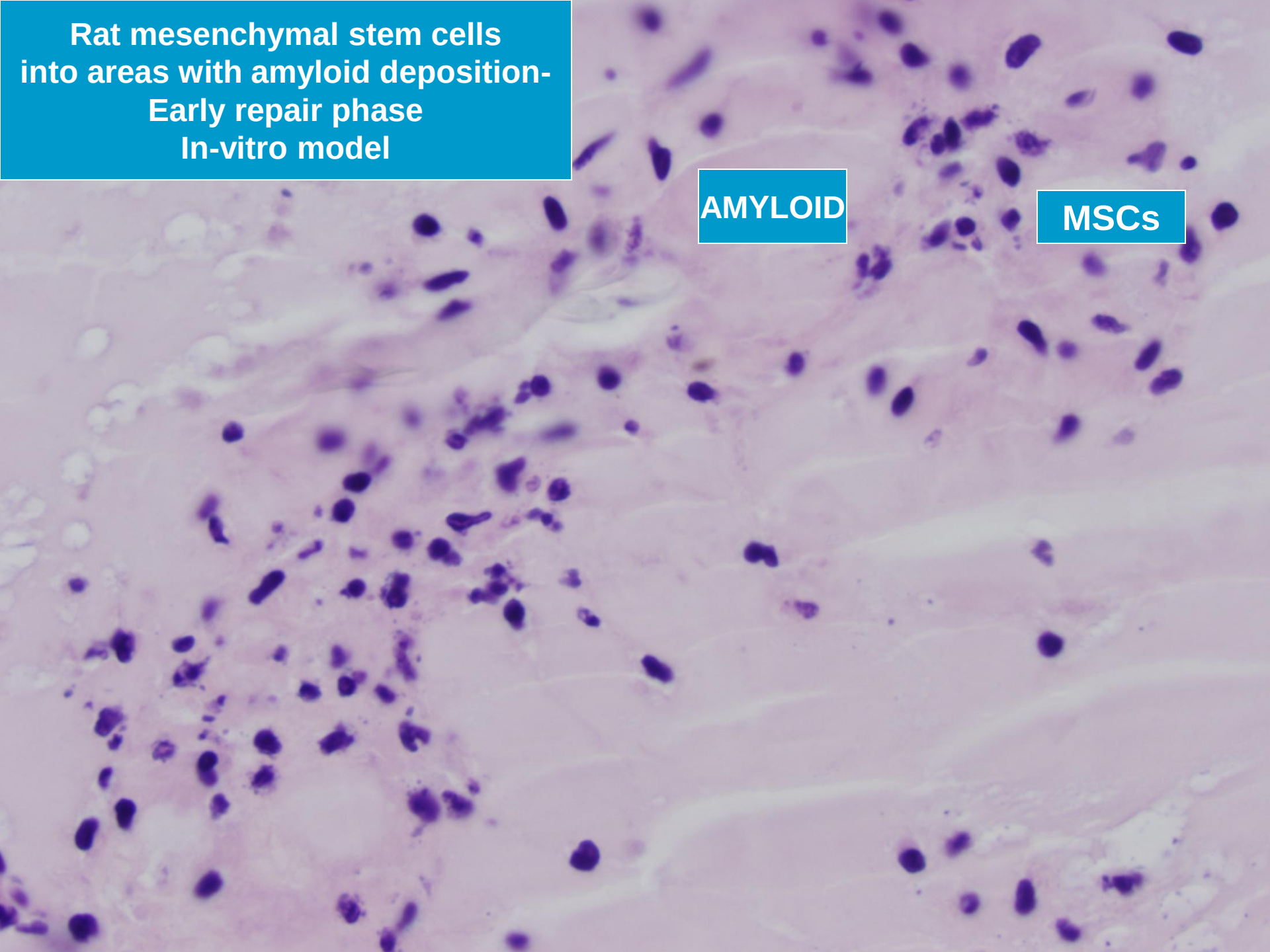




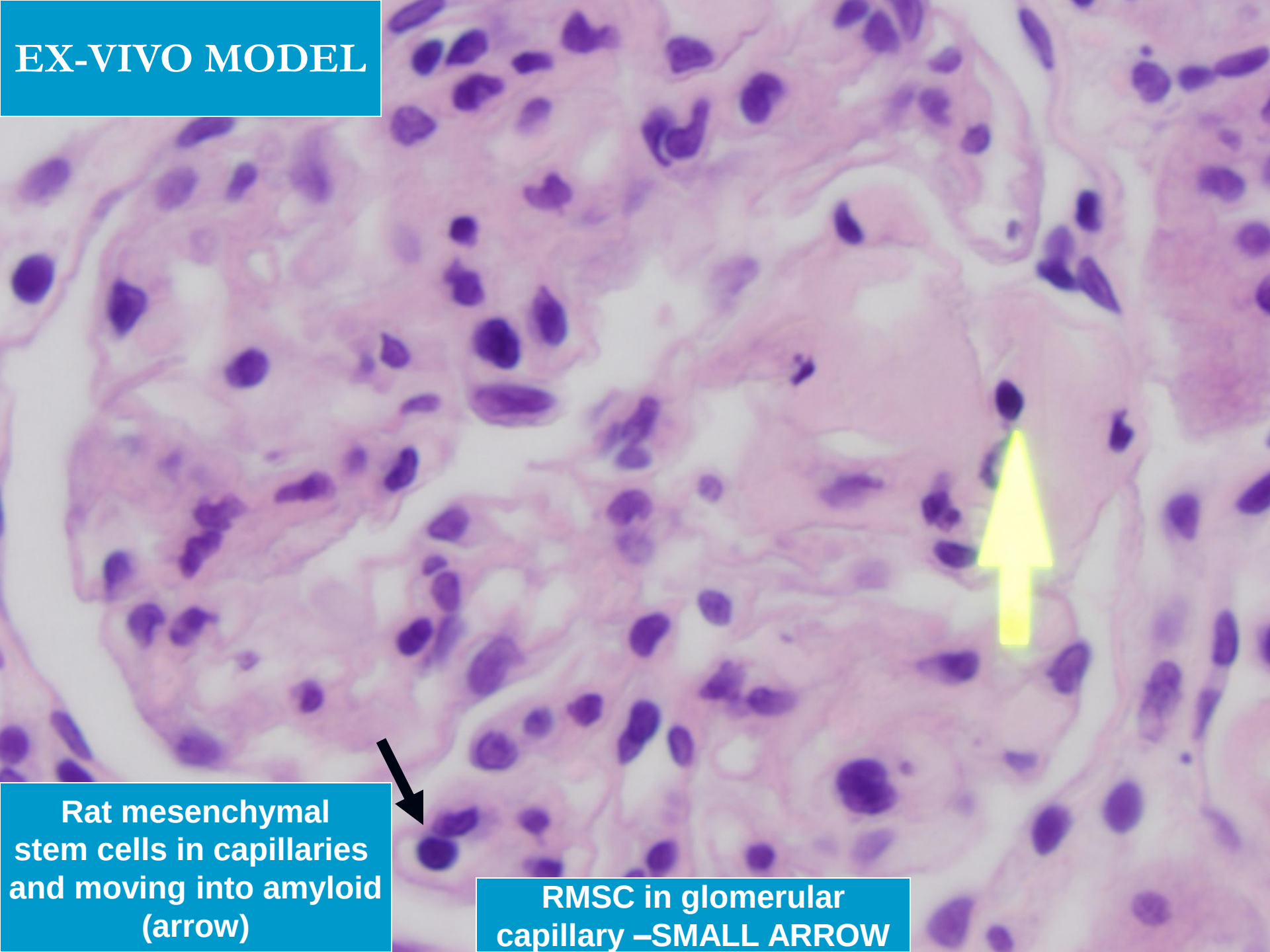
MTG5W ALLC4d RMSC-PKH2 4W



IN-VITRO 6D SYSTEM / STEM CELLS MIGRATE TO THE INJURED AREAS


Rat mesenchymal stem cells
