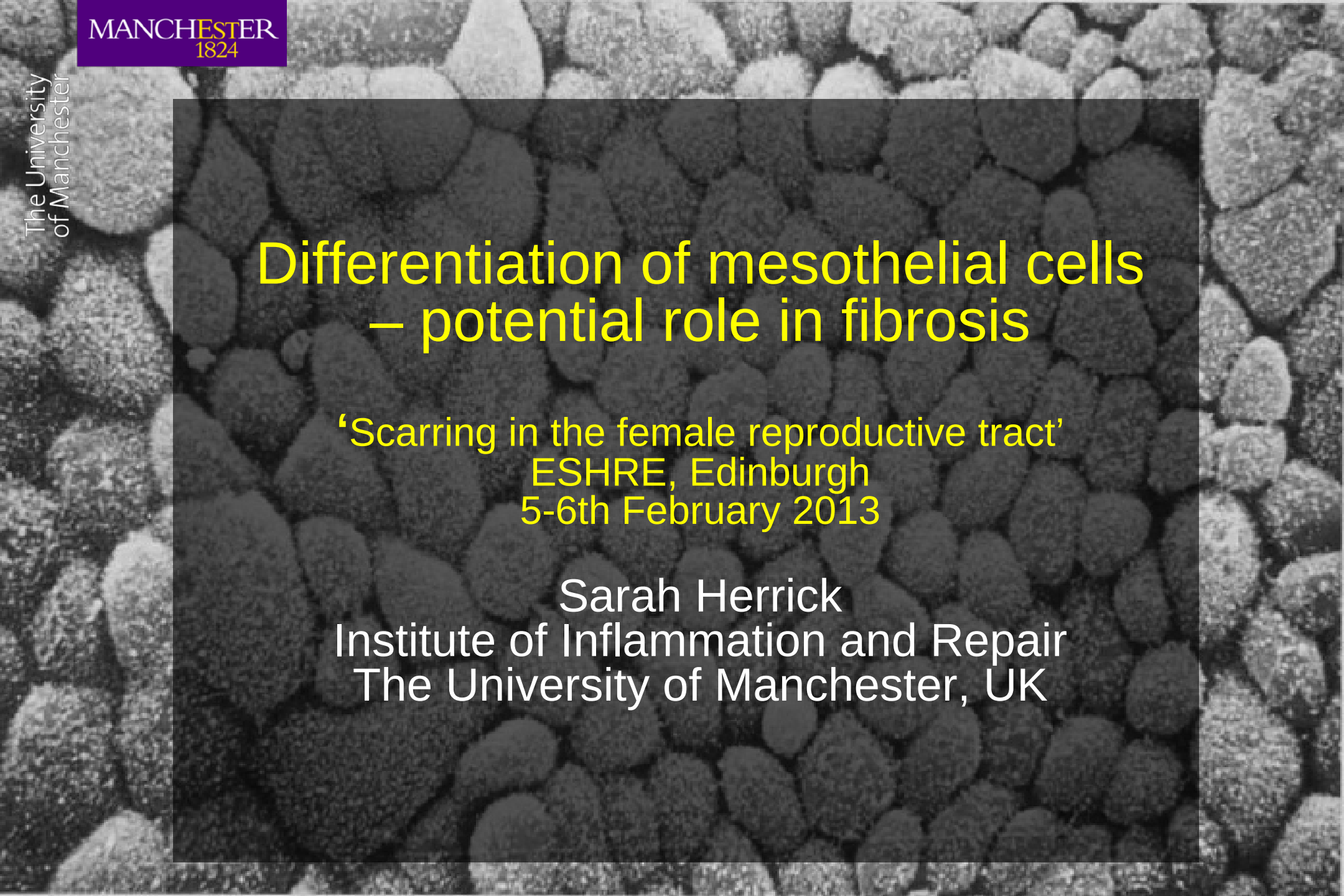


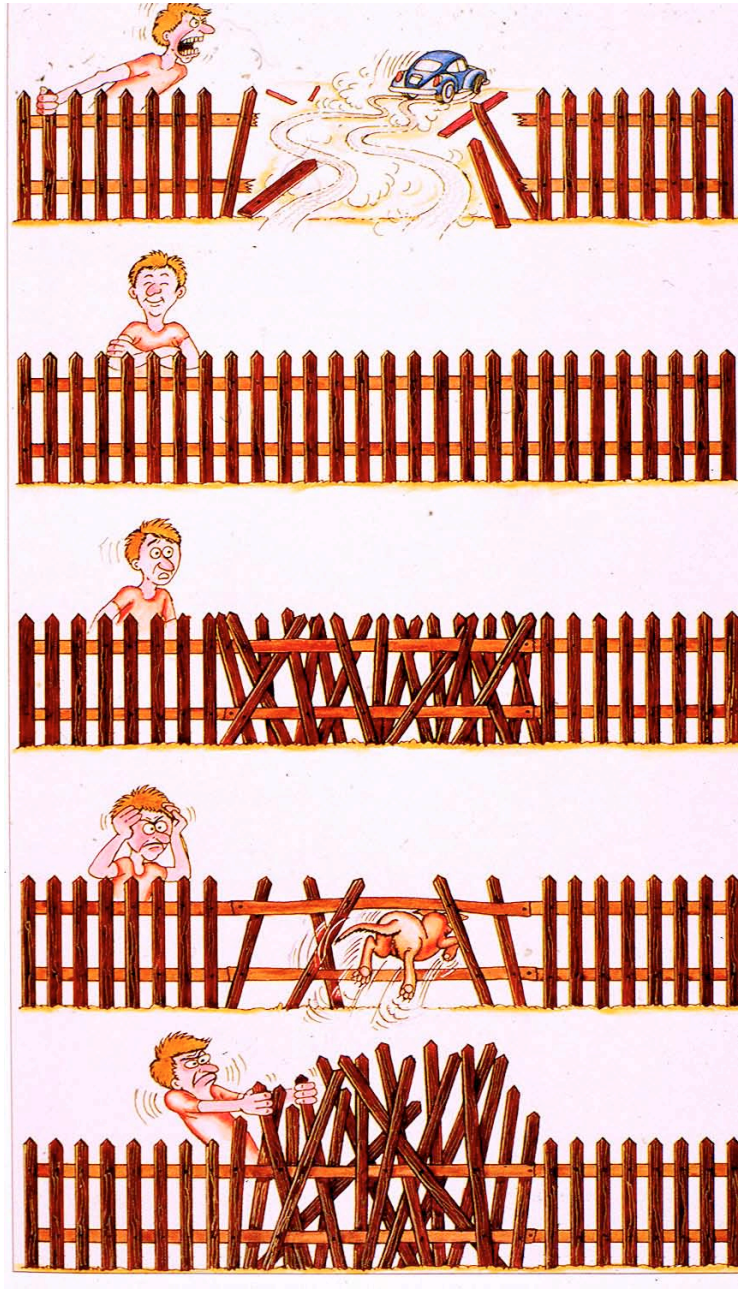
MANCHESTER 1824

The University of Manchester

Differentiation of mesothelial cells – potential role in fibrosis

‘Scarring in the female reproductive tract’
ESHRE, Edinburgh
5-6th February 2013

Sarah Herrick
Institute of Inflammation and Repair
The University of Manchester, UK

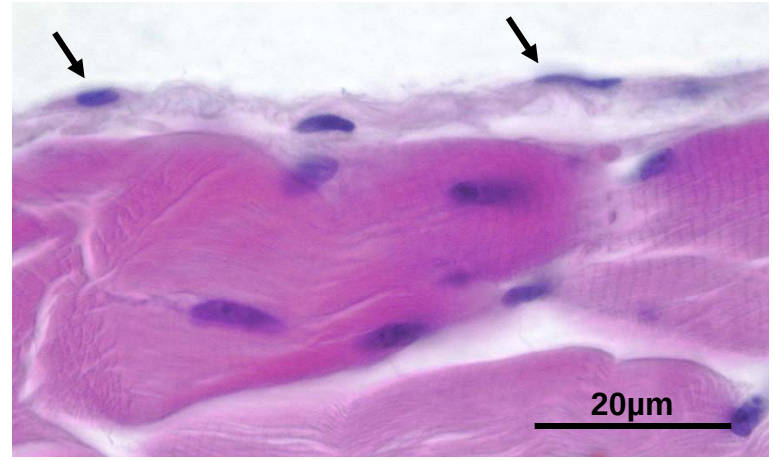


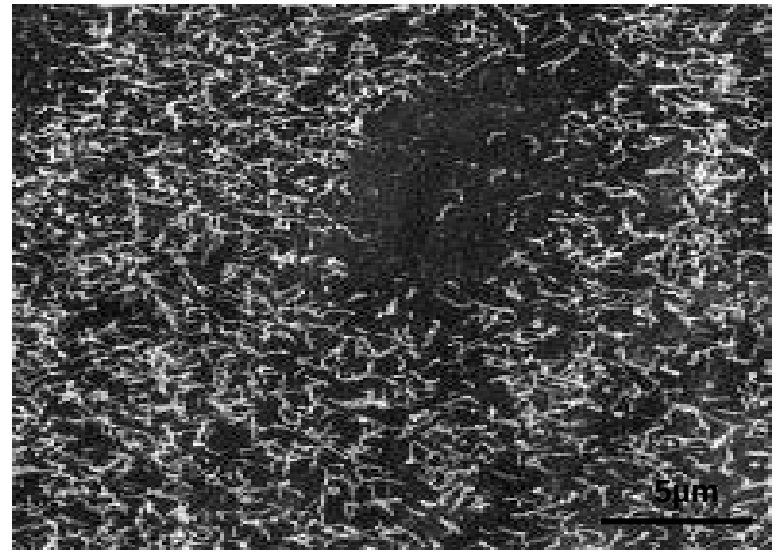
Repair scenarios

- After injury…
- the ideal is regeneration…
- but usually repair with scarring occurs…
- if inadequate and weak, chronic wounds form…
- or if excessive, hypertrophic/ fibrotic scarring results

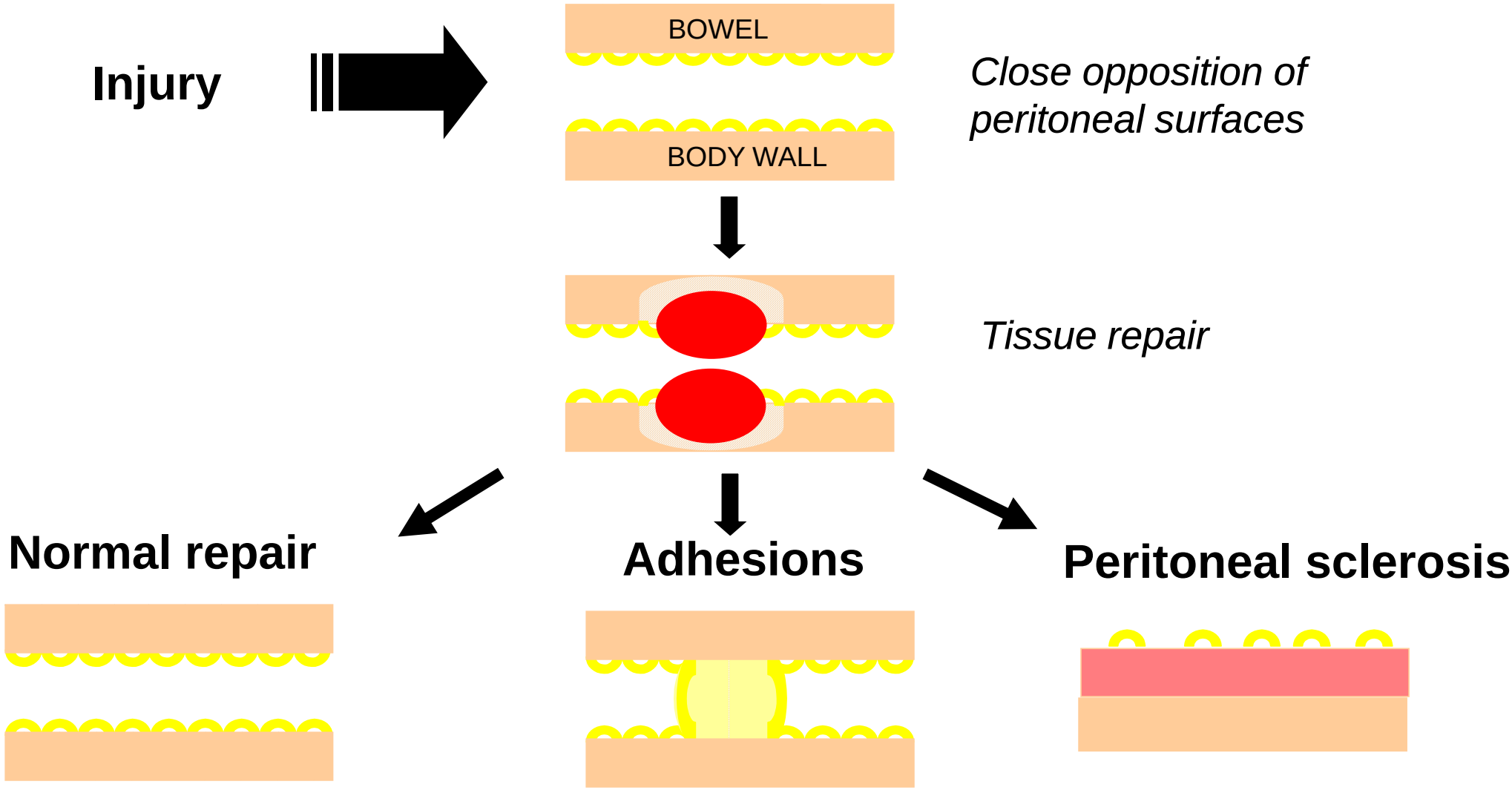
Importance of the mesothelium

- Mesothelium
  - single layer of squamous epithelium
  - mesodermally derived
  - all three serosal cavities
- Subserosal connective tissue
- Peritoneal fluid
- Functions
  - Non-adhesive barrier- surfactant and microvilli
  - Solute and fluid transport
  - Immune function
- Pathology





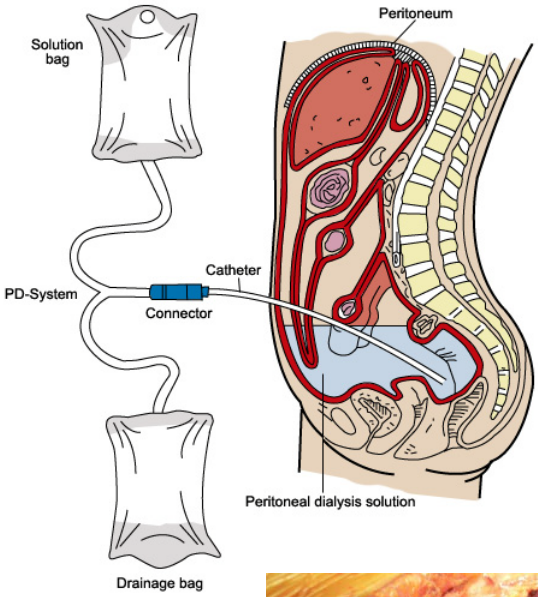
Peritoneal repair scenarios

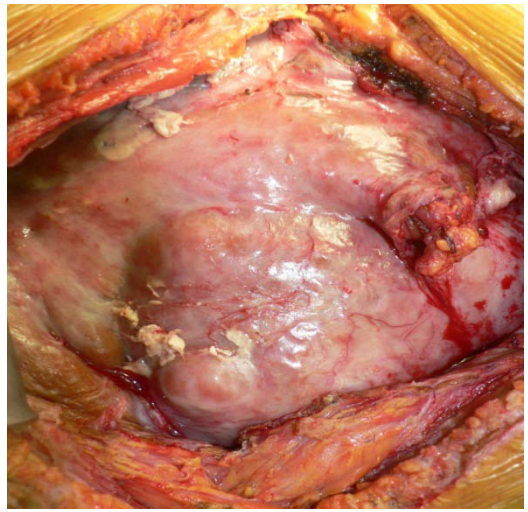


Abdominal/pelvic surgery often leads to adhesion formation

- Form after a range of peritoneal insults
- Complications :
  - Small bowel obstruction
  - infertility in women
  - difficulty of repeat surgery
  - chronic pelvic pain
- 5.5% of all abdominal repeat surgery directly due to adhesions - SCAR studies
- Reform after surgical adhesiolysis
- Limited preventative treatment – barrier devices
- Pharmaceutical interventions?

