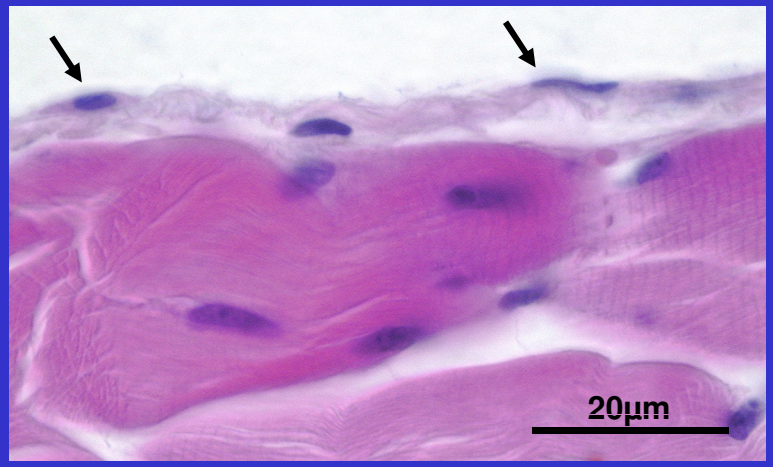
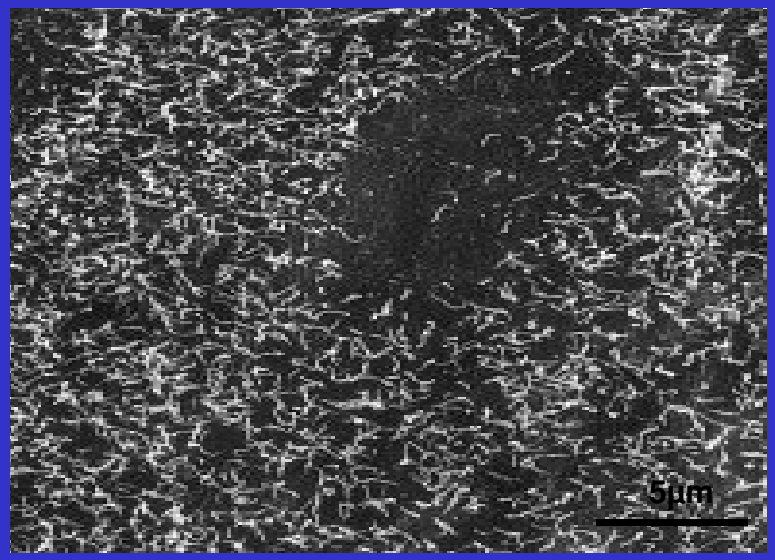

MANCHESTER
1824
The University of Manchester
In vitro models for adhesion research
ESHRE Campus Symposium
4-5th September 2009
Sarah Herrick
University of Manchester, UK

Peritoneum

- 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

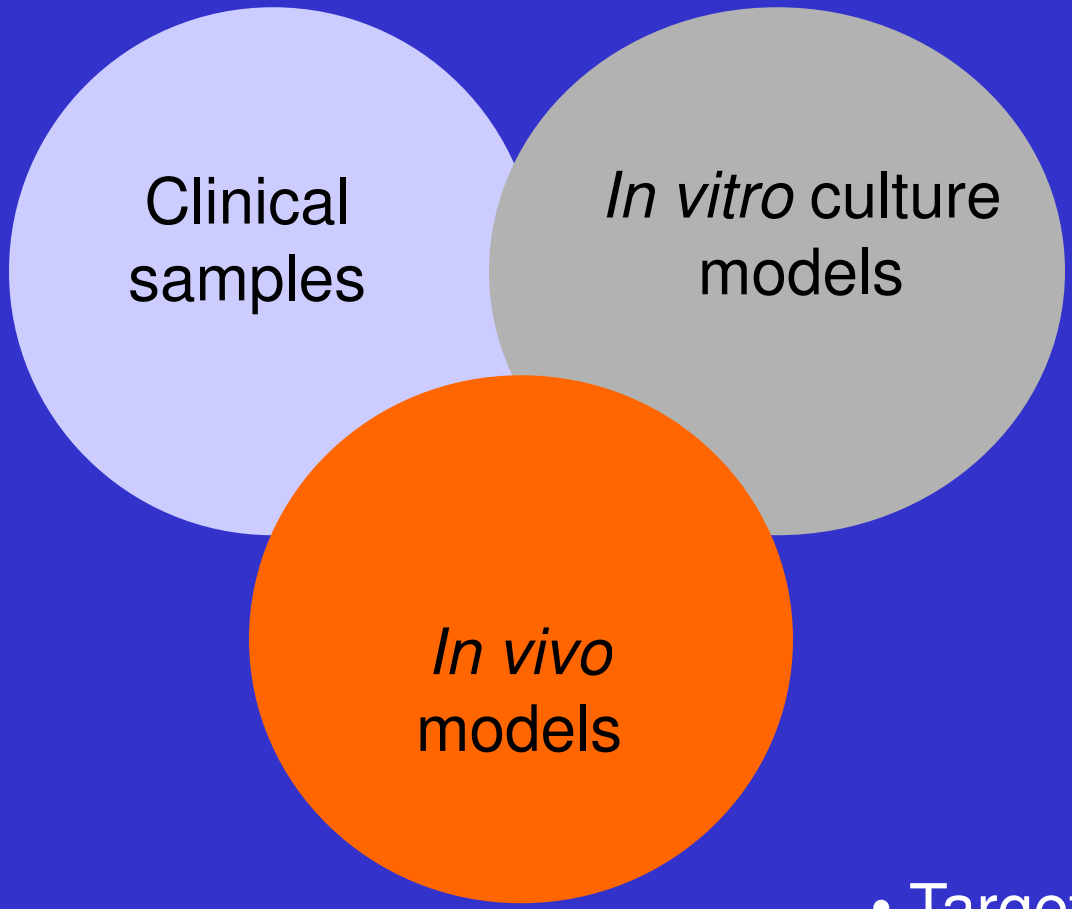




Peritoneal repair often leads to adhesion formation

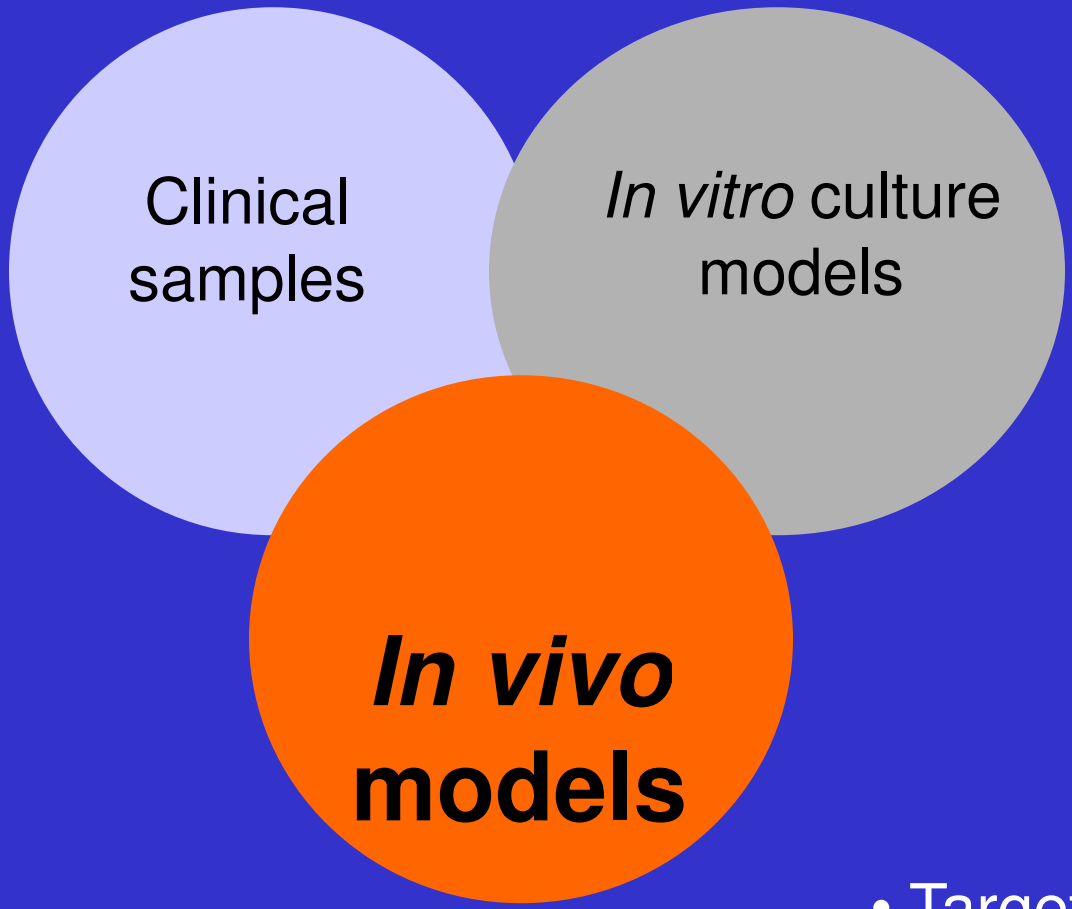
- Form after peritoneal insult
- Often involve the omentum
- Complications :
  - intestinal obstruction
  - infertility in women
  - difficulty in repeat surgery
  - chronic pelvic pain?
- Costs NHS £569 million over 10 year period- SCAR study
- Reform after surgical adhesiolysis
- No adequate treatment – barrier devices

Tools used to understand adhesion pathogenesis



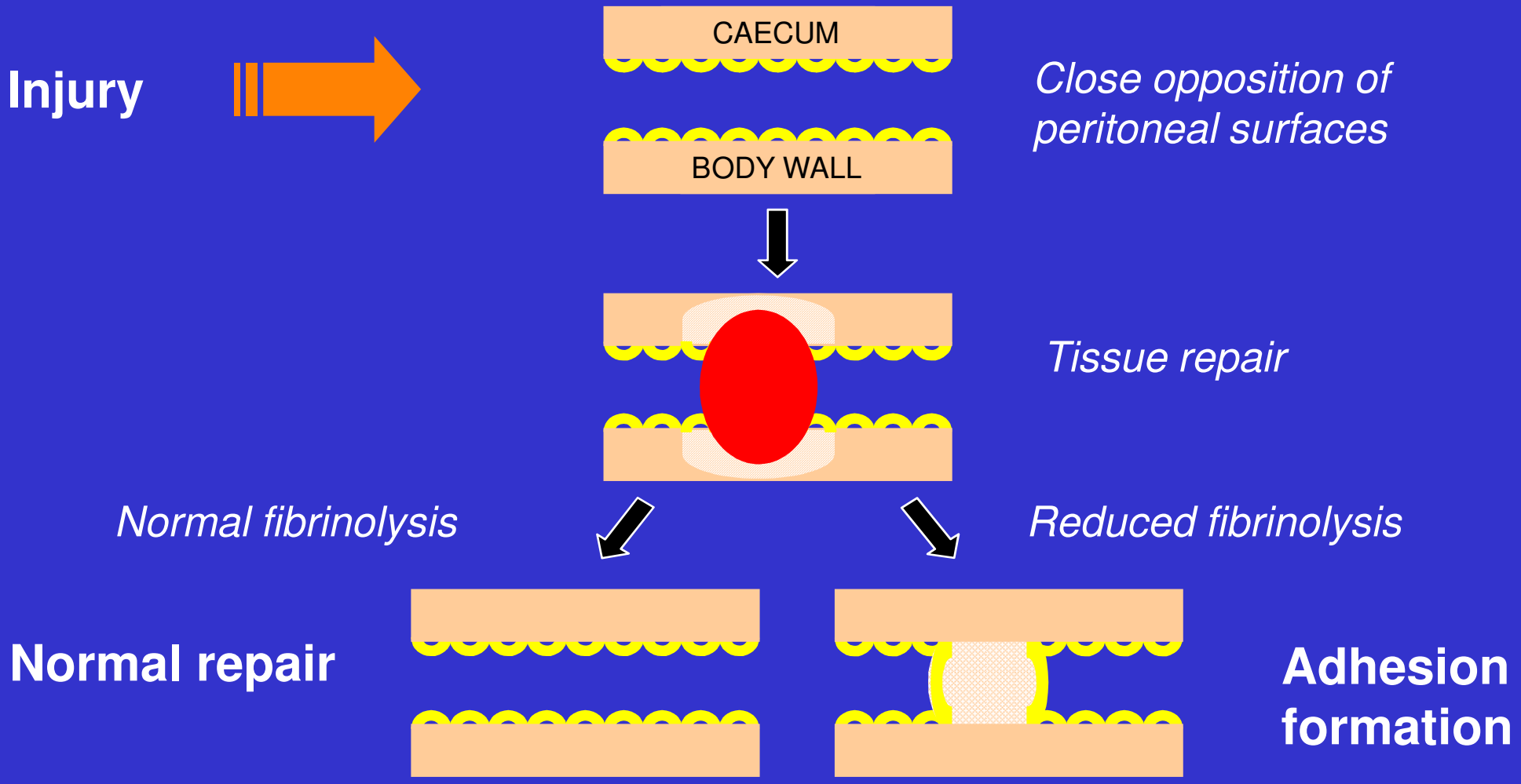
- Target identification
- Target validation