into areas with amyloid deposition-
Early repair phase
In-vitro model
AMYLOID
MSCs

EX-VIVO MODEL

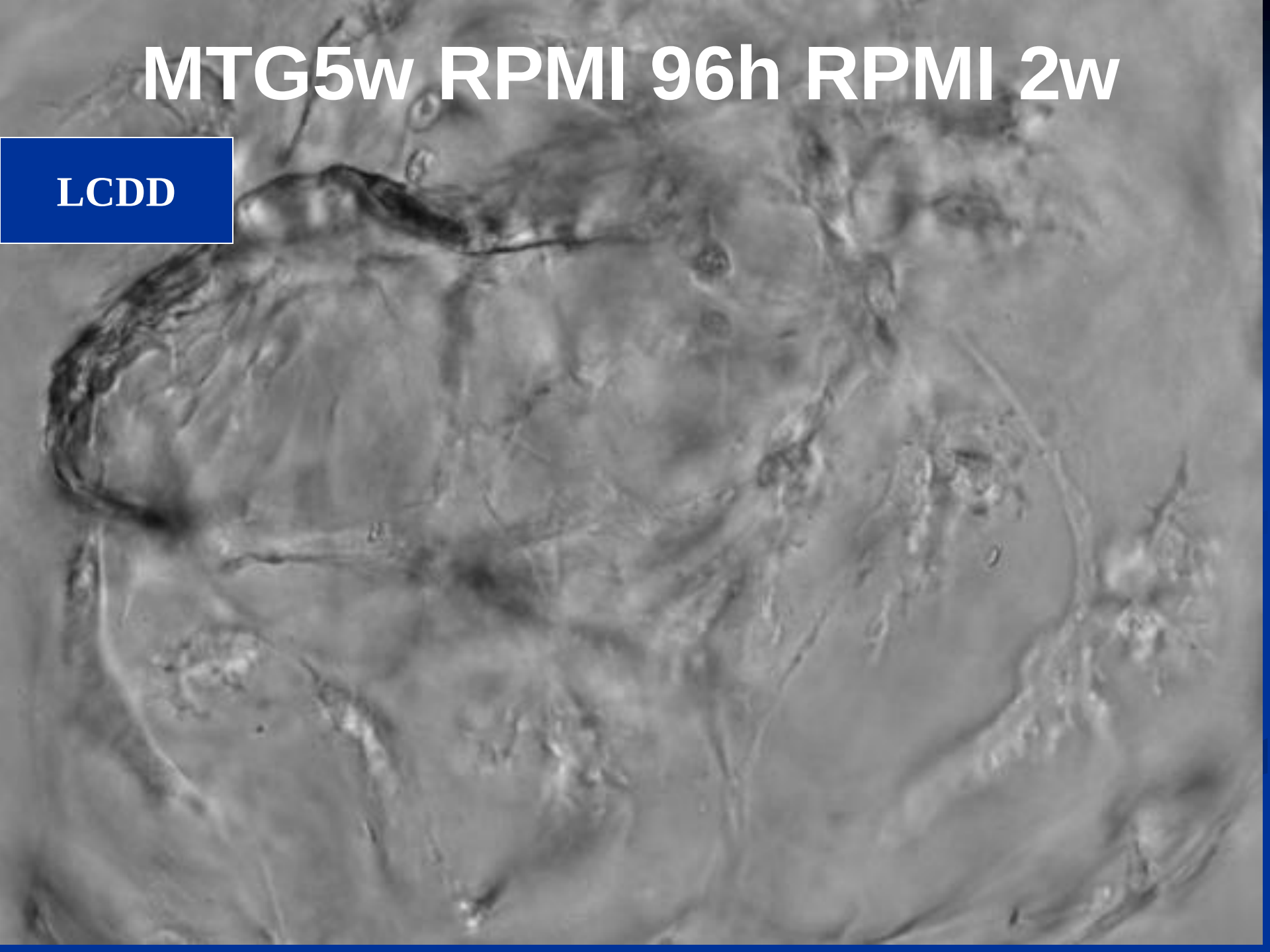


Rat mesenchymal stem cells in capillaries and moving into amyloid (arrow)