Peritoneal dialysis commonly results in peritoneal sclerosis



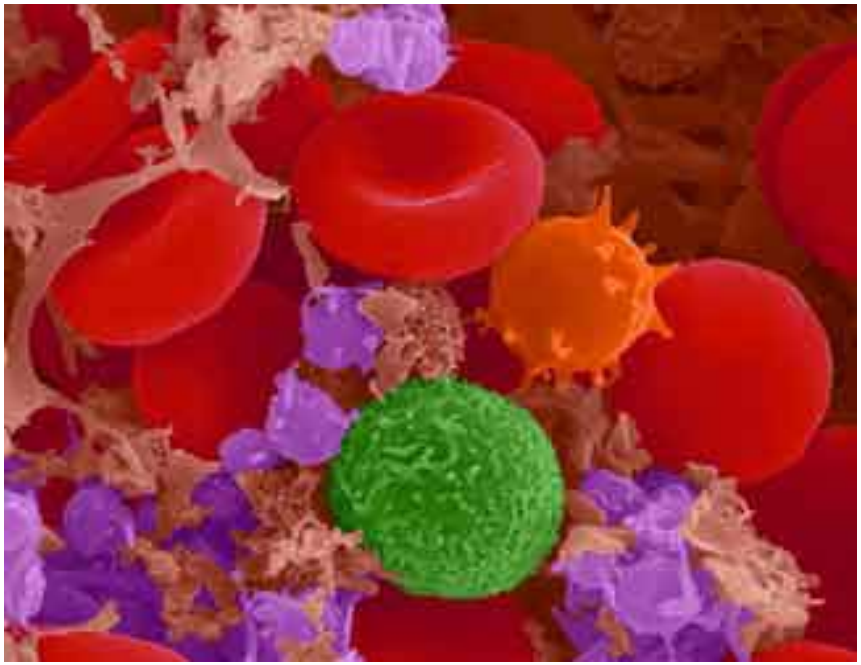


- Peritoneal dialysate – high glucose, low pH, high lactate
- Peritonitis
- Thickening of peritoneal membrane leads to ultrafiltration failure

- Rare condition of PD - Encapsulating Peritoneal Sclerosis (EPS)
- Cocooning of viscera by mass scar tissue leading to bowel obstruction
- Incidence linked to time on dialysis
  - 2 years – 1.9%
  - 8 years – 19.4%
- Surgical management
- Pathophysiology?

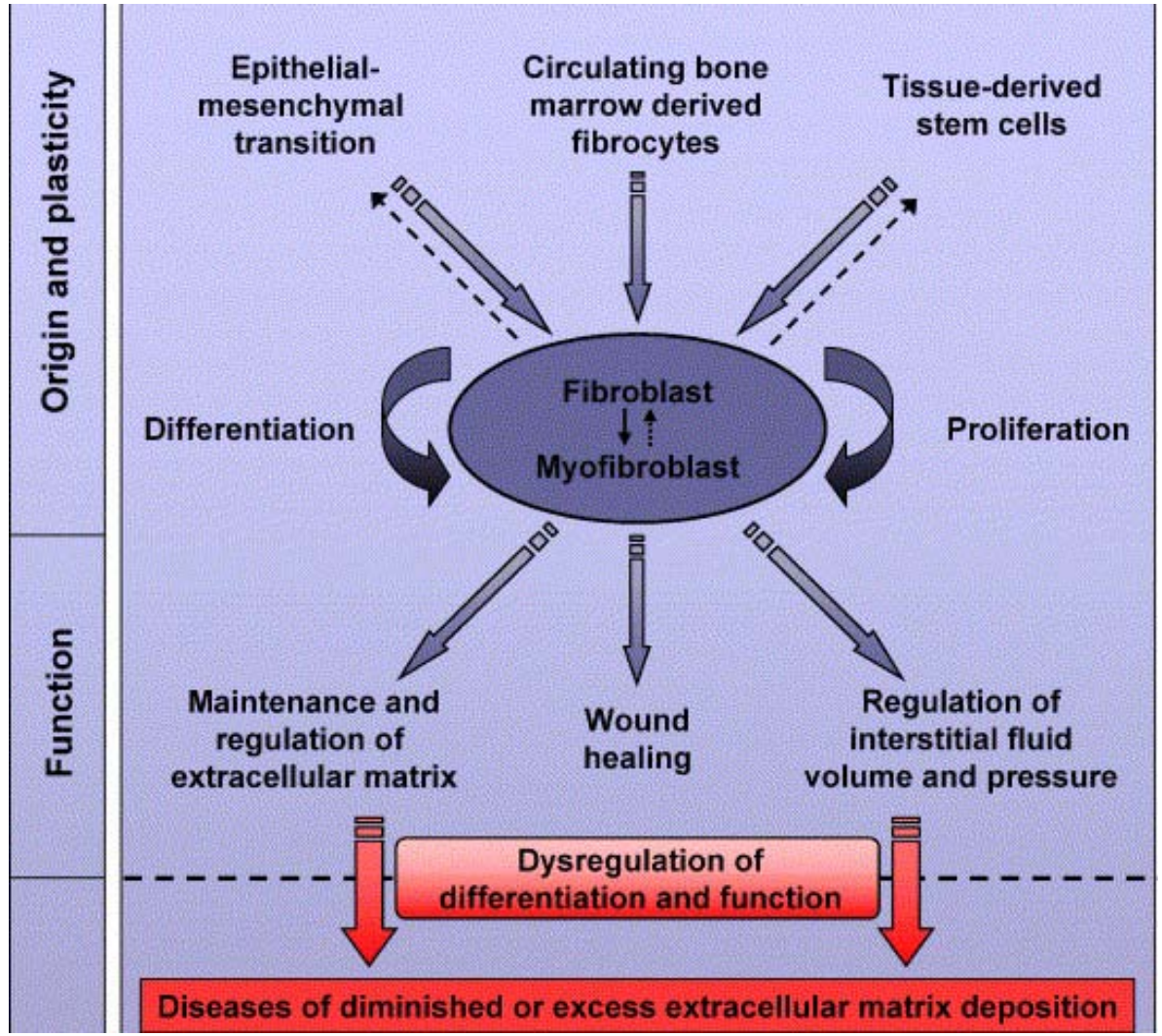
van Dellen D et al., BrJ Surg, 2011

How does tissue repair progress to fibrosis?



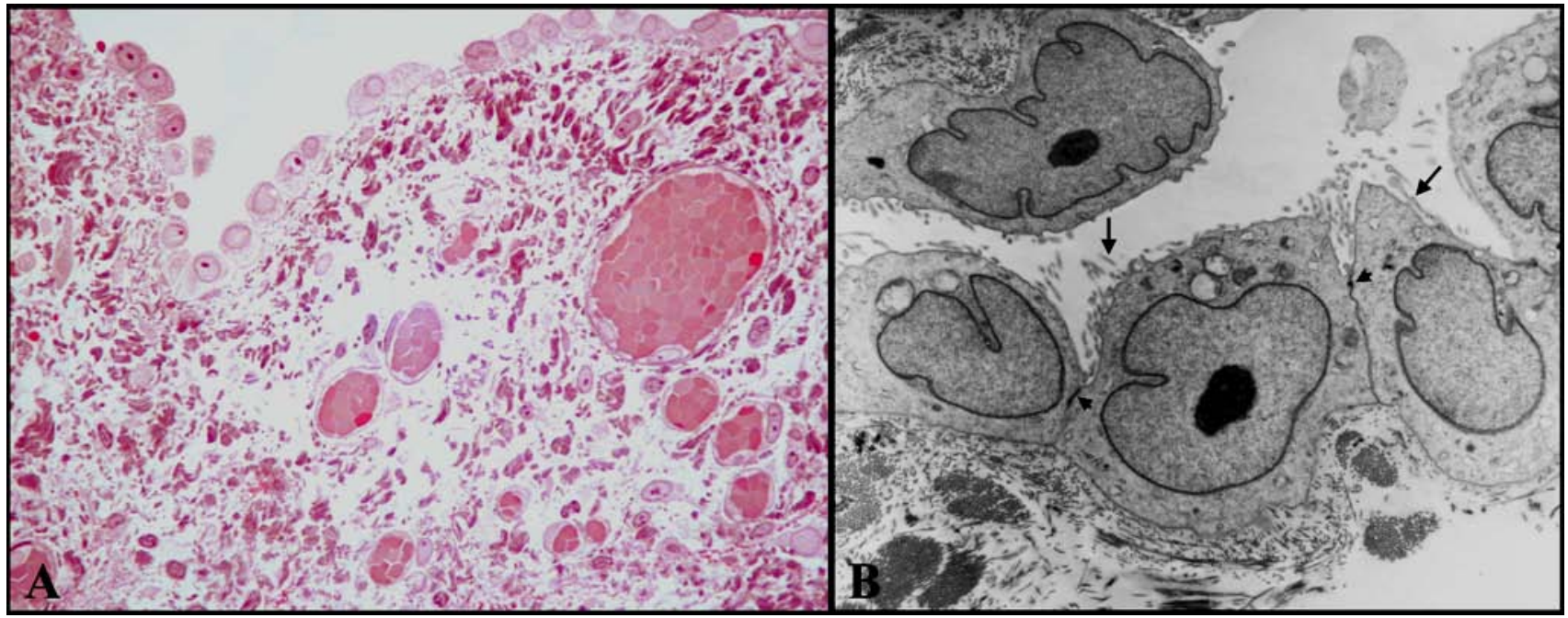
- Coagulation and inflammation
- Granulation tissue formation
- Re-epithelialisation
- Tissue contraction
- Remodeling and scar formation - ……fibrosis

Important role of the ‘fibroblast’ in tissue repair and fibrosis



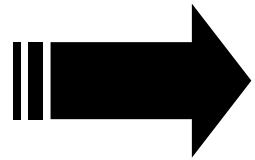
McAnulty R. Int J Biochem Cell Biol (2007)

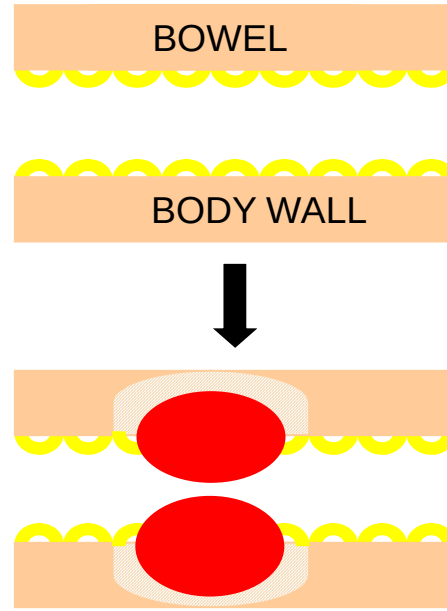
Does the mesothelium play a role in peritoneal fibrosis?