Tools used to understand adhesion pathogenesis

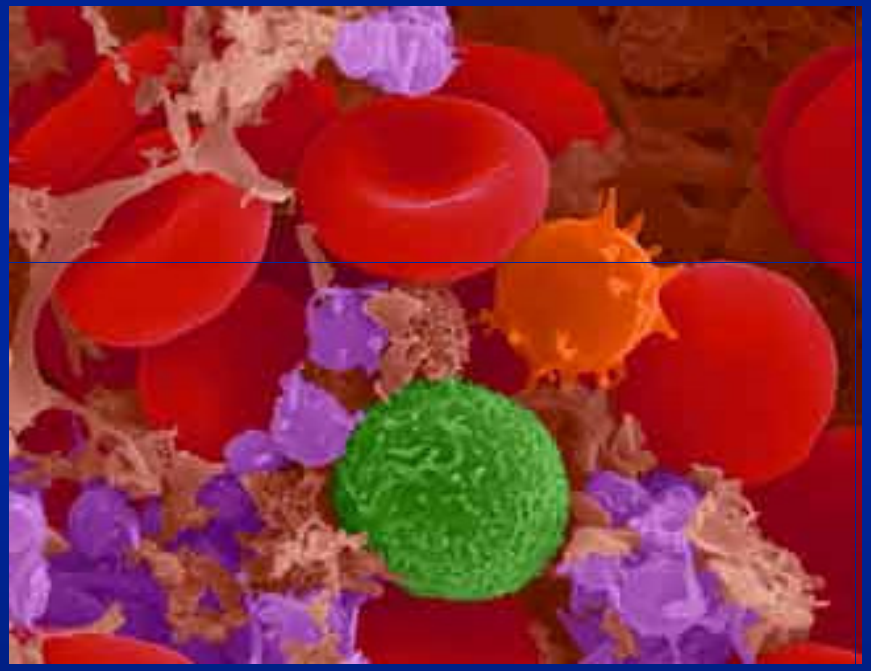


- Target identification
- Target validation

Pathogenesis of adhesion formation

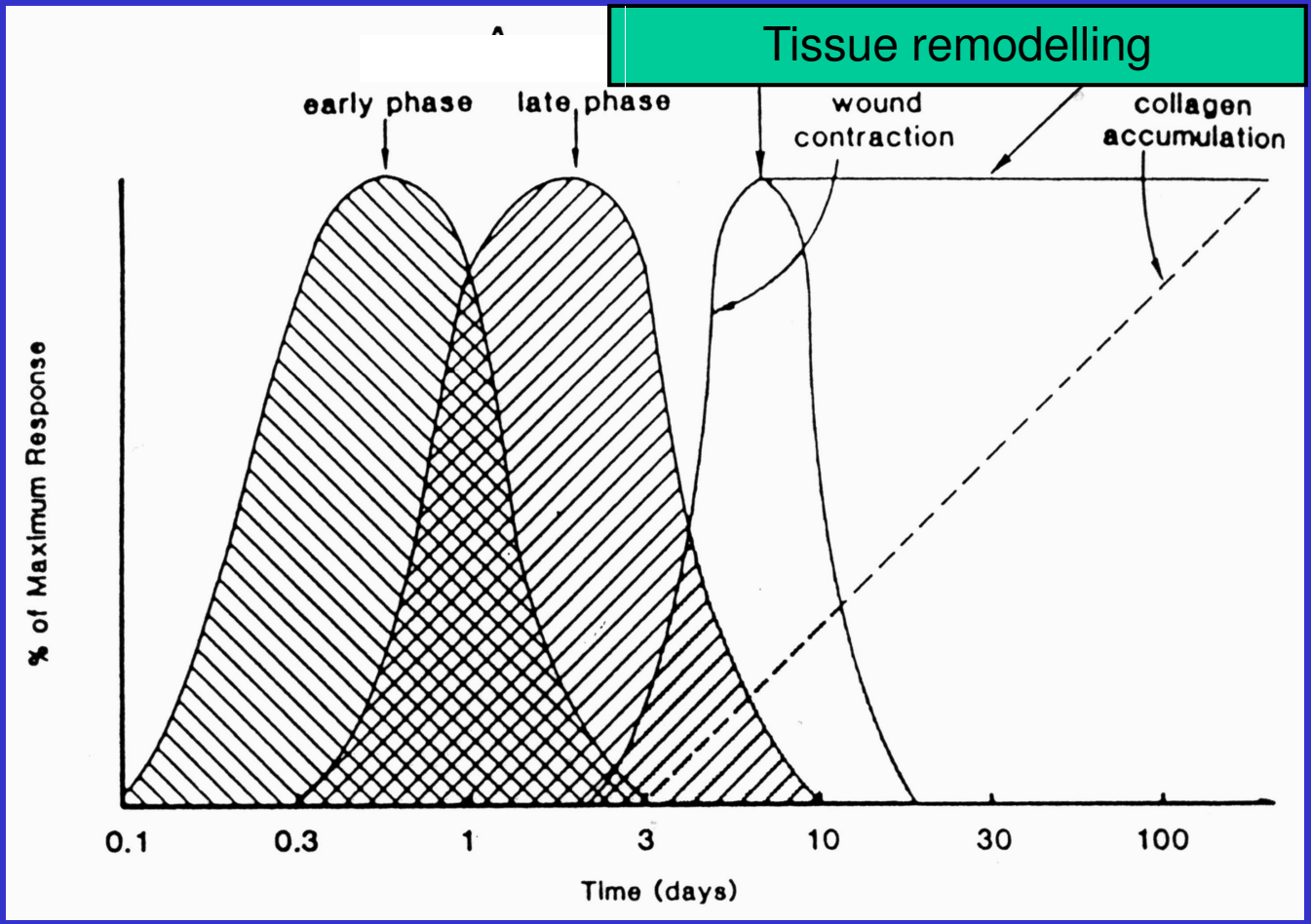


Phases of tissue repair



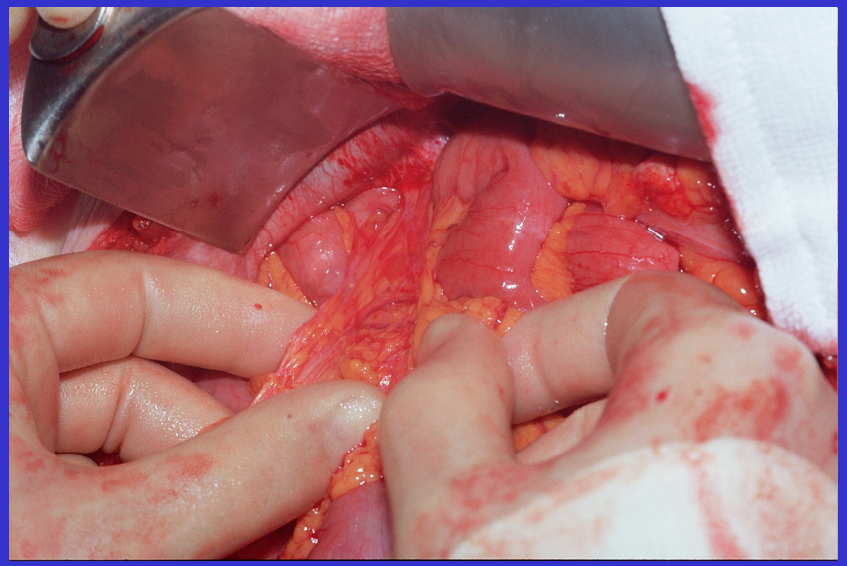
- Coagulation and inflammation
- Epithelialisation
- Granulation tissue
- Tissue contraction
- Remodeling and scarring/fibrosis

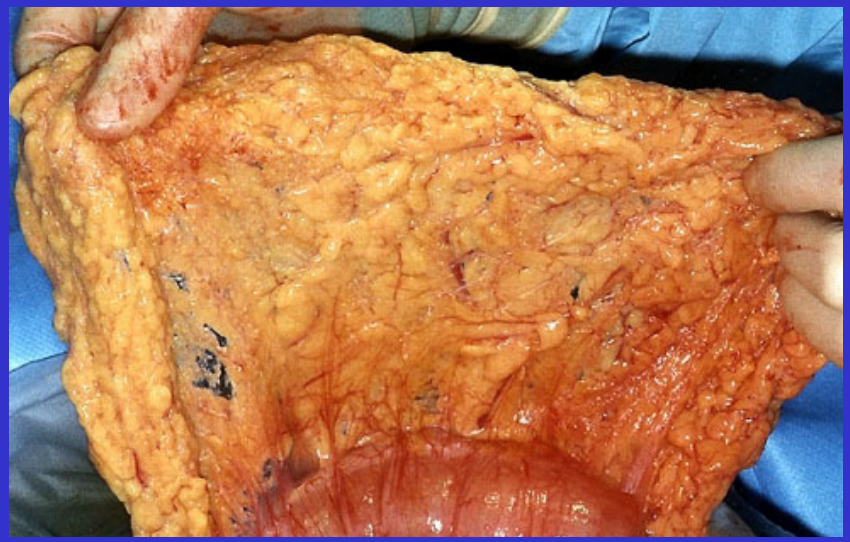
Temporal relationship of cellular phases of tissue repair



Understand the disease - clinical samples

- Sample collection
  - Blood/plasma
  - Peritoneal fluid/ lavage
  - Tissue (adhesion/peritoneum)
- Compare healthy/normal with adhesion samples
- Compare pre- and post- surgery
- Snap shot – set time points
- Hard to determine stage of adhesion development
- Patient variability
- Limited access and availability
- Not open to manipulation





Peritoneal fluid analysis

Journal of Surgical Research 154, 312–316 (2009)
doi:10.1016/j.jss.2008.05.012

Studies of TGF-β_{1-3} in Serosal Fluid During Abdominal Surgery and Their Effect on *In Vitro* Human Mesothelial Cell Proliferation

Peter Falk, B.Sc.,*[,1] Maria Bergström, Ph.D., M.D.,* Ingrid Palmgren, B.Sc.,* Lena Holmdahl, Ph.D., M.D.,* Michael E. Breimer, Ph.D., M.D.,* and Marie-Louise Ivarsson, Ph.D., M.D.†

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REPRODUCTIVE SURGERY

Preoperative predictors of postsurgical adhesion formation and the Prevention of Adhesions with Plasminogen Activator (PAPA-study): results of a clinical pilot study

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BJOG: an International Journal of Obstetrics and Gynaecology
September 2002, Vol. 109, pp. 1041–1049

Differential expression of matrix metalloproteinase and tissue inhibitor of MMP in serosal tissue of intraperitoneal organs and adhesions

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Members of the The Peritoneal Healing and Adhesion Multi University Study (PHAMUS)

REPRODUCTIVE BIOLOGY

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Peritoneal fluid concentrations of matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and transforming growth factor-beta in women with pelvic adhesions

Ying C. Cheong, M.B., B.Ch.,[a,b,d] Jenny B. Shelton, Ph.D.,[c] Susan M. Laird, Ph.D.,[c] Tin-Chiu Li, Ph.D., F.R.C.O.G.,[b] William L. Ledger, Ph.D., F.R.C.O.G.,[b] and Ian D. Cooke, Ph.D., F.R.O.C.G.[b]

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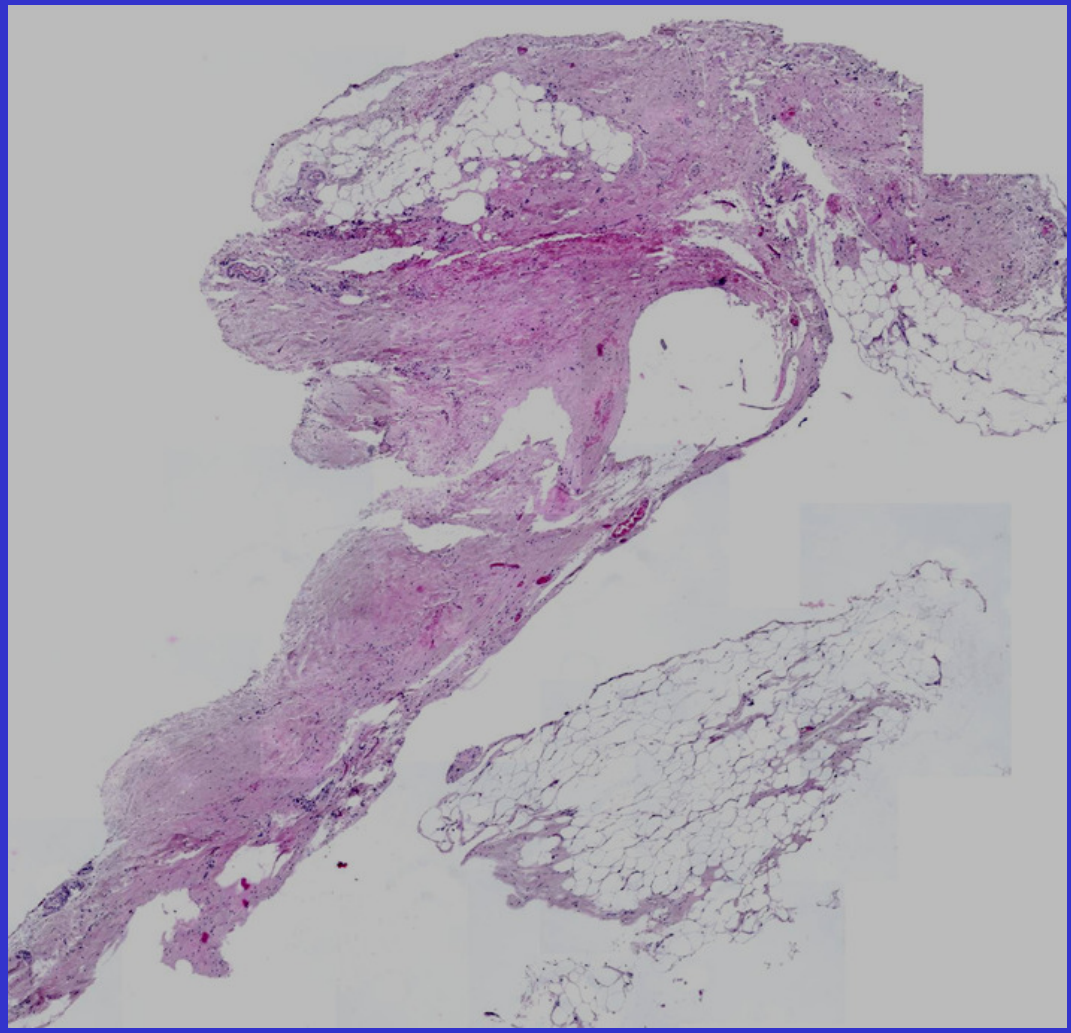
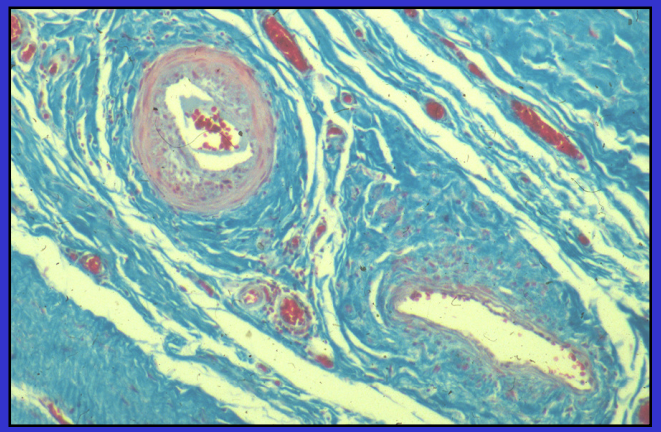
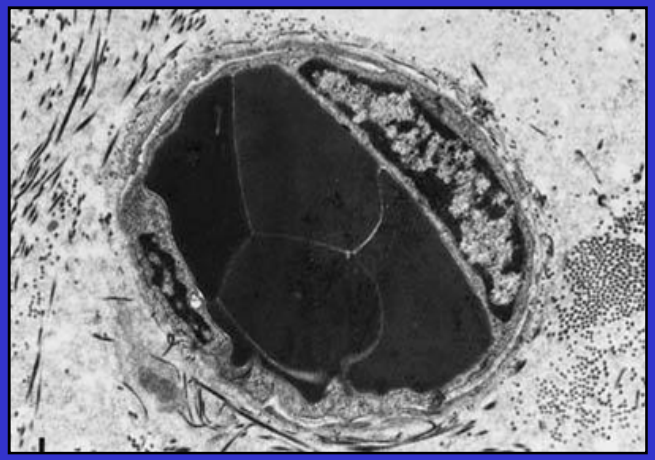
Do human peritoneal adhesions resemble scar tissue?

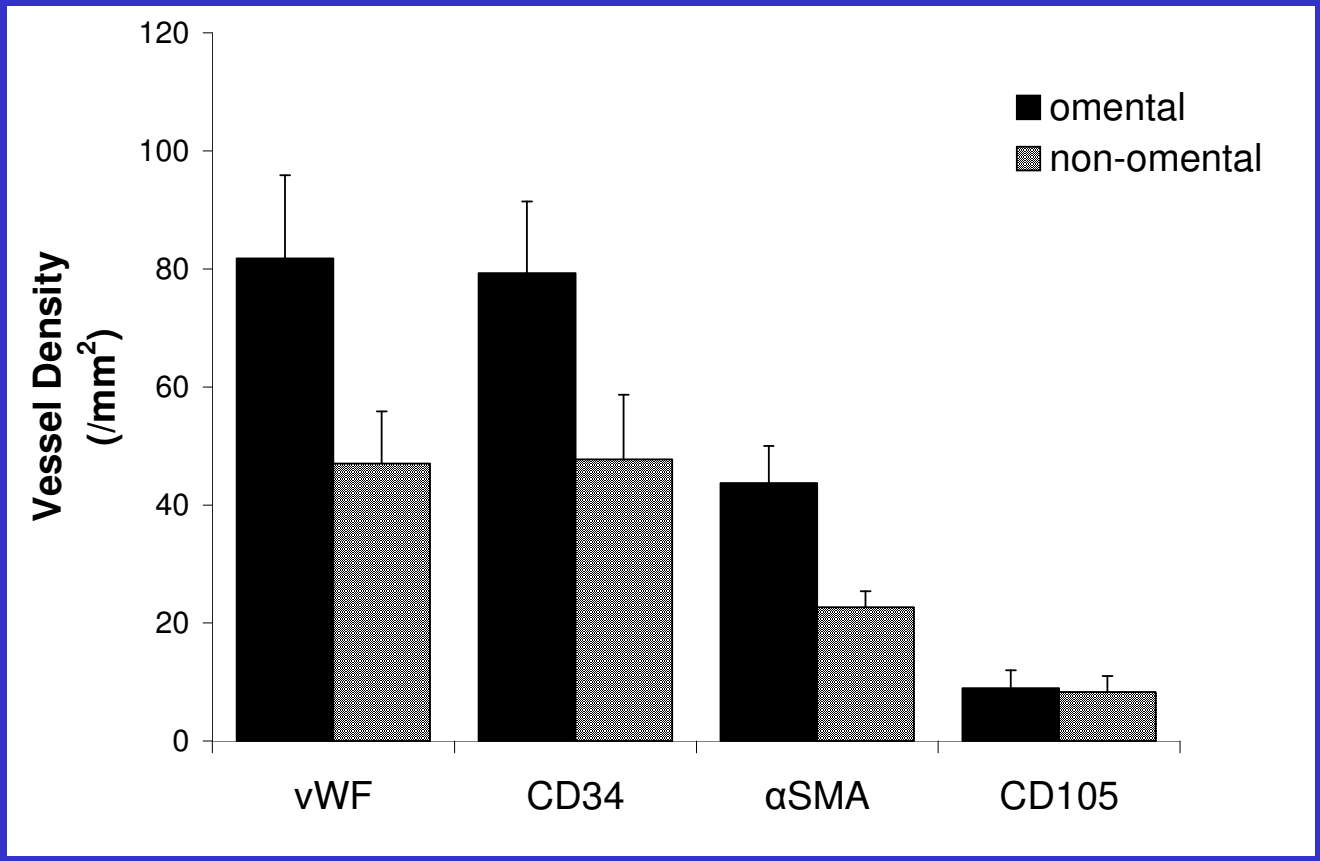
- Samples of adhesion and peritoneal tissue taken during abdominal surgery
- Location, size, type and number of adhesions recorded – visual record if possible
- Patient history recorded
- Tissue samples divided and processed:
  - histology
  - immunocytochemistry
  - electron microscopy



