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Experimental Studies on the Pro-thrombotic Effect of Inhaled Particles

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Respiratory effects of particles

Episodes of increased PM_{10} cause increase in respiratory morbidity such as:

- Respiratory symptoms
- Decrease in lung function
- Exacerbations of asthma in adults and children
- Hospitalization for bronchitis and pneumonia

Cardiovascular effects of particle exposure

- epidemiology & “clinical” studies:
  - increased particulate pollution associated with:
    - Heart rate variability ↕, arrhythmias without hypoxia or respiratory distress
    - Plasma viscosity ↑, C reactive protein ↑, fibrinogen ↑, factor VII ↑
    - PMN ↑, platelets↑, mast cells ↑, endothelial adhesion molecules ↑

Effects of particles exposure

Particles are significant contributor to morbidity and mortality

not only with regard to the respiratory tract,

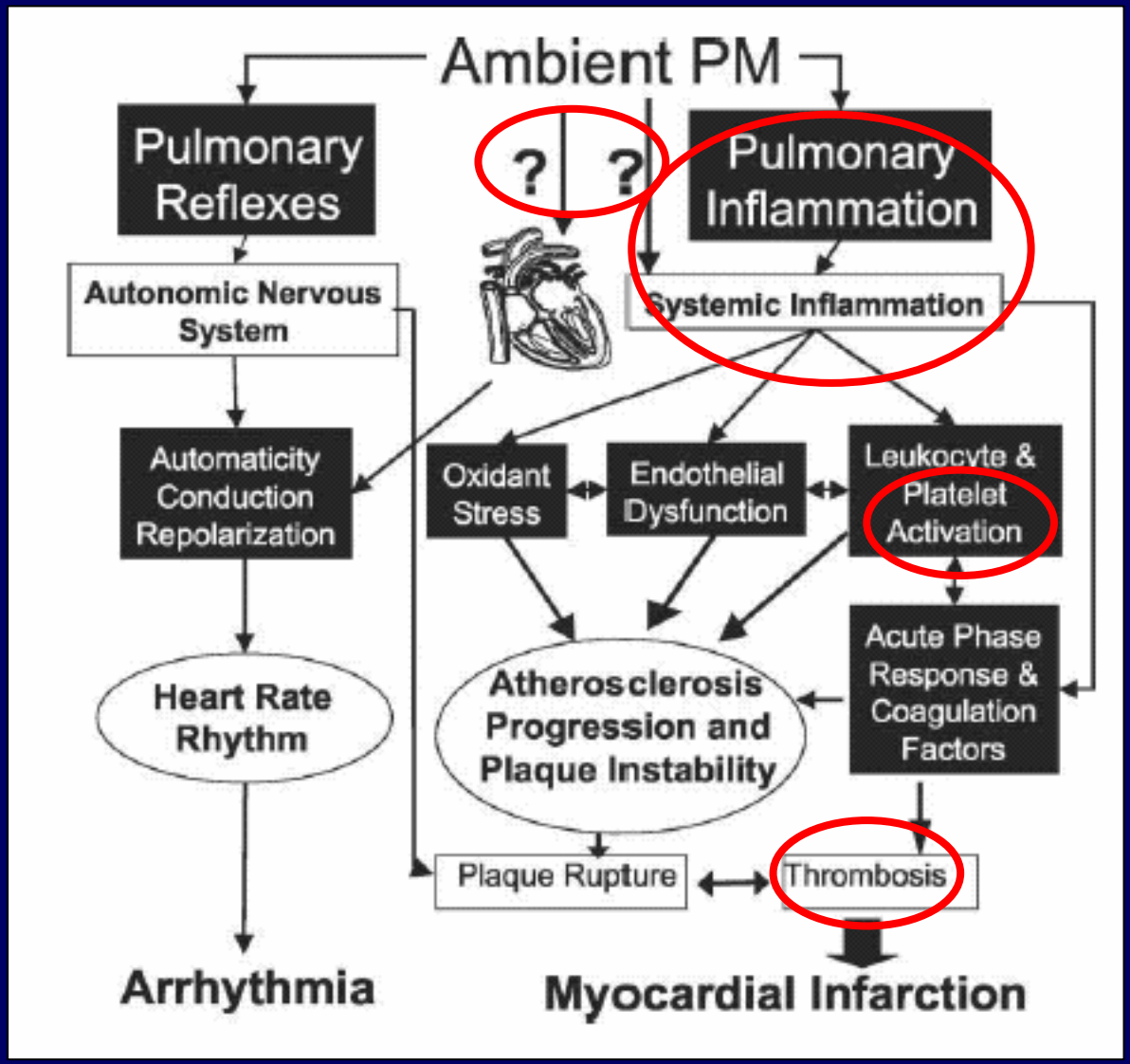
but also the cardiovascular system

Mechanisms?

- experimental toxicology:
  - which constituents of the particles?
  - by what mechanisms?

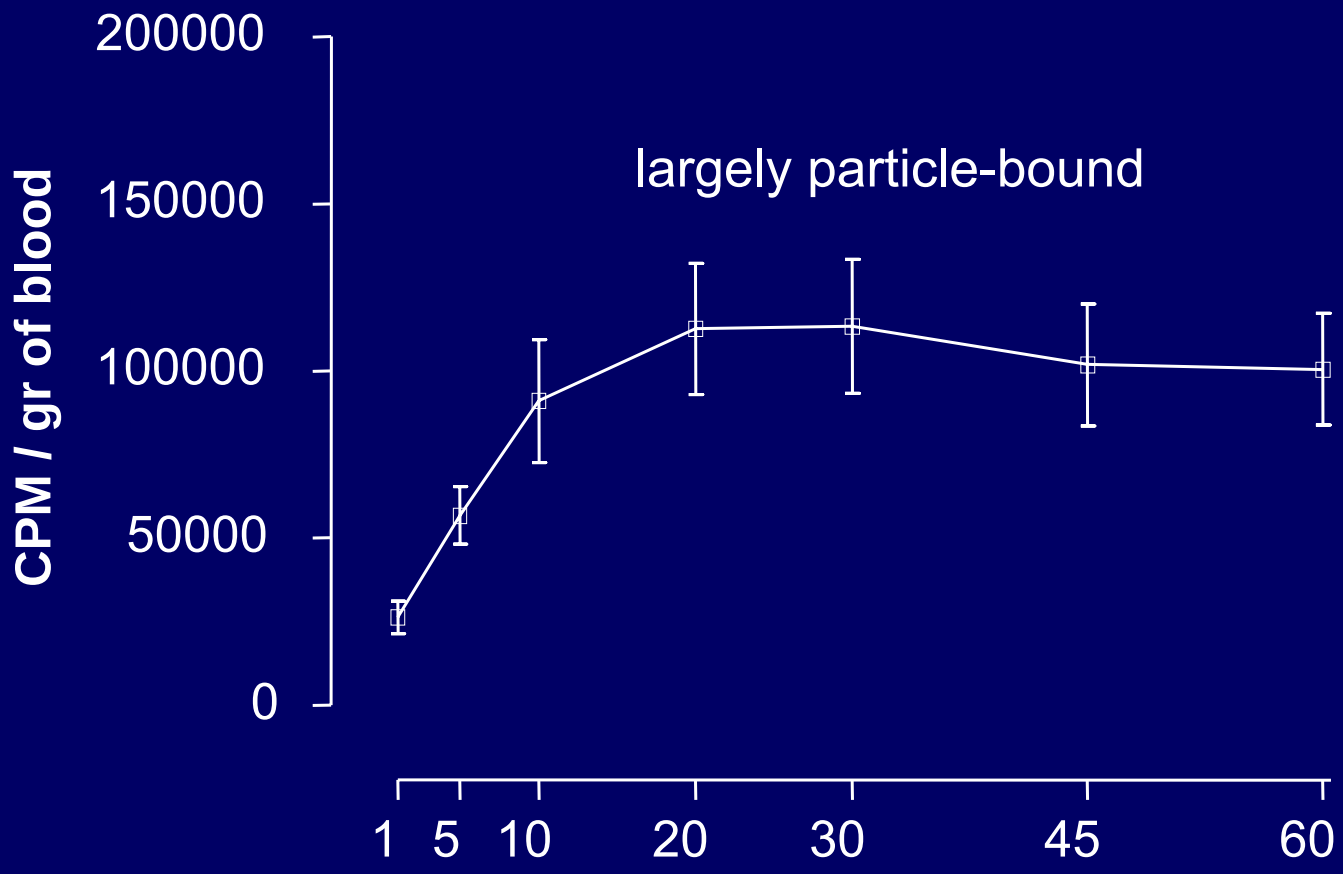
➔ “biological plausibility”?

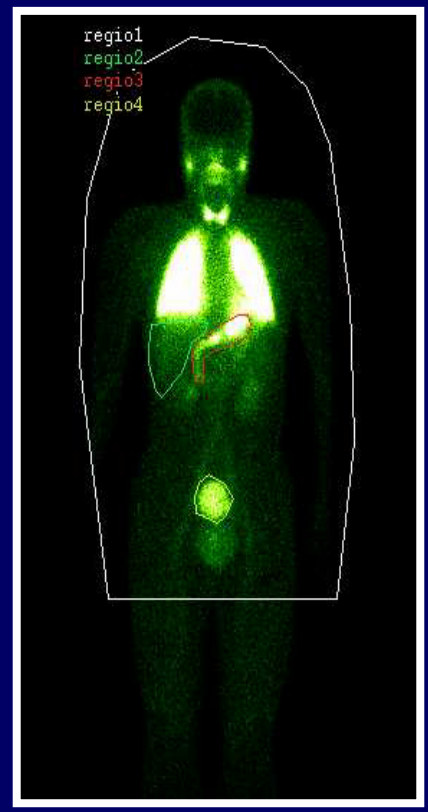
Brook RD *et al*. Air pollution and cardiovascular disease. A statement for health- care professionals from the expert panel on population and prevention science of the American Heart Association. *Circulation* 2004 (June 1); 109: 2655-71



Translocation:
Radioactivity in blood

Inhalation of ^{99m}Tc-carbon particles (“Technegas”)





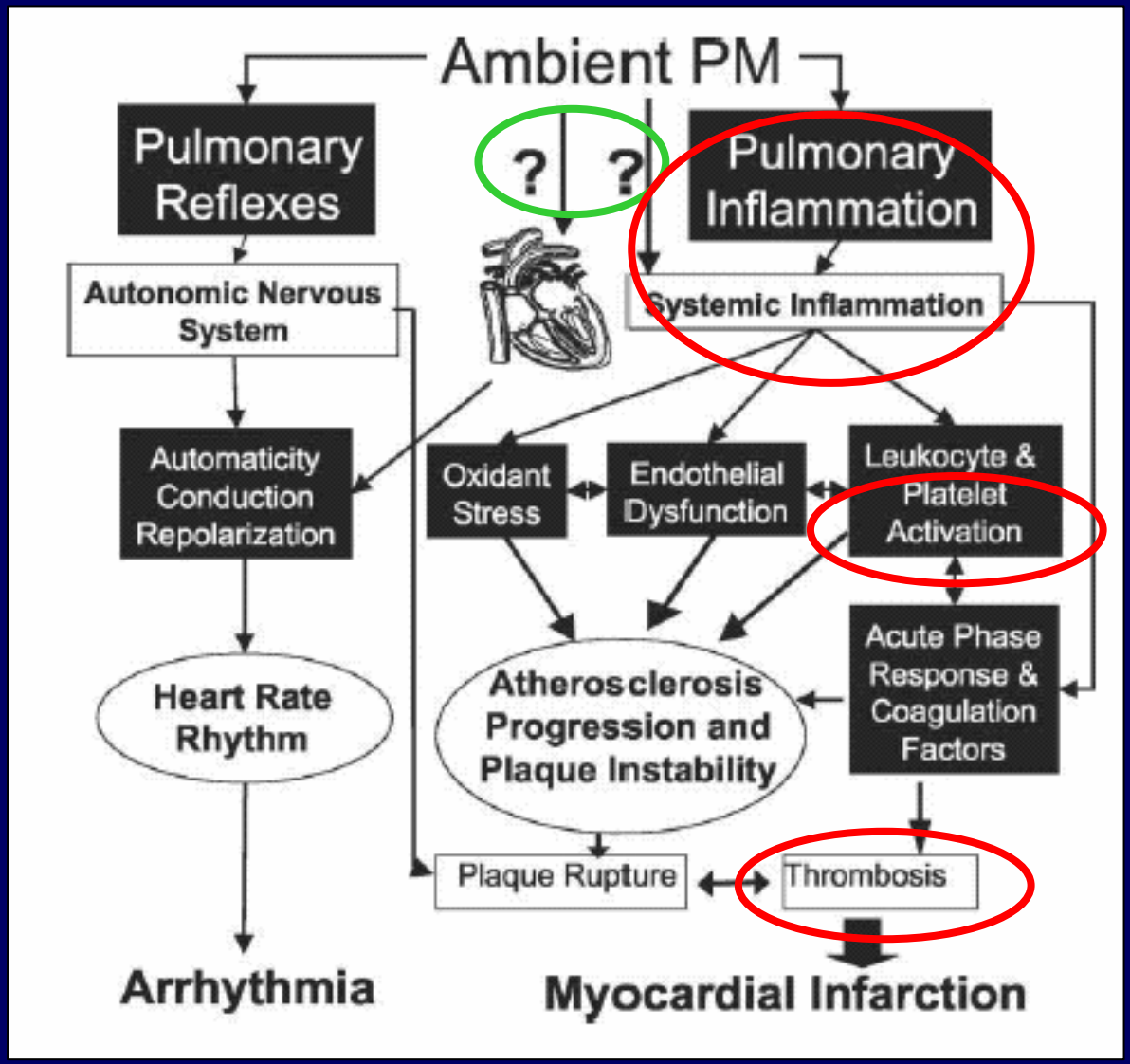
Nemmar *et al.* Circulation 2002,105, 411-4

Controversy going on …

- Geiser M et al. Ultrafine Particles Cross Cellular Membranes by Nonphagocytic Mechanisms in Lungs and in Cultured Cells *Environ Health Perspect* 113:1555–1560 (2005).