Peritoneal repair scenarios

Injury





Close opposition of peritoneal surfaces

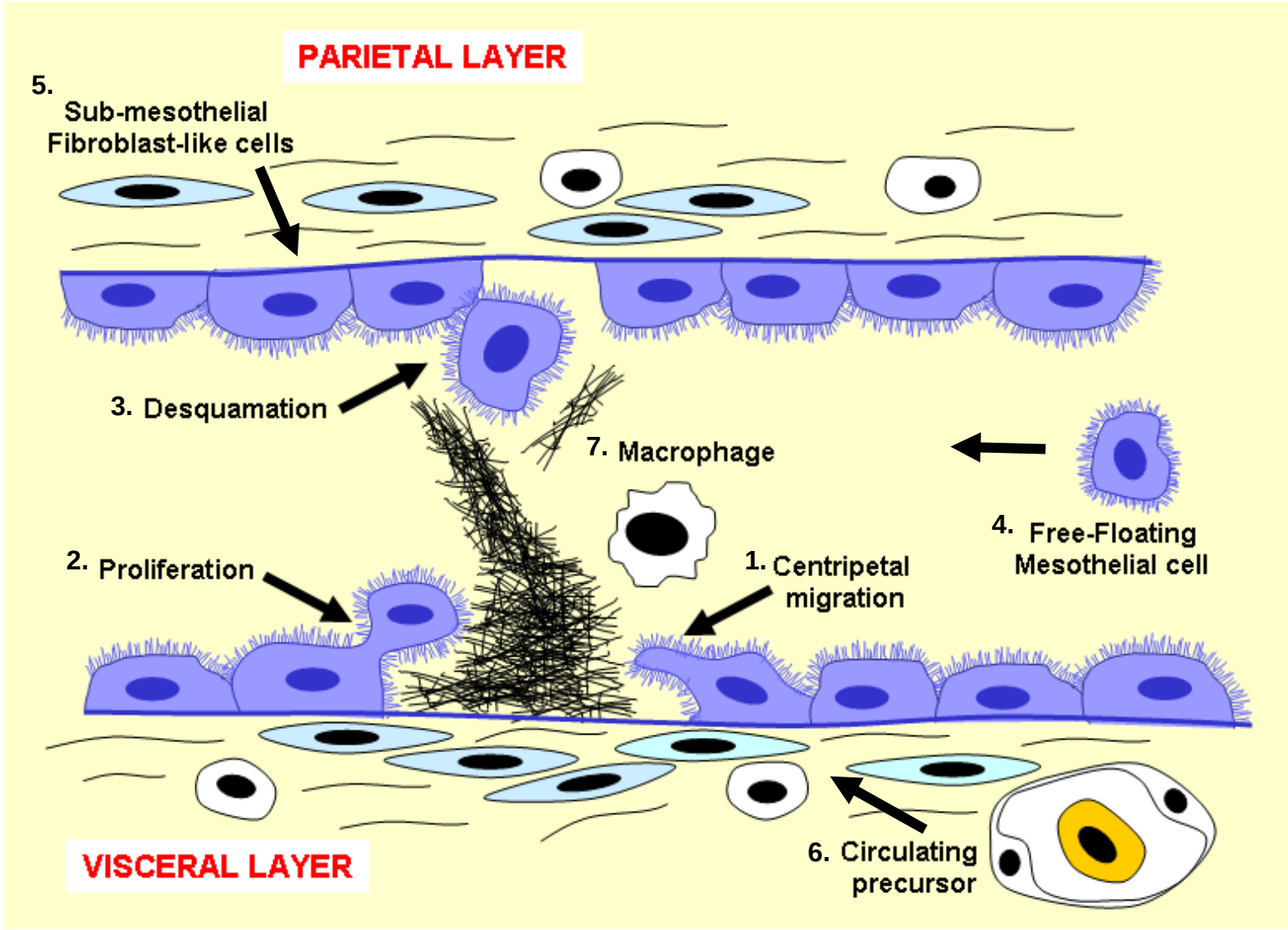
Tissue repair

Normal repair

Adhesions

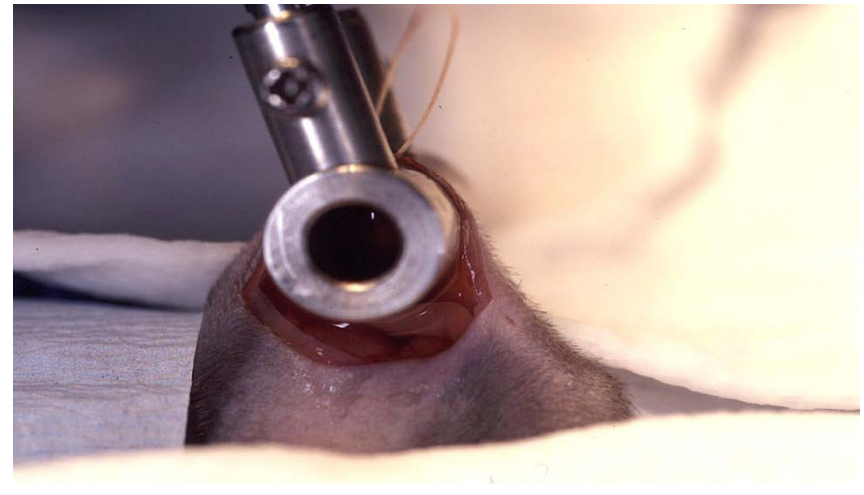
Peritoneal sclerosis

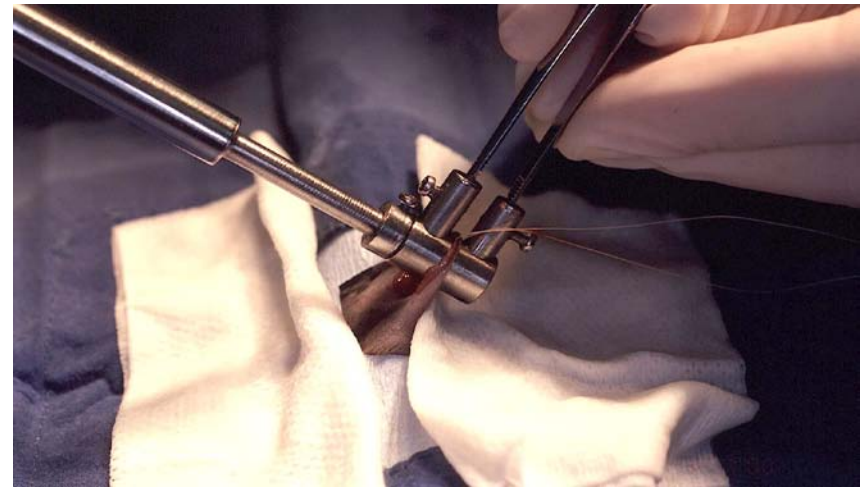
Proposed schemes of mesothelial regeneration



Rodent model of surgical injury

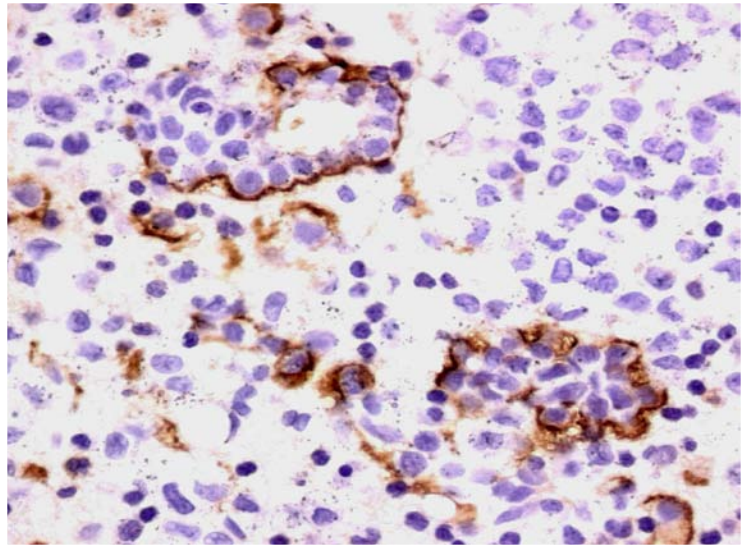
- Isolate and label rat cells
  - mesothelial cells
  - peritoneal lavage cells
  - peritoneal macrophages
  - lung fibroblasts
- Laparotomy and simple abrasion injury
- Intra-peritoneal injection of fluorescent Di-I labelled cells
- Assessed distribution of labelled cells post-injury

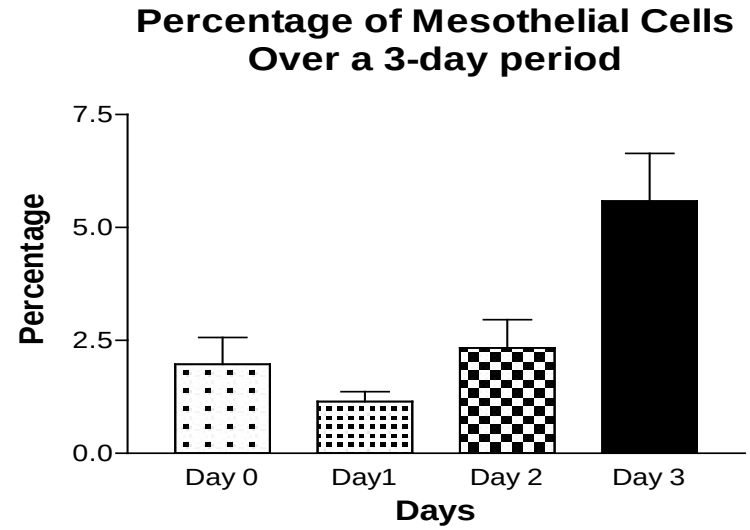




Mesothelial cells in serosal fluid increase after injury in rats

HBME+ve





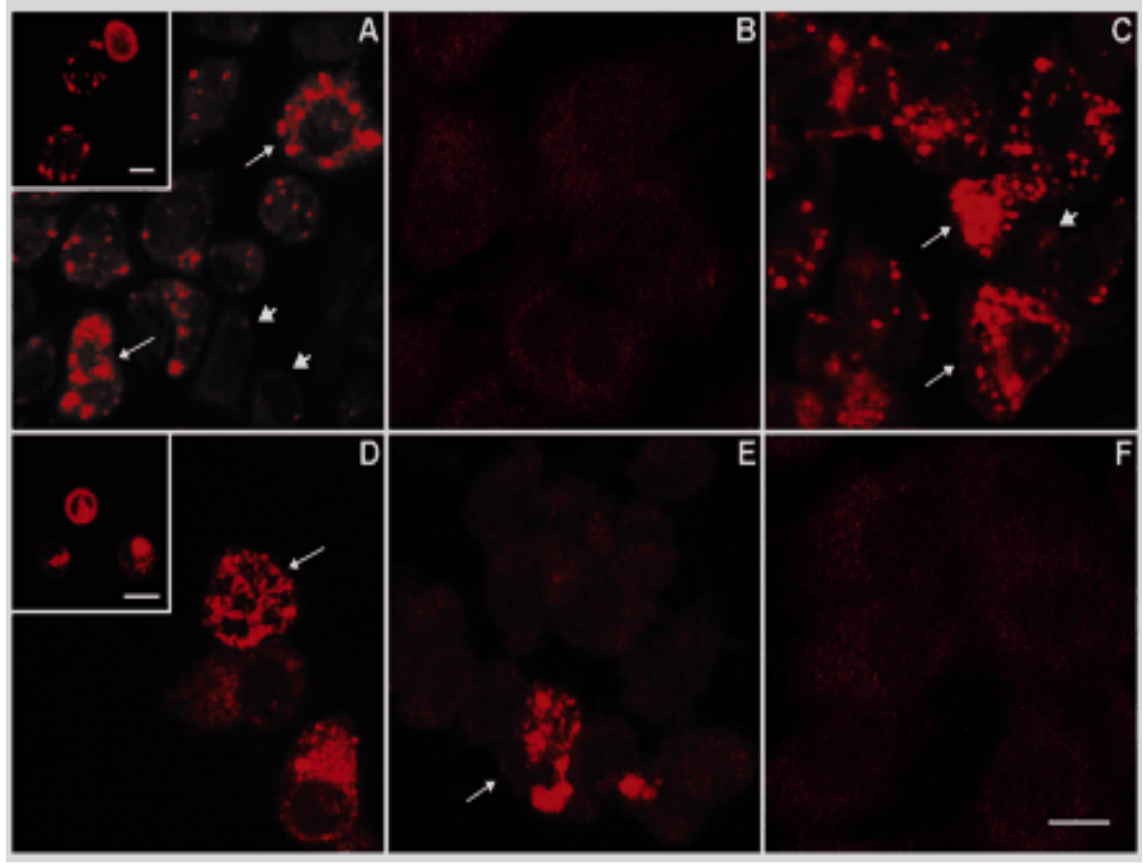
Foley-Comer et al., 2002. J Cell Sci 115, 1383