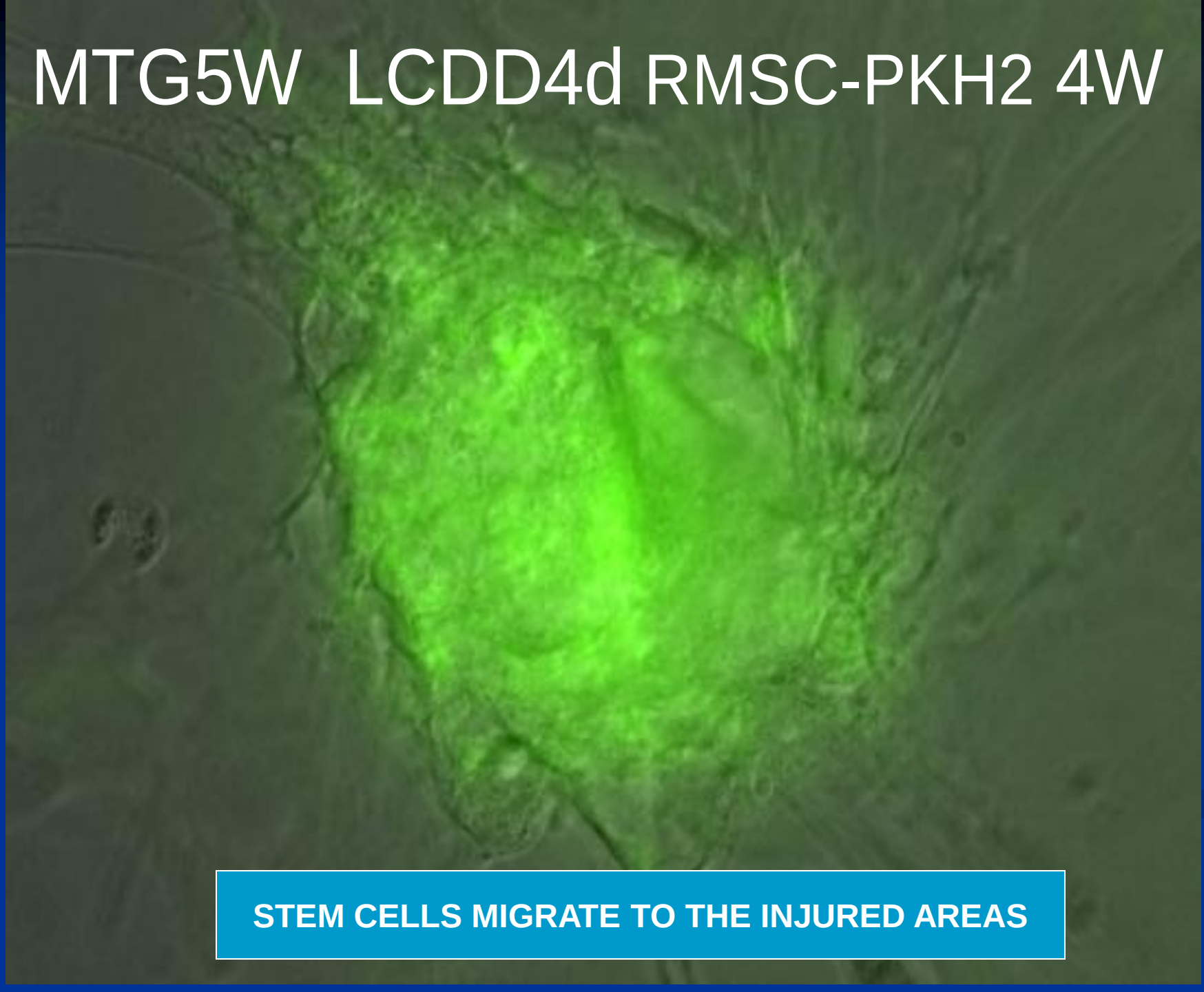
RMSC in glomerular capillary –SMALL ARROW

MTG5w RPMI 96h RPMI 2w

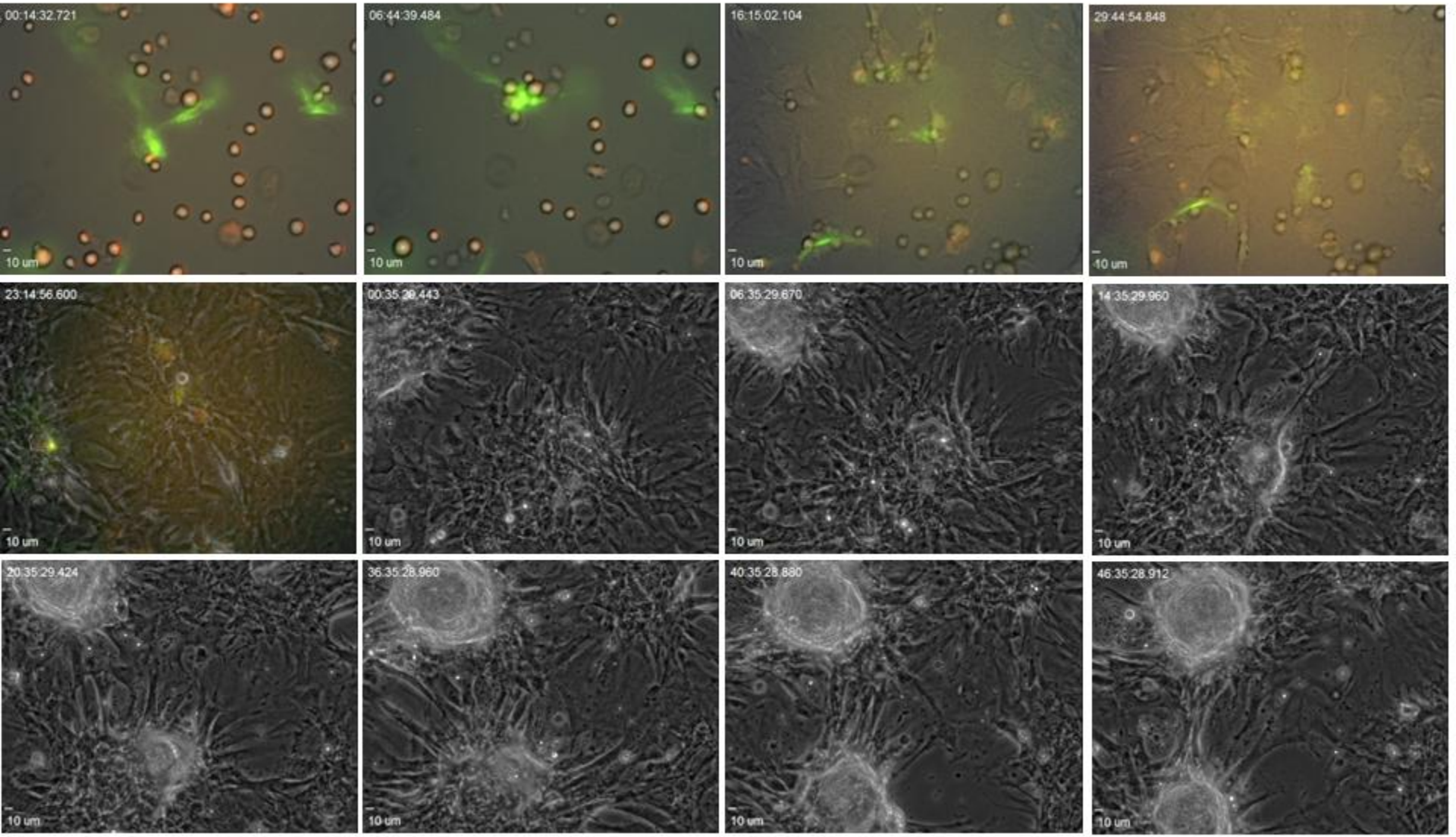
LCDD



MTG5W LCDD4d RMSC-PKH2 4W



STEM CELLS MIGRATE TO THE INJURED AREAS


00:14:32.721
10 um
06:44:39.484
10 um
16:15:02.104
10 um
29:44:54.848
10 um
23:14:56.600
10 um
00:35:29.443
10 um
06:35:29.670
10 um
14:35:29.960
10 um
20:35:29.424
10 um
36:35:28.960
10 um
40:35:28.880
10 um
46:35:28.912
10 um

METALLOPROTEINASES
Paradoxical issues

Can MMPs help prevent or resolve fibrosis in pathological conditions? Both??