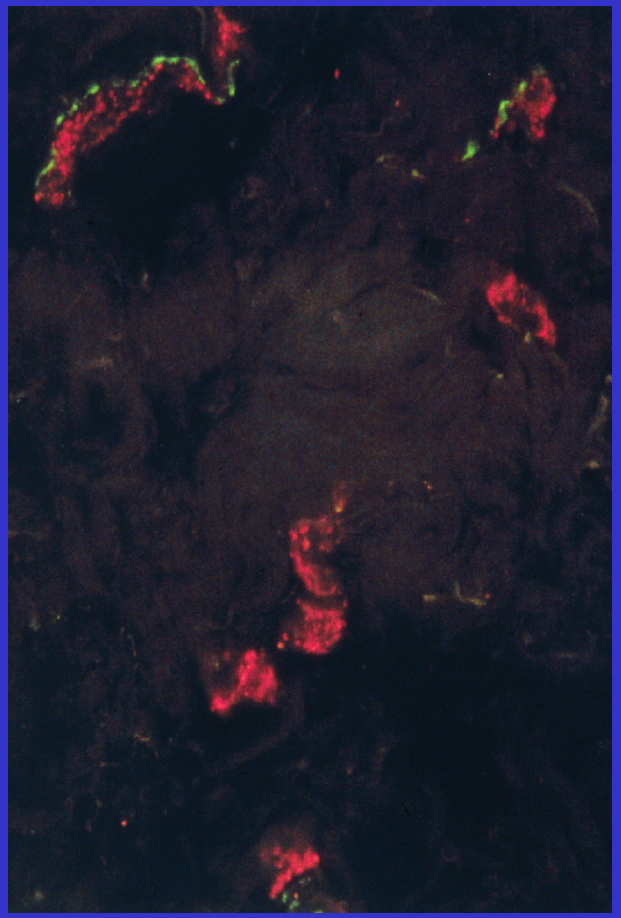
Human peritoneal adhesions are well vascularised

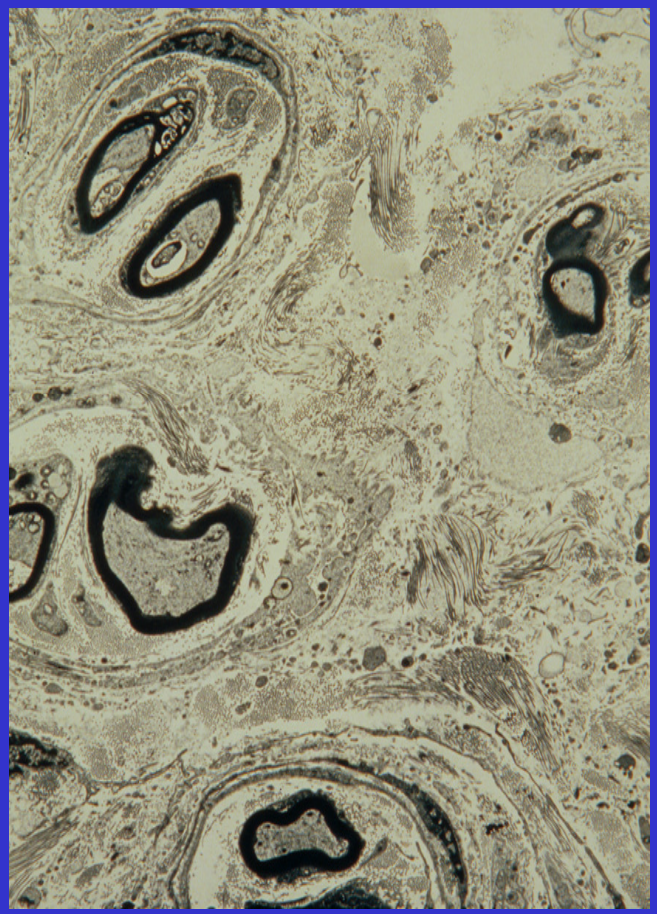


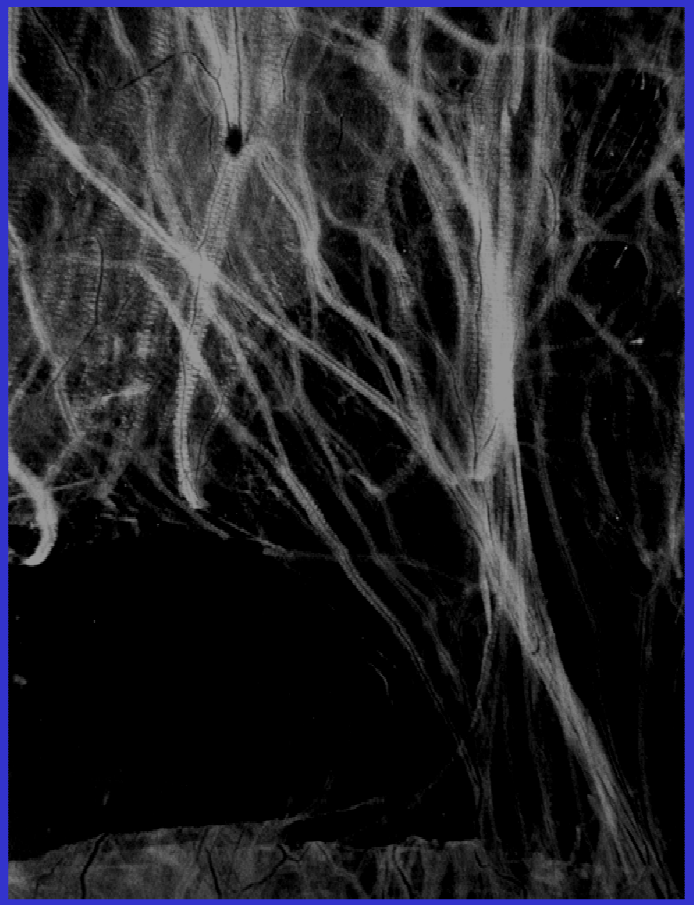




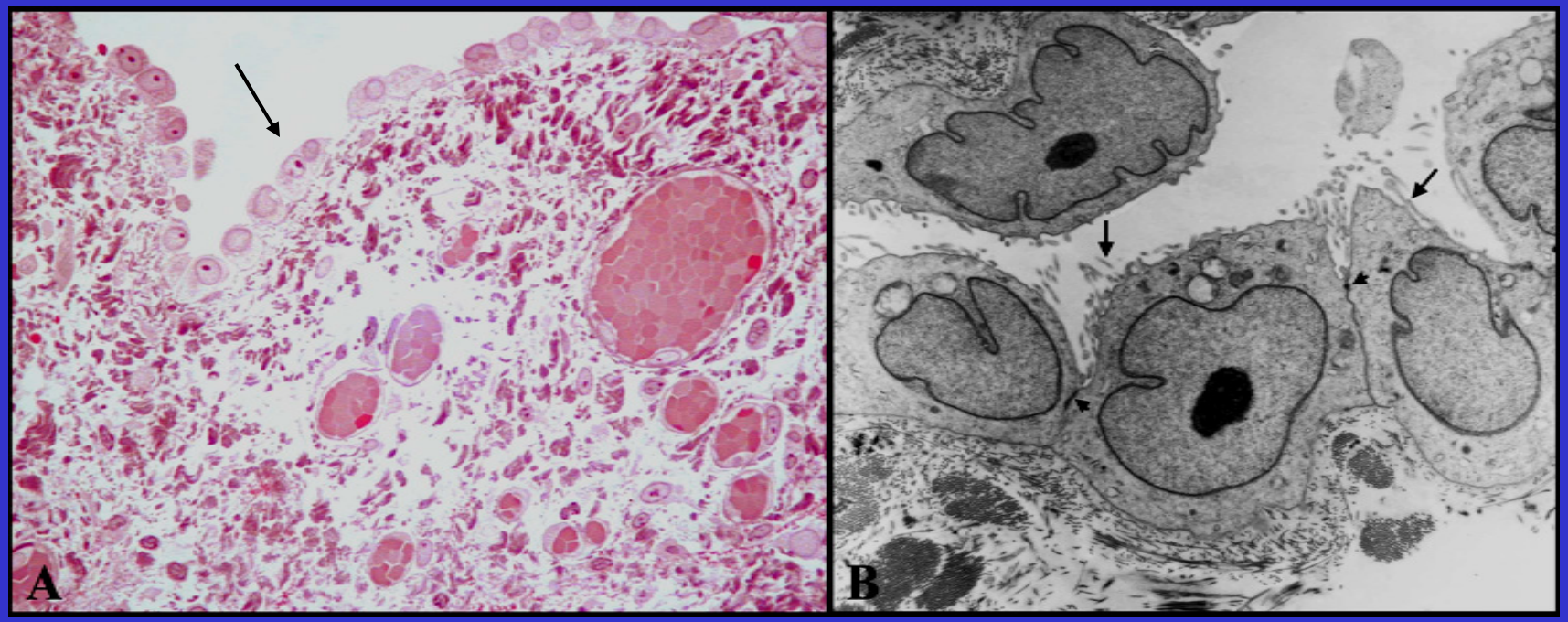
Human adhesions contain sensory nerves - relationship with pelvic pain?



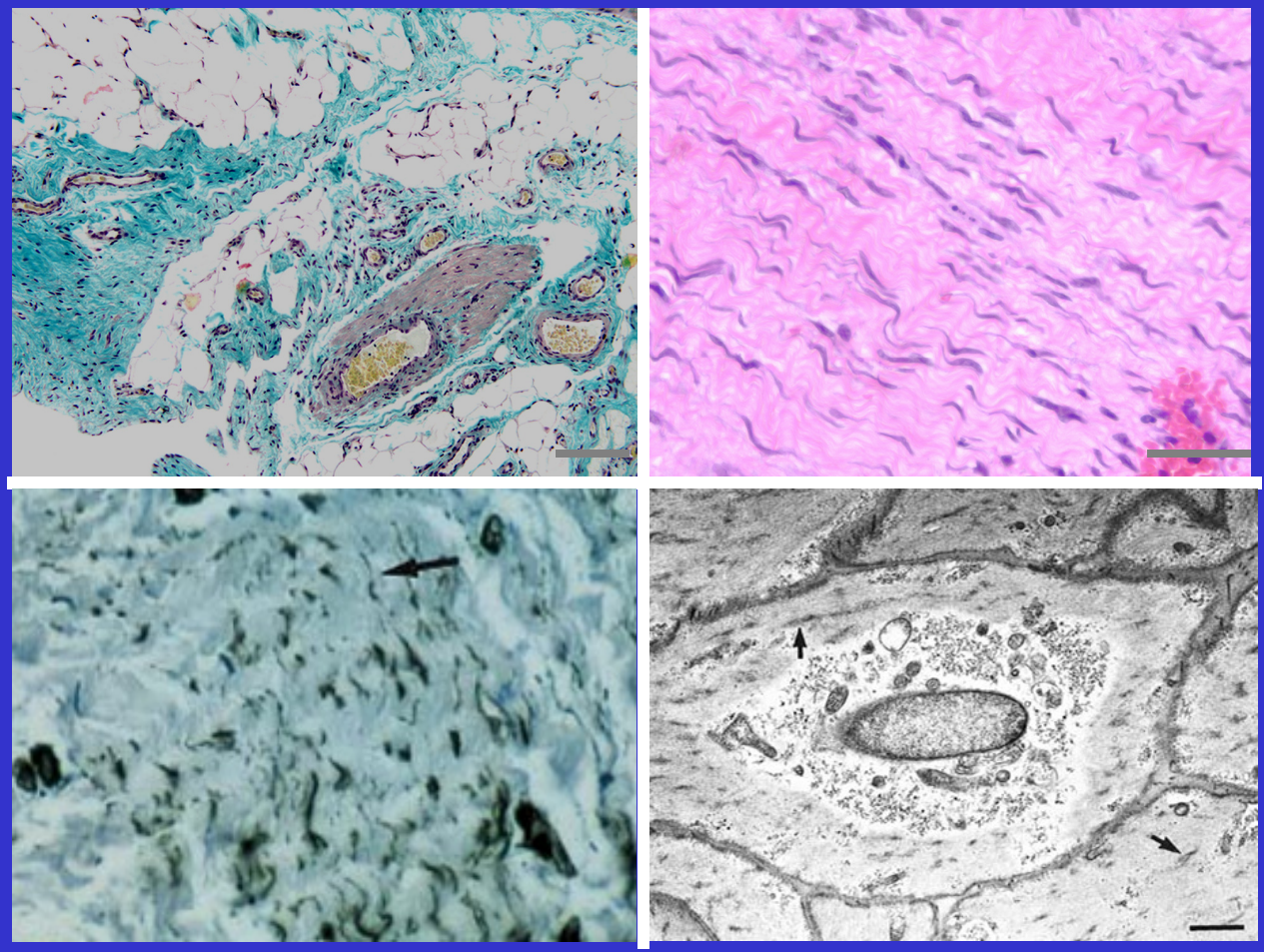




Mesothelial lining

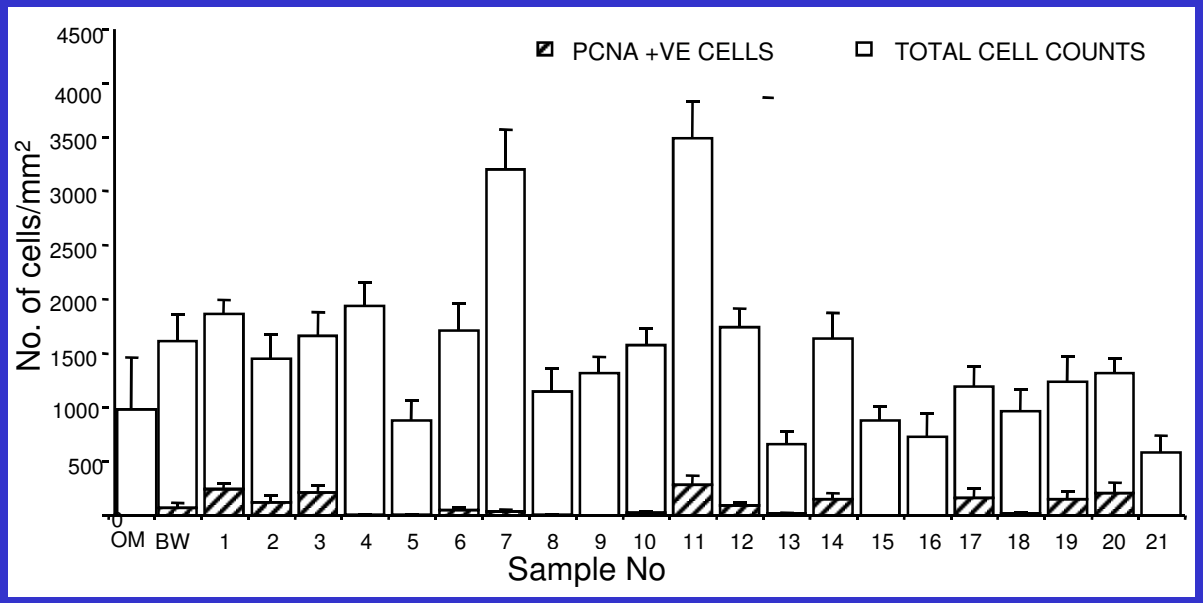


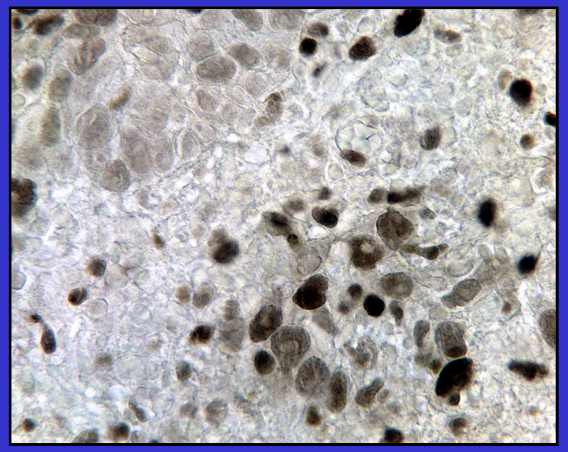
Human adhesions show the presence of α-smooth muscle actin-positive cells



