Cardiovascular Effects of Nanoparticles

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Over the past decades epidemiological and toxicological studies have provided a body of evidence that elevated levels of ambient particulate air pollution are associated with increased cardiovascular morbidity and mortality. Various cardiovascular risk factors, i.e. elevated heart rate, decreased heart-rate variability, arterial vasoconstriction, augmented systolic blood pressure, and increased plasma viscosity were associated with ambient particle exposure. These changes may result in detrimental consequences for cardiac function, especially in patients with ischemic heart disease, cardiac arrhythmias, and congestive heart failure. Related to those findings, recent toxicological studies have put special emphasis on adverse effects mediated by nanoparticles, the environmental source of which are primarily traffic related combustion processes. With increasing commercial interest in nanoparticles or nanotubes, the risks associated with occupational exposure will become a matter of concern. Recent investigations provide evidence that nanoparticles can be quickly translocated from the lungs into the circulation and to secondary target organs, such as liver, heart, spleen, and brain. Therefore, the following mechanisms mediating cardiovascular effects of inhaled nanoparticles are postulated: (A) pulmonary and/or systemic inflammatory responses leading to endothelial dysfunction and a pro-coagulatory state, (B) direct interactions of translocated nanoparticles with endothelium and/or blood constituents promoting thrombogenesis, (C) dysfunction of the autonomic nervous system mediated by direct reflexes from intrapulmonary receptors and/or by local or systemic inflammatory stimuli, (D) cardiac malfunction due to ischemic responses in the myocardium and/or altered ion-channel functions in myocardial cells. Available data from experimental particle instillations and in-vitro studies are supportive of hypothesis (A) and give evidence for inflammation-mediated enhanced thrombus formation and aggravation of atherosclerotic lesions. In a study designed to specifically address hypothesis (B), we were recently able to demonstrate that intra-arterial application of

nanoparticles significantly enhances platelet accumulation on the venular endothelium of healthy mice. Particle-induced platelet adhesion was strongly associated with deposition of fibrin and increased expression of von-Willebrand factor on the endothelial surface. Inflammatory parameters were not elevated, indicating that nanoparticles may have the potential to exert a pro-thrombotic effect in the vascular system without triggering inflammatory processes. To examine the hypothetical pathway (C) – dysfunction of the autonomic nervous system in response to nanoparticle inhalation – heart rate and heart-rate variability were studied in rats during a 24h exposure to carbonaceous nanoparticles. A mild but consistent increase in heart rate with a significant associated decrease in heart-rate variability was observed. These results point to a particle-induced alteration of cardiac autonomic balance, which is mediated by a sympathetic stress response. In summary, the current toxicological evidence is clearly supportive of adverse cardiovascular effects arising from nanoparticle exposure, but the available data are as yet too scarce to provide a comprehensive understanding of the different pathophysiological pathways involved.

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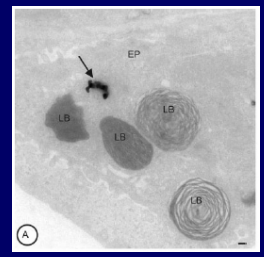