Let's review literature on collagenases…

- MMP-9- cleaves collagen IV- degradation of collagen IV activates EMT
- MMP-9 is also a profibrotic agent because it activates TGF-β and endothelin-1
- MMP-2 necessary and sufficient to induce tubular cell EMT in vitro
- transgenic mice overexpressing MMP-2 display fibrotic lesions
- MMPs 2 and 9 HAVE NO MATRIX-DESTRUCTION ACTIVITY ON ALTERED MESANGIUM, since it contains predominantly TENASCIN
- MMP-7 catabolizes TENASCIN- MCs produce MMP-7 but cannot release it in LCDD

MESANGIAL REPAIR

- May need to "provide" new MESANGIAL CELLS TO REPOPULATE THE INJURED MESANGIUM
- May need to clean the "debris" in the mesangium and DISPOSE OF the altered, expanded mesangial matrix
- Need to "heal" the mesangial matrix- replace by normal matrix in terms of biochemical composition to restore functionality (? by stem cells)
- **Need to eliminate or control the injurious agent/s**
- **Need to return to MESANGIAL HOMEOSTASIS**

LITERATURE

- **2006- Kunter et al (Floege's group, Germany)** Transplanted mesenchymal stem cells accelerate glomerular healing in experimental glomerulonephritis. J Am Soc Nephrol 17: 2202-2212
- **2008- Wong et al (Malaysia)- Differentiation of human mesenchymal stem cells into mesangial cells in post-glomerular injury murine model.** Pathol 40: 52-57
- **2013/14- Wong et al- In vitro differentiation of mesenchymal stem cells into mesangial cells when co-cultured with injured mesangial cells.** Cell Biol Int 2014 e-pub Dec 2013
- **2011- Herrera GA, Turbat-Herrera, EA, Teng, J:** Glomerular repair: Present status and future expectations. **MSCs differentiate into mesangial cells.** Exp Mod Renal Dis (Herrera GA, Ed) In Contrib Nephrol #169, pp351-362

Kunter et al.: Transplanted mesenchymal stem cells accelerate glomerular healing in experimental glomerulonephritis. J Am Soc Nephrol 2006; 17: 2202-2206

- Anti-Thy1.1 model of mesangioproliferative GN with mesangiolysis
- … **PARACRINE EFFECT OF MESENCHYMAL STEM CELLS GOVERNS THE REPAIR THAT TAKES PLACE IN GLOMERULI and possibly recruitment of chemoattractants and "feeders" for circulating hematopoeitic stem cells-**

  conclusions reached at day 6 of repair process

  … results **DID NOT SUPPORT** those of Nagaya et al.: MSCs improve cardiac function by transforming into cardiac myocytes (2004); Imasawa et al.: Bone marrow-derived cells differentiate into glomerular mesangial cells (2001)- Both studies showing MSCs differentiating into mature cellular elements and behaving as such

MESENCHYMAL STEM CELLS GROWTH FACTORS PRODUCTION

- Vascular endothelial growth factor (VEGF)
- Transforming growth factor-β (TGF-β)
- Fibroblast growth factor (FGF)

- Do not appear to secrete PDGF-BB, a significant growth factor needed for cellular proliferation in the mesangium and for mesangial development

Wong et al- Differentiation of mesenchymal stem cells into mesangial cells in post-glomerular injury murine model (2008)

MSCs isolated from bone marrow

Athymic mice infused with anti-mouse mesangial cell sera inducing a self-limiting glomerulonephritis with **MESANGIOLYSIS and mesangial cell apoptosis**

Then hMSCs were injected

hMSCs differentiated into mesangial cells with proliferative phenotype (Ki 67 +)

Wong, et al- MSCs differentiate into mesangial cells when co-cultured with injured mesangial cells (2014)

- MSCs obtained from bone marrow
- Mesangial cells injured with hydrogen peroxide
- **Injured mesangial cells plated and then co-cultured with MSCs for 7 days**
- Immunophenotyping / Contraction studies with angiotensin to prove differentiation
- **MSCs differentiated into MCs**

Introducing MSCs into the damaged mesangium

- Rat MCs cultured on Matrigel loaded plates (10% FBS/RPMI) until confluence / MCs made quiescent (incubated 48 hours in 0.5% FBS/RPMI)
- Incubated with LCDD and AL-Am LCs (10ug/ml) purified from the urine of patients with these diseases (renal biopsy proven) for 96 hours
- **Rat mesenchymal stem cells LABELED with PKH-2 fluorescein or Texas Red & placed into wells of GLCs-treated MCs for up to 5 weeks in the in-vitro and for up to 3 weeks in the ex-vivo experimental platforms**
- Observe process using a 6D living cell model to monitor events taking place at specified intervals with sequential photos and using IF and EM in the ex-vivo platform