Incorporation of isolated free-floating mesothelial cells on denuded surface

Mesothelial cells
day 8

Fibroblasts
day 8

Lavage cells
day 8



Macrophages
Day 2

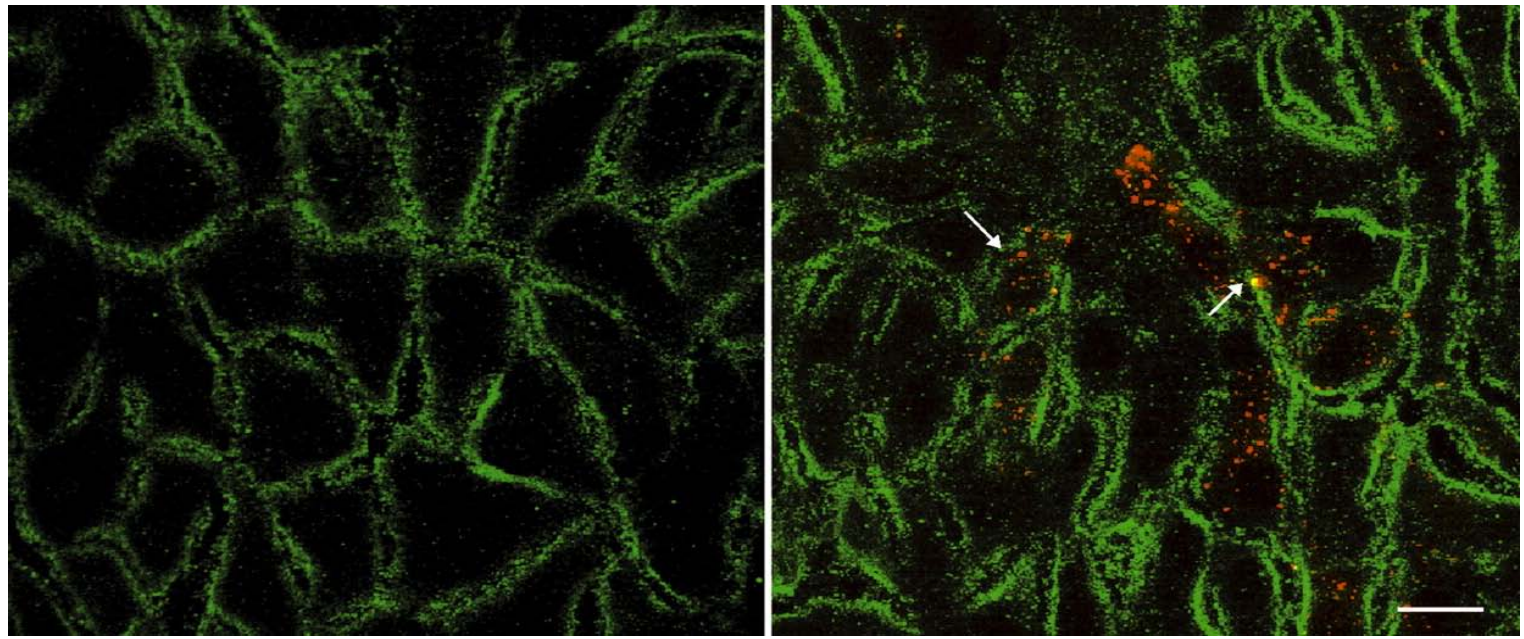
Macrophages
Day 5

Macrophages
Day 8

Formation of junctional components by incorporated mesothelial cells

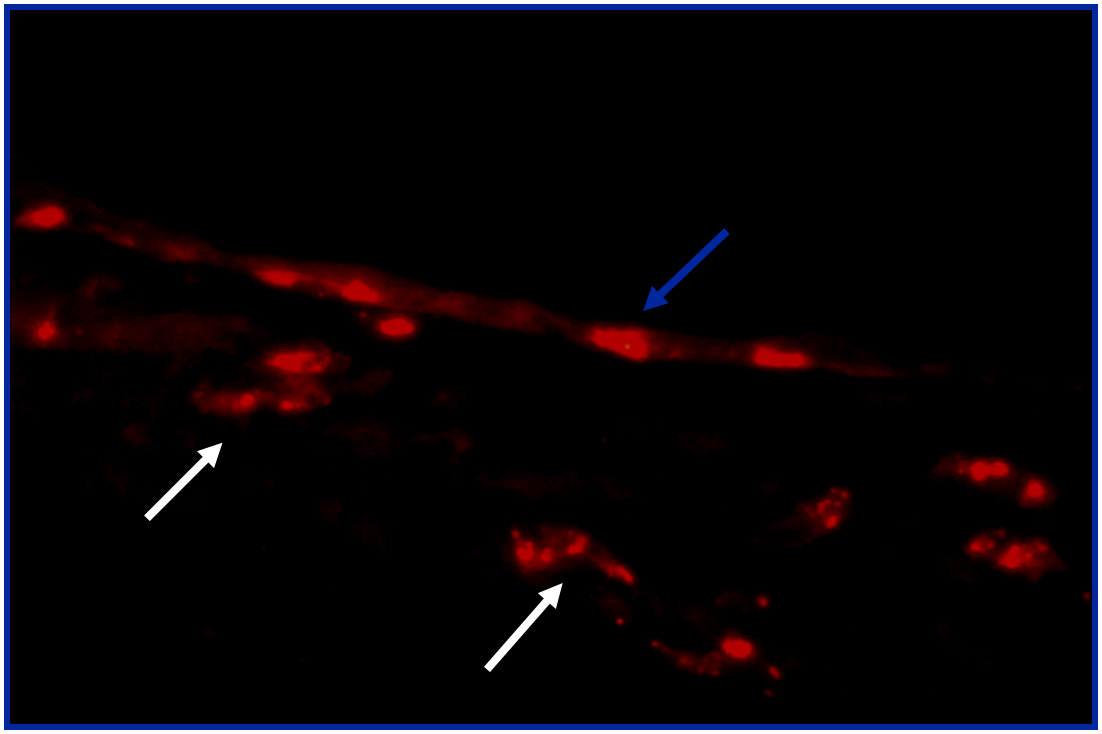
DiI-fibroblasts

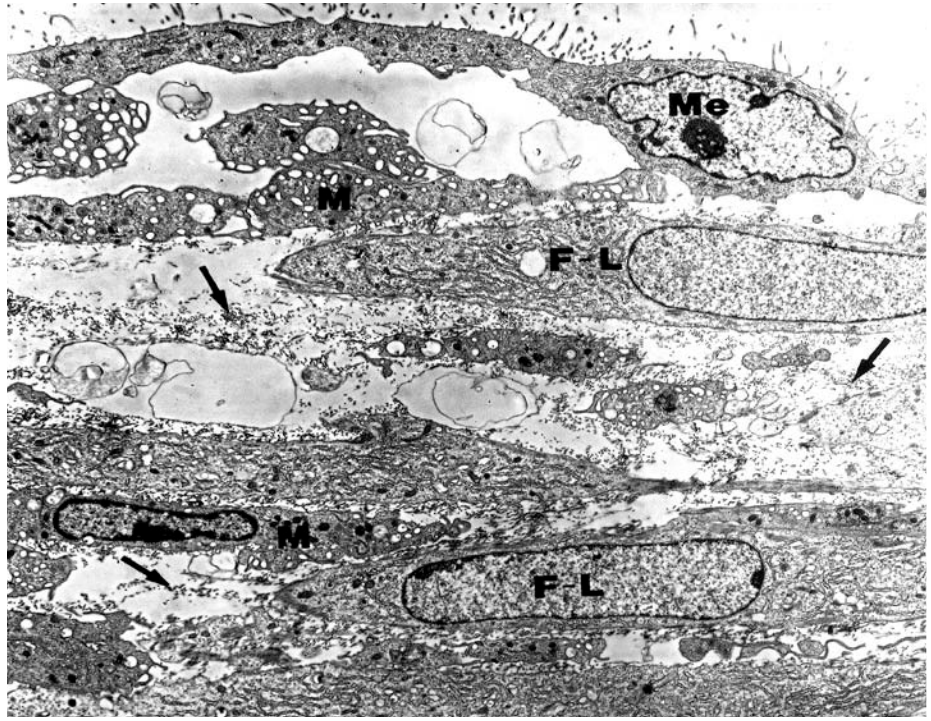
DiI-mesothelial cells



Foley-Comer et al., 2002. J Cell Sci 115, 1383

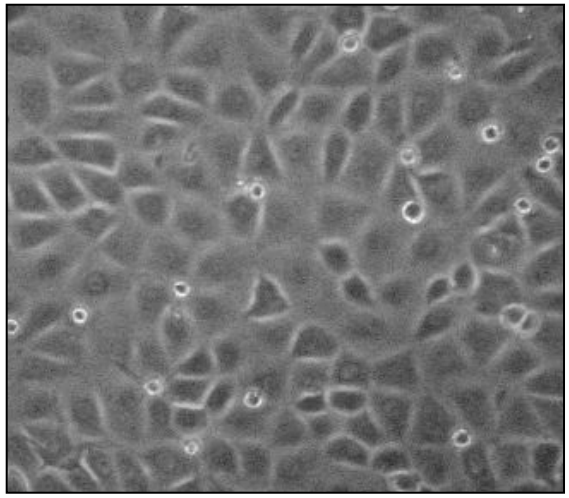
Di-I labelled mesothelial cells incorporate into multiple layers





Epithelial-Mesenchymal Transdifferentiation (EMT) of mesothelial cells *in vitro*

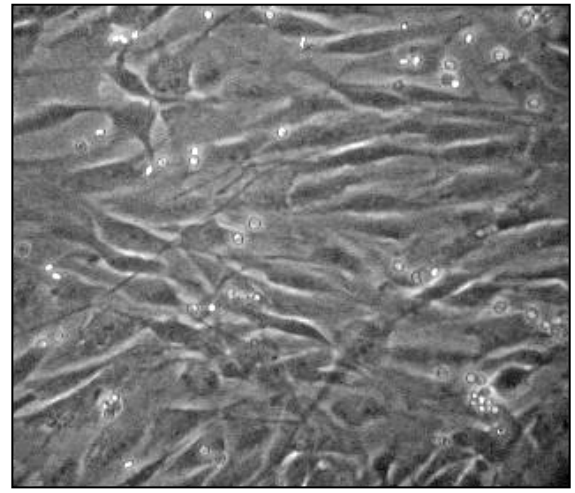
Epithelial



HGF/TGF-β1/EGF
Sub-culture
Fibrin/collagen gel
Dialysis fluid
Menstrual effluent

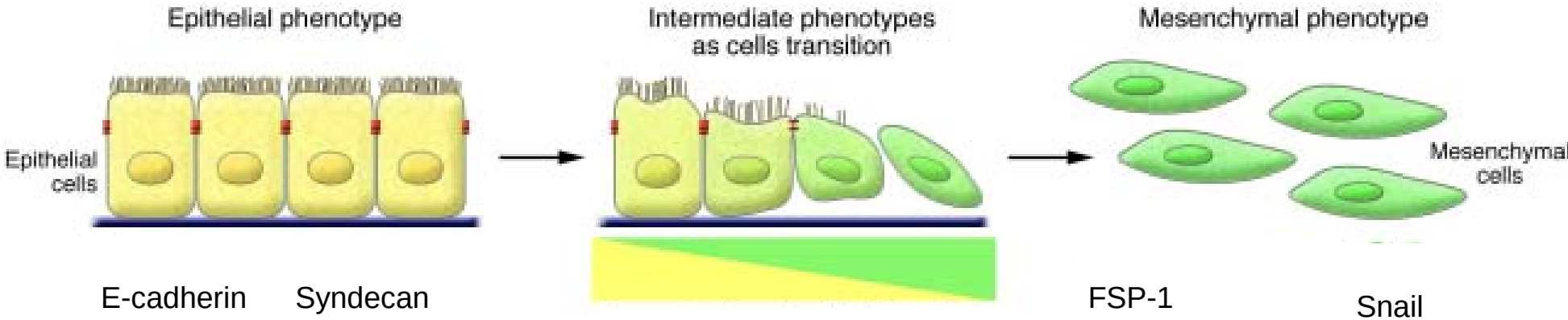
EMT

Mesenchymal



epithelial – fibroblast - myofibroblast - smooth muscle-like phenotype

Pathway to epithelial-mesenchymal transdifferentiation



E-cadherin
Cytokeratin
ZO-1
Laminin
Entactin

Syndecan
MUC 1
Desmoplakin
Collagen IV
msR200

Progressive loss of epithelial markers and gain of mesenchymal markers

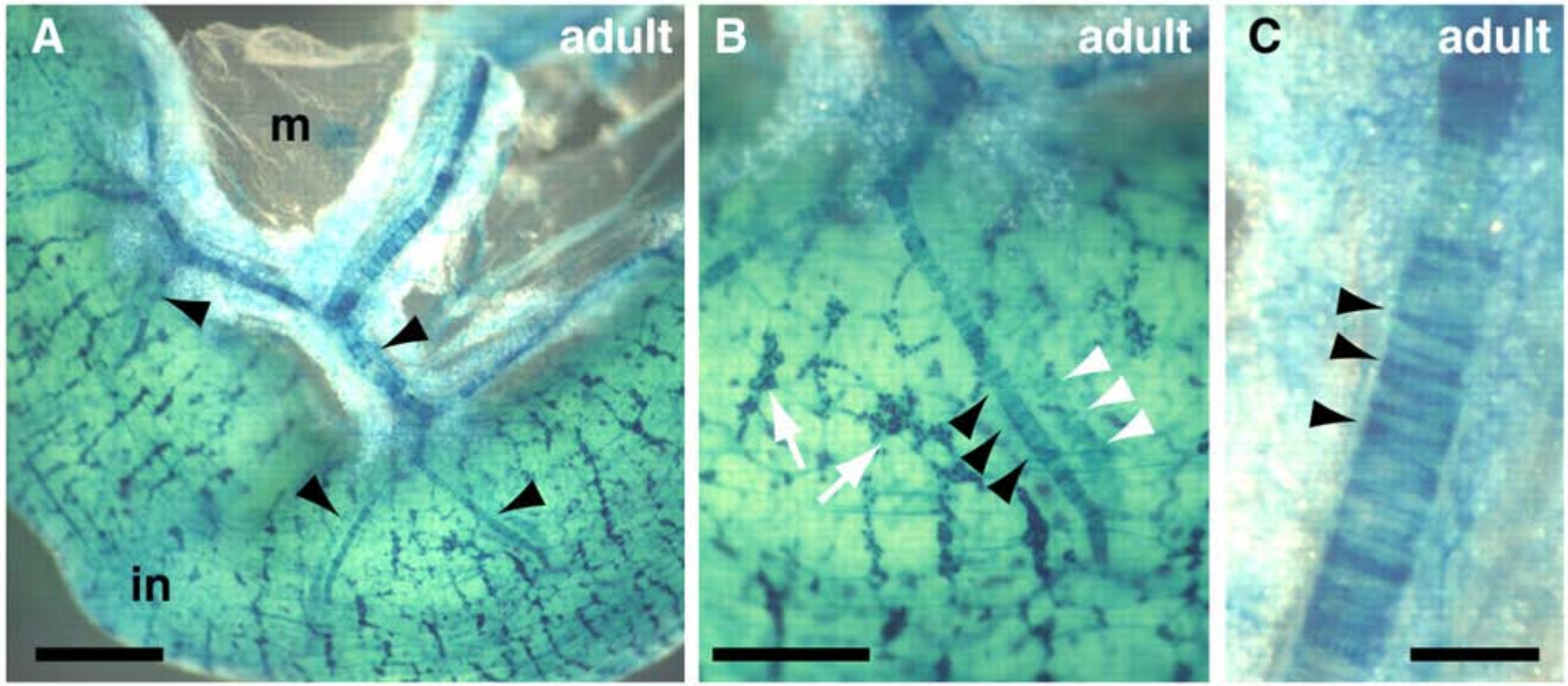
FSP-1
N-cadherin
Vimentin
Fibronectin
B-catenin
Syndecan 1
miR10b

Snail
Slug
Twist
α –SMA
FOXC2
LEF-1

Kalluri R and Weinberg RA, J Clin Invest 199 (2009)

Does mesothelial cell EMT occur *in vivo?*

Mesothelium EMT contributes to blood vessels of the heart, lung, mesentery and gut during development

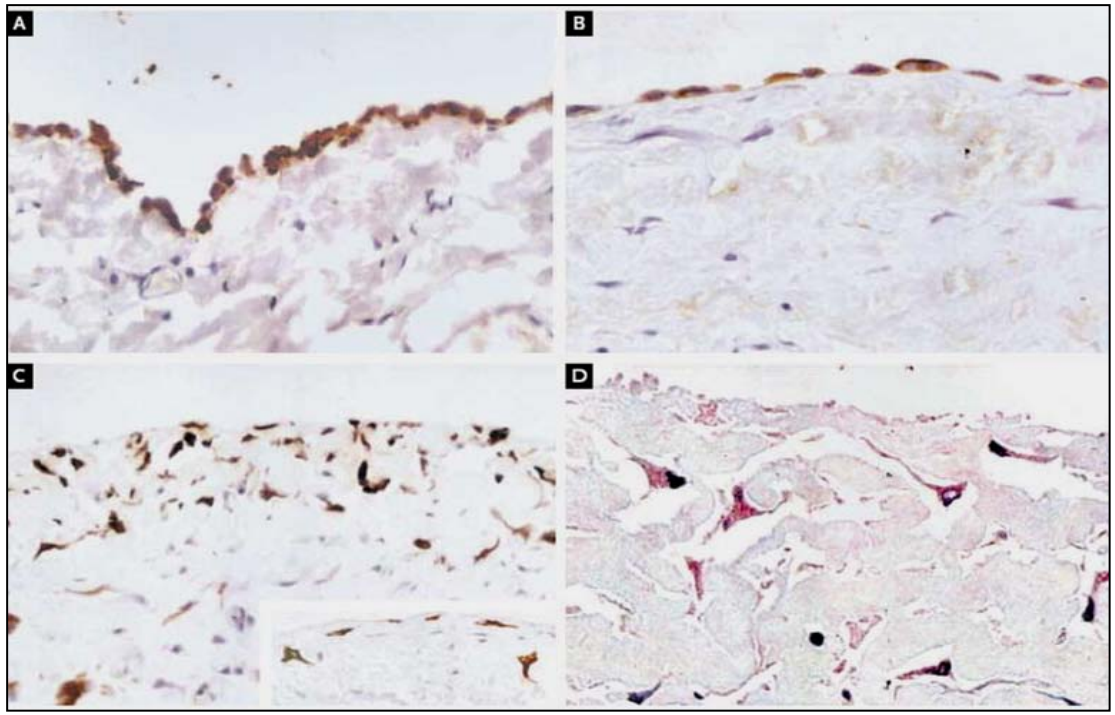


Wilm B et al. Development 2005;132:5317-5328

Mesothelial cell EMT occurs with injury in adult

Cytokeratin control

Cytokeratin 6 mth dialysis



Cytokeratin 8mth dialysis

ICAM1 8mth dialysis

Peritoneal dialysis (CAPD)

↓

Mesothelial cell damage/stress

↓ ← **TGF-ß1**

(Myo)fibroblast formation

↓

Extracellular matrix deposition

↓

Peritoneal sclerosis

.