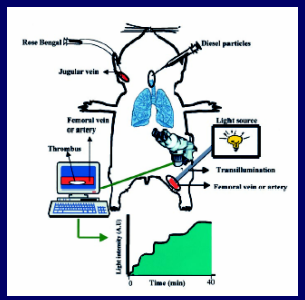

Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis

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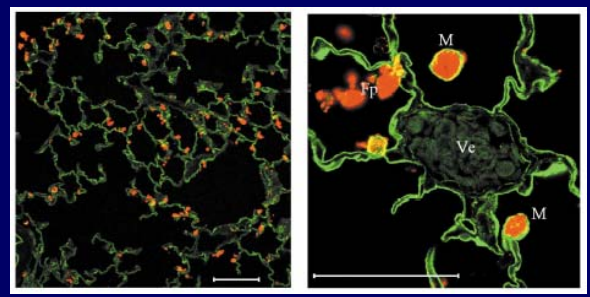
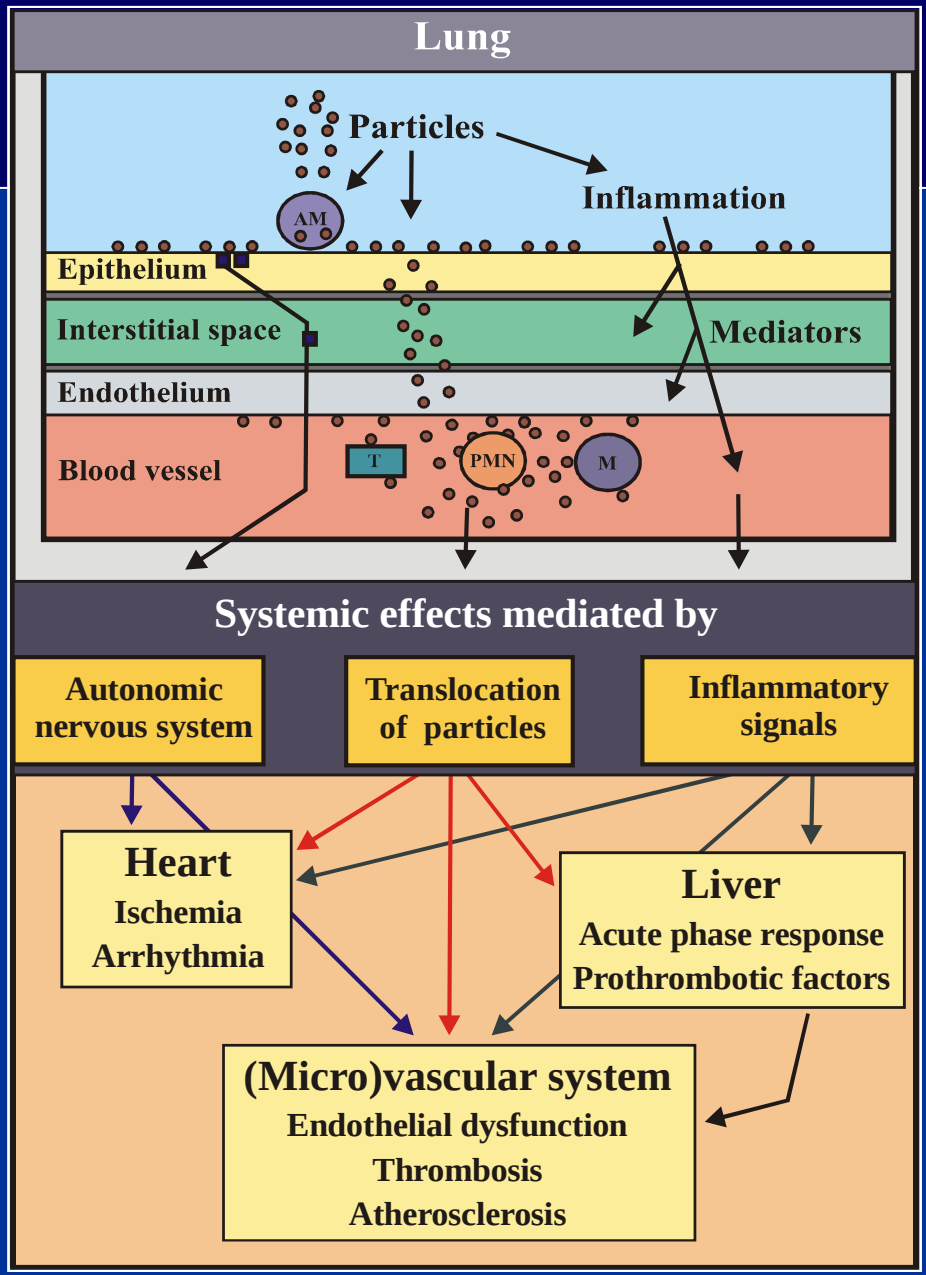
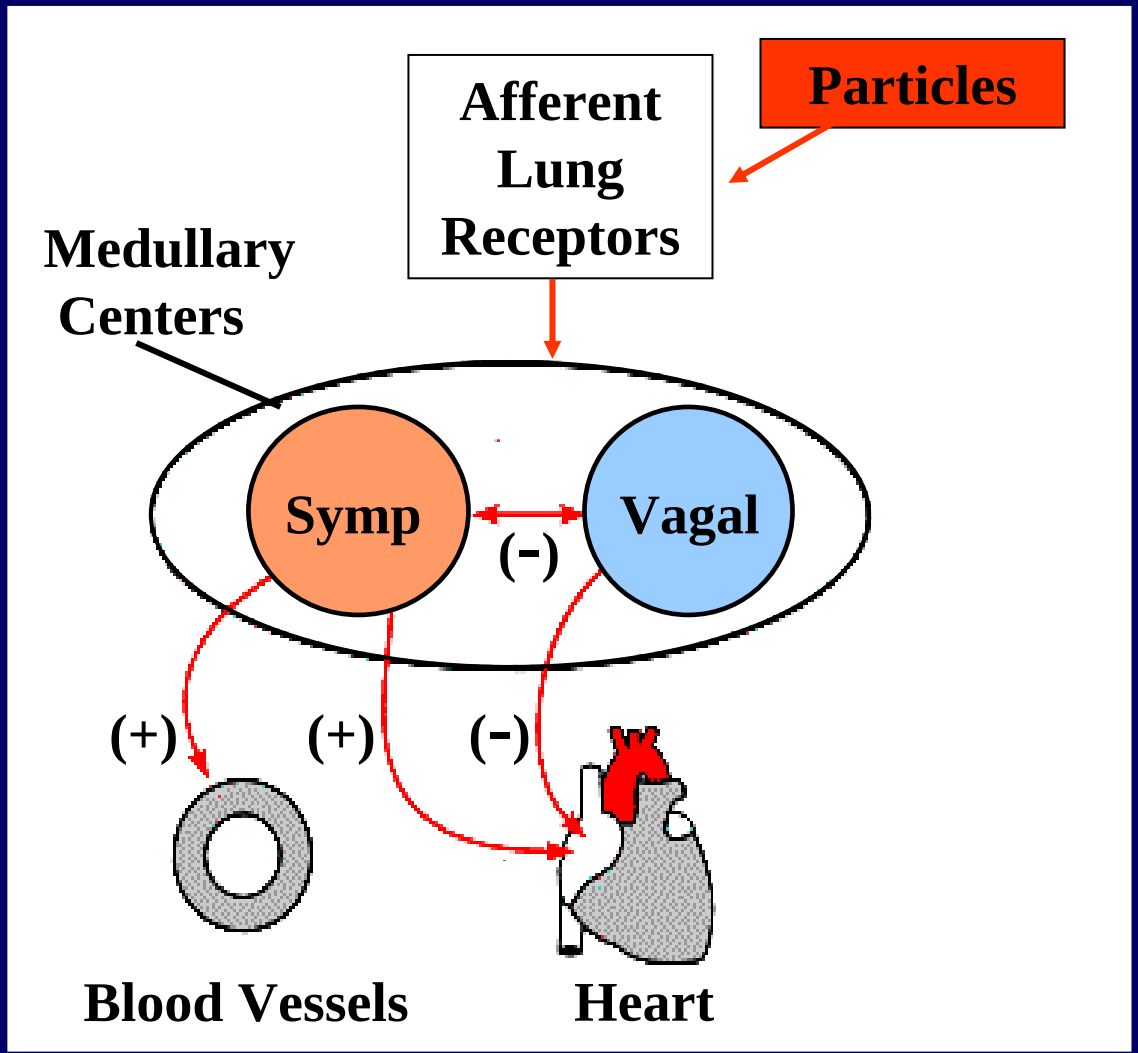