Research team

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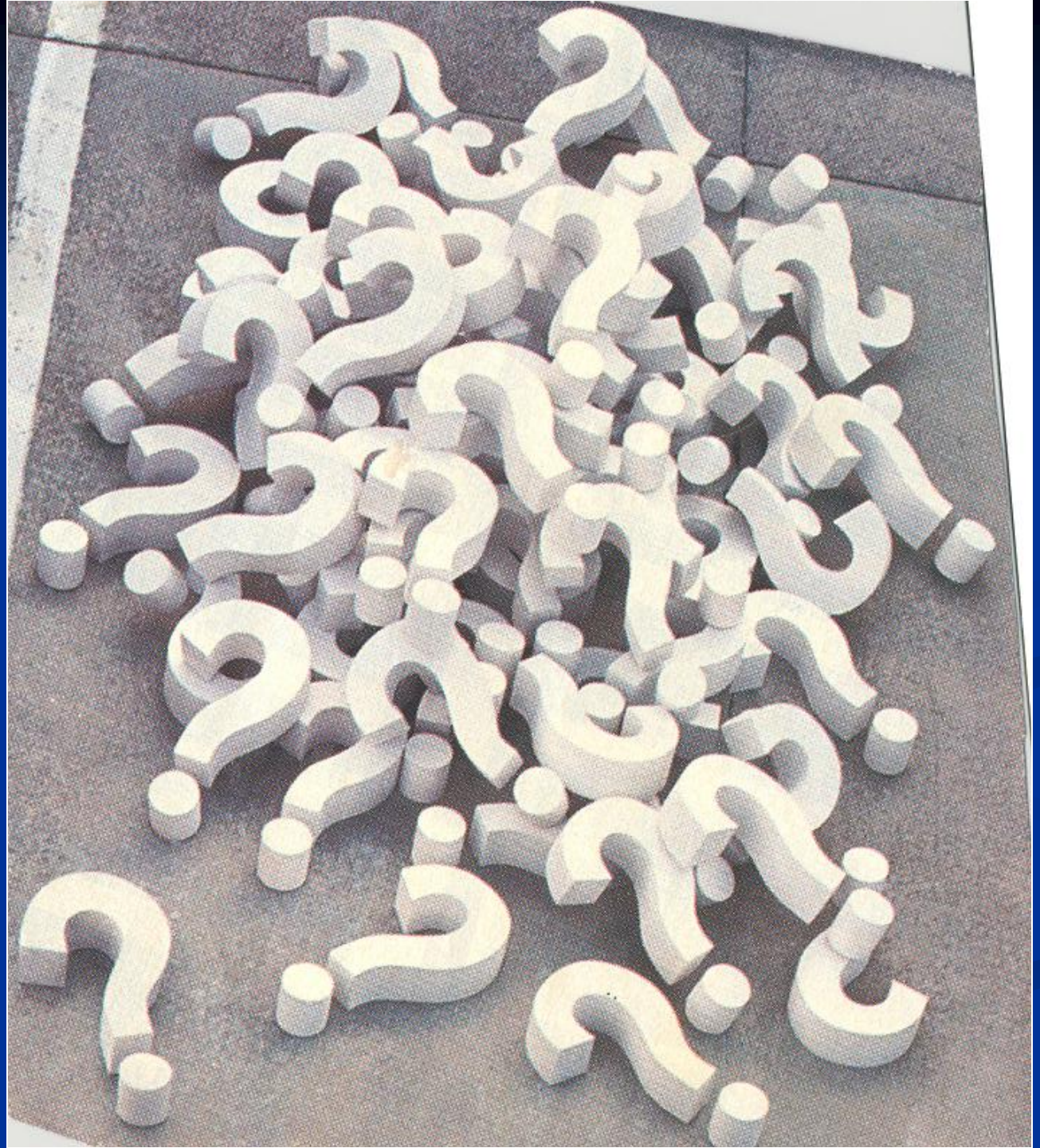
- Raymond Comenzo MD

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