Yanez-Mo et al., (2003) NEJM 348:403-413;
Yang et al. (2003). Kidney Int 63: 1530-1539

Transforming Growth Factor (TGF)-β

Fibrogenic cytokine:

- Wound healing
- Fibrosis
- Growth and development

3 mammalian isoforms:

- TGF-β1, TGF-β2 and TGF-β3

Secreted in latent form

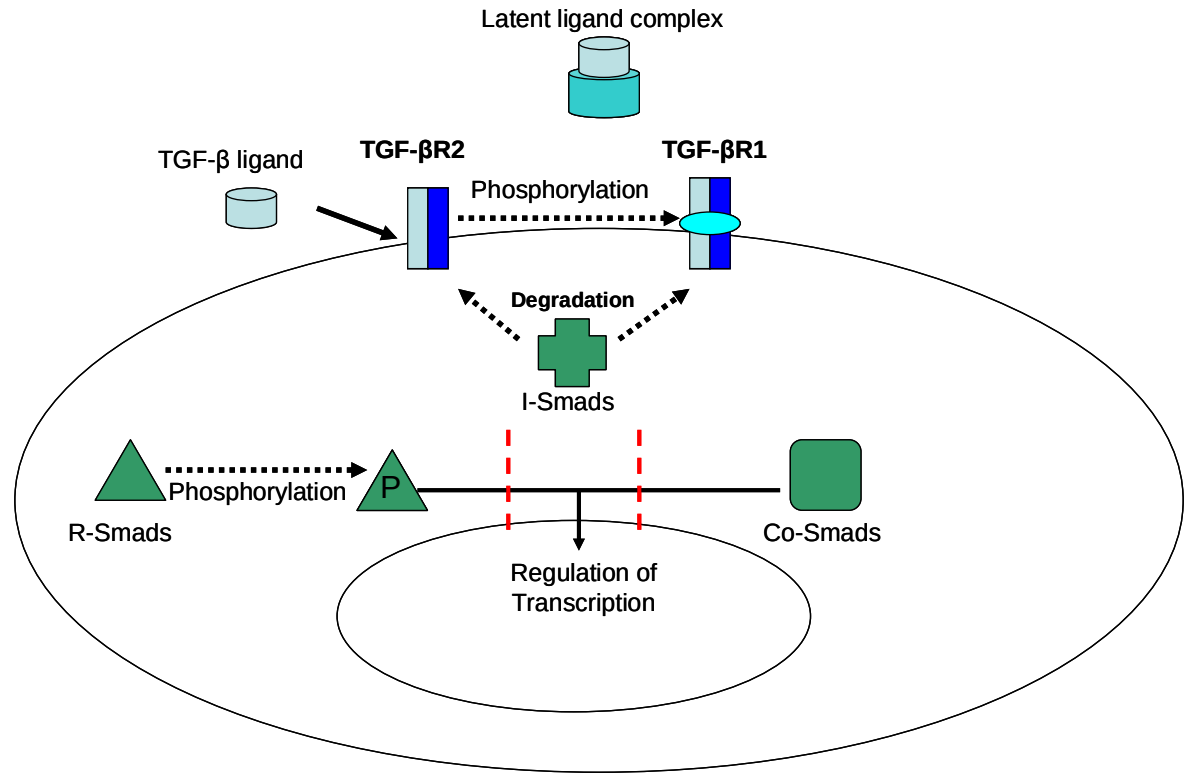
- Activation

Once activated can signal through

- TGF-βRI
- TGF-βRII
- SMAD pathway

Functions

- Induction of myofibroblasts
- Increase in ECM production
- Upregulates protease inhibitors

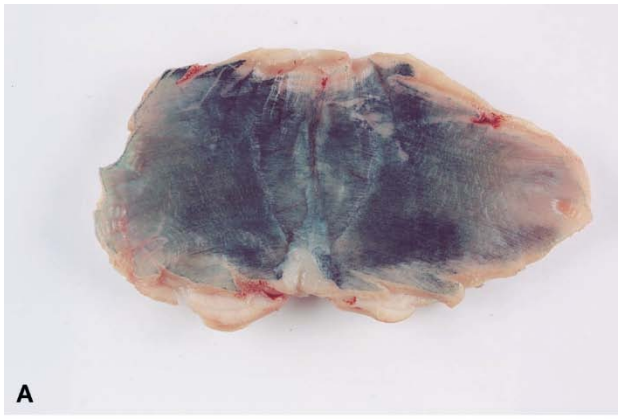


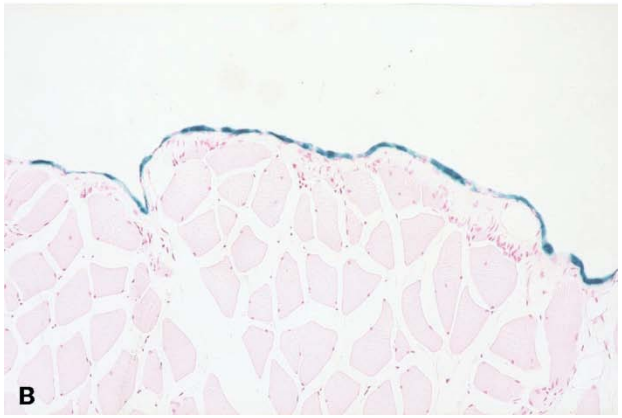
Mouse model of peritoneal fibrosis

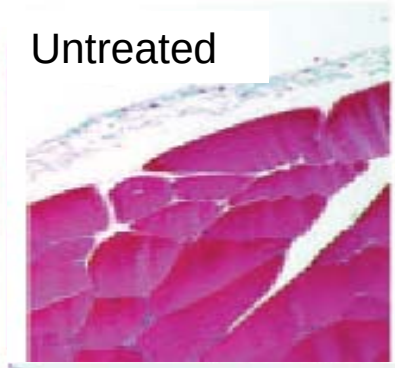
- Peter Margetts, McMaster University, Canada

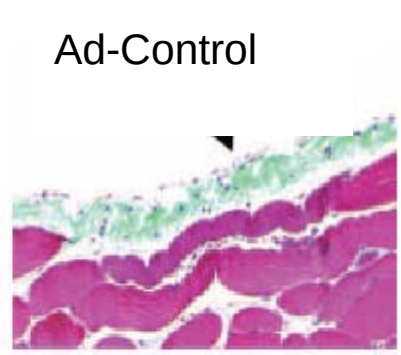
Adenovirus delivery of TGF-β1 – 20days – transient peritoneal fibrosis

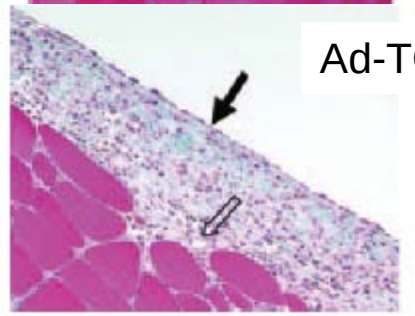
Expression extended by helper-dependent adenovirus – 70days – severe fibrosis/EPS

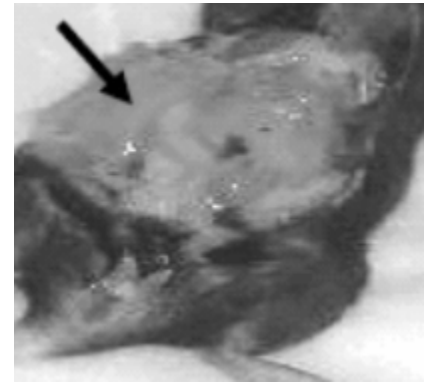


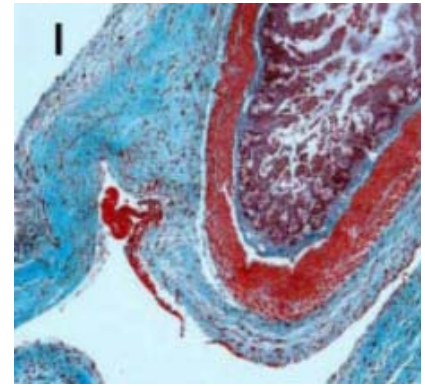






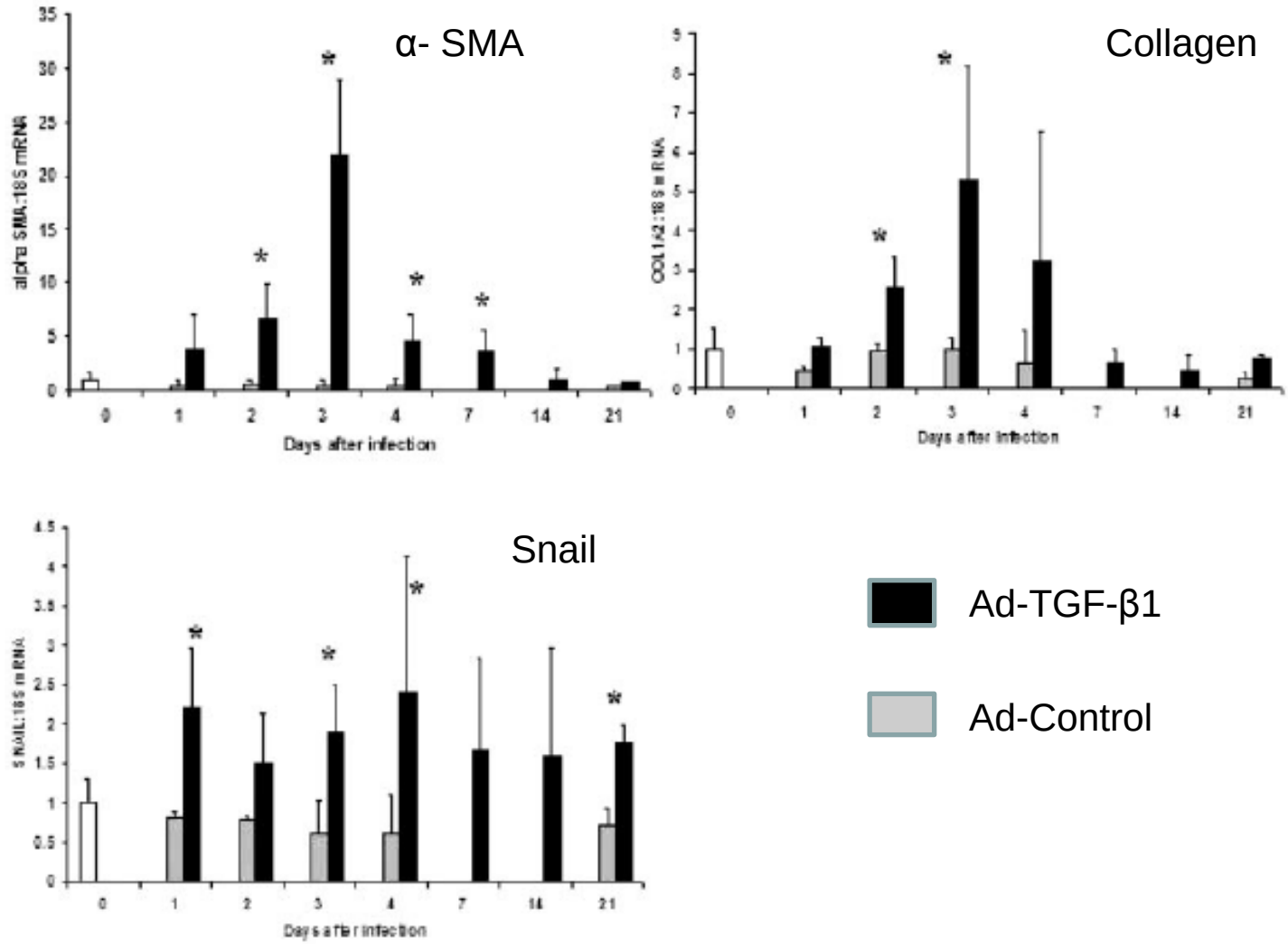






Liu L et al., Perit Dial Int 29; 508 (2009);
Margetts P et al., J Am Soc Nephrol 12; 2029 (2001) and J Am Soc Nephro 16,425 (2005)

Peritoneal expression of mesenchymal markers after adenovirus infection



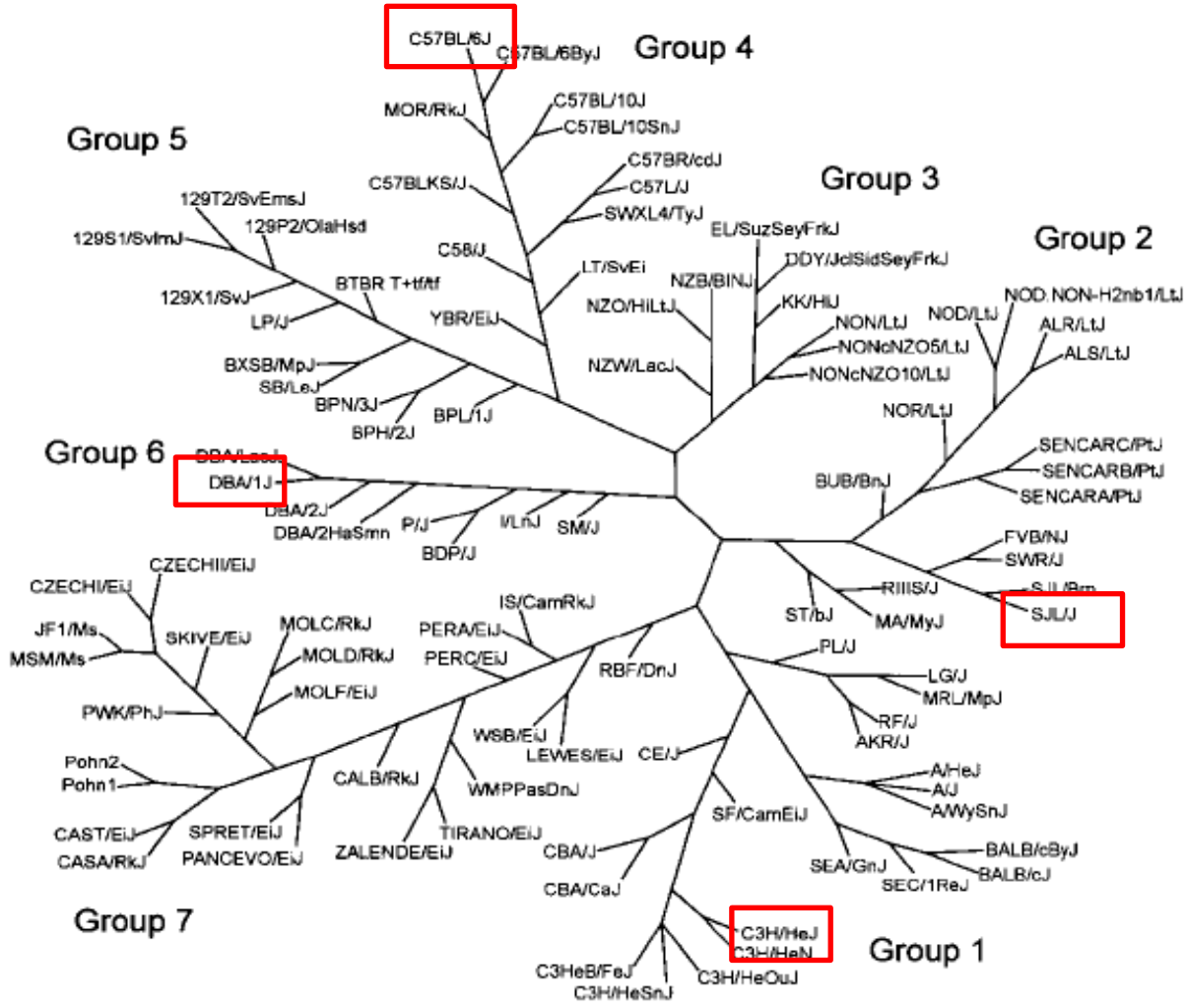
Margetts P J et al. JASN 2005;16:425-436

Is there a genetic predisposition to peritoneal fibrosis?