- Mills et al., Do inhaled carbon nanoparticles translocate directly into the circulation in man? *Am J Respir Crit Care Med* Articles in Press. Dec 9, 2005.

Brook RD *et al*. Air pollution and cardiovascular disease. A statement for health- care professionals from the expert panel on population and prevention science of the American Heart Association. *Circulation* 2004 (June 1); 109: 2655-71



Methods

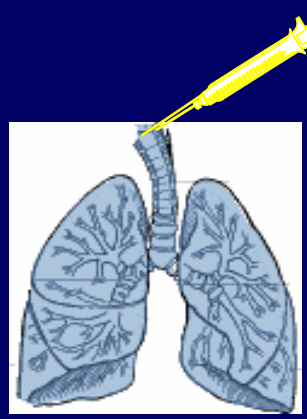
Pulmonary inflammation

- **DEP 5 - 500 µg/animal or vehicle were i.t. instilled to hamster**

1 hour

Bronchoalveolar lavage (BAL)

Saline



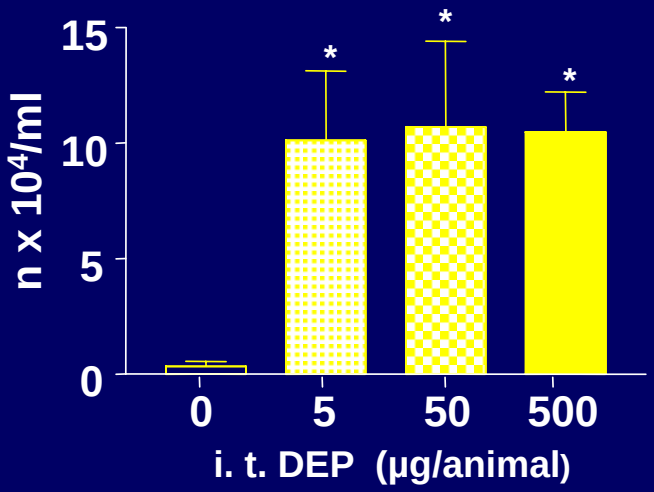
- Cells
- Proteins
- Histamine

Nemmar A. *et al*. Am J Respir Crit Care Med, 2002, 166, 998–1004
Nemmar A. *et al*. Circulation, 2003,107, 1202-8

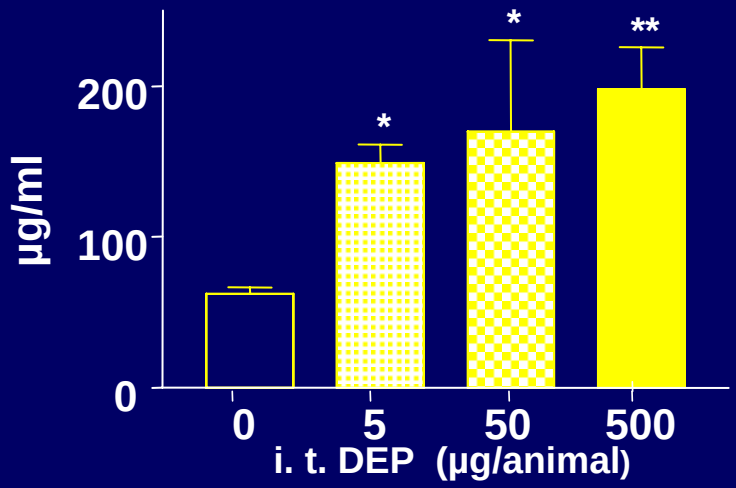
Results

Pulmonary inflammation (BAL)

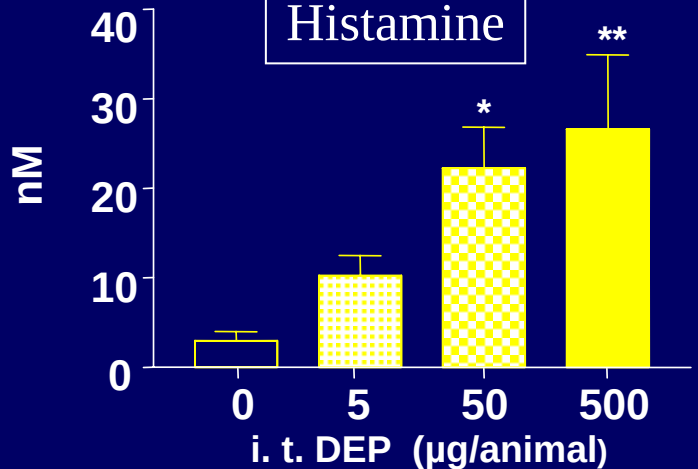
Neutrophils



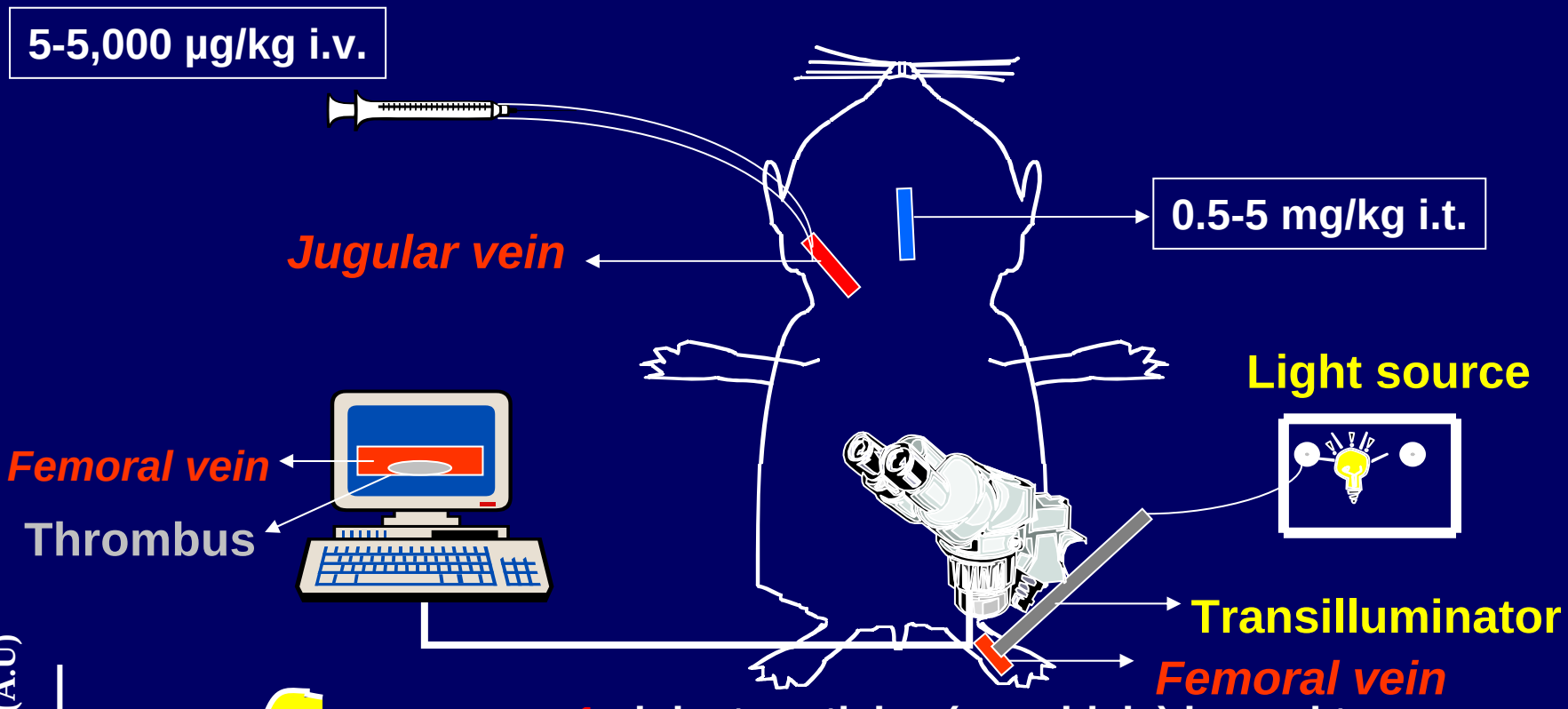
Proteins

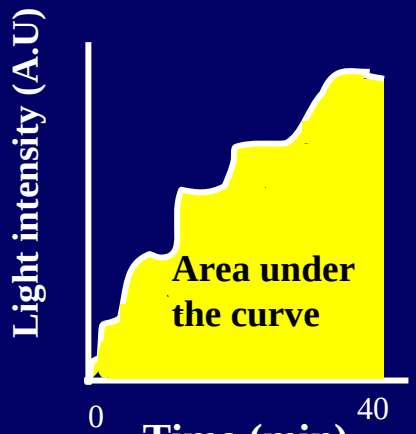


Histamine



Thrombus Formation: Methods





1. inject particles (or vehicle) i.v. or i.t.
2. 10 min later, inject Rose Bengal i.v.
3. 2 min transillumination (540 nm)
   → oxidative damage to endothelium
4. follow thrombus formation during 40 min
5. BAL (protein, LDH, cells) *(only after i.t.)*

Thrombus Formation: Materials & animals

- Polystyrene microspheres of 60 nm ø
  - unmodified: neutral
  - carboxylate-modified: negatively charged
  - amine-modified: positively charged

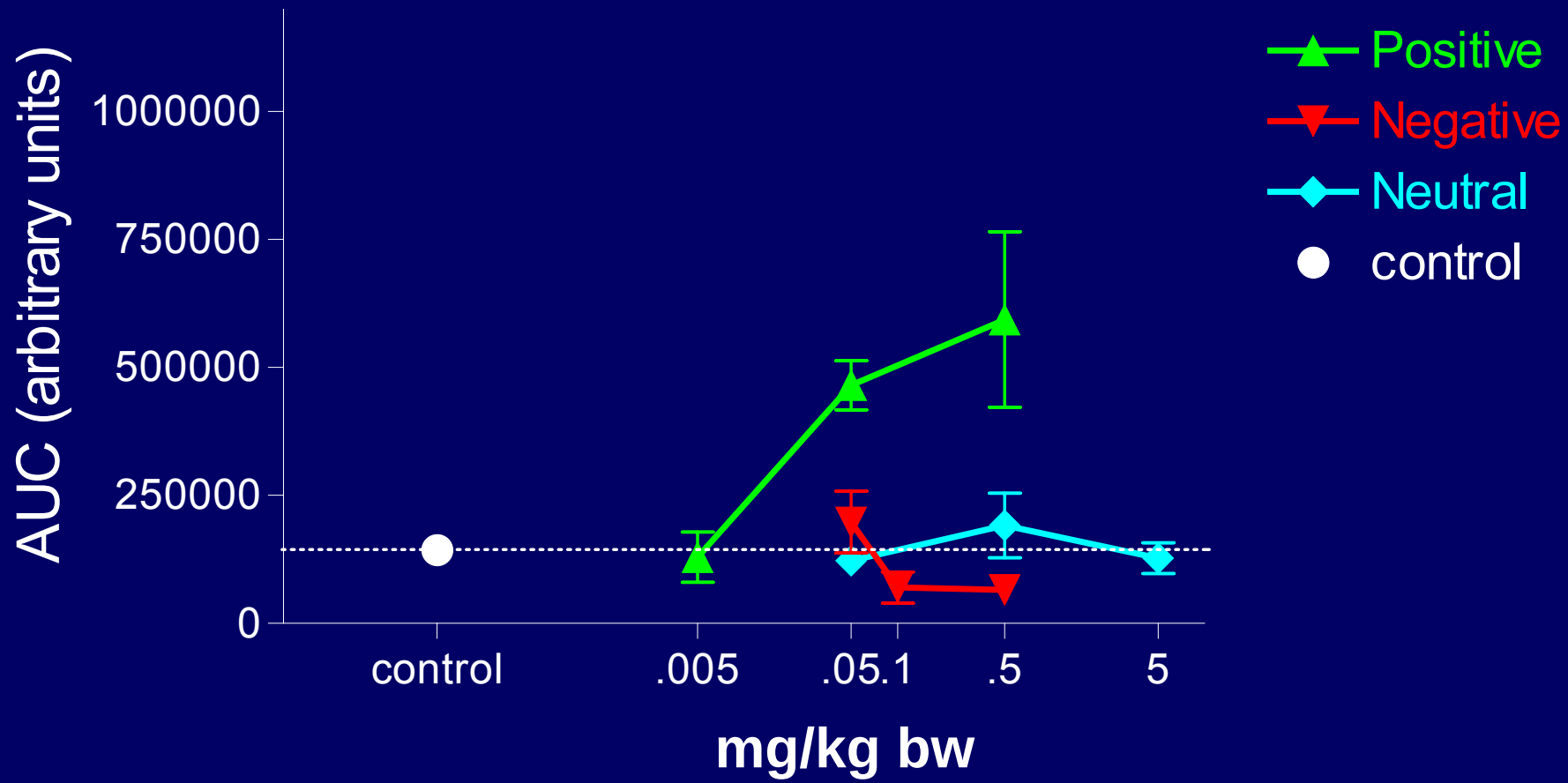
  suspended in NaCl 0.9%

  sonicated + vortexed immediately before administration
- Hamsters (100-150g), anaesthetized