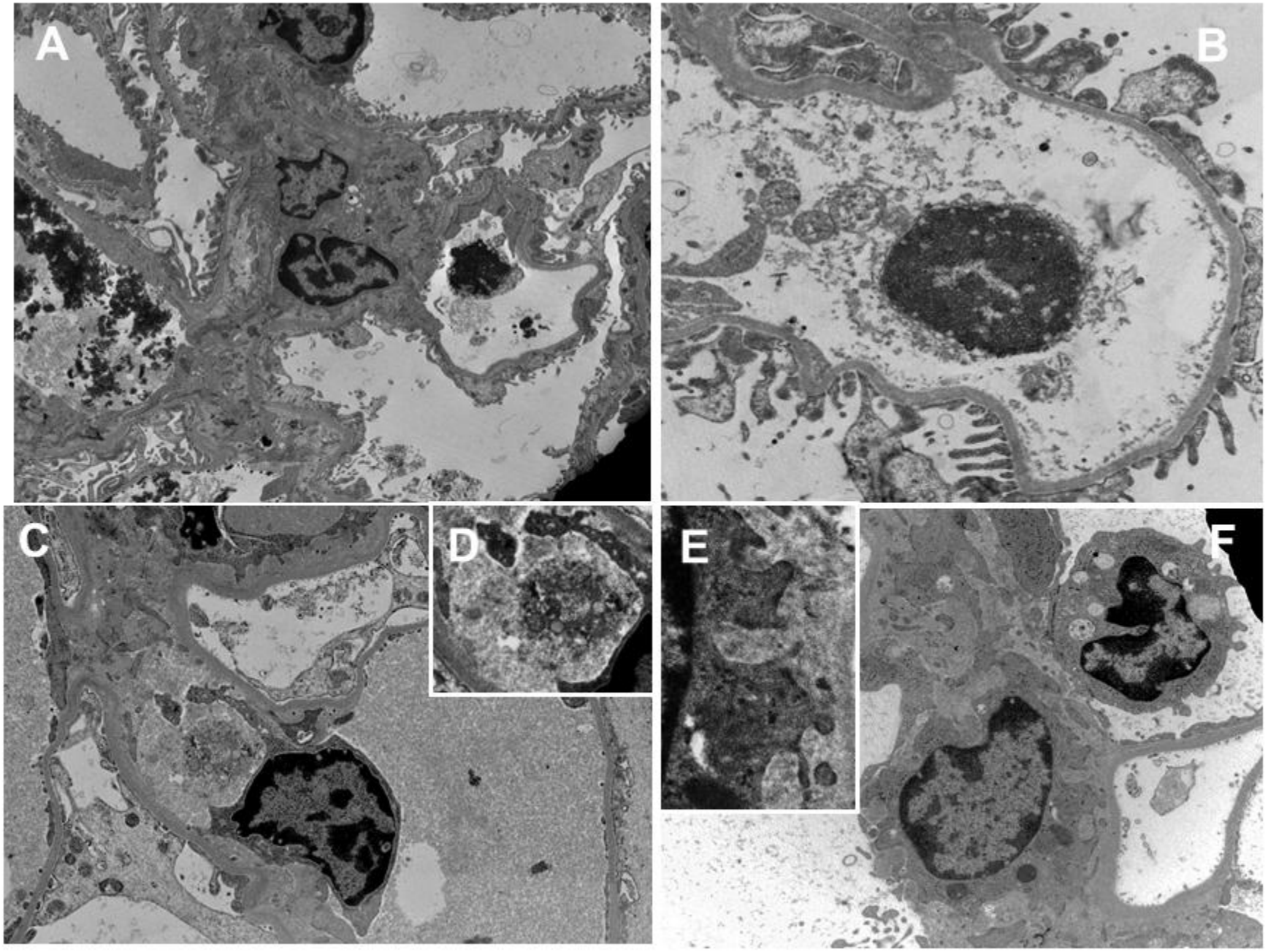
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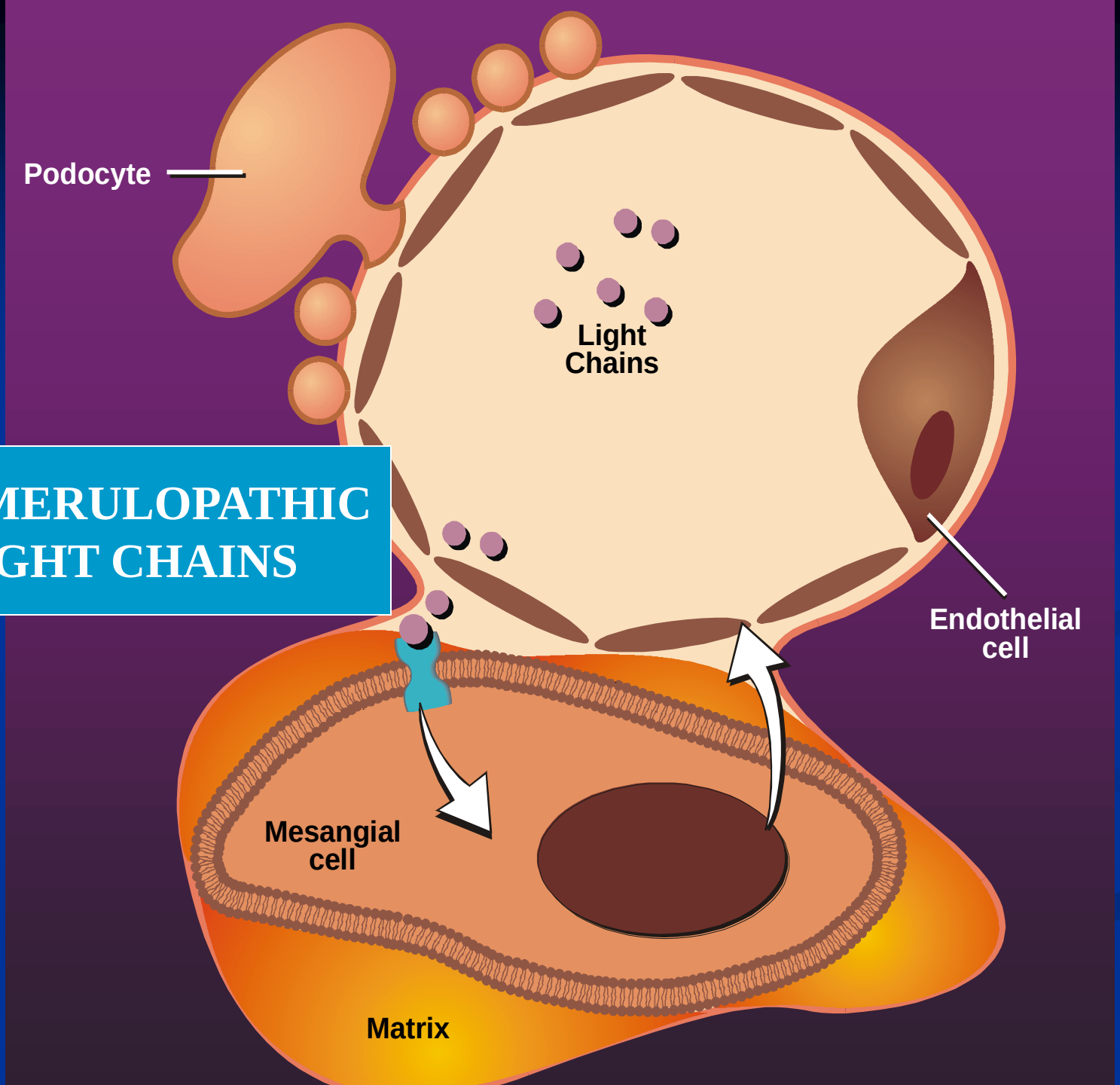
- Luan Truong MD