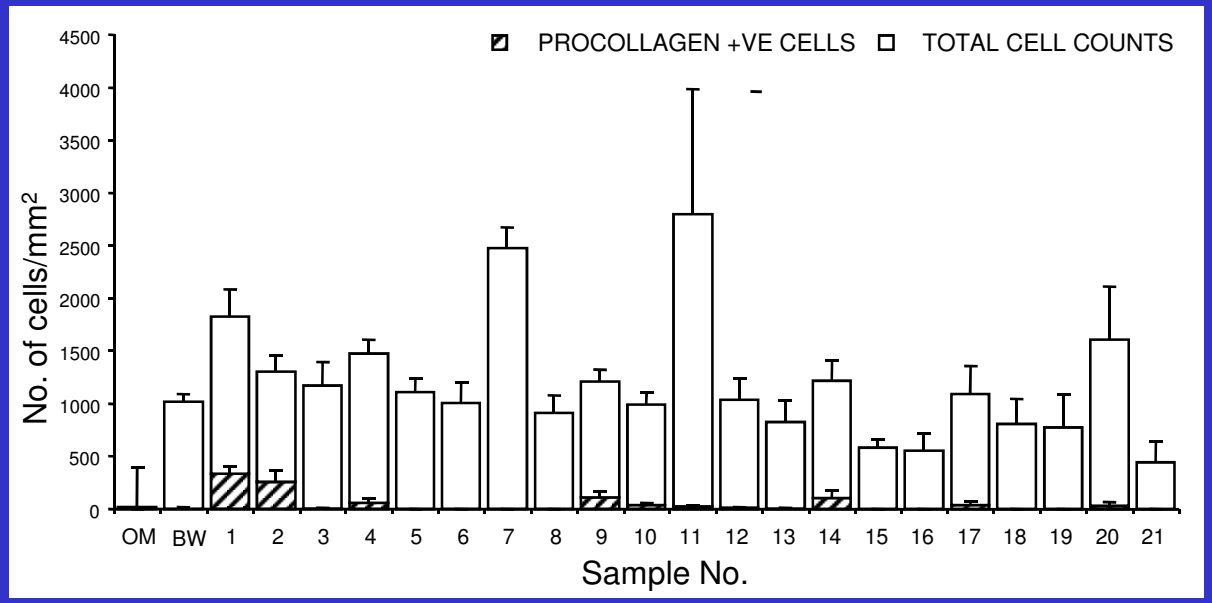
Herrick et al., J Pathology 2000, 192: 67-72; Sulaiman et al., Ann Surg 2001, 234: 256-261

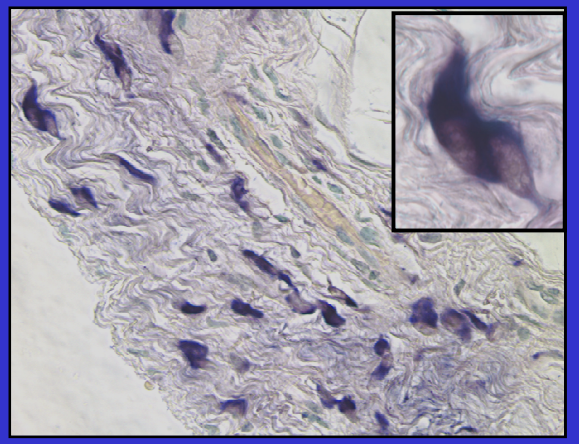
Long-term remodelling of human adhesions





Cell proliferation





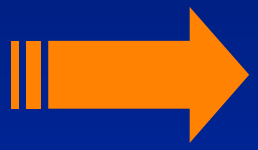
Pro-collagen type 1 gene expression

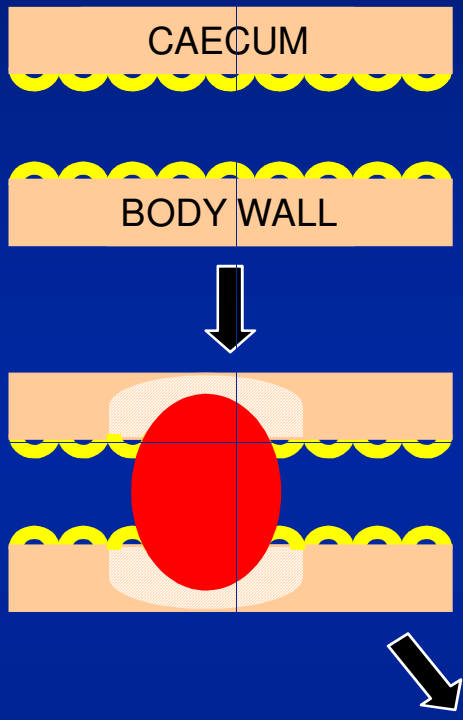
In vitro evidence for a difference between peritoneal and adhesion fibroblasts

- Increased α-smooth muscle cell actin in adhesion fibroblasts compared with peritoneal fibroblasts (Saed et al., 2004, Fertil Steril)
- Decreased apoptosis and increased proliferation of adhesion fibroblasts compared with peritoneal fibroblasts under hypoxia (Saed et al., 2002, Fertil Steril)
- Increased production of TGF-β1 and ECM by adhesion fibroblasts compared with peritoneal fibroblasts (Saed et al., 2001, Fertil Steril)

Pathogenesis of adhesion formation

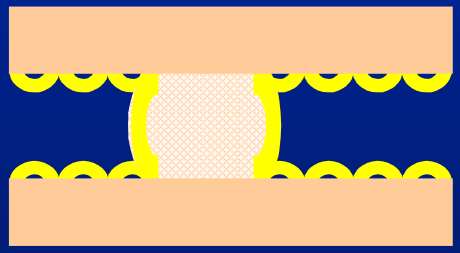
Injury





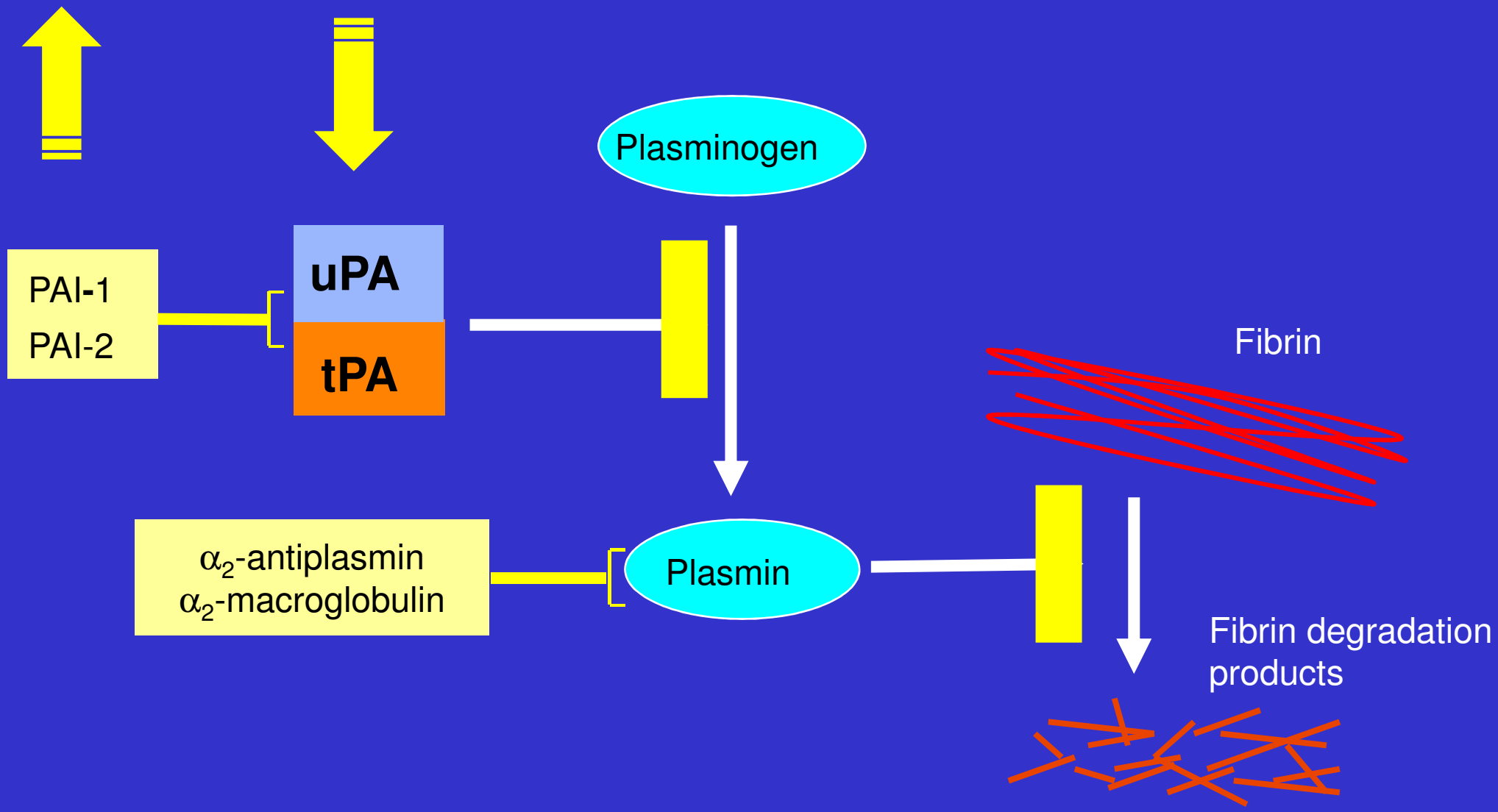
Close opposition of peritoneal surfaces

Reduced fibrinolysis

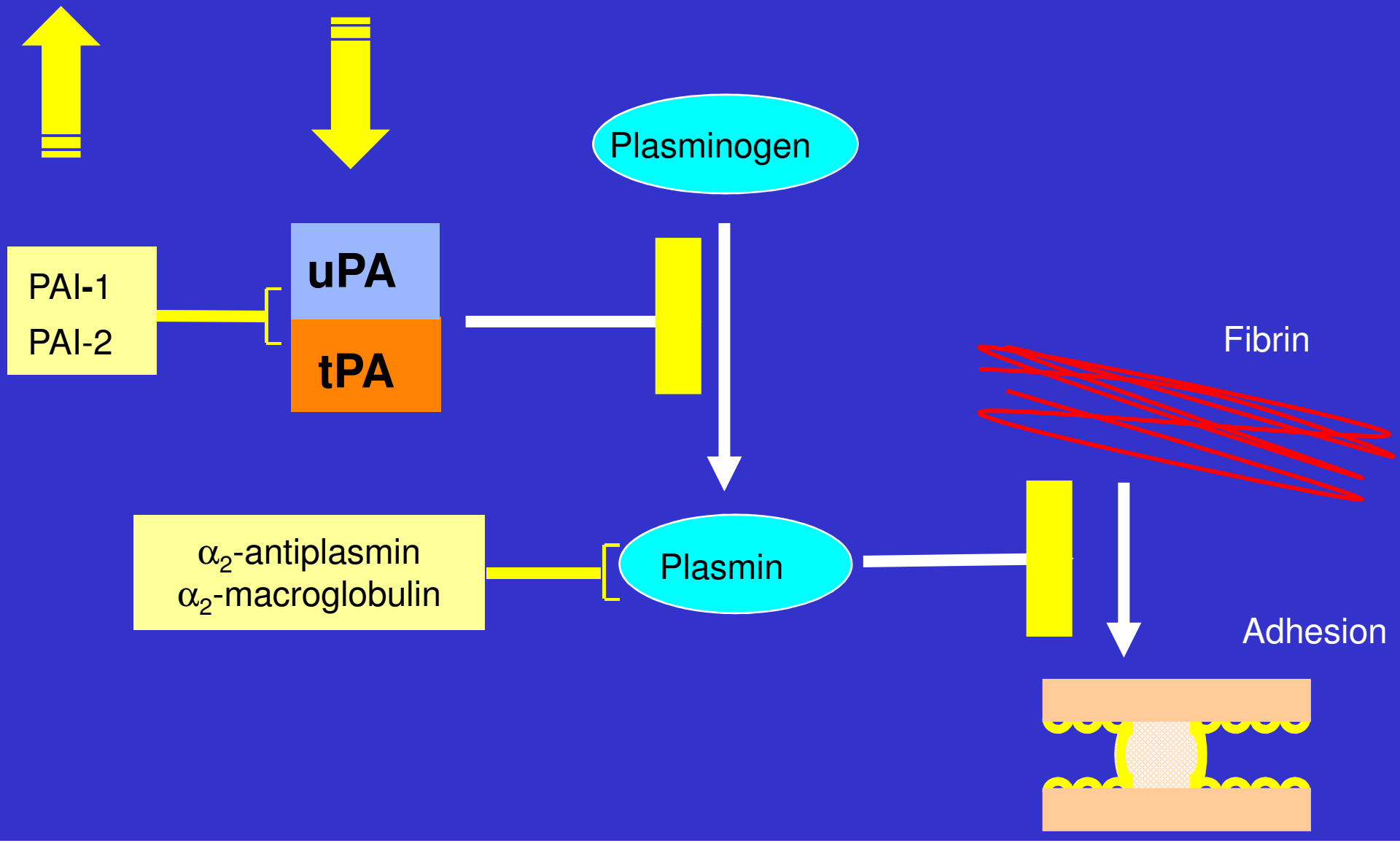


Adhesion formation

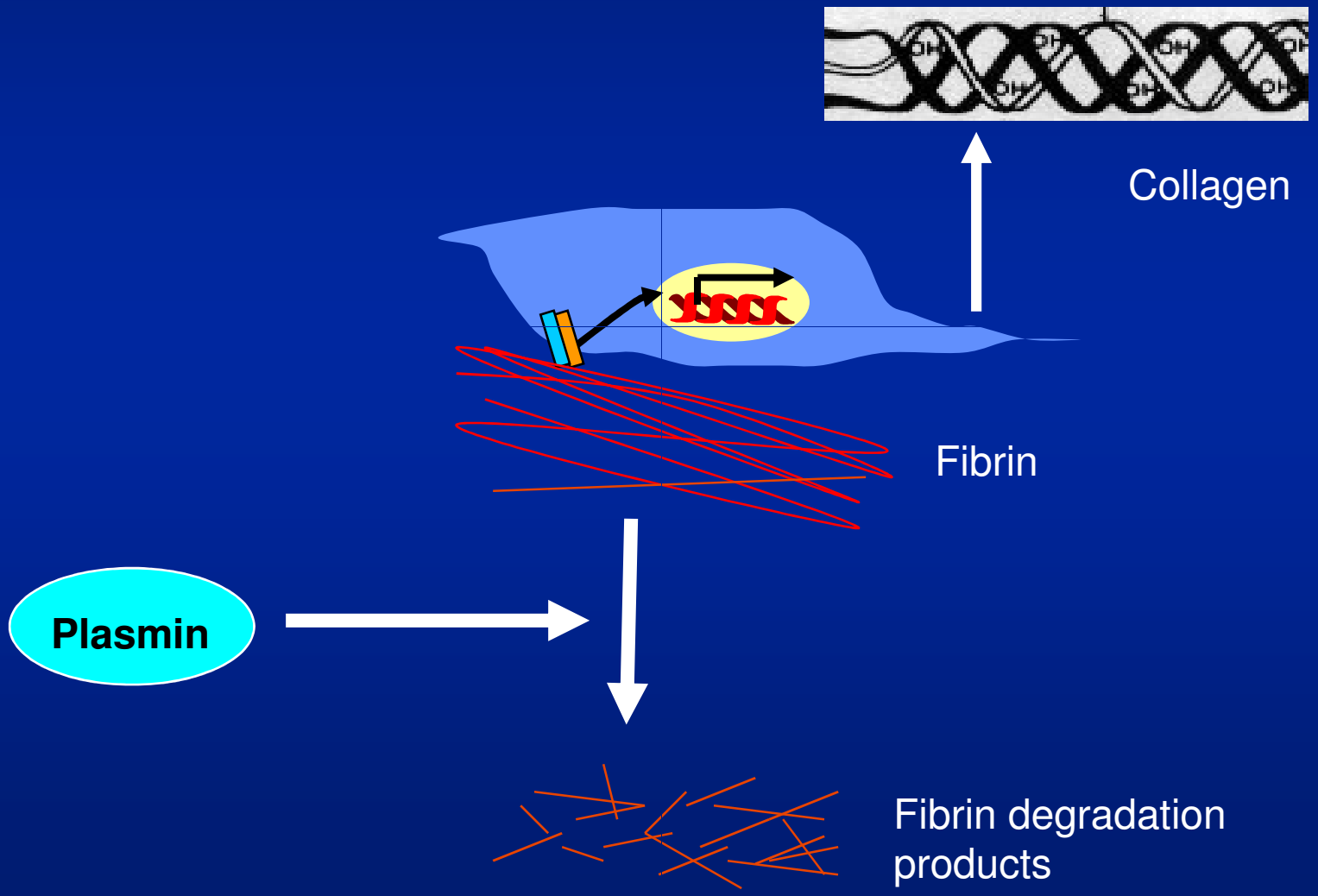
Fibrinolytic system



Reduced fibrinolytic system induces adhesion formation?