  n=3-4 per day, including 1 vehicle control

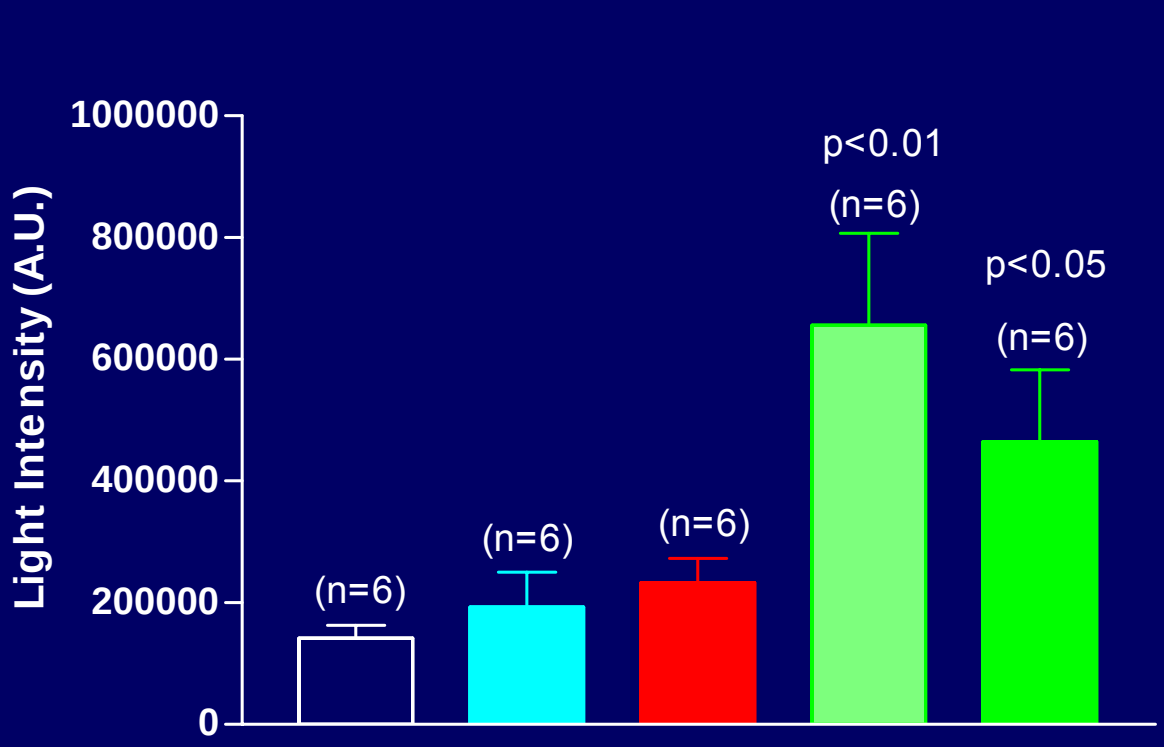
Thrombus Formation: Results

particles i.v.



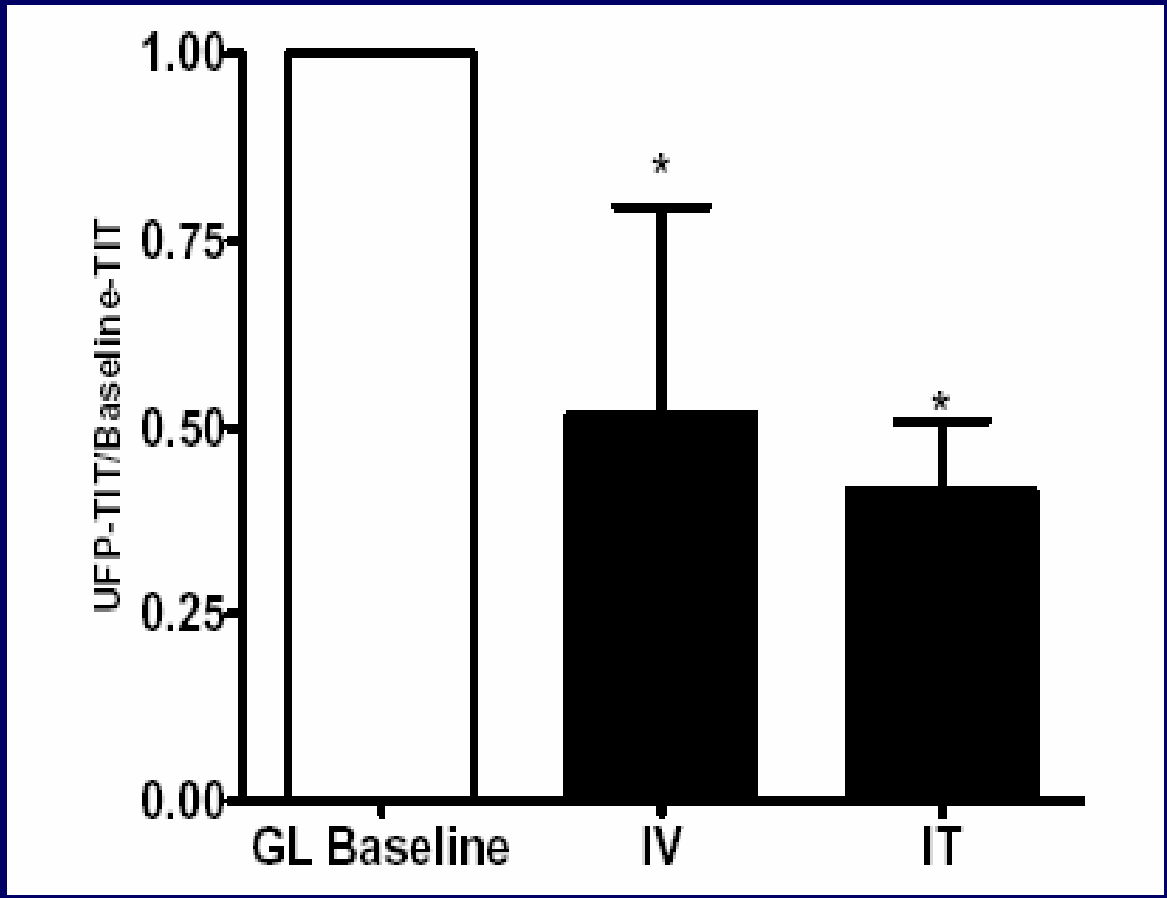
Thrombus Formation: Results

particles i.t.



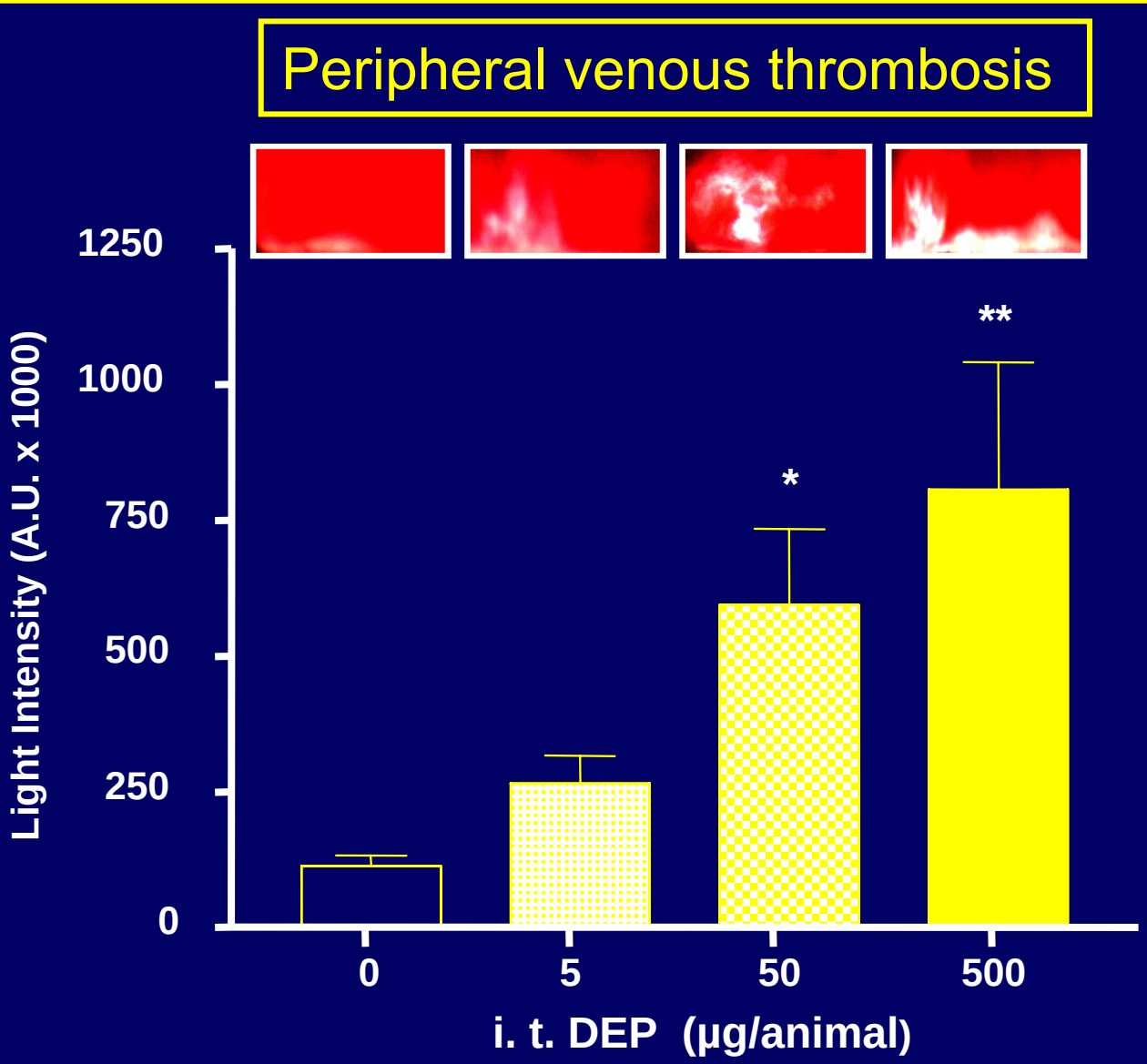
Control
Neutral 5mg/kg
Negative 5mg/kg
Positive 0.5mg/kg
Positive 5mg/kg

Silva VM, Corson N, Elder A, Oberdörster G. The rat ear vein model for investigating in vivo thrombogenicity of ultrafine particles (UFP). *Tox Sci*, 2005, 85, 983-9