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E
F

GLOMERULOPATHIC LIGHT CHAINS

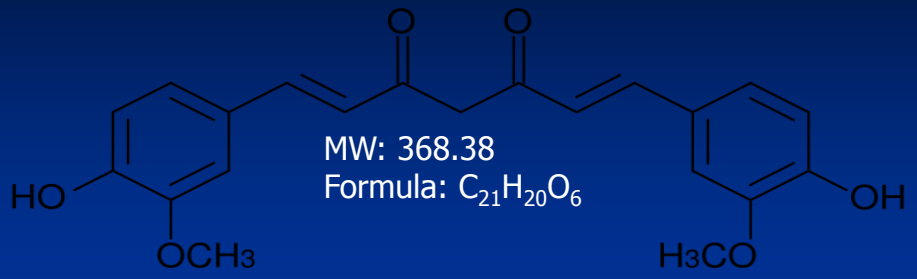


Curcumin: The most powerful compound in botanical medicine. The worlds most important herbal extract.

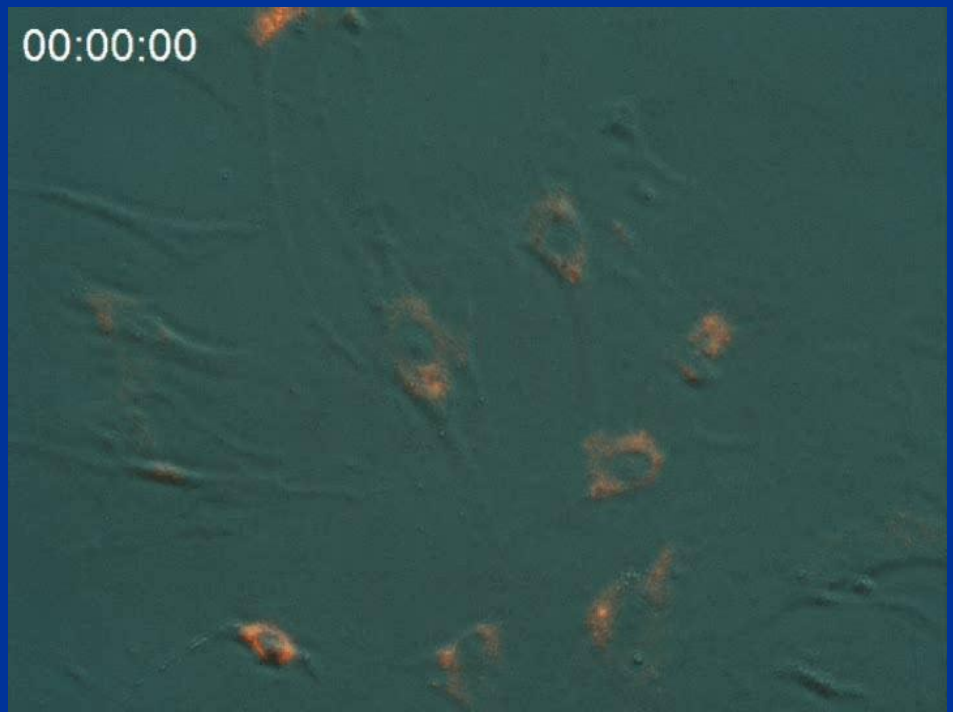




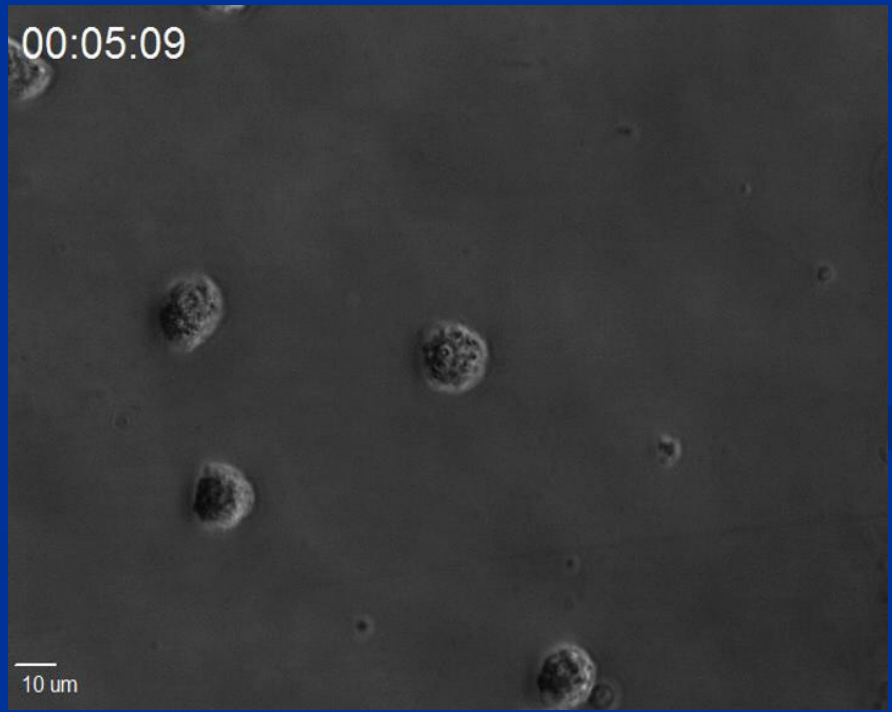