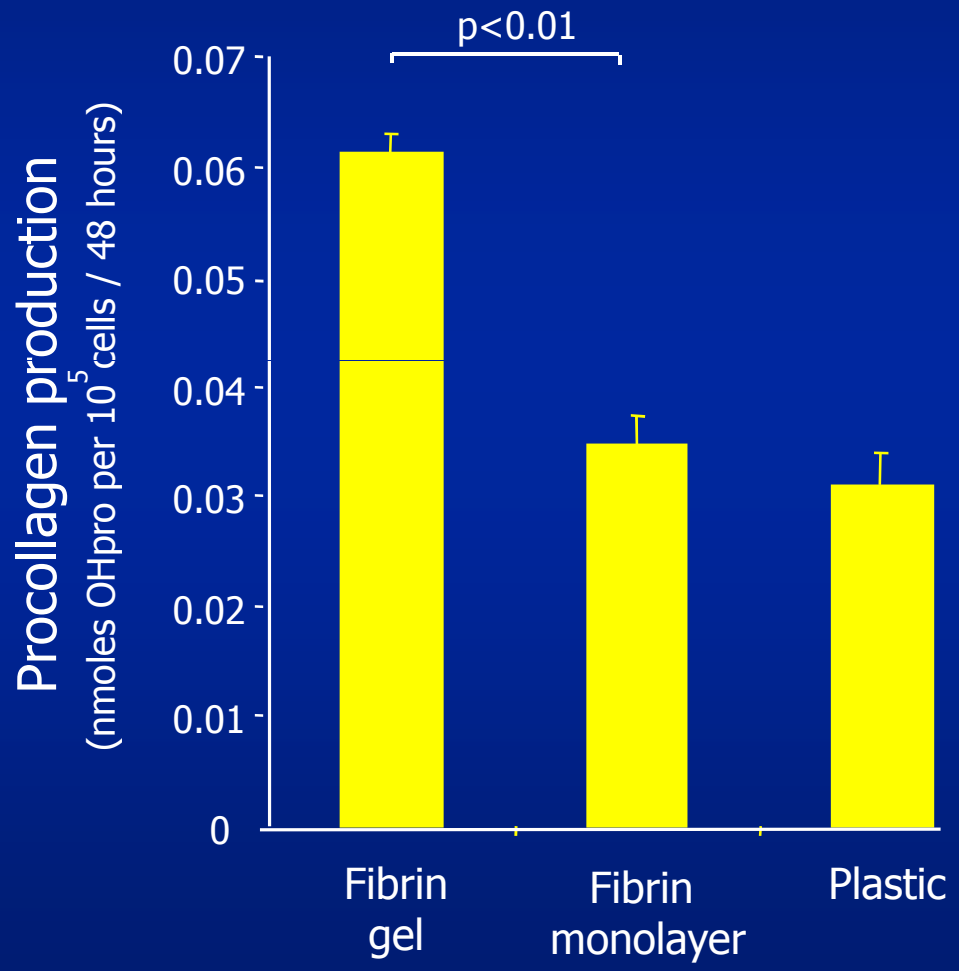


Role of fibrin matrix in adhesion formation

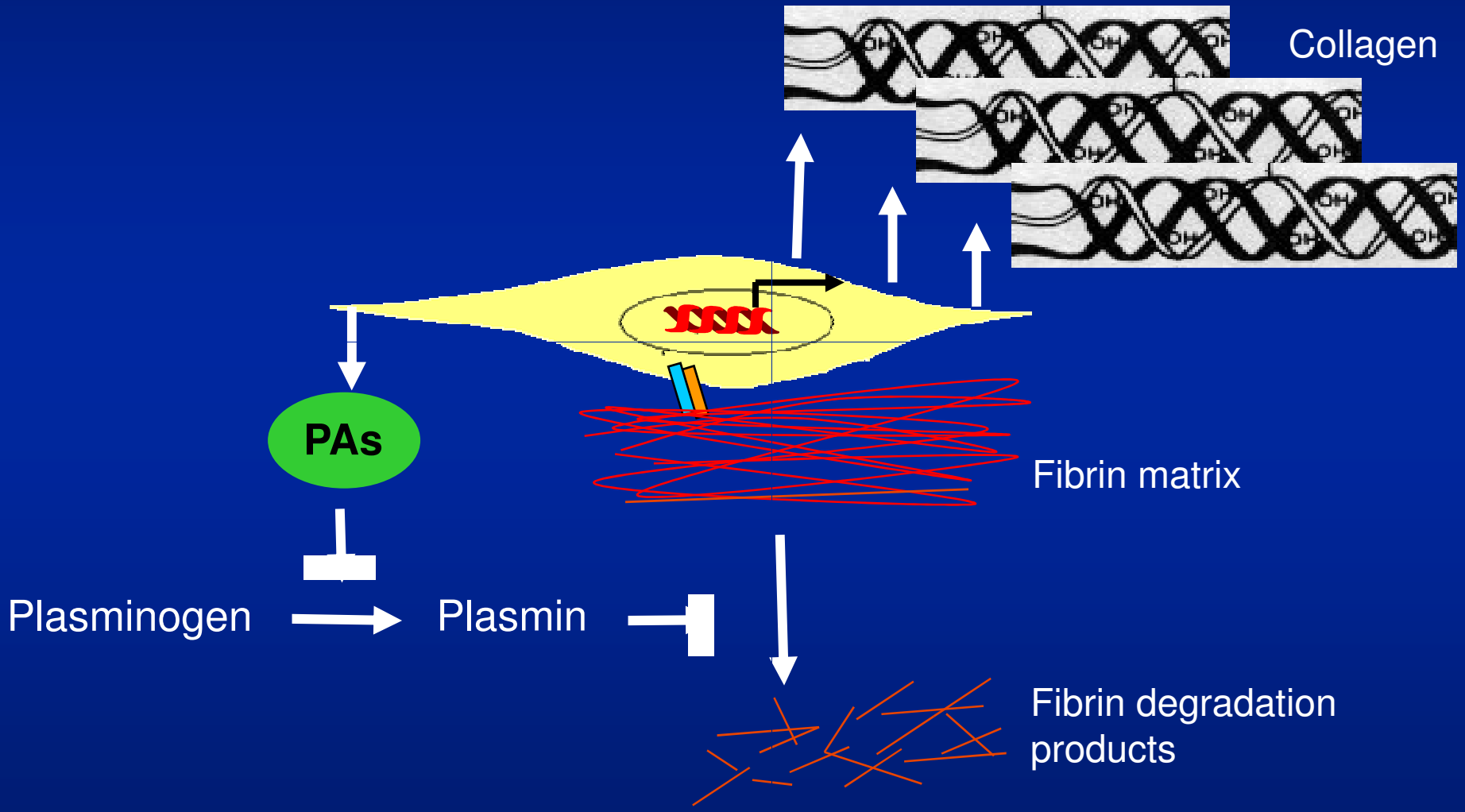


Increased collagen production by fibroblasts in fibrin gel

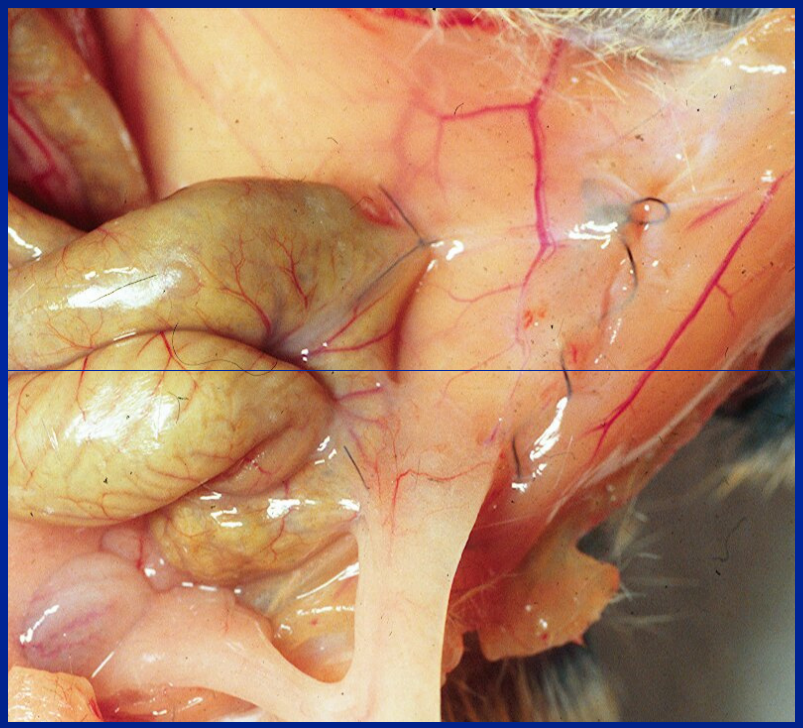
- Fibroblast isolation from tissue
  - –Collagenase digestion
  - –Explant culture
- Combine cultured fibroblasts with fibrinogen solution
- Add thrombin and culture +/- fibrinolytic inhibitors
- Analyse fibroblast proliferation and collagen turnover

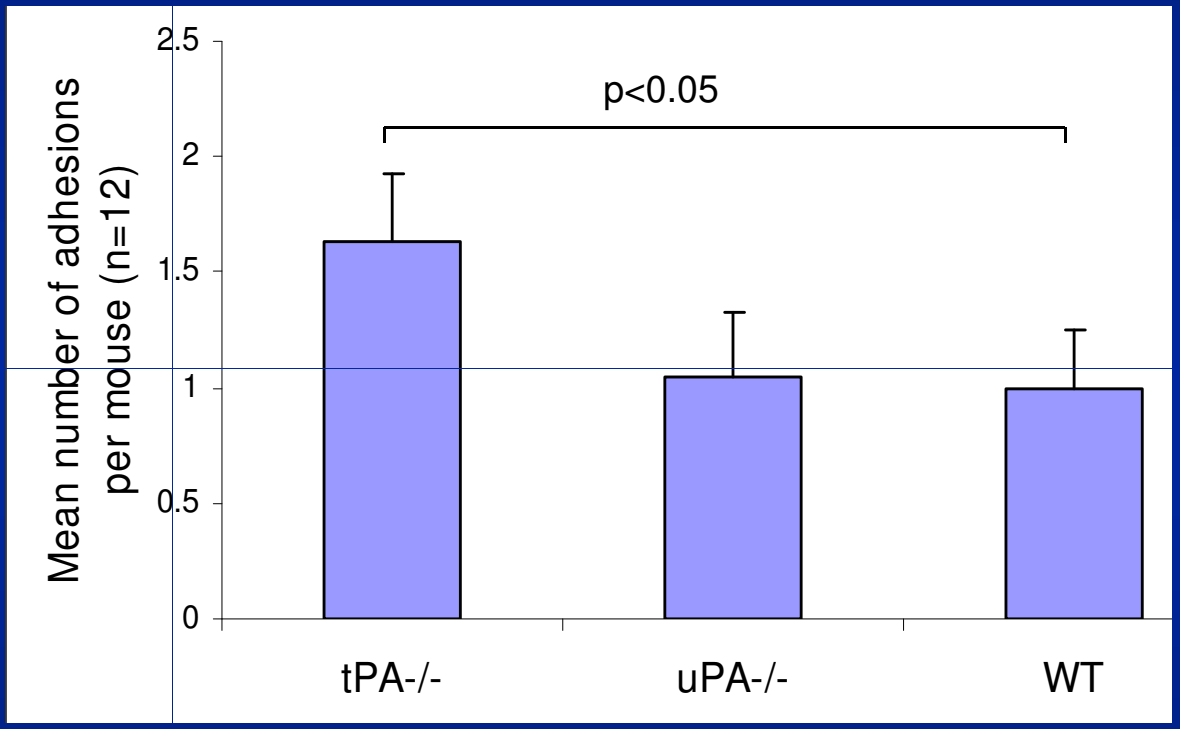


Reduced fibrinolysis and fibrin persistence enhance collagen deposition in vitro



tPA-deficient mice show increased adhesion formation

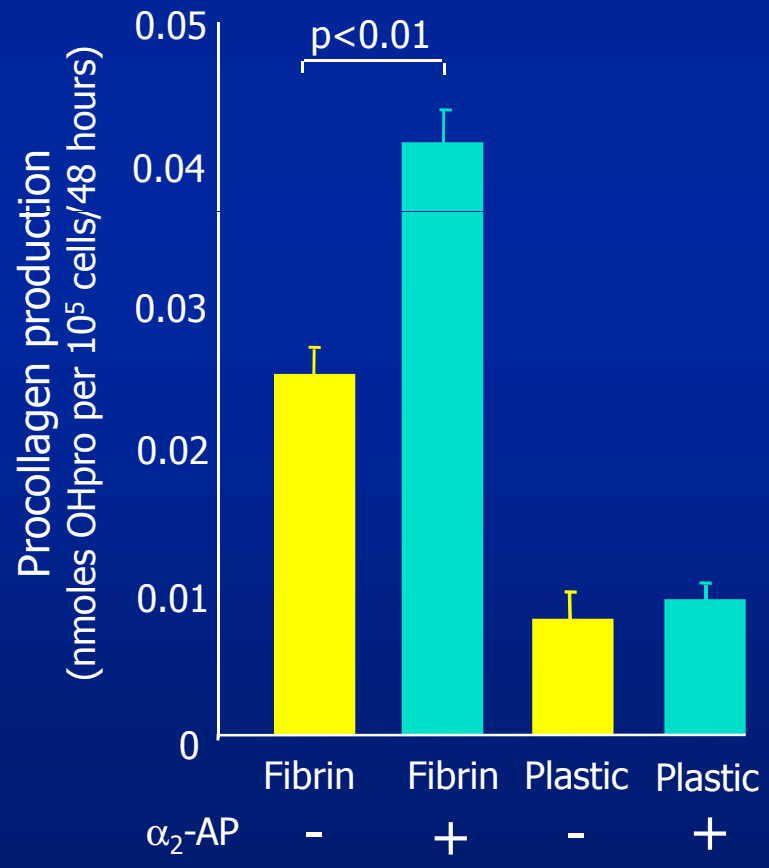




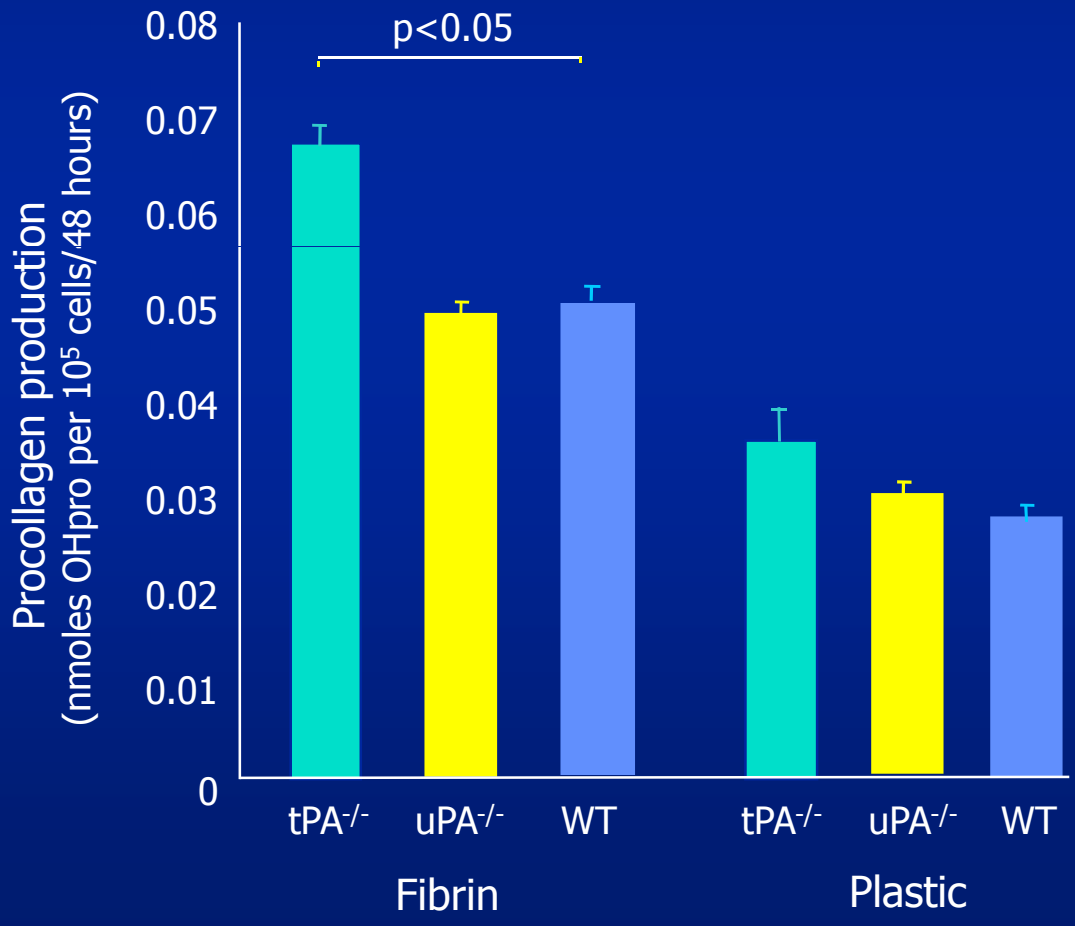
- tPA-deficient mice are more susceptible to adhesion formation following a surgical or chronic inflammatory episode