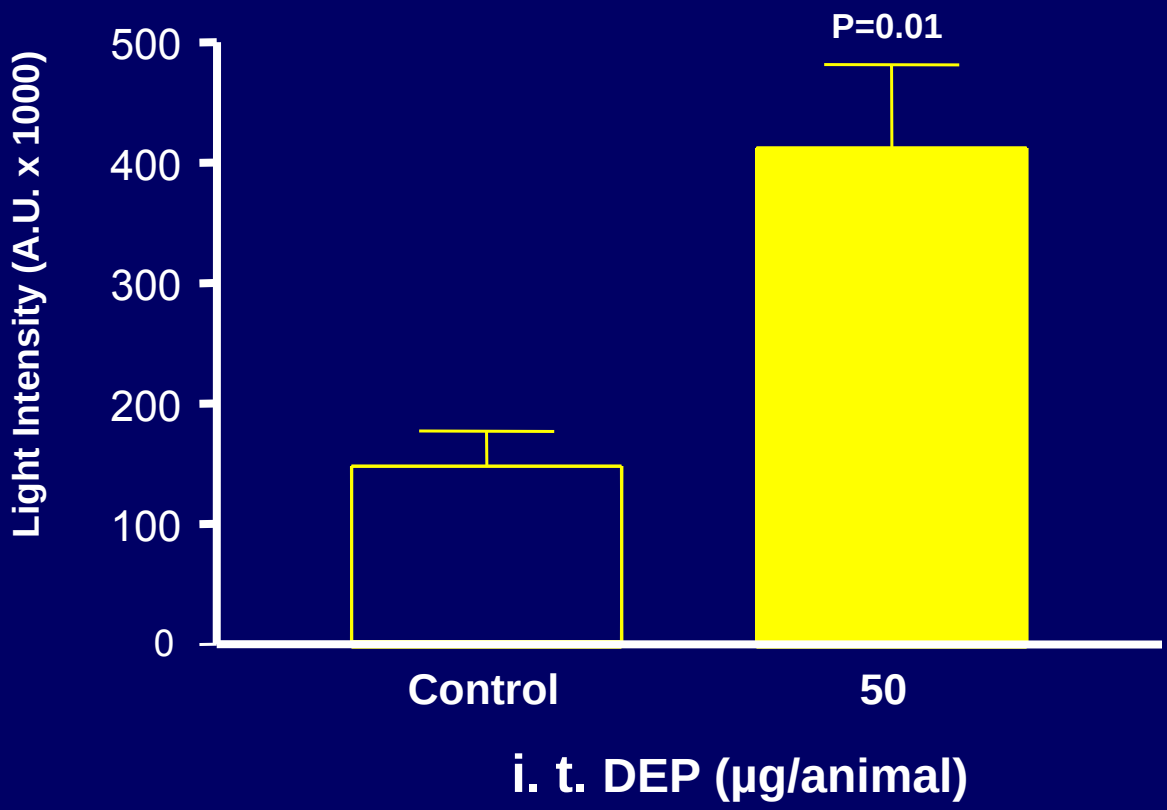
Thrombus Inducing Time (TIT)
Aminated (+) Polystyrene UFPs (60 nm) injected iv or it 10-15 min after baseline (without Rose Bengal)

Thrombus Formation: Results



Thrombus Formation: Results

Peripheral arterial thrombosis



Thrombus Formation & Pulmonary Inflammation
Summary & conclusion

- Within 1 hour after their deposition in the lungs, (Polystyrene - DEP)
  - ➢ cause pulmonary inflammation
  - ➢ aggravate thrombosis

Additional questions

The kinetics of

- Pulmonary inflammation?
- Platelet function following exposure to particles?

- And how is thrombogenicity affected by pulmonary inflammation?

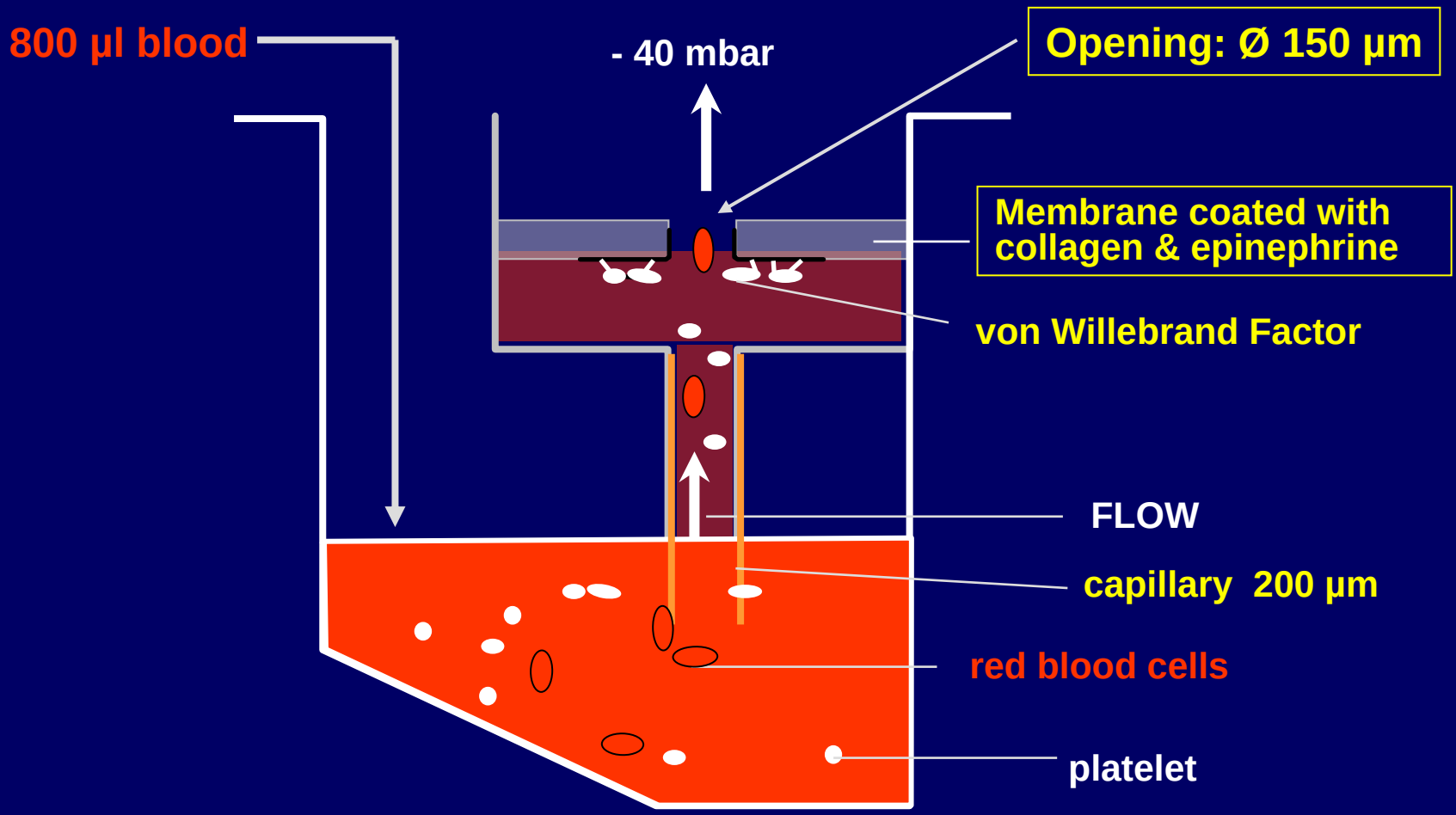
Methods

- Time effects: 1, 6 and 24h after DEP exposure
- Hamsters i.t. instilled with DEP
  - (50 µg/animal, n= 4-6)
- Endpoints assessed
  - Lung inflammation (PMN)
  - Thrombosis (+ Platelet activation)
  - Histamine concentrations in BAL & in plasma

Methods

Platelet activation

Platelet function analysis
PFA-100®



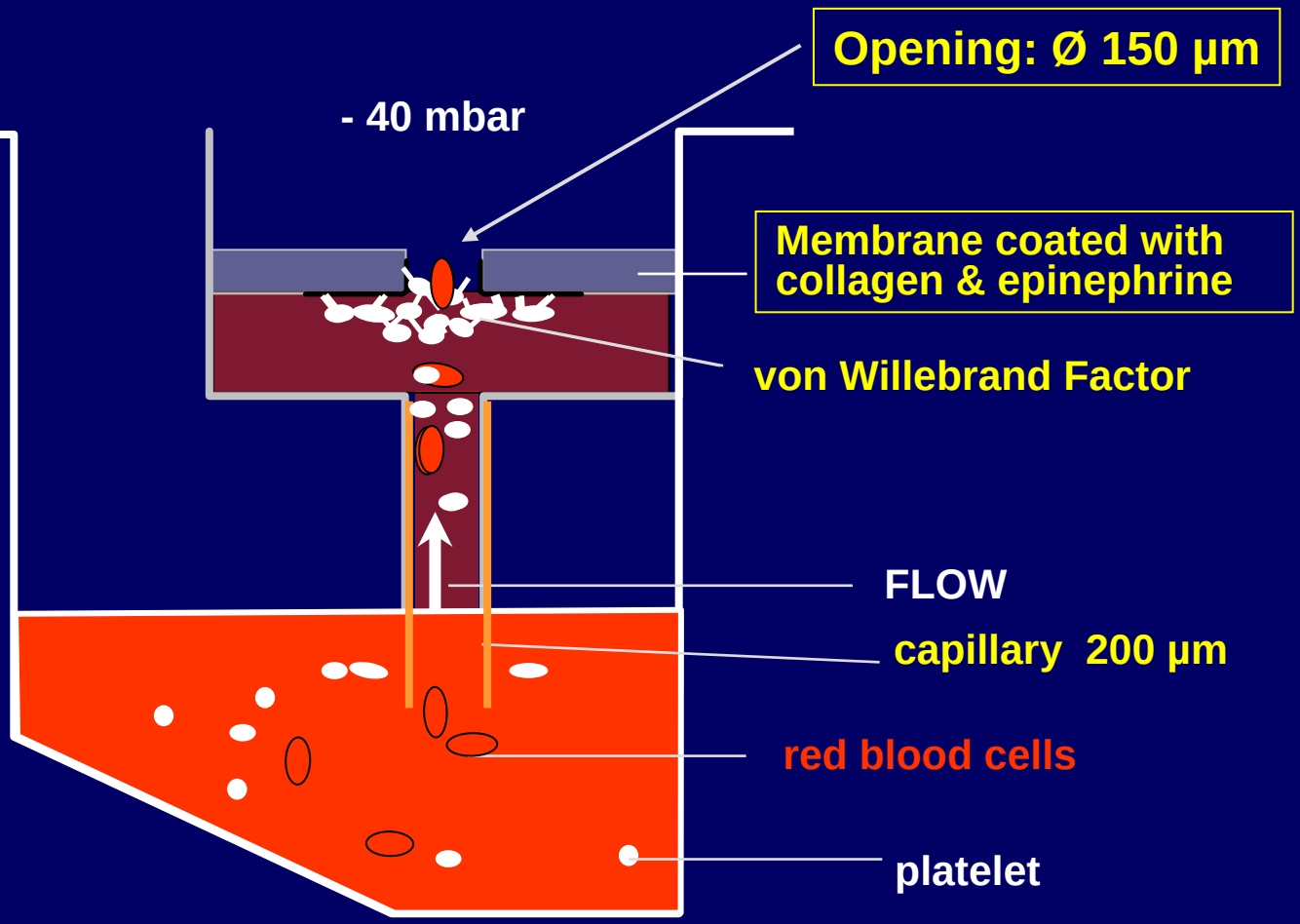
Methods

Platelet activation

Platelet function analysis PFA-100®

t_0 → $t_{closure}$

closure time



Methods

Platelet function analysis

ex vivo

i.t. DEP (50 µg/animal) or saline

blood

blood collection

5, 15, 30 and 60 min

in vitro

DEP (0.1 - 5 µg)