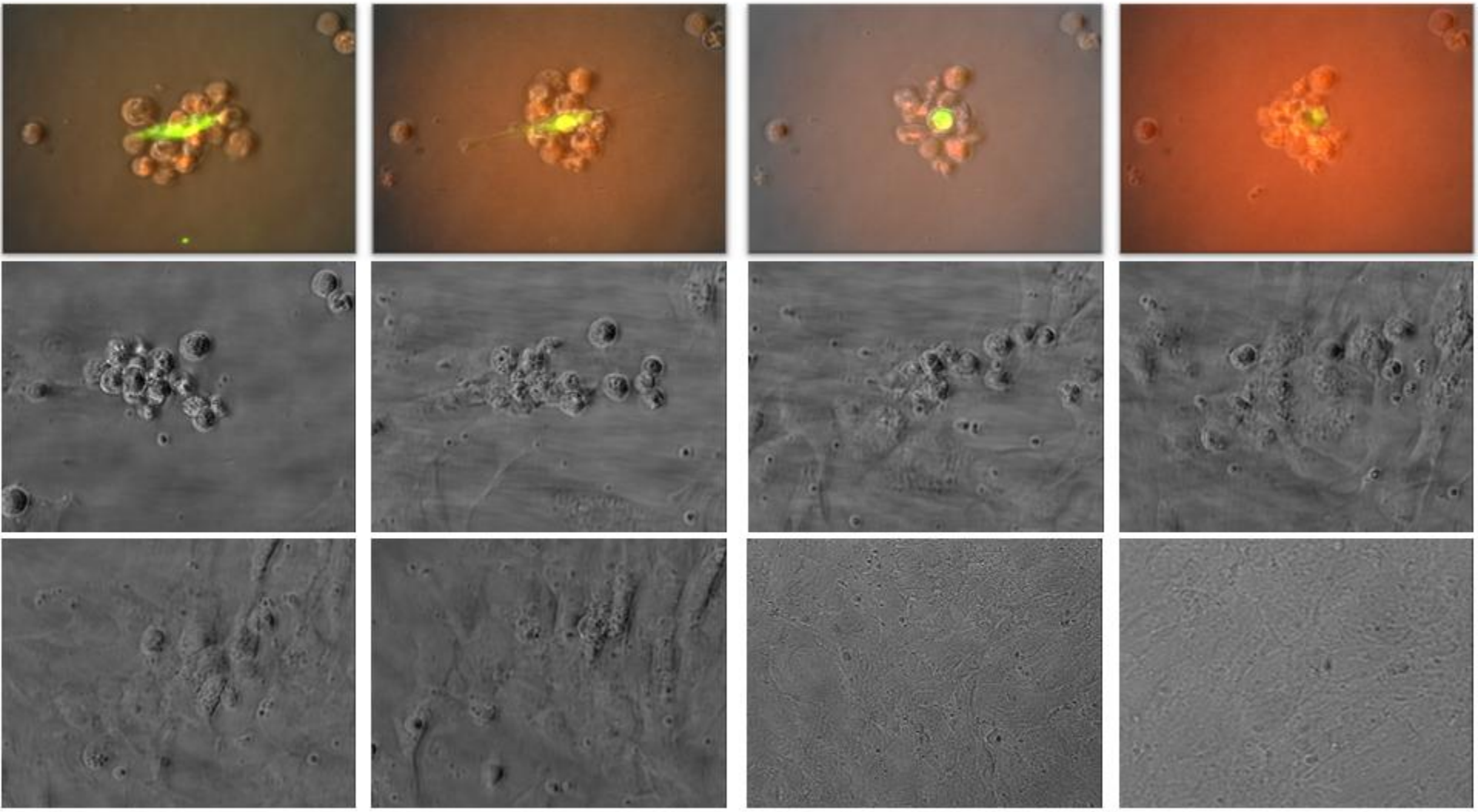


Enhances LCDD-LC induced RMC (Caspase-3) apoptosis

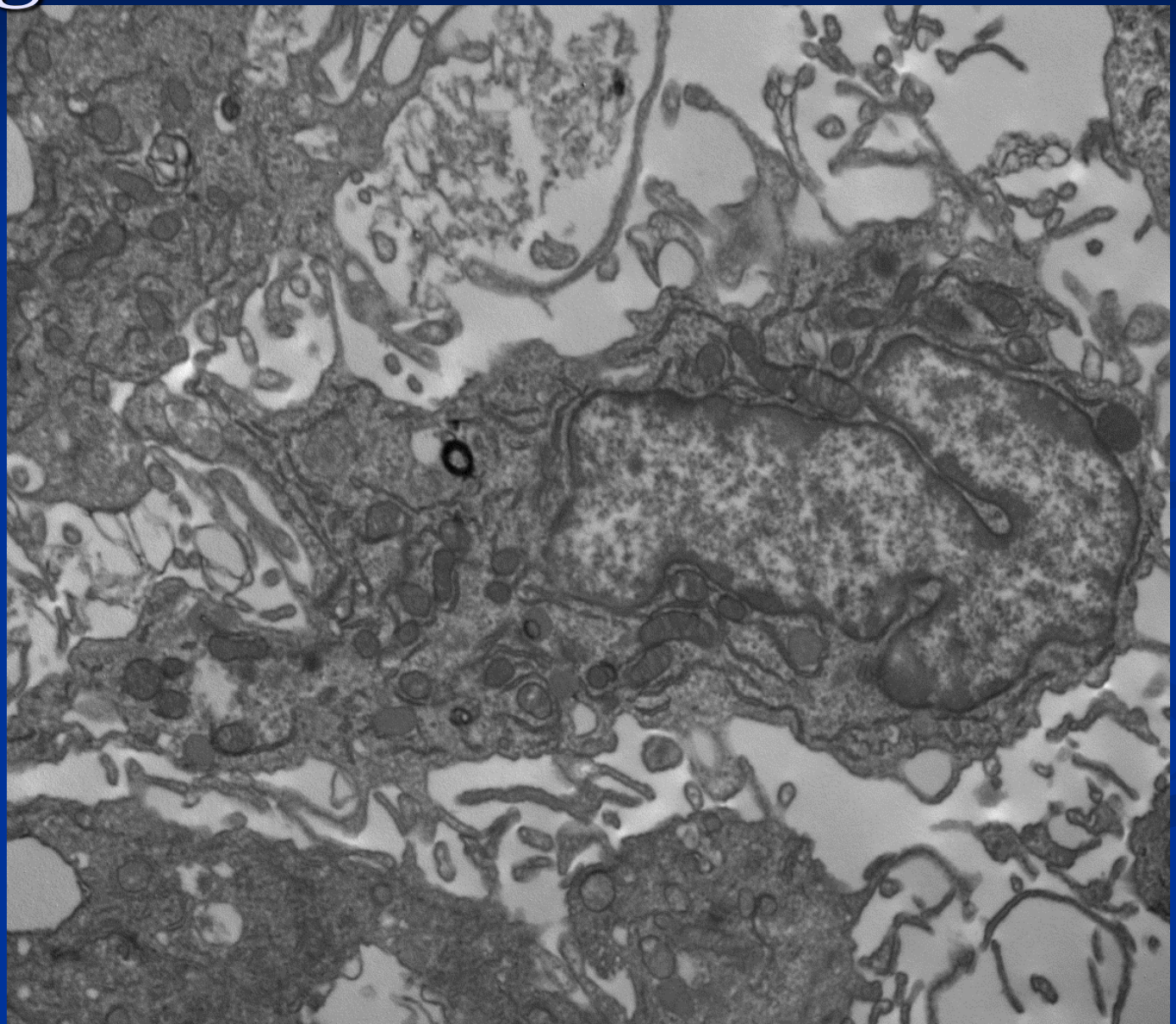


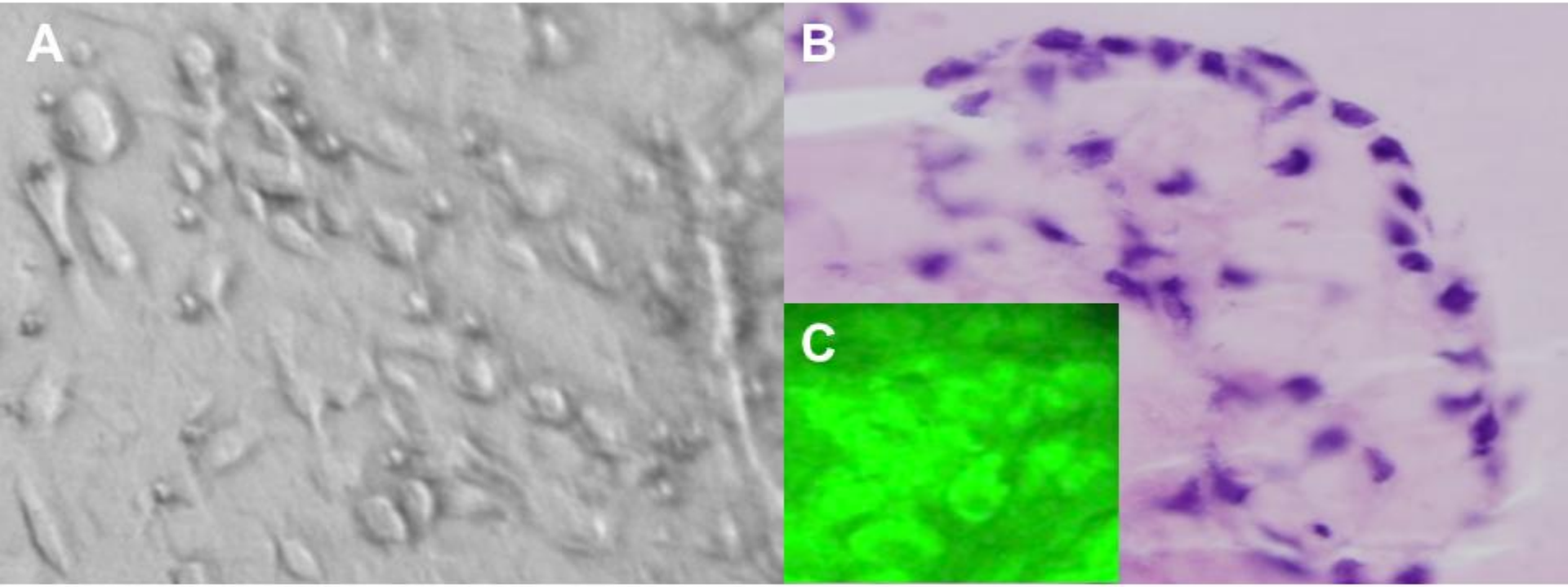
Enhances RMSC phagocytosis of LCDD-LC- induced apoptotic bodies





Teng-15-02 C57BL6 Mouse MSC




A
B
C