Increased collagen production by fibroblasts in fibrin gels with reduced fibrinolysis

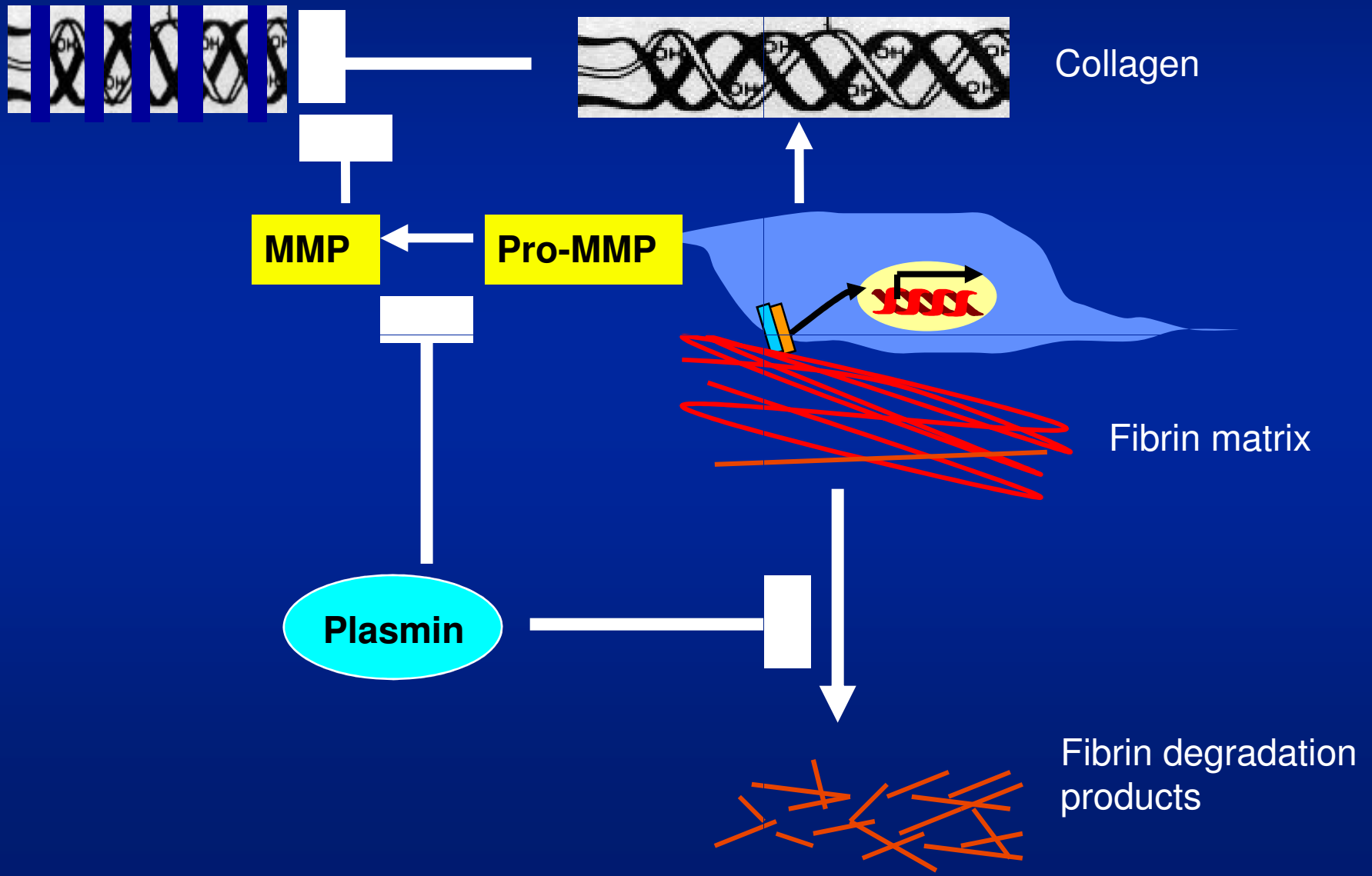
Plasmin inhibitor



PA-deficient murine cells

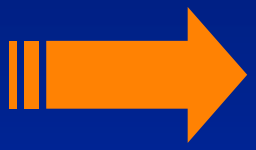


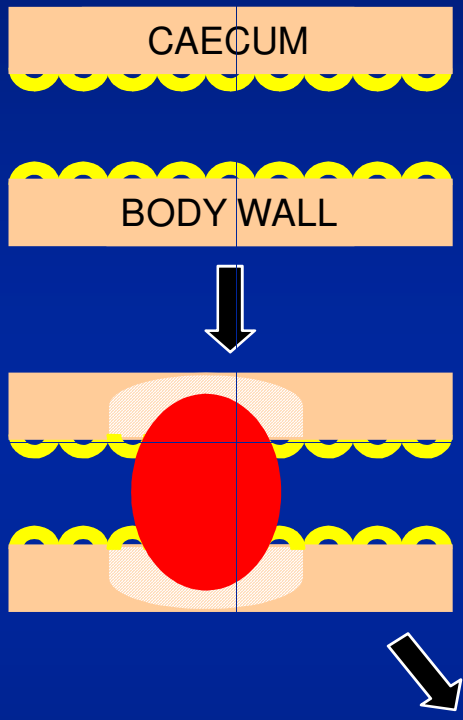
Both increased collagen gene expression and decreased degradation involved



Pathogenesis of adhesion formation

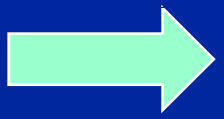
Injury





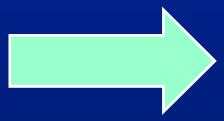
Close opposition of peritoneal surfaces

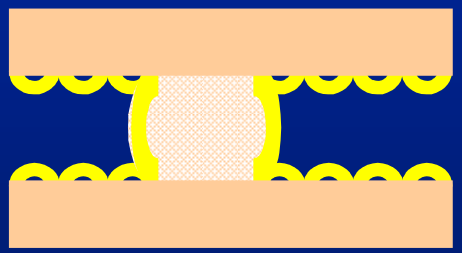
Inward migration of cells



Reduced fibrinolysis

Enhanced collagen deposition

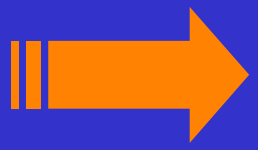


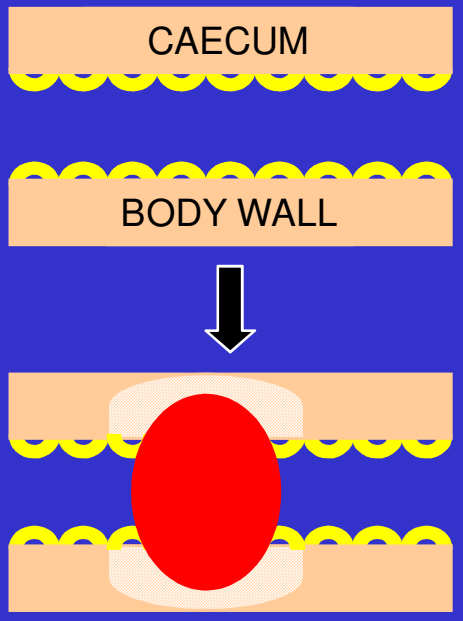


Adhesion formation

Pathogenesis of adhesion formation

Injury



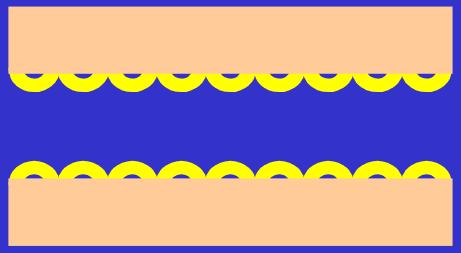


Close opposition of peritoneal surfaces

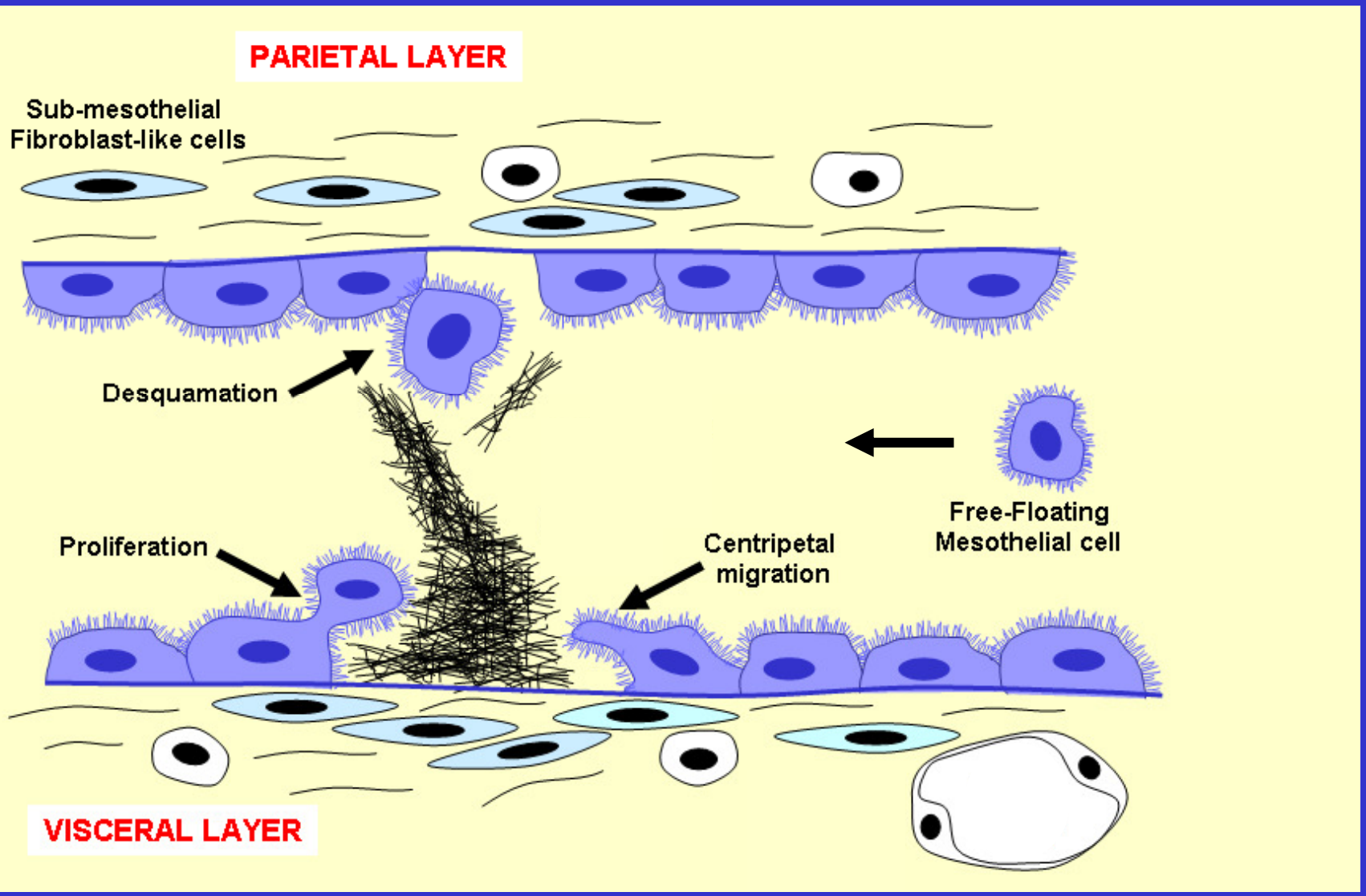
Tissue repair

Normal fibrinolysis

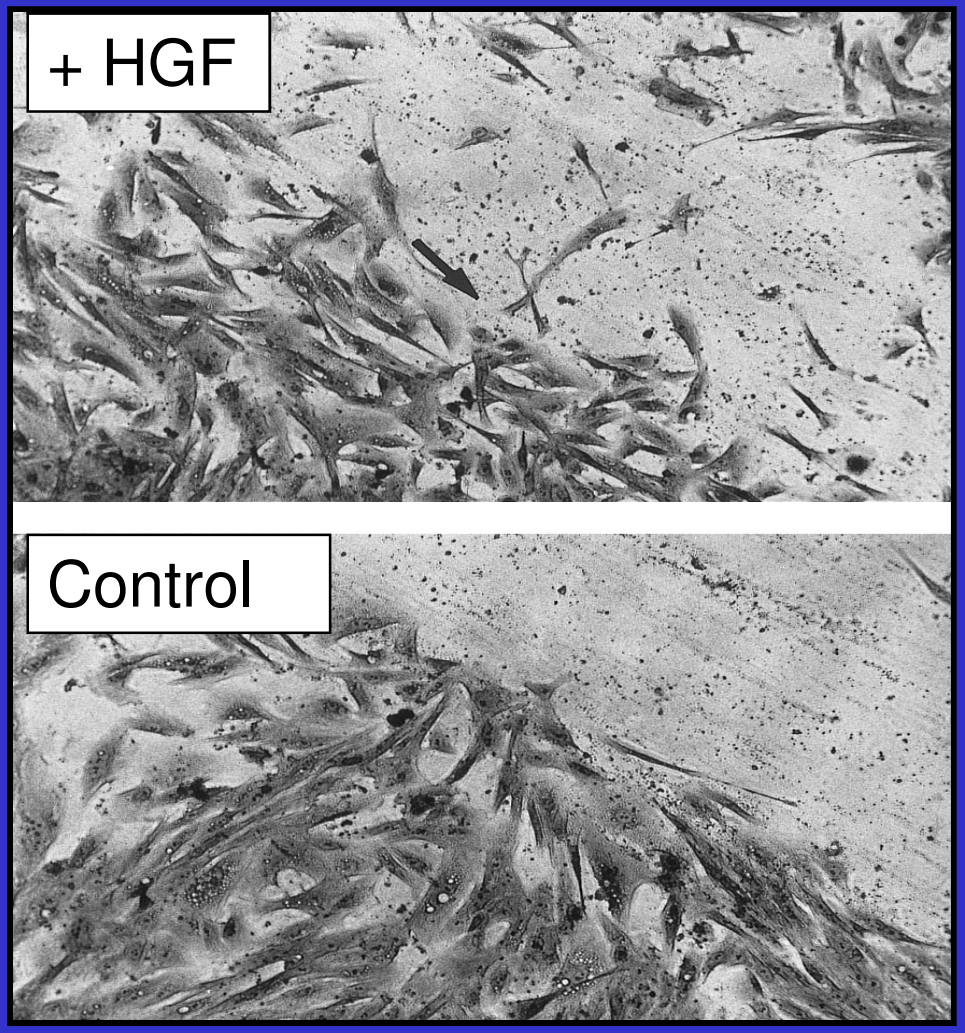
Normal repair



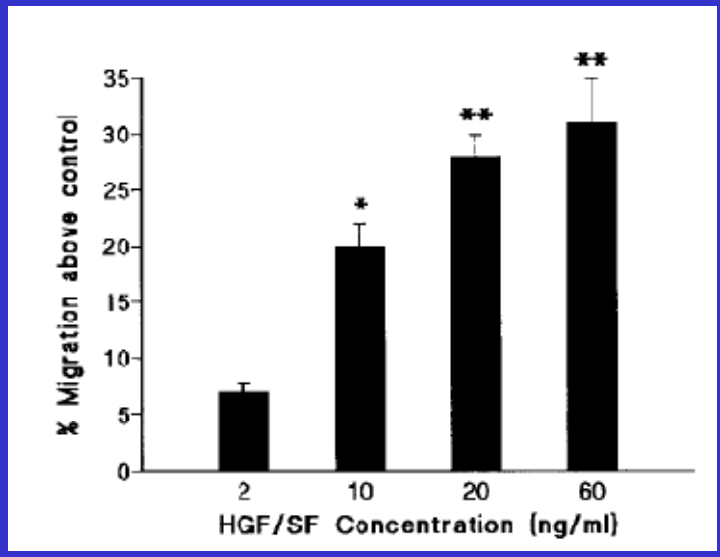
Proposed scheme of mesothelial regeneration



Hepatocyte growth factor (HGF) increases mesothelial regeneration in a scratch wound model