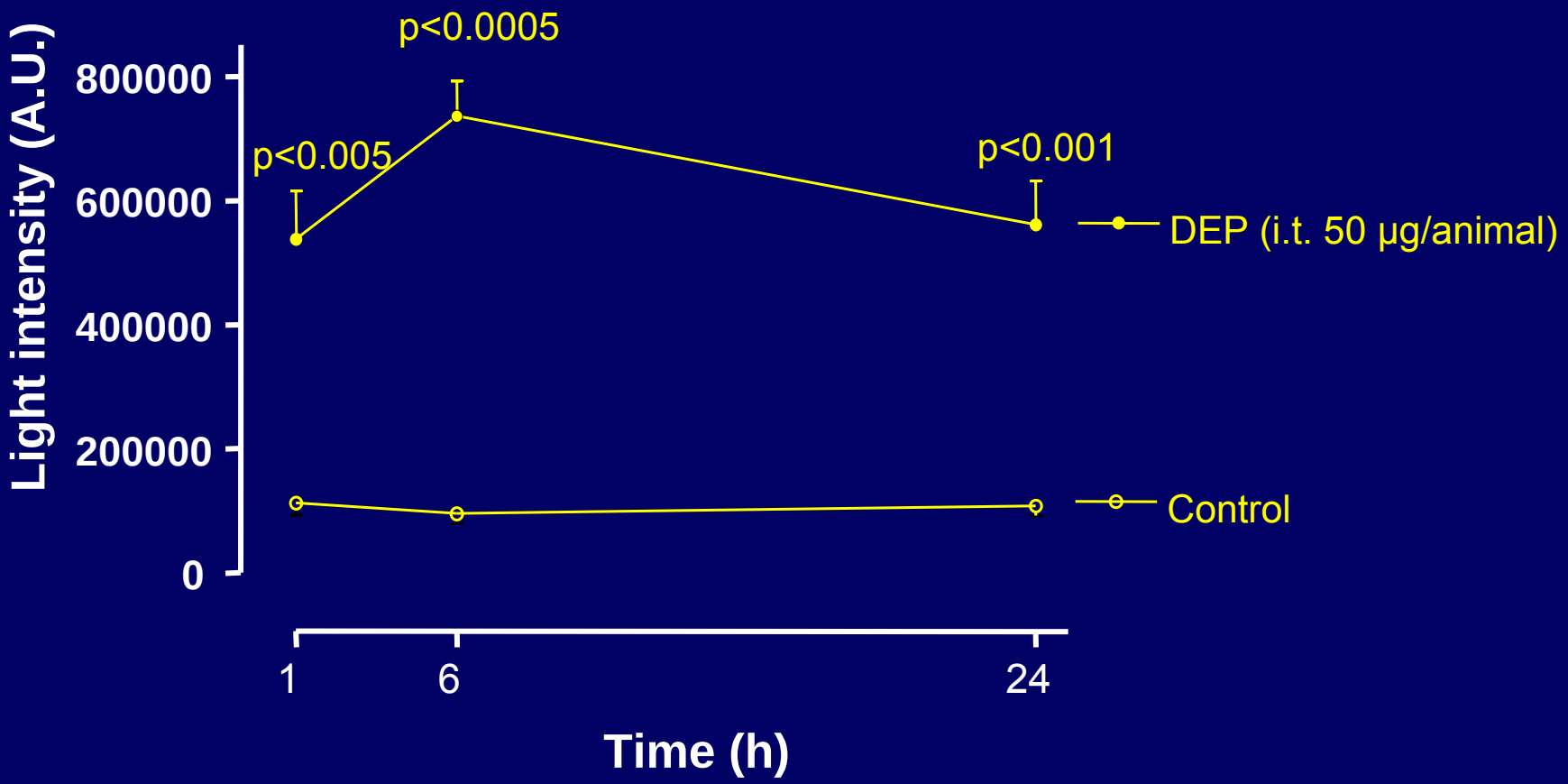
+

1ml hamster

5 min incubation

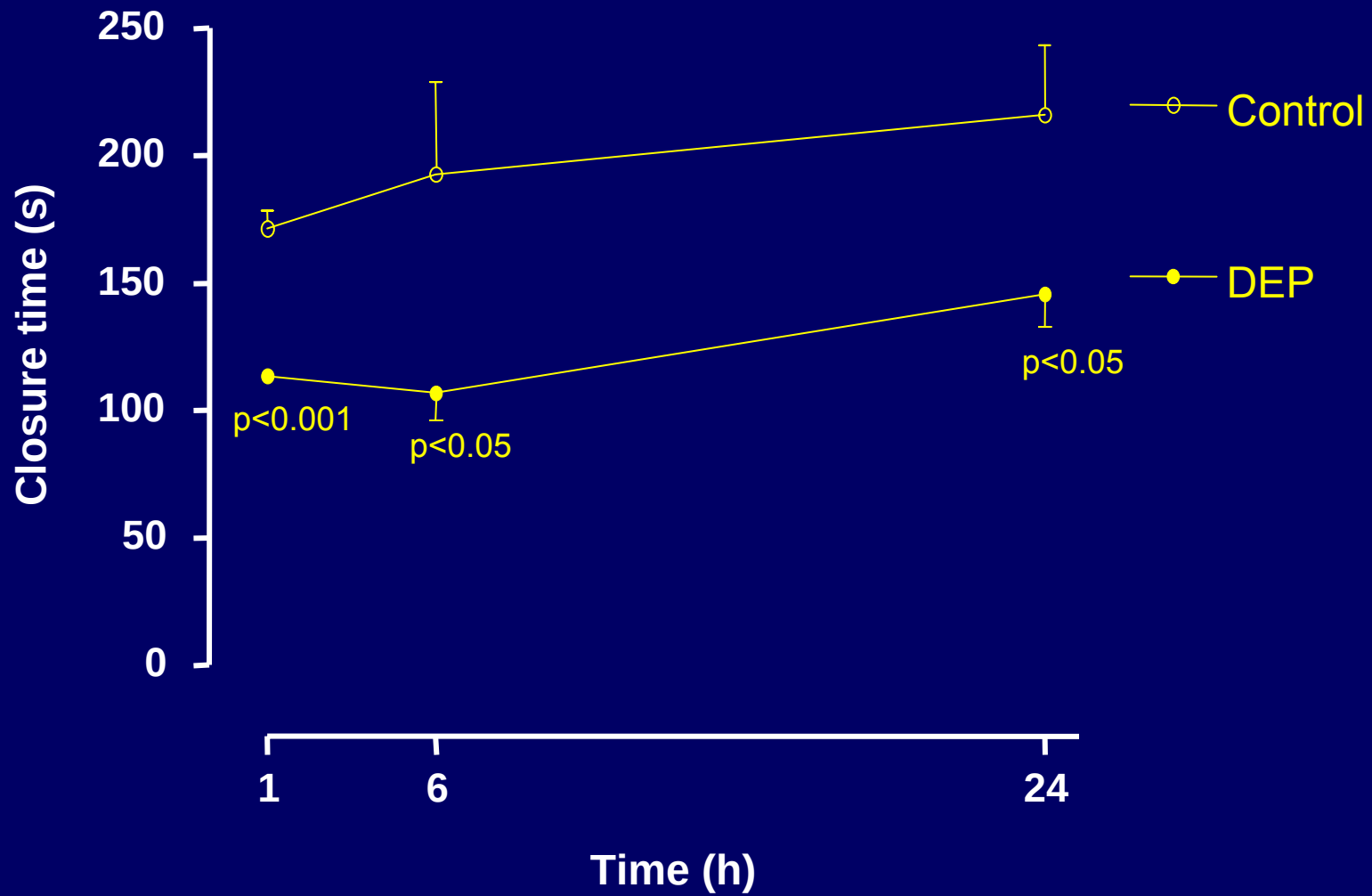
Time effect of i.t. DEP

Thrombosis *in vivo*

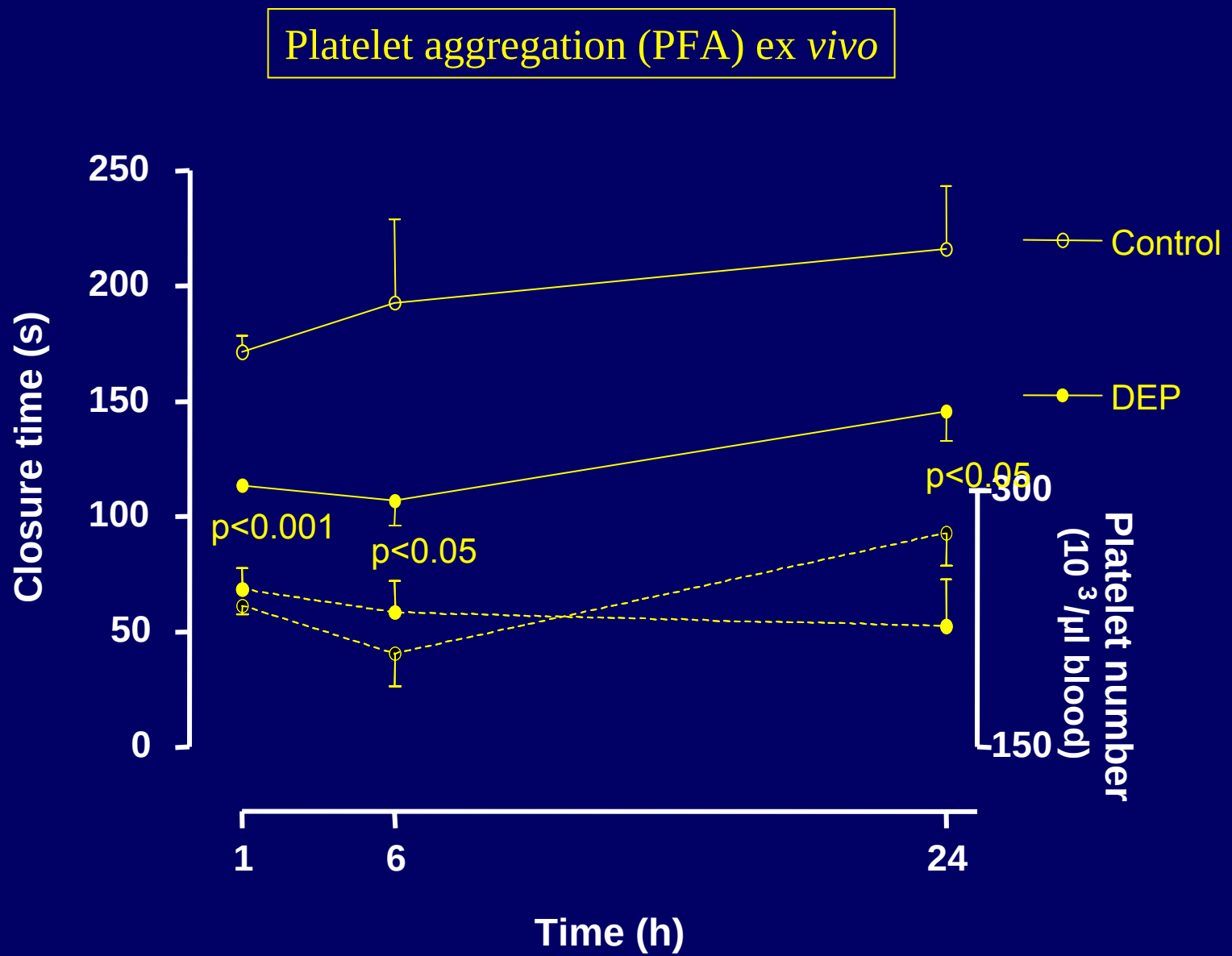


Time effect of i.t. DEP

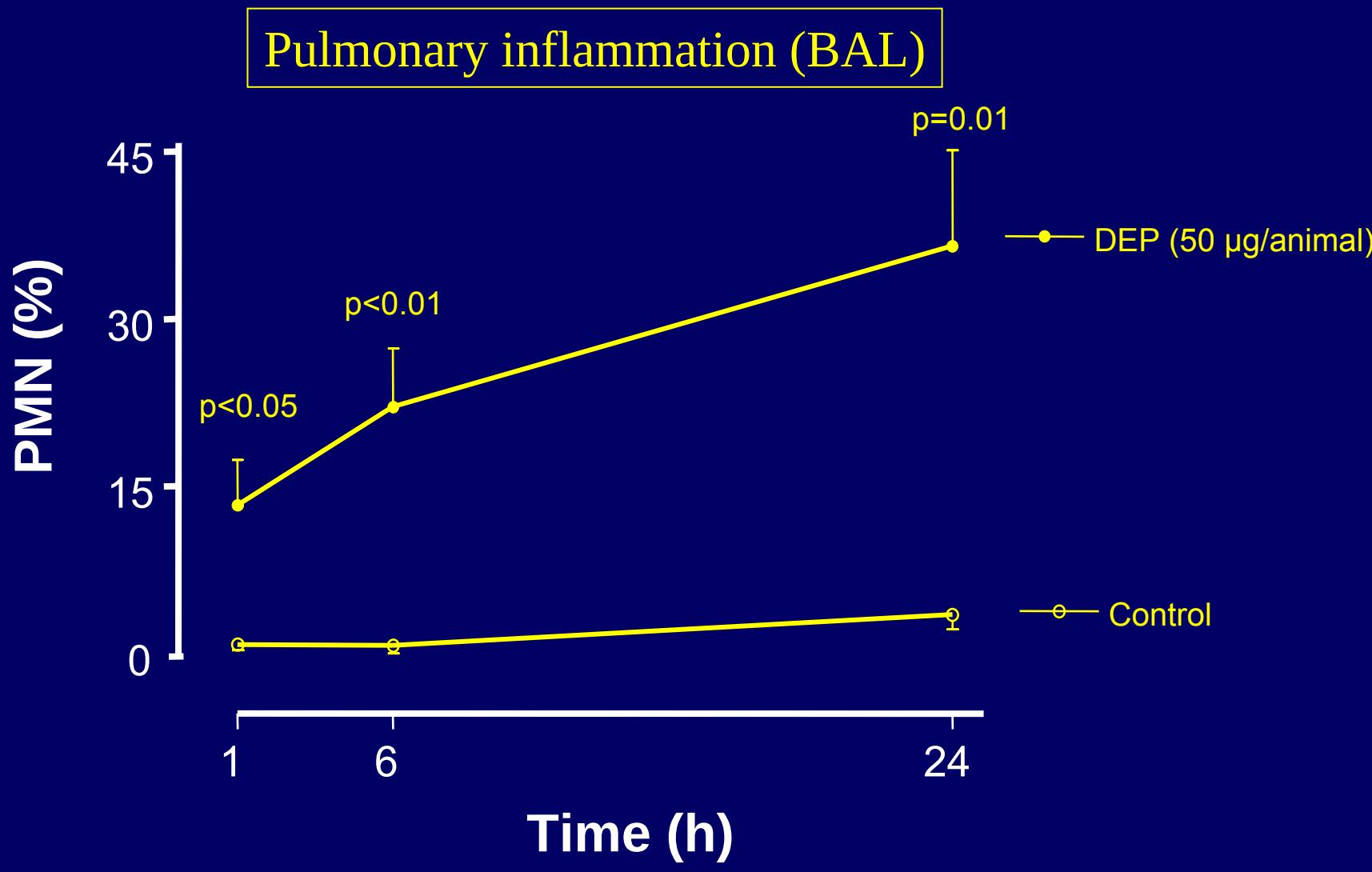
Platelet aggregation (PFA) ex *vivo*



Time effect of i.t. DEP

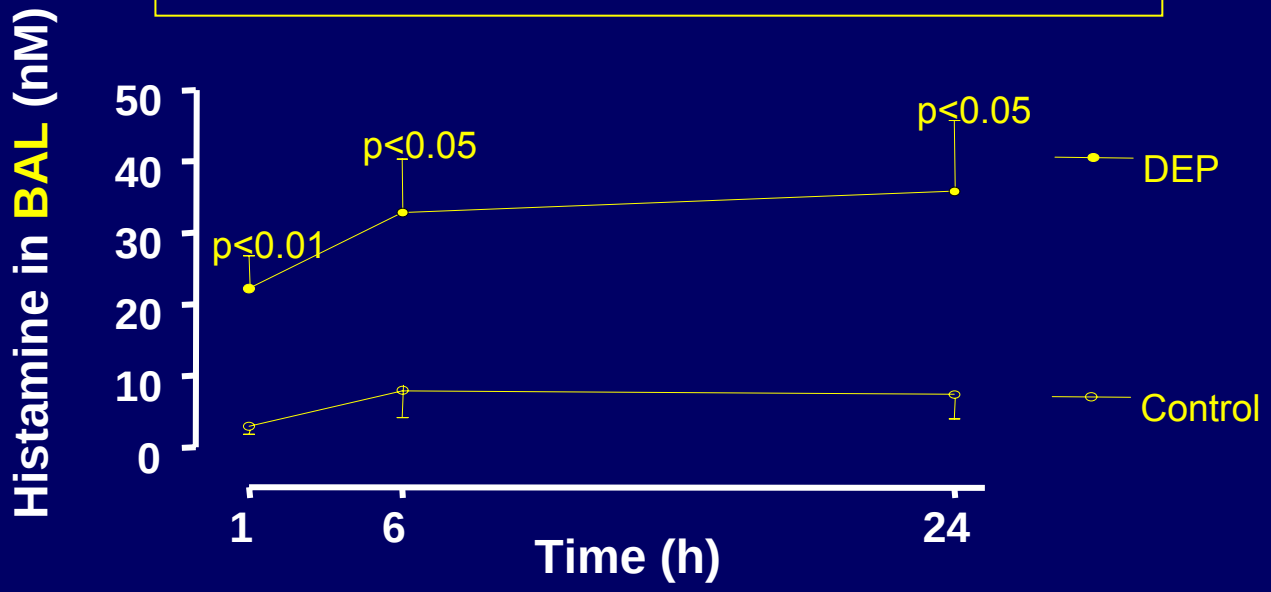


Time effect of i.t. DEP



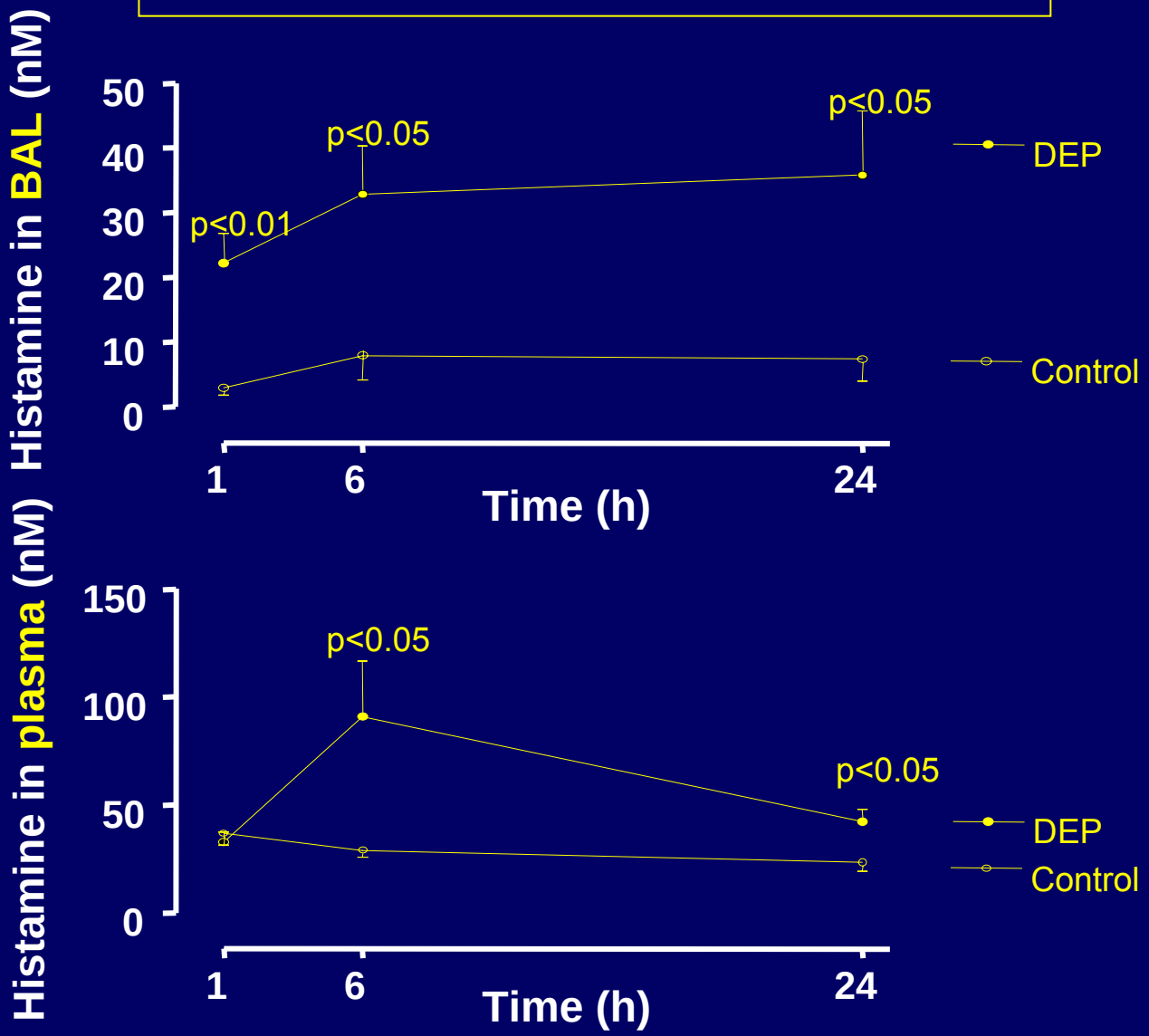
Time effect of i.t. DEP *in vivo*

Histamine measurements



Time effect of i.t. DEP *in vivo*

Histamine measurements



Time effect of i.t. DEP: Summary

- I.t. instillation of DEP leads to a significant prothrombotic effect and lung inflammation, which persist up to 24 h.