Mouse strain differences in the fibrotic response

Strain	Susceptibility	Fibrosis Type	Reference
C57BL/6J	Susceptible	Pulmonary	Haston et al., 1996
		Hepatic	Hillebrandt et al., 2002
		Renal	Kato et al., 2008
C57BL/6	Susceptible	Pulmonary Intestinal Hepatic Renal	Schrier et al., 1983 Skwarchuk and Travis, 1998 Knight et al., 2007 Puri et al., 2010
	Intermediate	Hepatic	Shi et al., 1997
	Resistant	Renal	Sugimoto et al., 2007
		Cardiac	Faulx et al., 2005
BALB/c	Susceptible	Hepatic	Shi et al., 1997
	Resistant	Pulmonary Hepatic Renal	Schrier et al., 1983 Knight et al., 2007 Puri et al., 2010
BALB/cJ	Susceptible	Hepatic	Hillebrandt et al., 2002
A/J	Susceptible	Cardiac	Faulx et al., 2005
	Resistant	Hepatic	Hillebrandt et al., 2002

Mouse family tree – Jackson Labs



Petkov P. et al., *Genome Res.* 2004 14: 1806-1811

Animal model of peritoneal fibrosis - mouse strain differences

C57BL/6J

DBA/2J

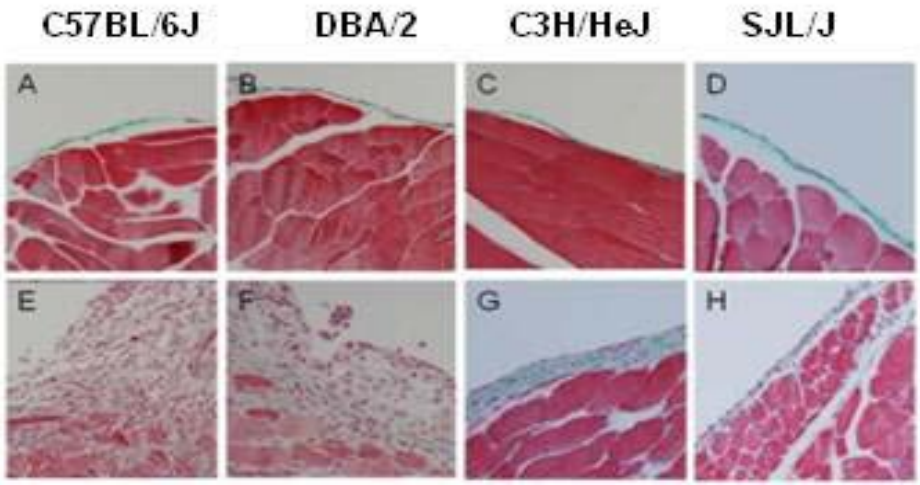
C3H/HeJ

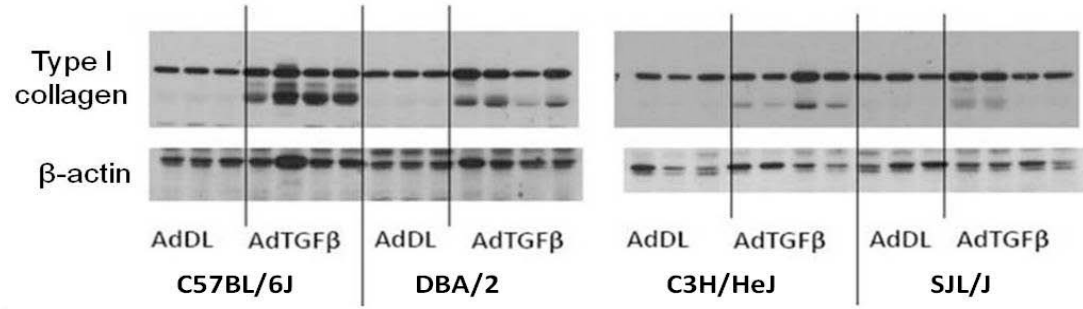
SJL/J

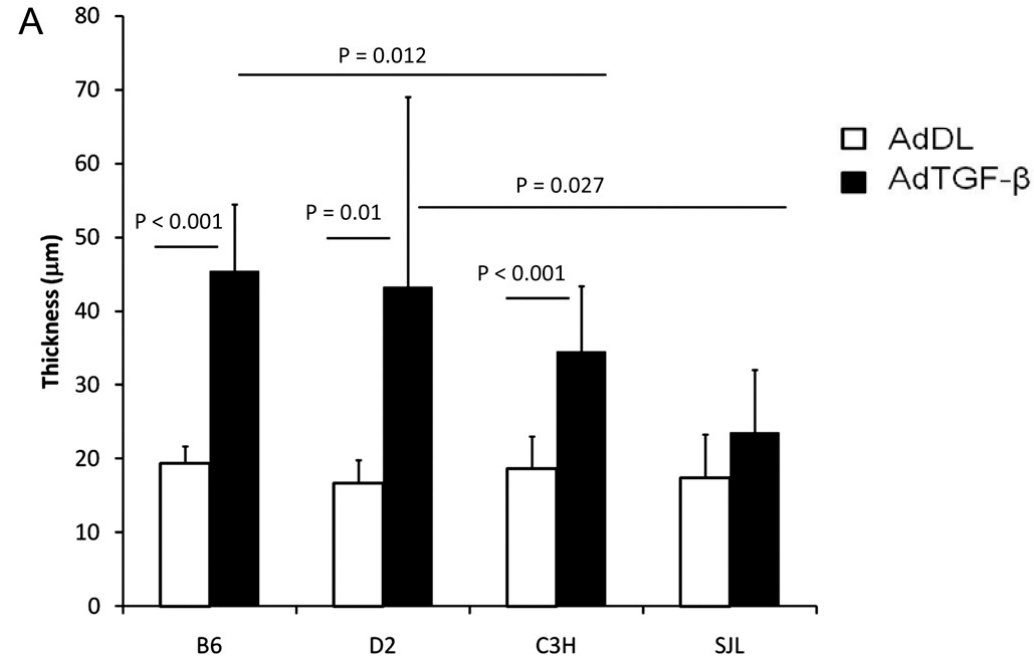
- Intraperitoneal injection of TGF-β1 expressing adenovirus administered to mice.
- After 4 or 10 days peritoneal tissue and omental tissue samples collected and analysed for fibrogenic differences

Louise Walkin, PhD student

Mouse stain differences in the development of peritoneal fibrosis







C57BL/6J = fibrotic
SJL/J = resistant

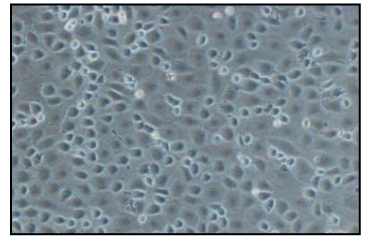
Margetts P *et al.,* Nephrol Dial Transplant. (2012)

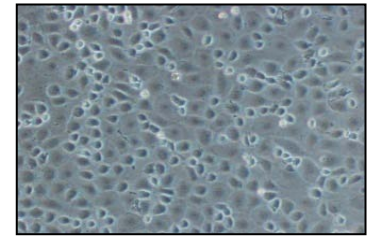
Mesothelial cell culture – mouse strain differences?

The Jackson Laboratory - JAX®
- Male , 8 weeks

C57BL/6J

SJL/J





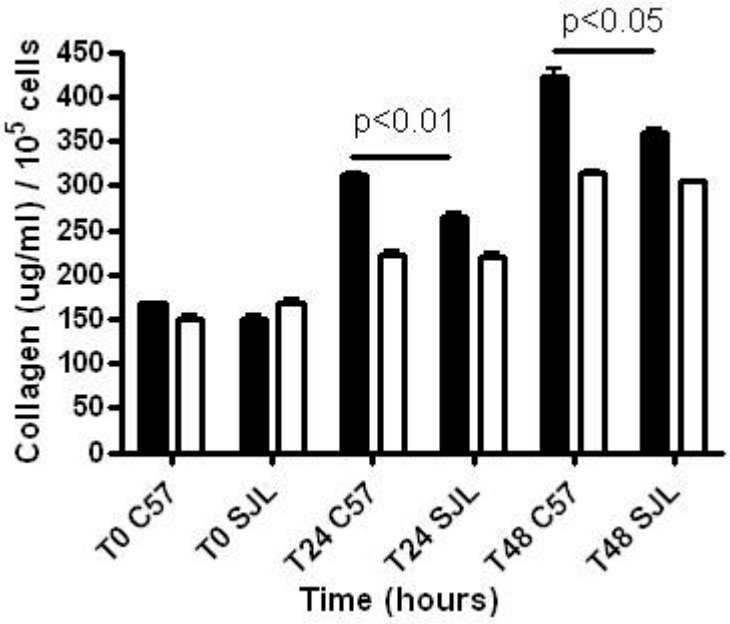
Isolation & culture

+/- 1ng/ml TGF-β1

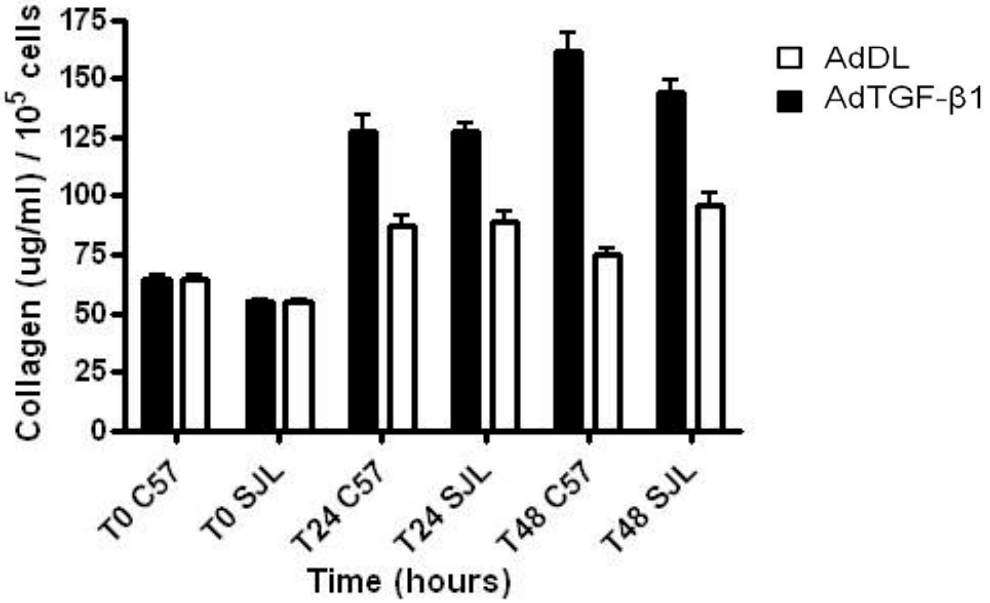
- Collagen production at 0, 24, 48 hours

Mouse strain difference in mesothelial cells – collagen production

Mesothelial cells



Fibroblasts



EMT markers?

Inhibition of EMT to prevent peritoneal fibrosis

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Inhibition of Transforming Growth Factor-Activated Kinase 1 (TAK1) Blocks and Reverses Epithelial to Mesenchymal Transition of Mesothelial Cells

Raffaele Strippoli[1,2], Ignacio Benedicto[3,4], Maria Luisa Perez Lozano[3,5], Teijo Pellinen[1], Pilar Sandoval[3,5], Manuel Lopez-Cab

OPEN ACCESS Freely available online

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Fibrin-Induced Epithelial-to-Mesenchymal Transition of Peritoneal Mesothelial Cells as a Mechanism of Peritoneal Fibrosis: Effects of Pentoxifylline

Cheng-Chung Fang[1,2], Jenq-Wen Huang[2], Ren-Shi Shyu[3], Chung-Jen Yen[2], Cheng-Hsiang Shiao[4], Chih-Kang Chiang[2], Rey-Heng H

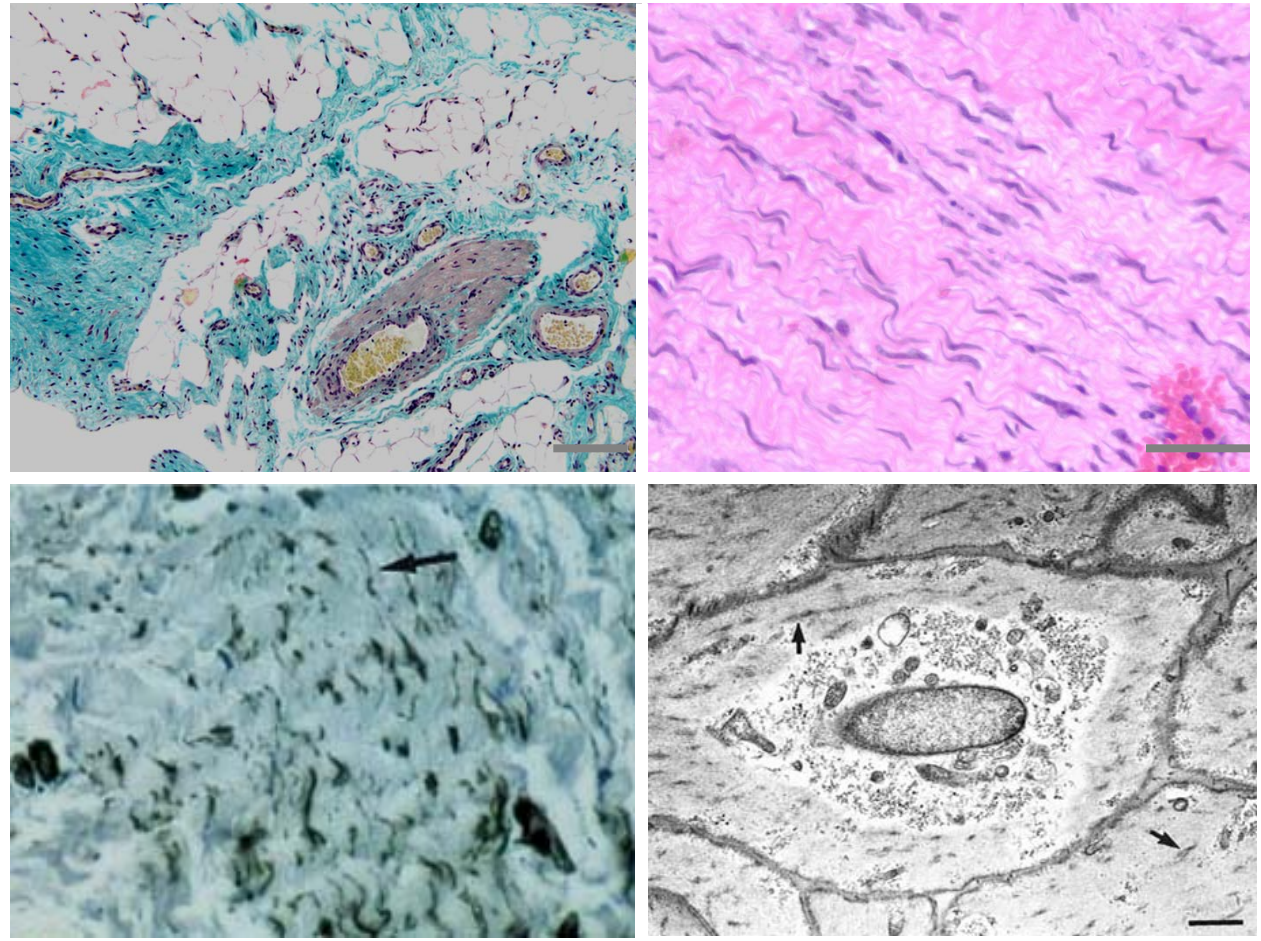
Nephrol Dial Transplant (2010) 25: 1098–1108
doi: 10.1093/ndt/gfp618
Advance Access publication 12 January 2010

2010

BMP-7 blocks mesenchymal conversion of mesothelial cells and prevents peritoneal damage induced by dialysis fluid exposure