- Mesothelial cells isolated from omentum by trypsin digestion
- Subjected to scratch wound when confluent monolayer
- +/- HGF (dose response)
- Analyse cell migration and proliferation



JOURNAL OF SURGICAL RESEARCH 61, 250–255 (1996)
ARTICLE NO. 0112

In Vitro Analysis of Peritoneal Adhesions in Peritonitis

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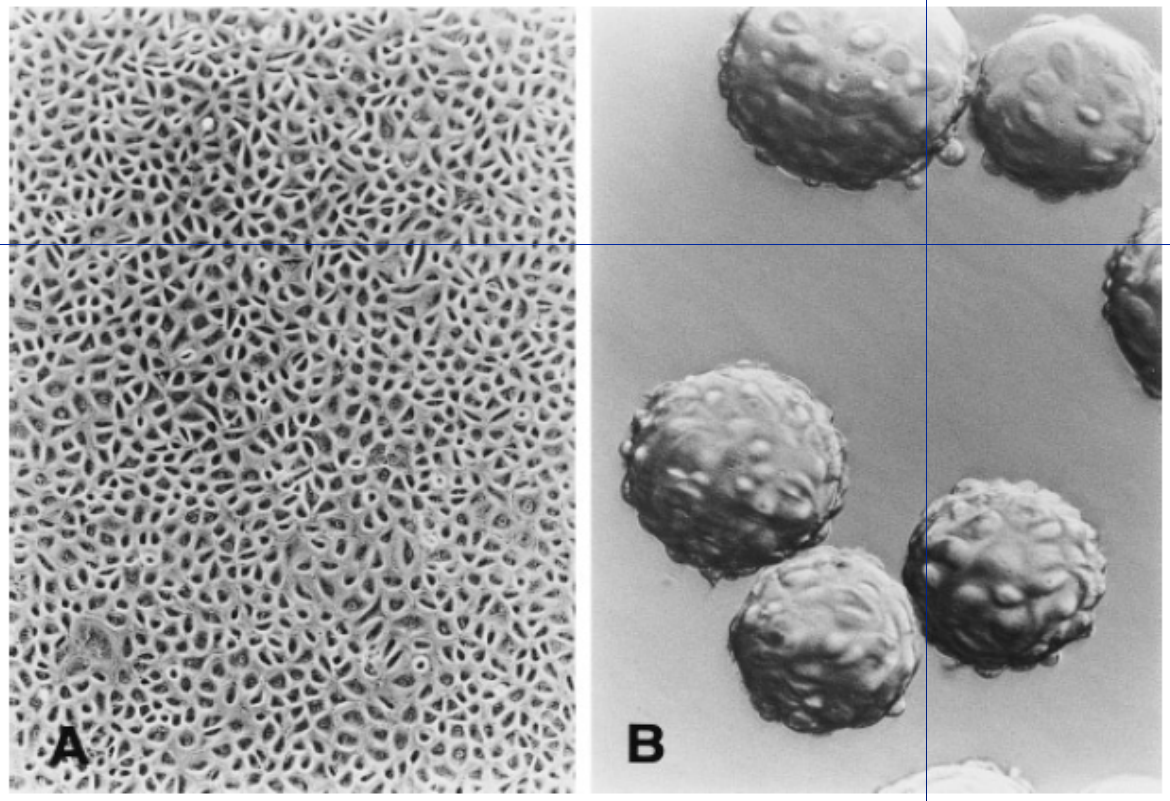

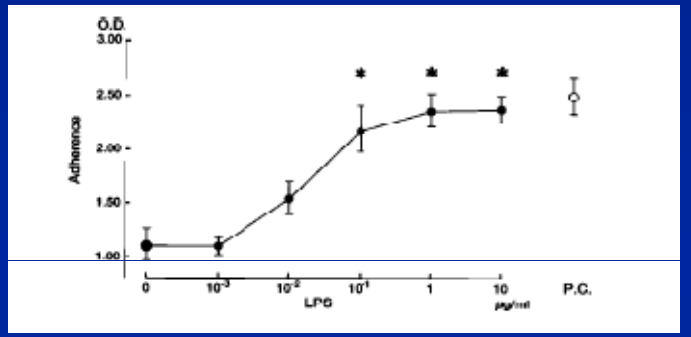

LPS increases bead adhesion

^{125}I-Albumin Permeability of Mesothelial Monolayers

	^{125}I albumin permeability (mean ± SEM %)
Control	3.95 ± 0.26
LPS pretreated (1.0 μg/ml, 24 hr)	8.23 ± 0.56*

LPS increases permeability to ^{125}I Albumen

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

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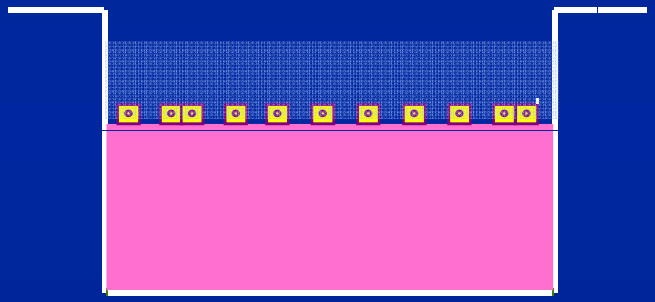
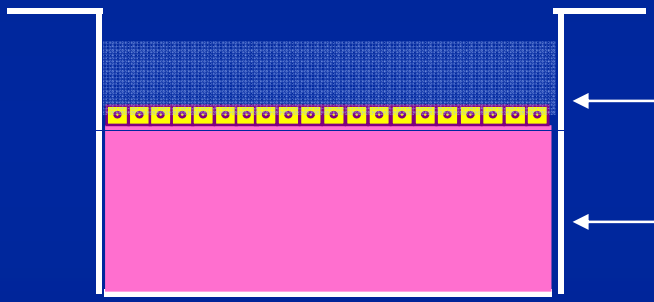
30 mins

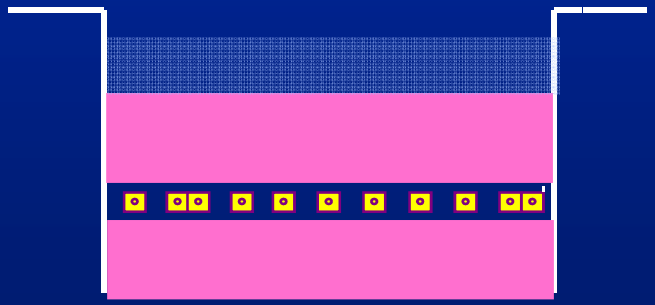
14 days

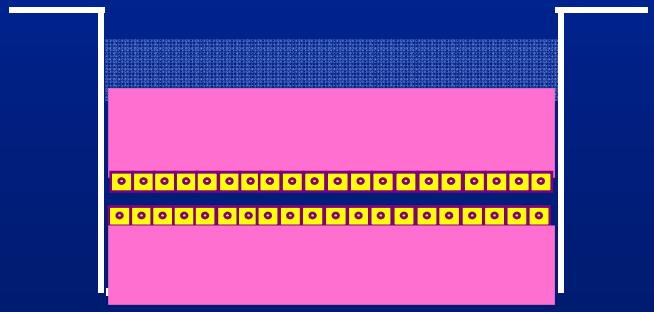
On gel



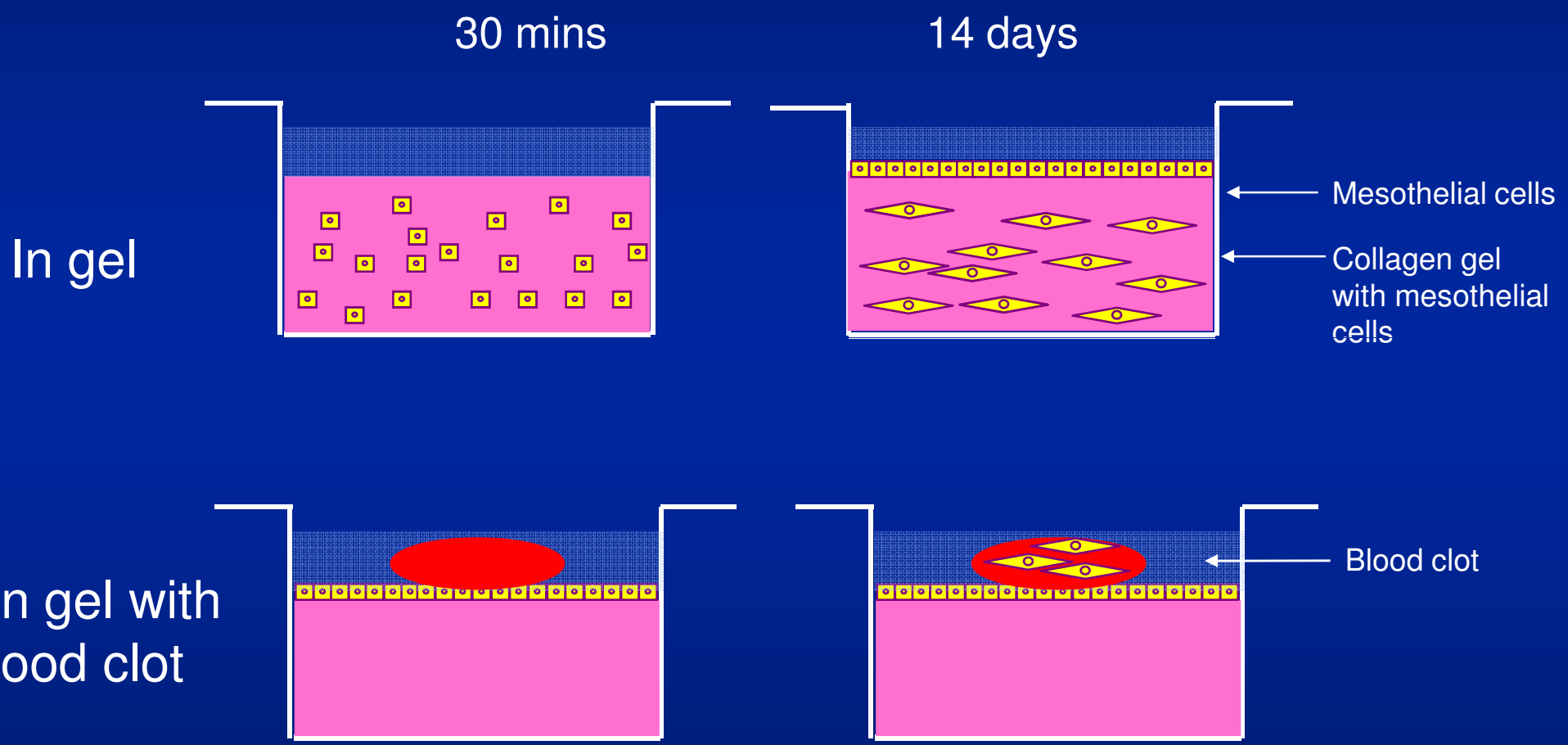


Between gels



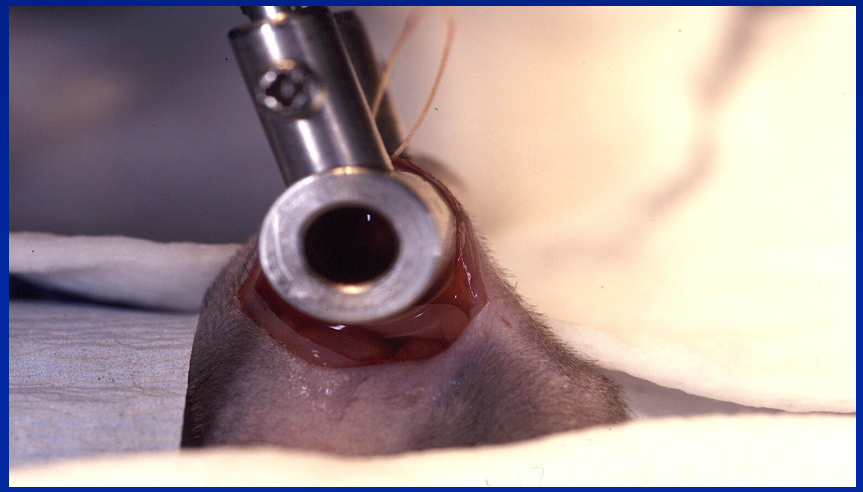


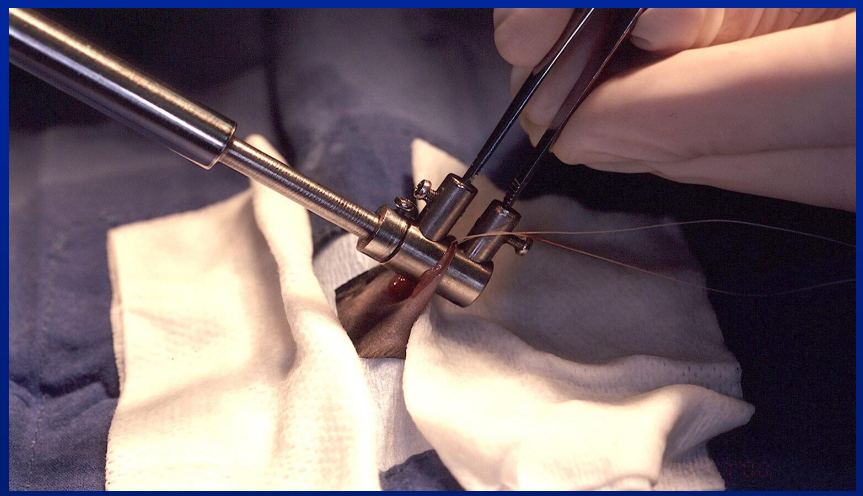
Epithelial or mesenchymal?



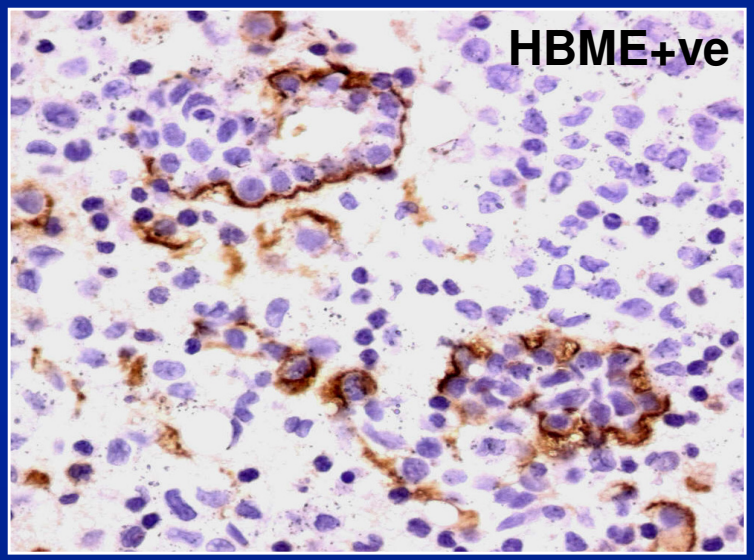
Rodent model of peritoneal repair

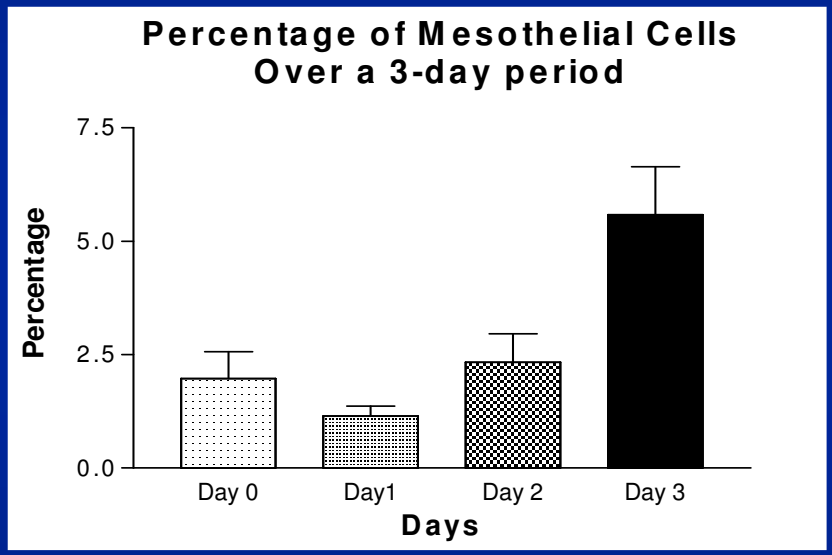
- Male Lewis rats (7-10 wk)
- Laparotomy and peritoneal abrasion injury
- Peritoneal injection of fluorescent labelled rat cells:
  - Cultured mesothelial cells
  - Cultured lung fibroblasts
  - Peritoneal lavage cells
  - Peritoneal macrophages
- Distribution of fluorescent cells assessed on wound imprints at set time points post-injury