- Histamine concentrations were increased in BAL at all time points but in plasma, histamine levels were only increased at 6 and 24h and **not at 1h.**
- Effects of i.t. DEP on pulmonary inflammation and peripheral thrombosis (at 6 & 24 h) can be blocked by pretreatment with diphenhydramine, dexamethasone or cromoglycate

Interpretation

- We conclude that:
  - Pulmonary inflammation and peripheral thrombosis are correlated at 6 and 24h.
  - At 1h, the prothrombotic effect does not appear to result from pulmonary inflammation.

This is compatible with direct platelet activation by “particles” (or its constituents) having penetrated into the circulation

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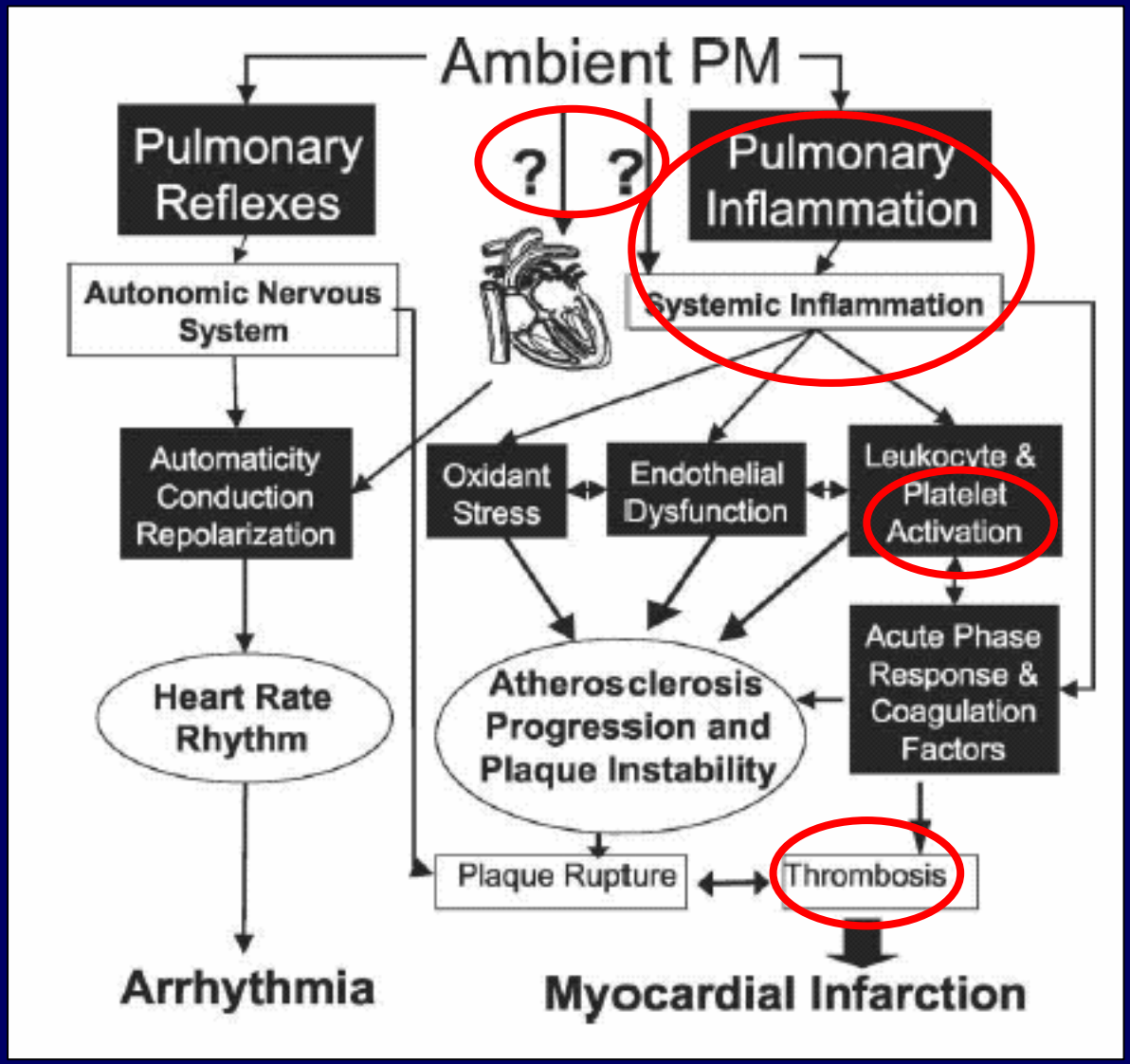

Acknowledgements

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D. Dinsdale

Sponsors:

- FWO
- OT-KULeuven

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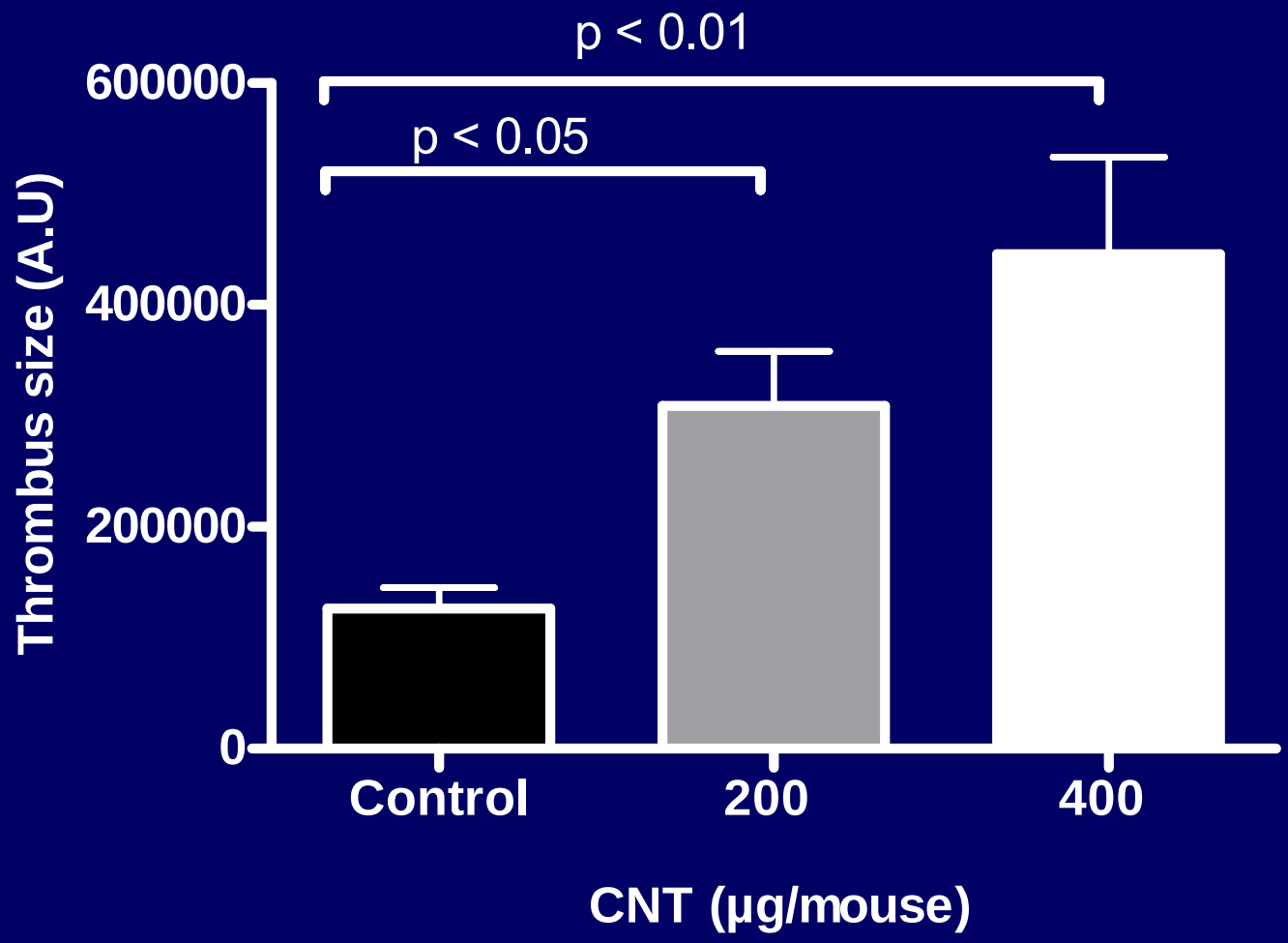
Thank you for your attention

Some recent data - unpublished

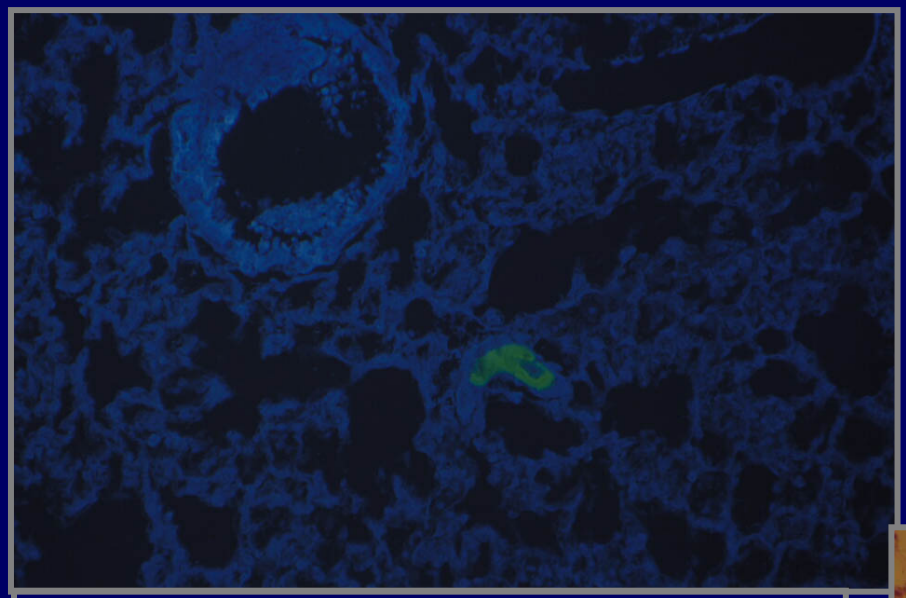
- Pro-thrombotic effect of carbon nanotubes
  (24 hr after dosing)
- Thrombotic effect of quantum dots (iv dosing)

Pro-thrombotic effect of carbon nanotubes



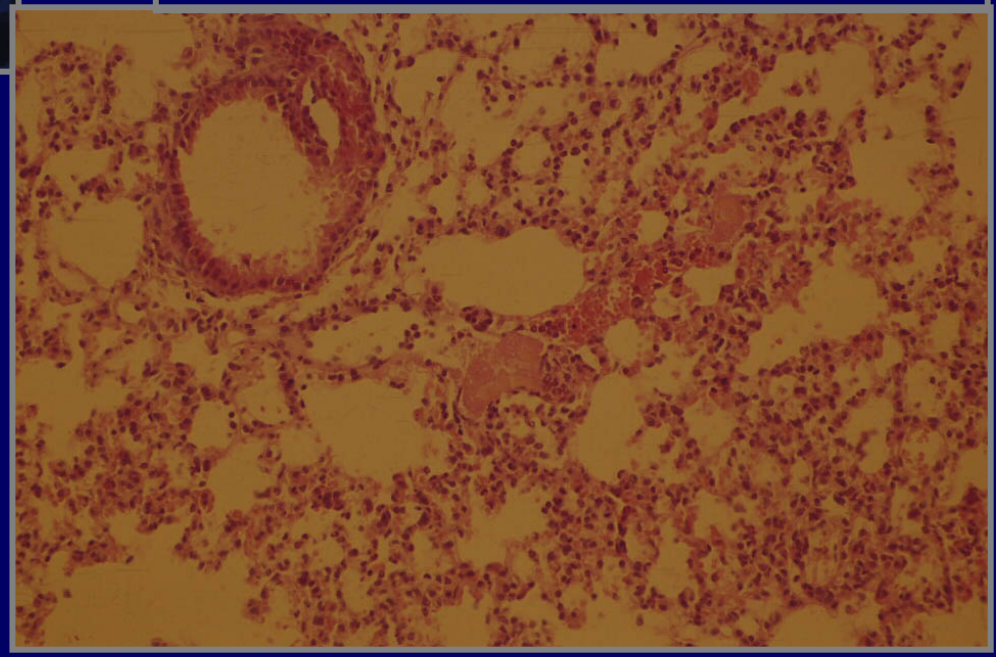
Mouse dosed iv with:

- Biocompatible carboxylated quantum dots
- 4 µg/animal



Non stained - fluorescence

Hematoxylin



Mechanisms of particle-induced lung inflammation and vascular thrombosis?

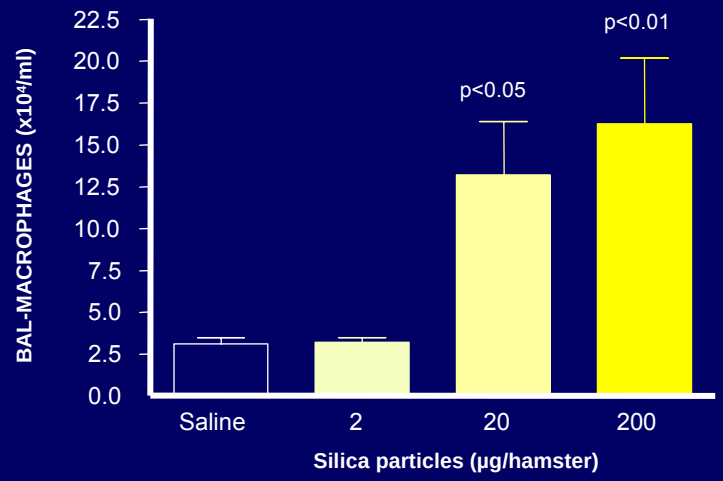
Nemmar *et al.* Am. J. Respir. Crit. Care Med. 2005; 171:872-89

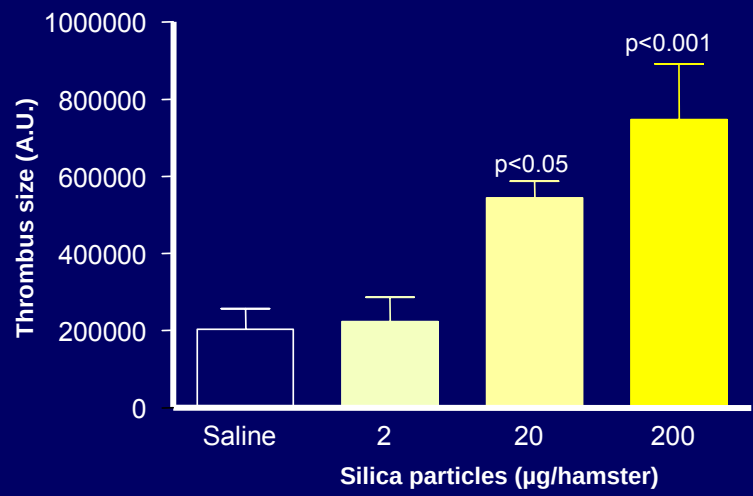
Silica particles

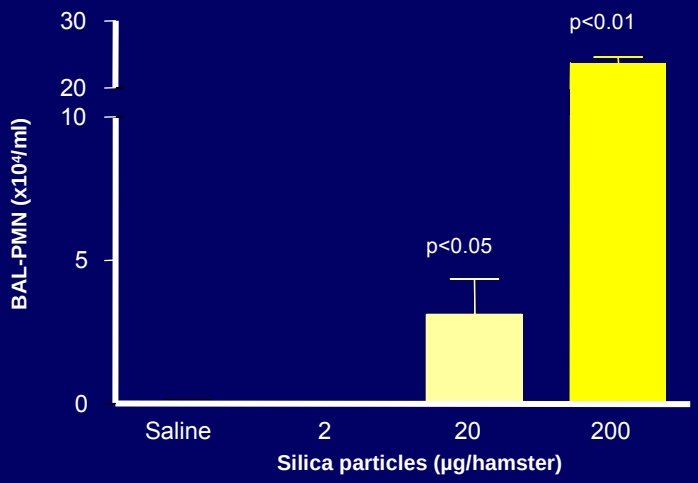
Pulmonary inflammation

24 h

Peripheral thrombosis







Silica particles

- Strategy of depletion:
  - I.t. clodronate liposomes: pulmonary macrophages (↓ 70 %)
  - I.p. cyclophosphamide: PMN depletion (↓ 80 %)