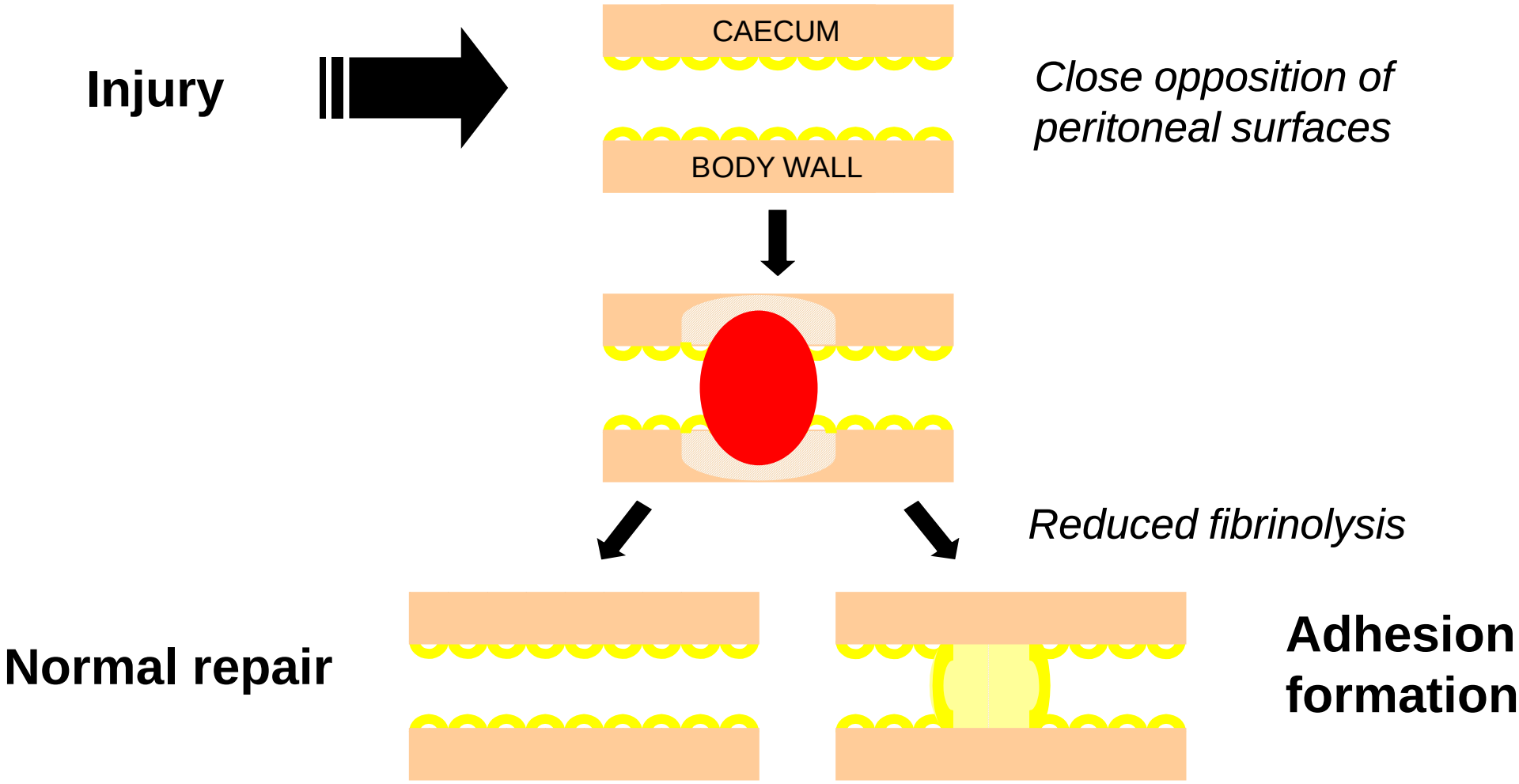
Jesús Loureiro[1], Margot Schilte[2], Abelardo Aguilera[1], Patricia Albar-Vizcaíno[1], Marta Ramírez-Huesca[1], M. Luisa Pérez-Lozano[1], Guadalupe González-Mateo[3], Luiz S. Aroeira[3], Rafael Selgas[3], Lorea Mendoza[4], Alberto Ortiz[5], Marta Ruíz-Ortega[5], Jacob van den Born[2], Robert H.J. Beelen[2] and Manuel López-Cabrera[1,6]

Human adhesions show the presence of myofibroblasts and clusters of smooth muscle



Herrick et al., J Pathology 2000, 192: 67-72; Sulaiman et al., Ann Surg 2001, 234: 256-261

Does mesothelial cell EMT occur in the pathogenesis of adhesion formation?



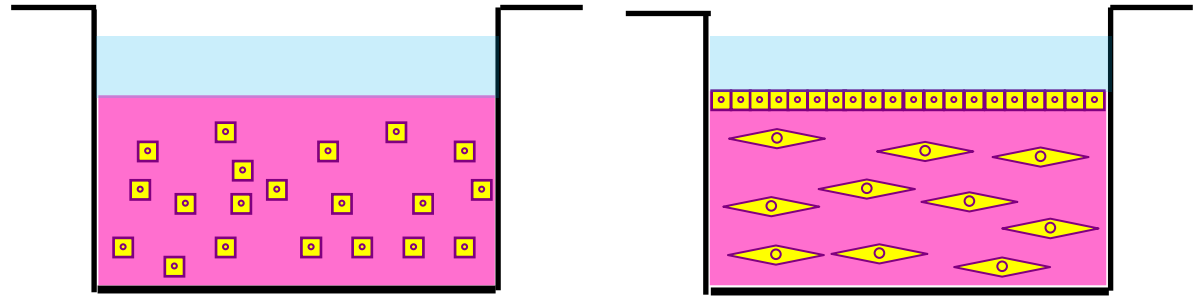
Journal of Surgical Research 82, 28–33 (1999)
Article ID jsre.1998.5449, available online at http://www.idealibrary.com on IDEAL®

Remodeling of Peritoneal-like Structures by Mesothelial Cells: Its Role in Peritoneal Healing

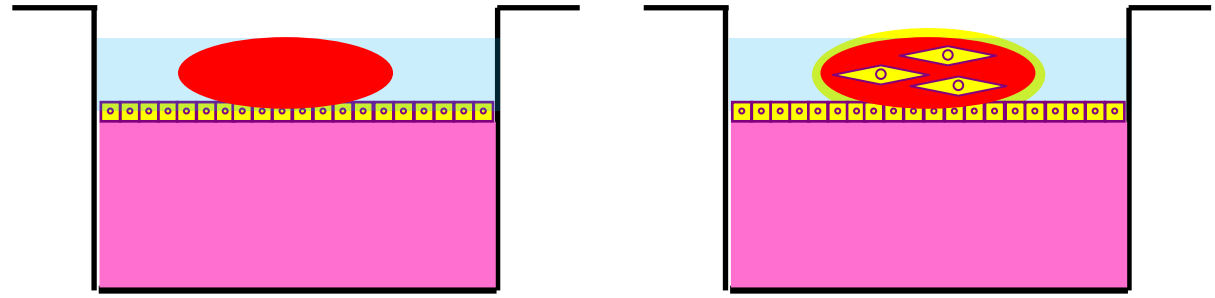
Fernando Bittinger,*,[1] Caroline Schepp,* Christoph Brochhausen,* Hans Anton Lehr,* Mike Otto,* Holger Köhler,* Carsten Skarke,* Siegfried Walgenbach,† and C. James Kirkpatrick*

*Department of Pathology, and †Department of Surgery, Johannes Gutenberg University, Mainz, Federal Republic of Germany

In collagen gel



On gel with blood clot

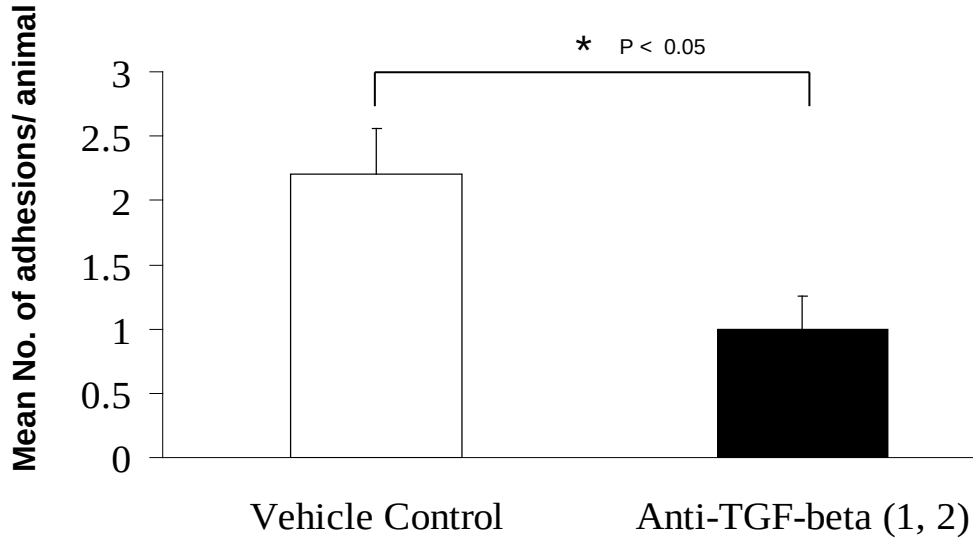


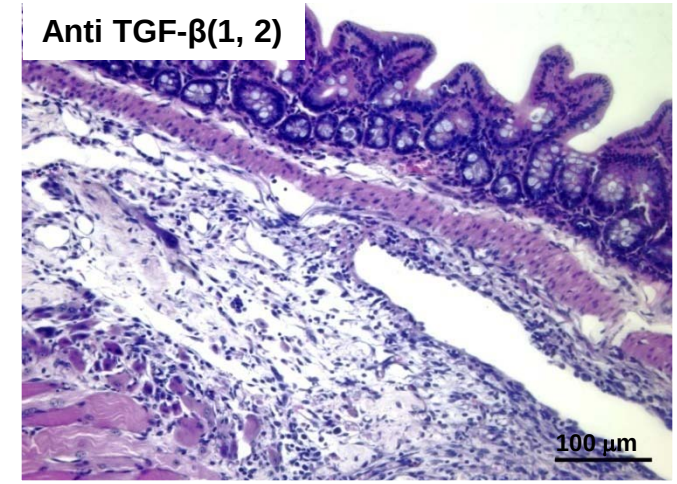
30 mins

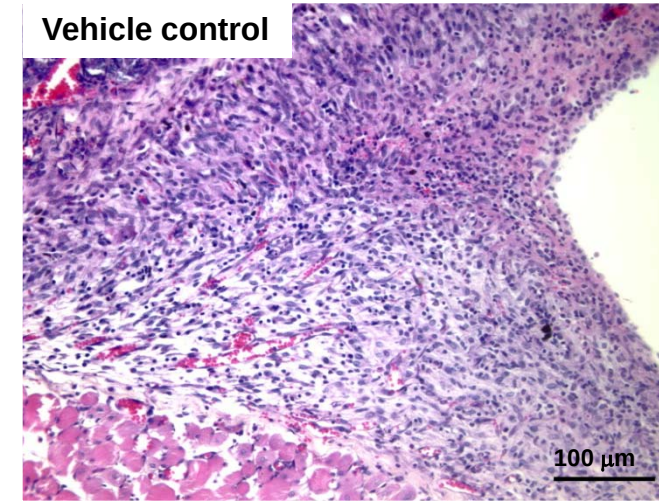
14 days

Inhibition of TGF-β1 and 2 reduces adhesion formation in murine model

- Neutralising antibody to TGF- β1 and 2 given topically on day of surgery and i.p 4hrs pre-surgery, post-surgery and every 4hrs for 24hrs
- Significant reduction of adhesions by blocking TGF- β1 and -β2 by 7 days

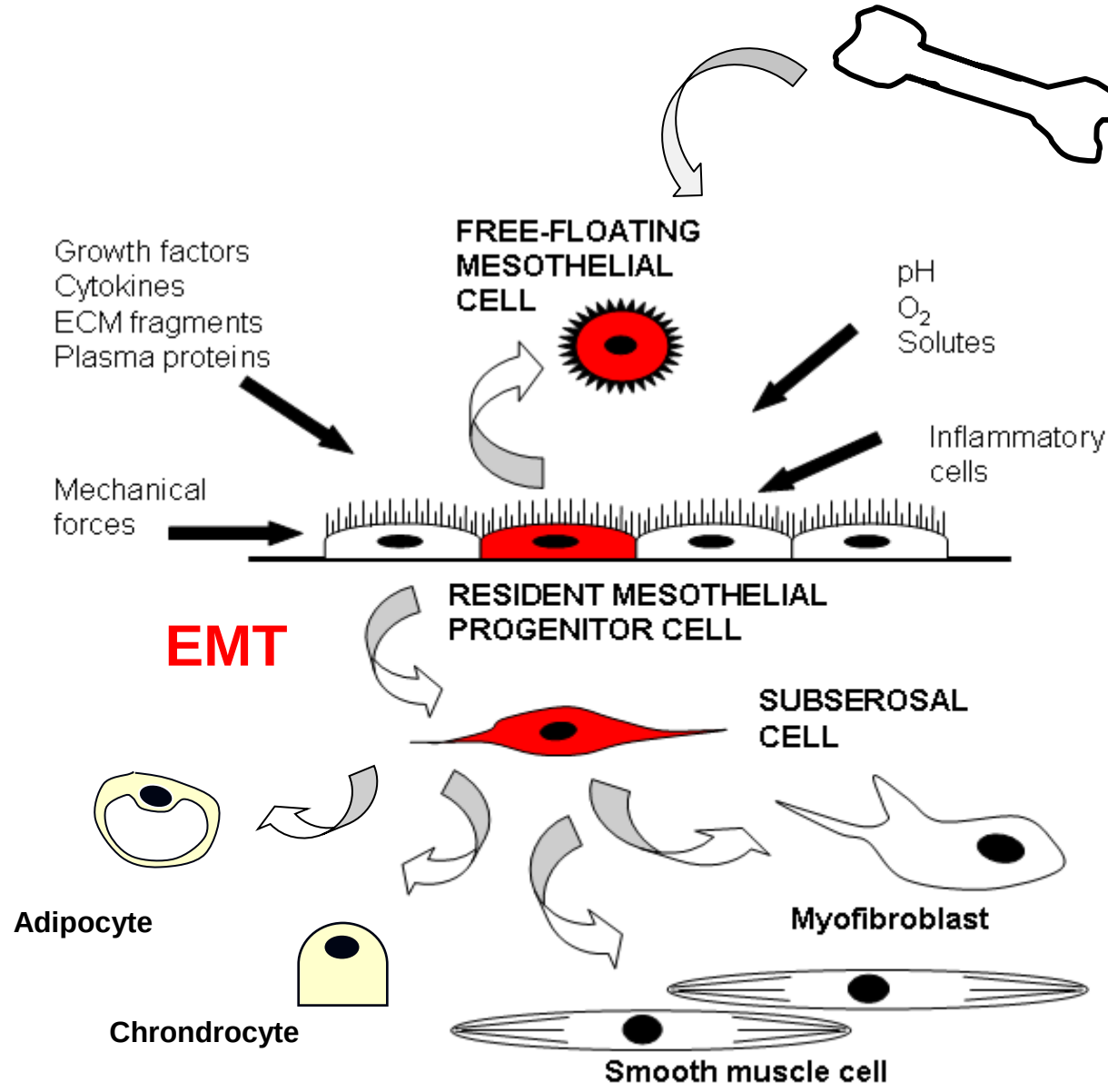




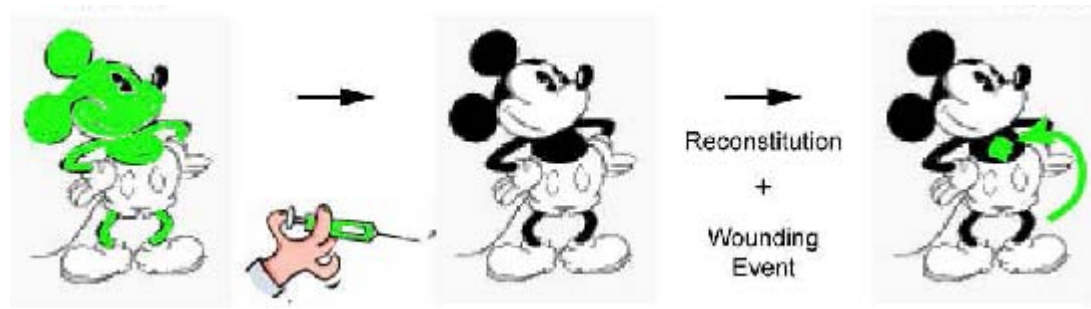


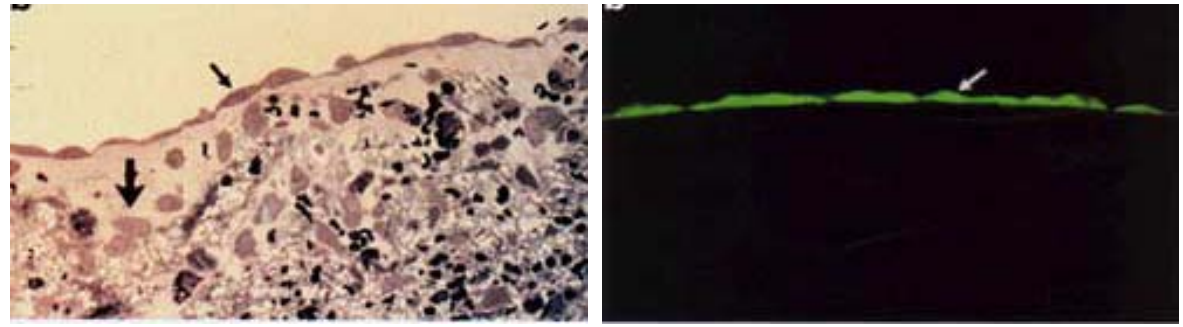
Gorvy et al. Am J Pathol 2005, 1005-1019.