Mesothelial cells in serosal fluid increase after injury in rats

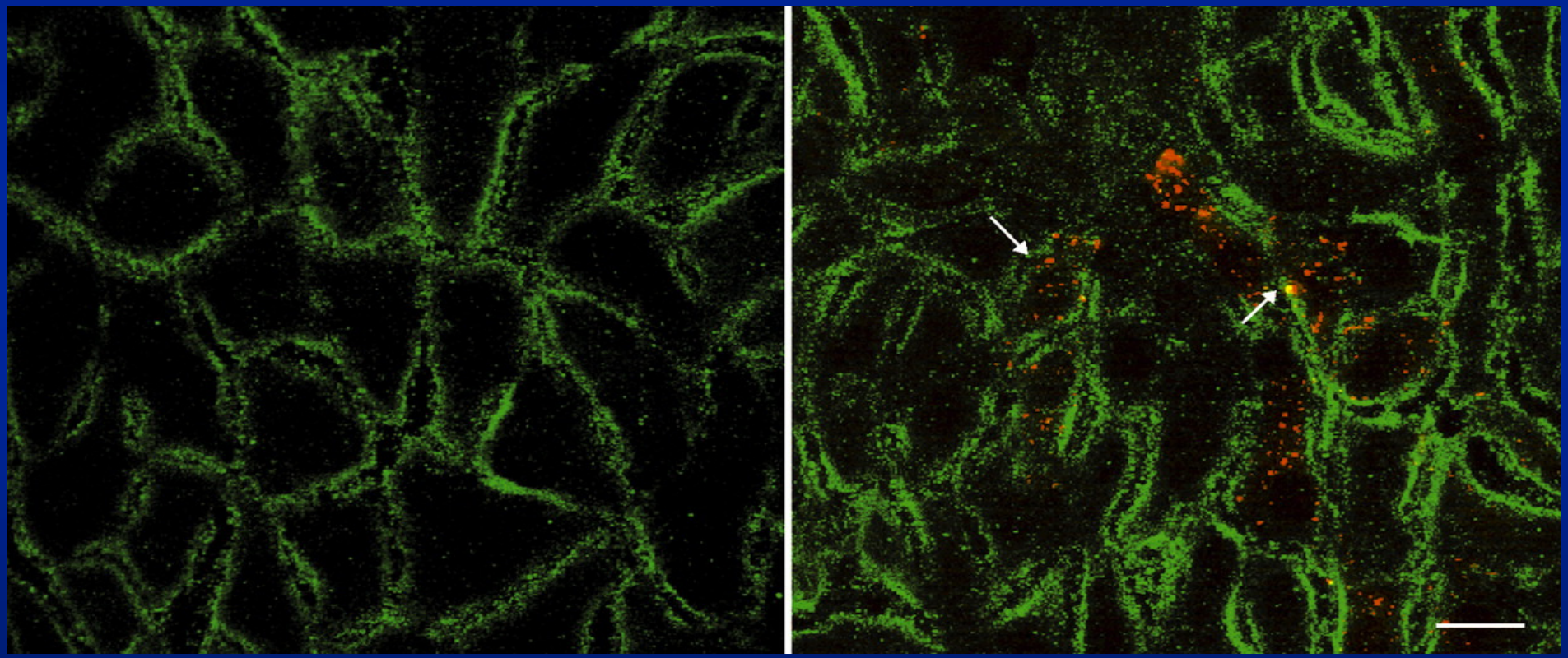




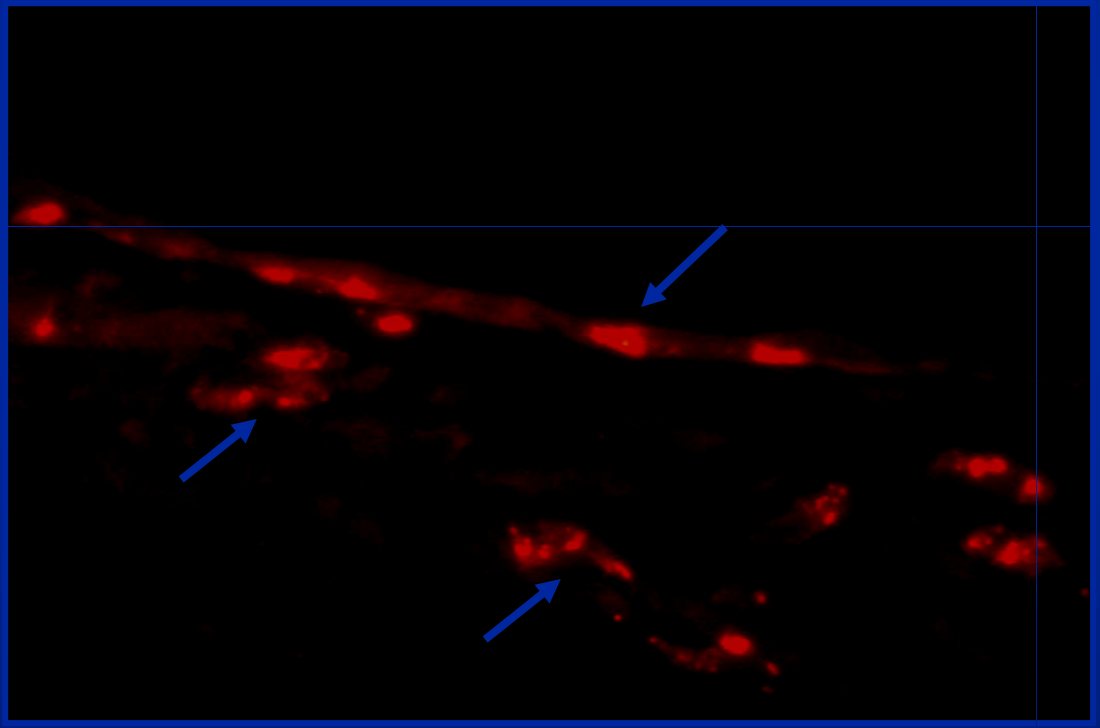
Incorporation of isolated free-floating mesothelial cells on denuded surface

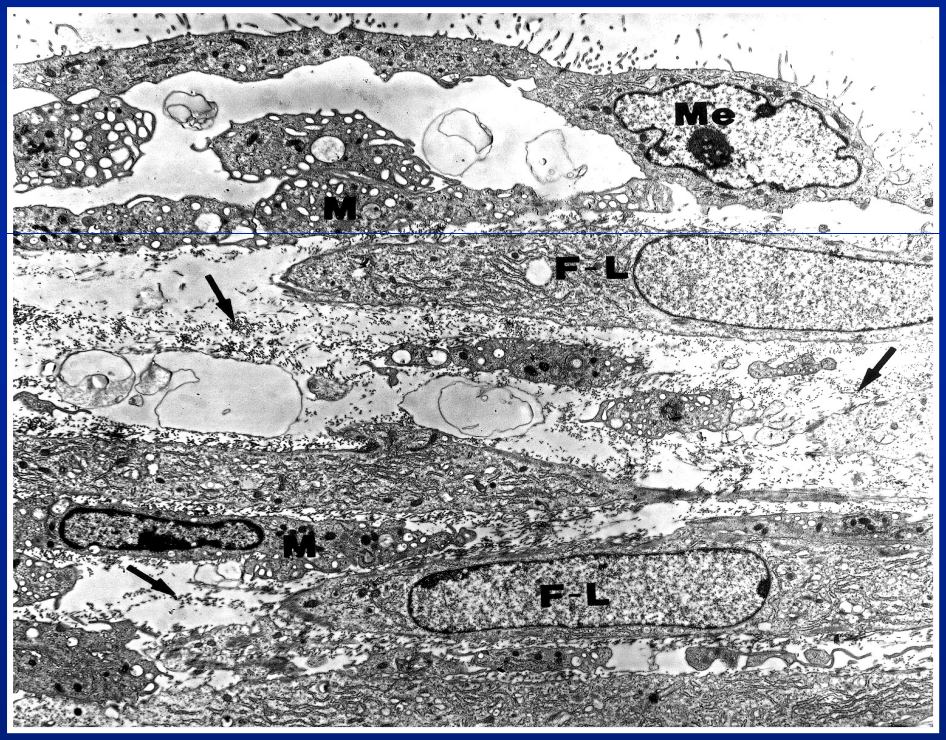
DiI-fibroblasts 8 days

DiI-mesothelial cells 8 days



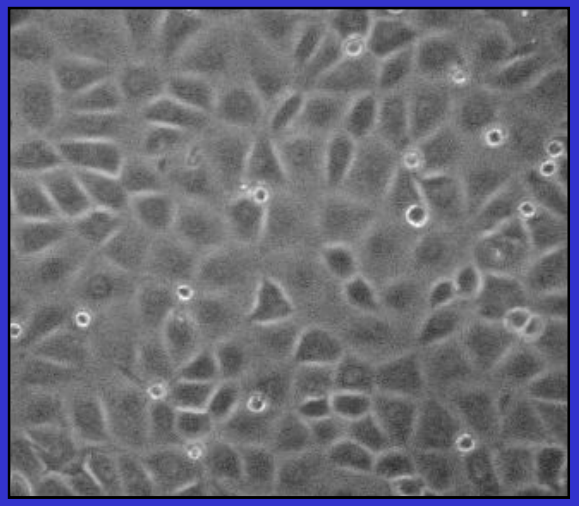
Di-I labelled mesothelial cells incorporate into multiple layers





Epithelial-mesenchymal transdifferentiation (EMT) of mesothelial cells *in vitro*

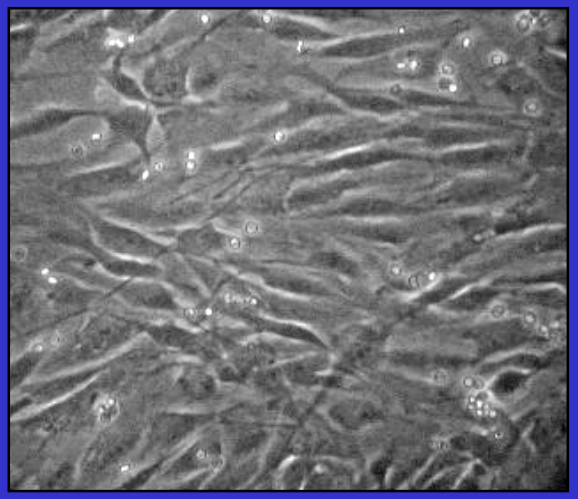
Epithelial



HGF/TGF- β1
Sub-culture
Fibrin/Collagen gel
Menstrual effluent

EMT

Mesenchymal

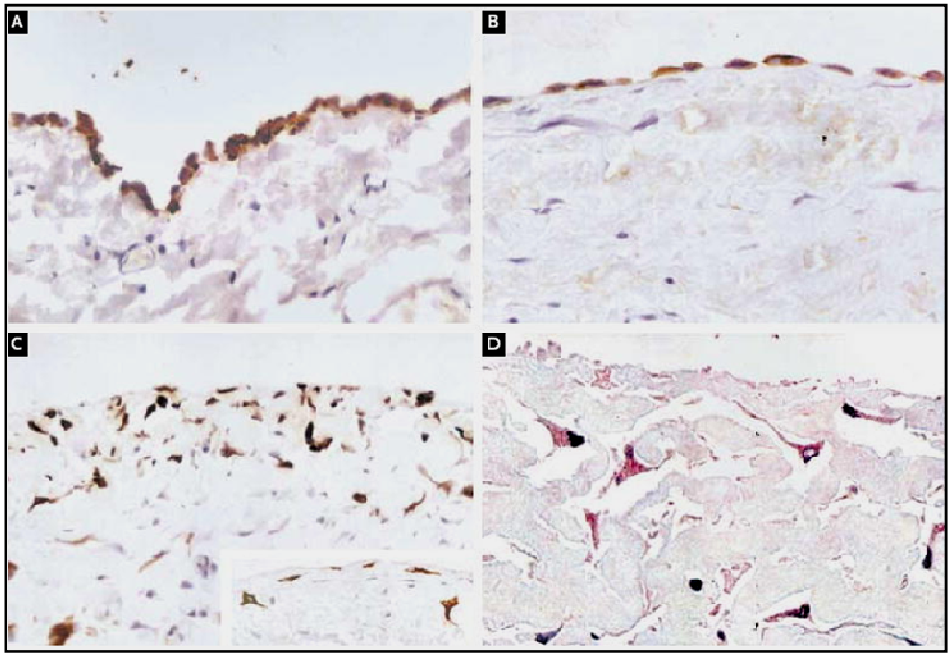


- Loss of intracellular junctions
- Change in intermediate filament expression
- Change in cell morphology
- Increase in myofibroblast and smooth muscle-like phenotype

EMT of mesothelial cells *in vivo*

Cytokeratin control

CK 6 mth dialysis



CK 8mth dialysis

ICAM1 8mth dialysis

Peritoneal dialysis (CAPD)

↓

Mesothelial damage/stress

↓ ← TGF-ß1

↑ Snail ↓ E-cadherin

↓

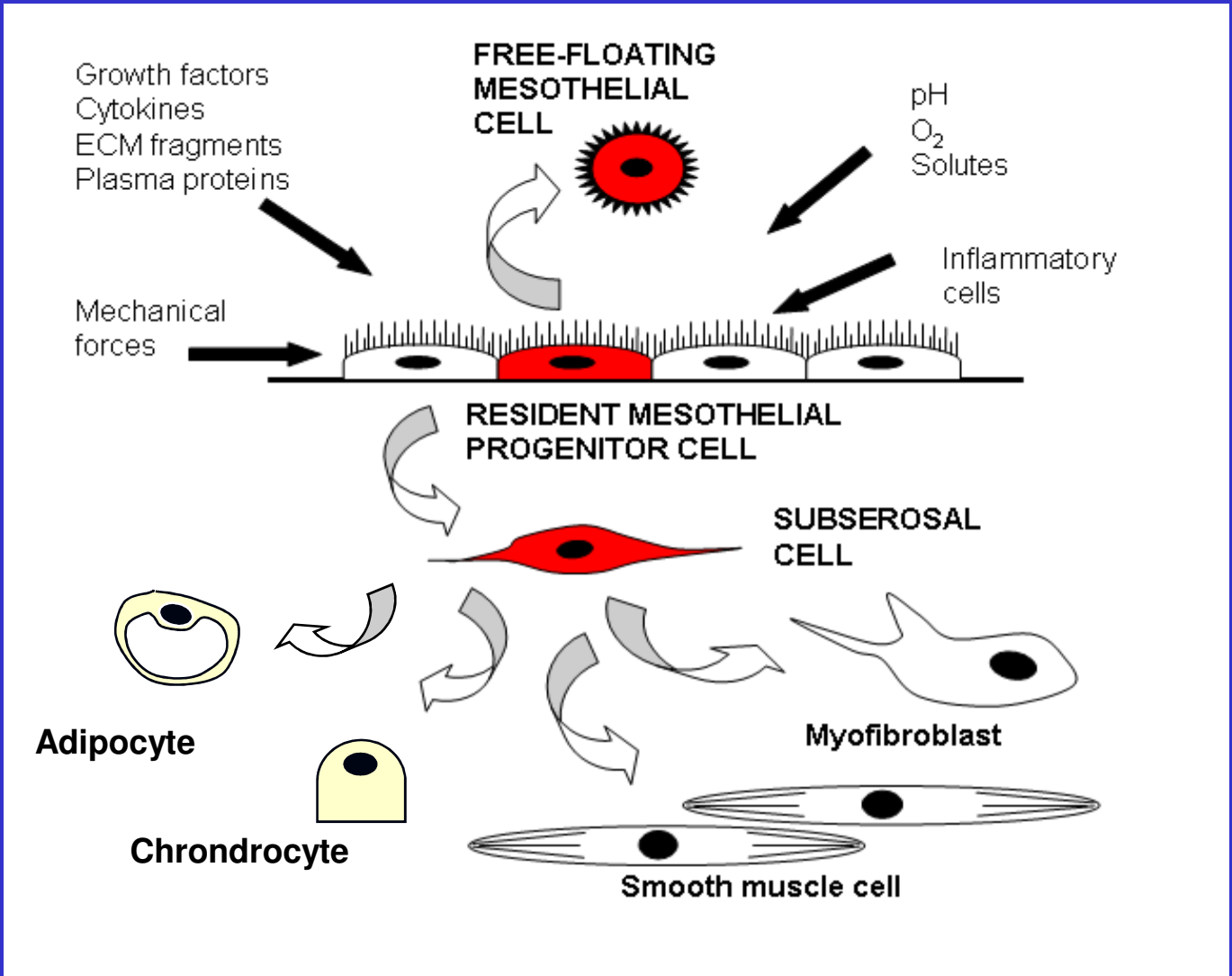
(Myo)fibroblast formation

↓

Peritoneal sclerosis

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

Possible role of a mesothelial progenitor cell in adhesion formation?



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