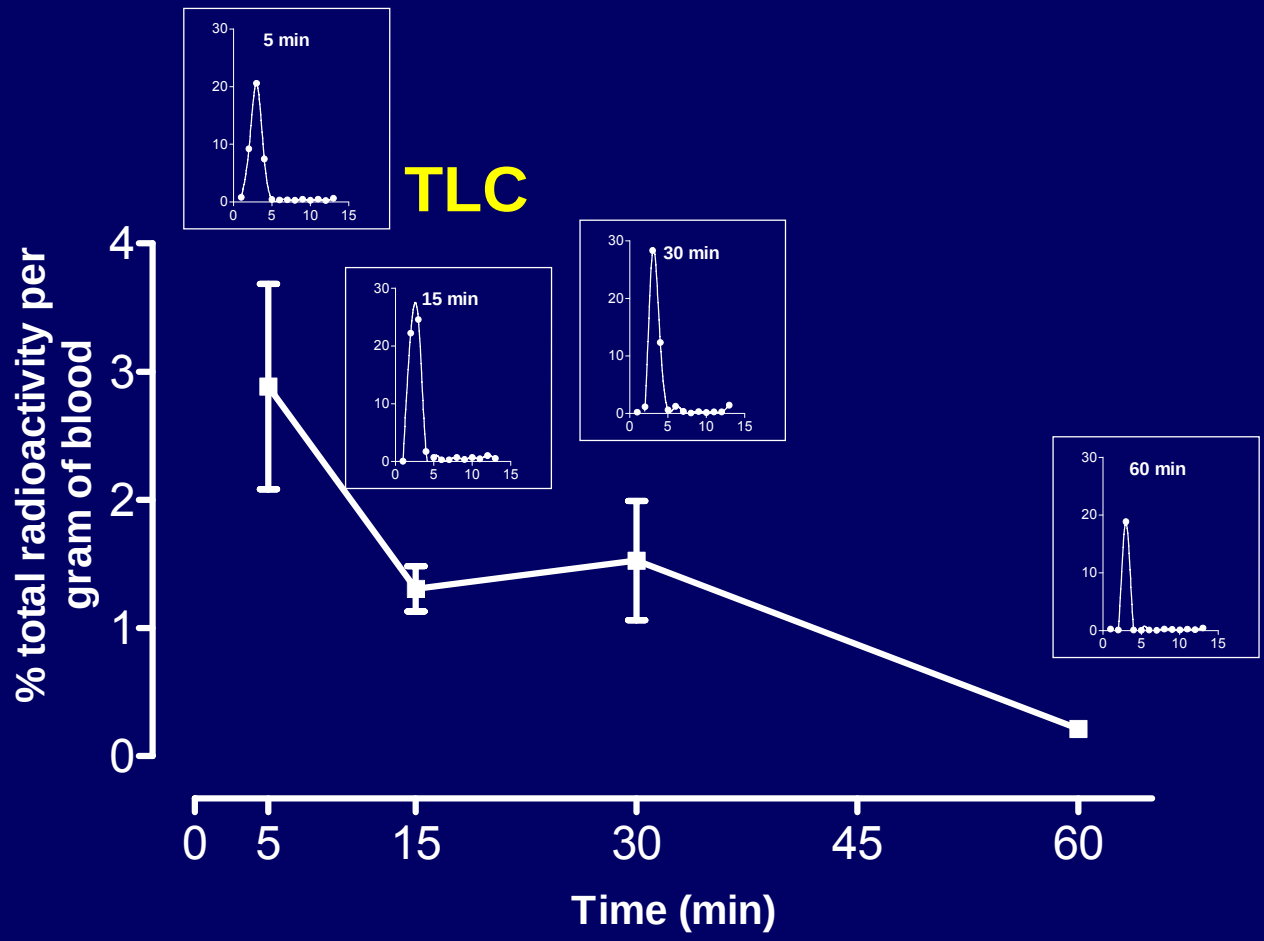
Pretreatment	Lung inflammation	peripheral thrombosis
Clodronate	inhibition	inhibition
Cyclophosphamide	inhibition	inhibition

- Additional results:
  - Elastase increases in BAL and plasma (+ partial but significant reduction of thrombosis after i.t. pretreatment with MeOSuc-AAPV-CMK, a specific neutrophil elastase inhibitor)

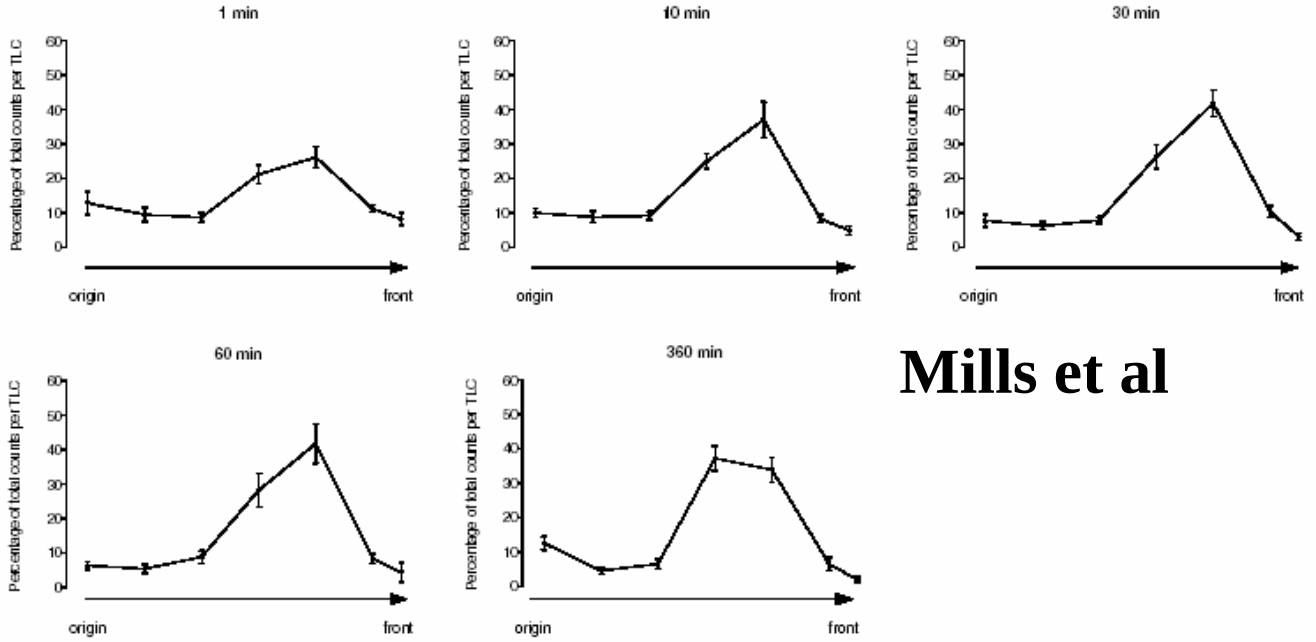
- Critical role of pulmonary macrophage-neutrophil cross-talk releasing neutrophil elastase into the blood circulation.
- Elastase, triggering activation of circulating platelets, may then predispose platelets to initiate thrombotic events on mildly damaged vasculature.

Translocation: Radioactivity in blood

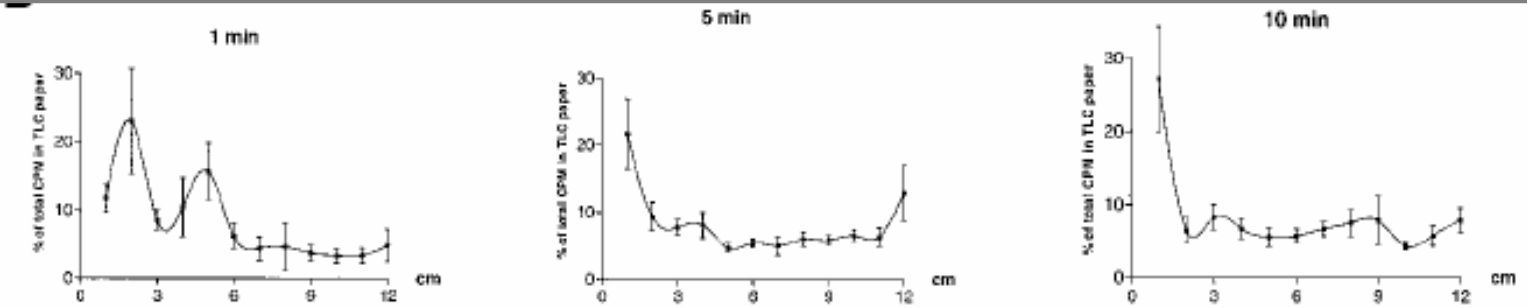
intratracheal instillation of ^{99m}Tc-albumin nanocolloid particles (80 nm) in hamsters 0



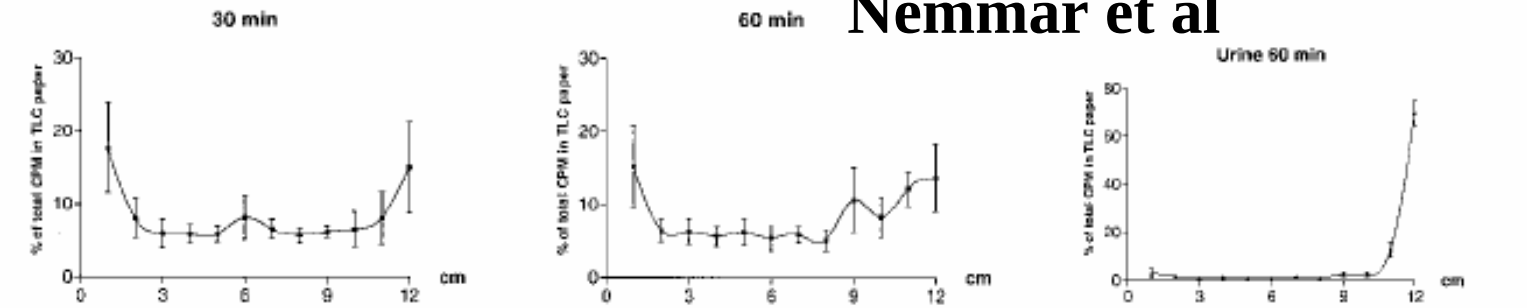
Nemmar *et al.* Am. J. Respir. Crit. Care Med. 2001;164:1665-8.

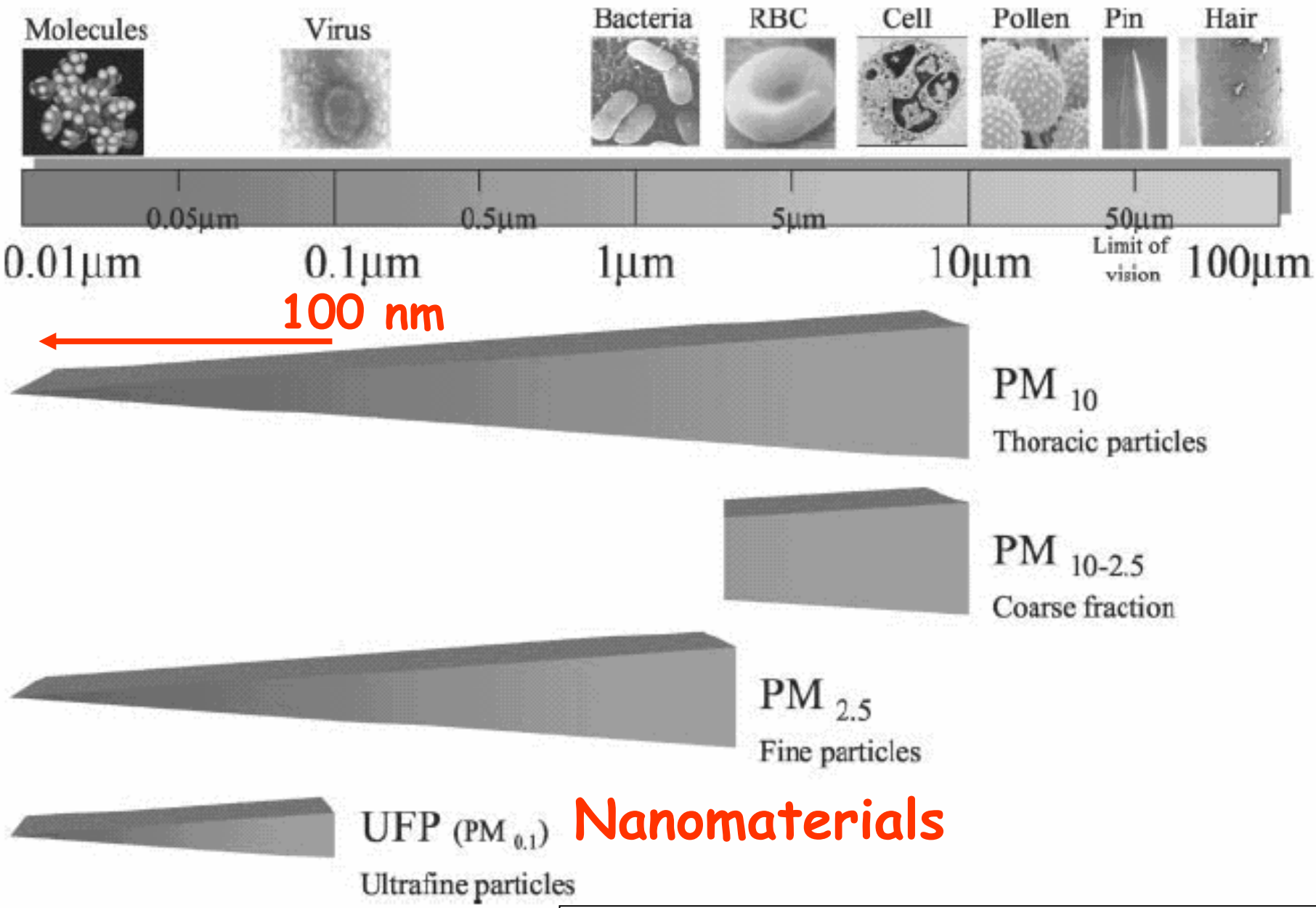


Mills et al



Nemmar et al





from Brook *et al*. Circulation 2004, 109, 2655-71)