Source and plasticity of mesothelial cells?



Evidence for a bone marrow source of regenerating mesothelial cells





Campbell et al., (2000) J Vasc Res. 37; 364- 371

Renal Failure, 32(9): 1081–1087, (2010)

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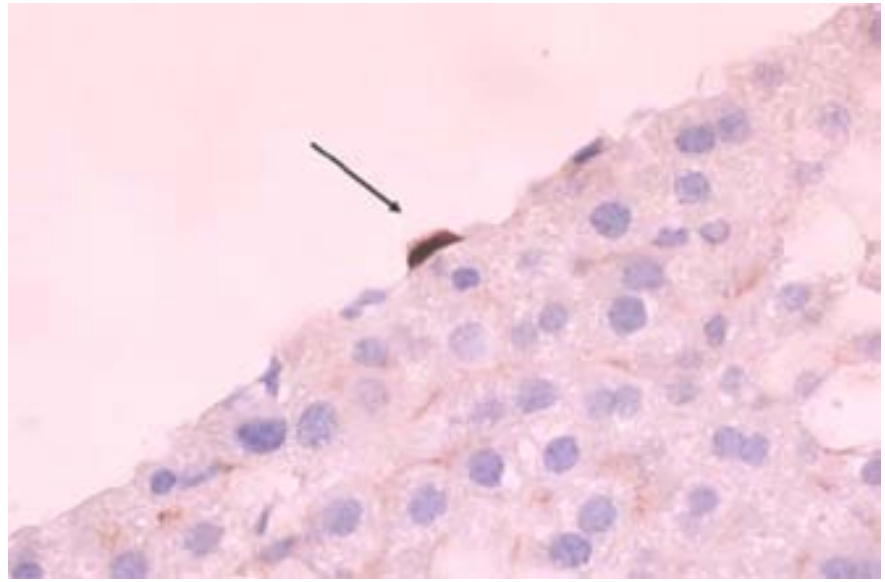
LABORATORY STUDY

Potential role of bone marrow-derived cells in the turnover of mesothelium

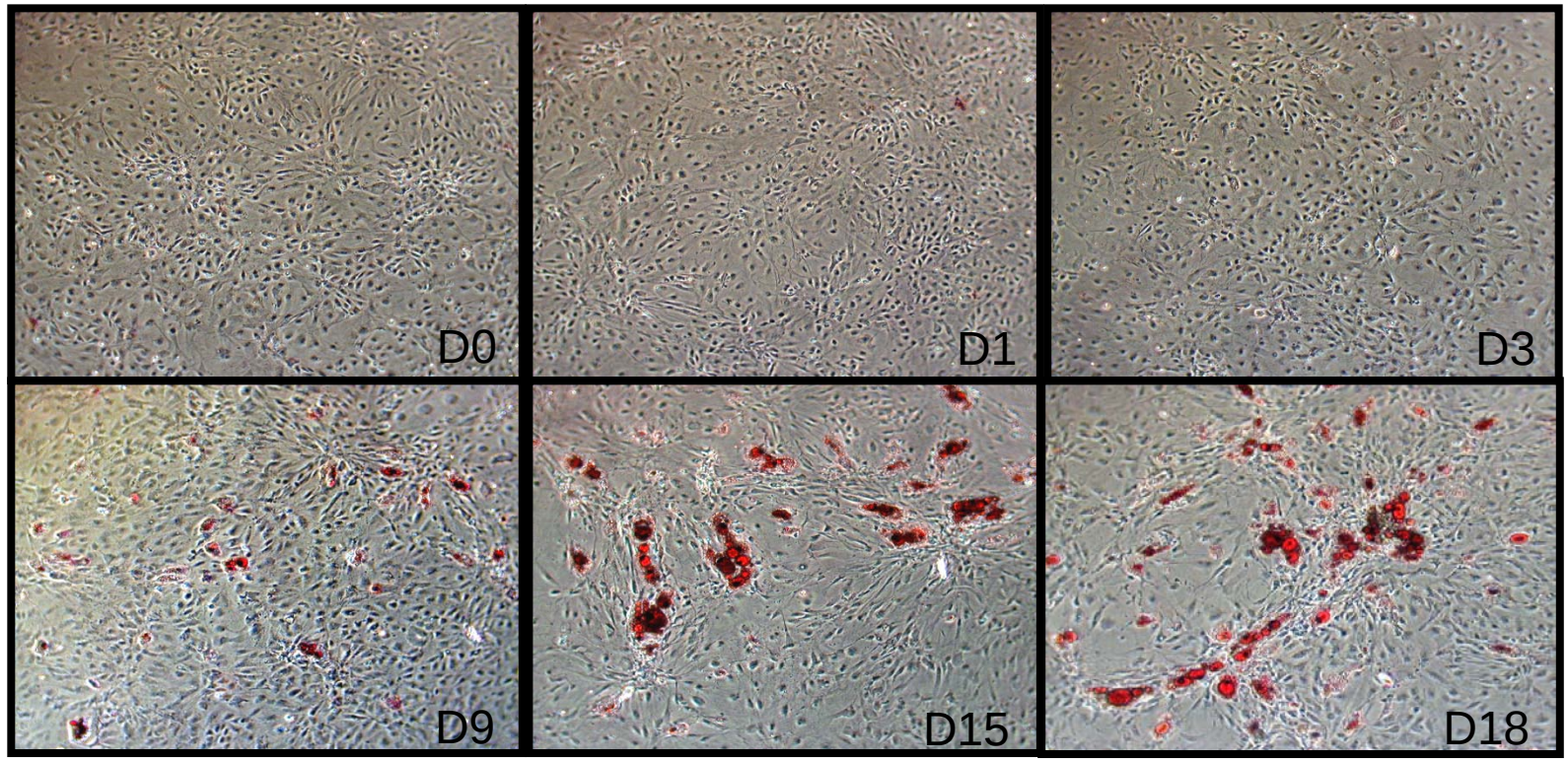
Kuo-Su Chen,[1] Chao-Hung Wang,[2] Tzung-Hai Yen,[1] Jim-Ray Chen,[3]
Ming-Jui Hung,[2] and Ching-Yuang Lin[4]



- GFP- bone marrow transplant into irradiated mice
- Found 2.2% of mesothelium GFP-labelled after 6 months
- Bone-marrow derived cells contribute to normal mesothelial turnover
- After injury?

Stimulated rat mesothelial cells accumulate lipid during adipogenic differentiation

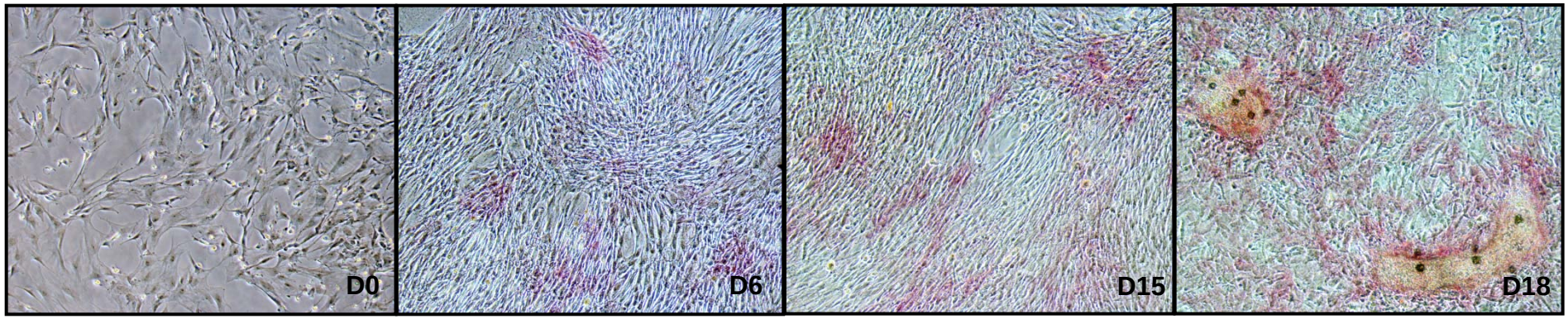
- Steve Mutsaers, UWA, Perth



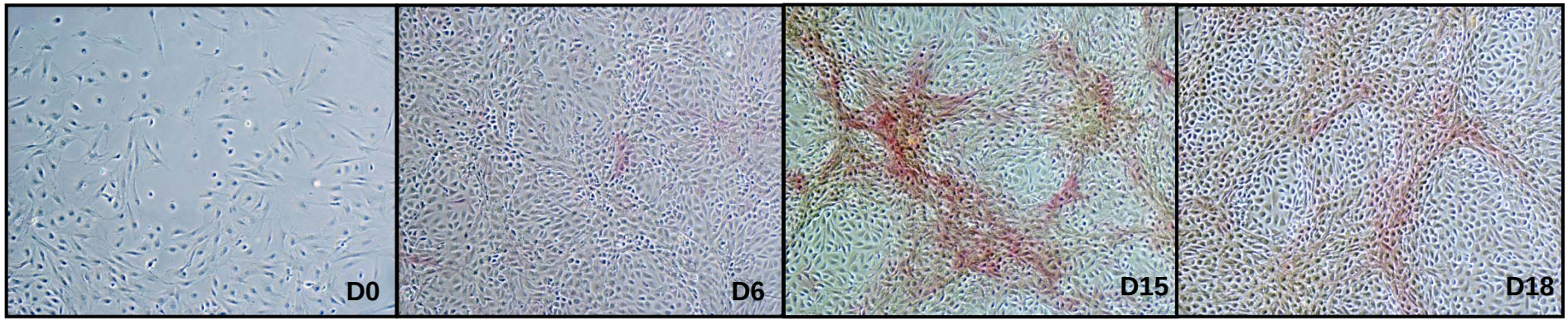
Lansley S. et al., J Cell Mol Med 2011;15(10):2095-105.

Stimulated rat mesothelial cells express alkaline phosphatase during chrondrogenic differentiation

Bone Marrow Mesenchymal Cells (BMMC)

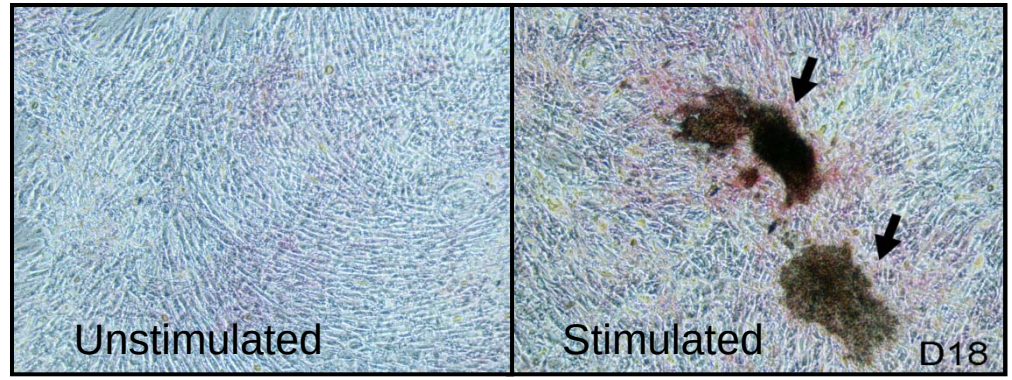


Mesothelial Cells

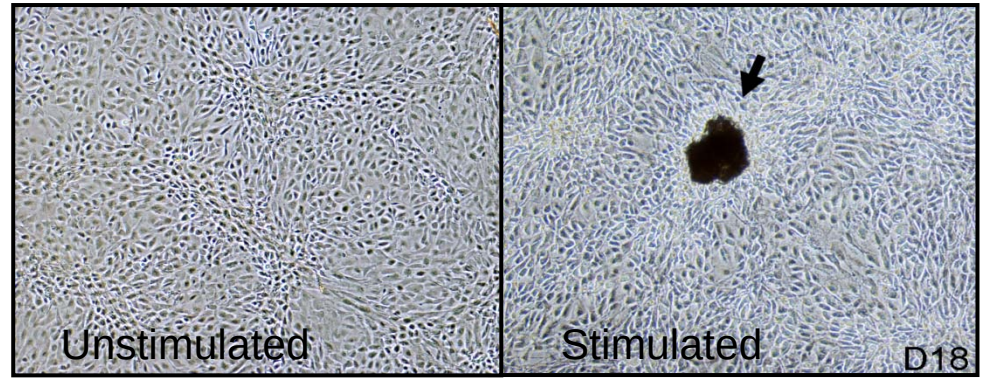


Stimulated rat mesothelial cells form mineralized bone nodules – Von Kossa staining

Bone Marrow Mesenchymal Cells (BMMC)



Mesothelial Cells



Lansley S. et al., J Cell Mol Med 2011;15(10):2095-105.

Conclusions

- Mesothelial cells undergo EMT during peritoneal fibrosis - adhesion formation?
- Inhibit EMT prevents peritoneal fibrosis – adhesion formation?
- Mesothelial cells show multipotential ability in culture – in people?
- Apparent bone marrow source of mesothelial cells – do these cells have stem cell-like properties?

- Need to perform cell tracking studies – which promoter?
- Use primary human mesothelial cells – source?
- Multidisciplinary approach?

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