Cardiovascular Effects of Nanoparticles

Potential pathophysiological pathways associated with nanoparticle exposure



Effects on the Autonomic Nervous System



Do inhaled nanoparticles alter the autonomic balance?

Parameters:

Heart Rate

Heart Rate Variability

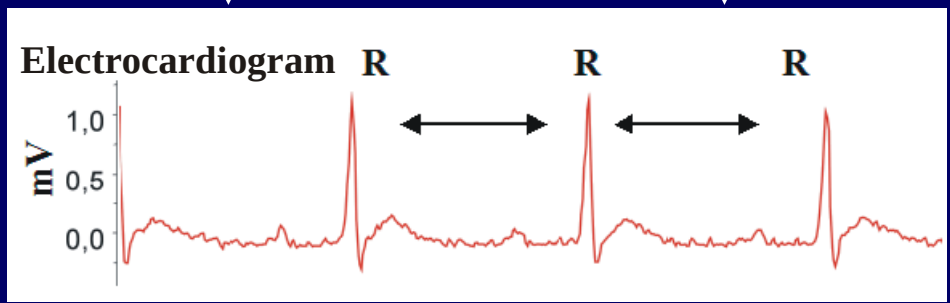
Experimental Approach – Telemetric Measurement of Heart Rate and Heart Rate Variability

Clean air

Receiver

np-CB

Receiver



Exposure for 24h

Baseline	Exposure	Recovery

Carbon black

Spark discharging

38 nm

180 µg·m^{-3}

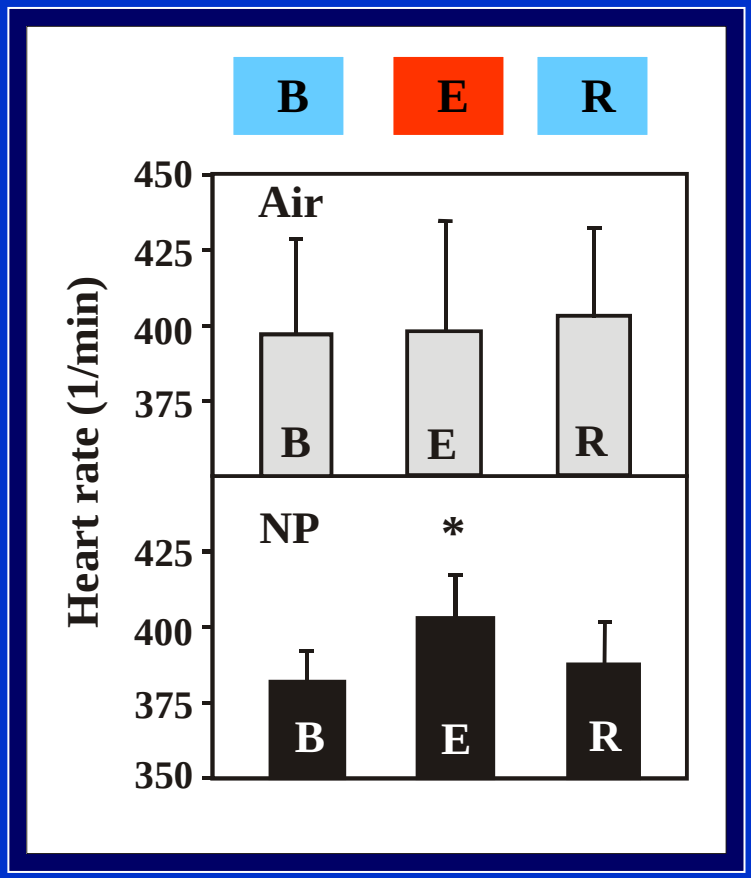
1.6 10^{7} cm^{-3}

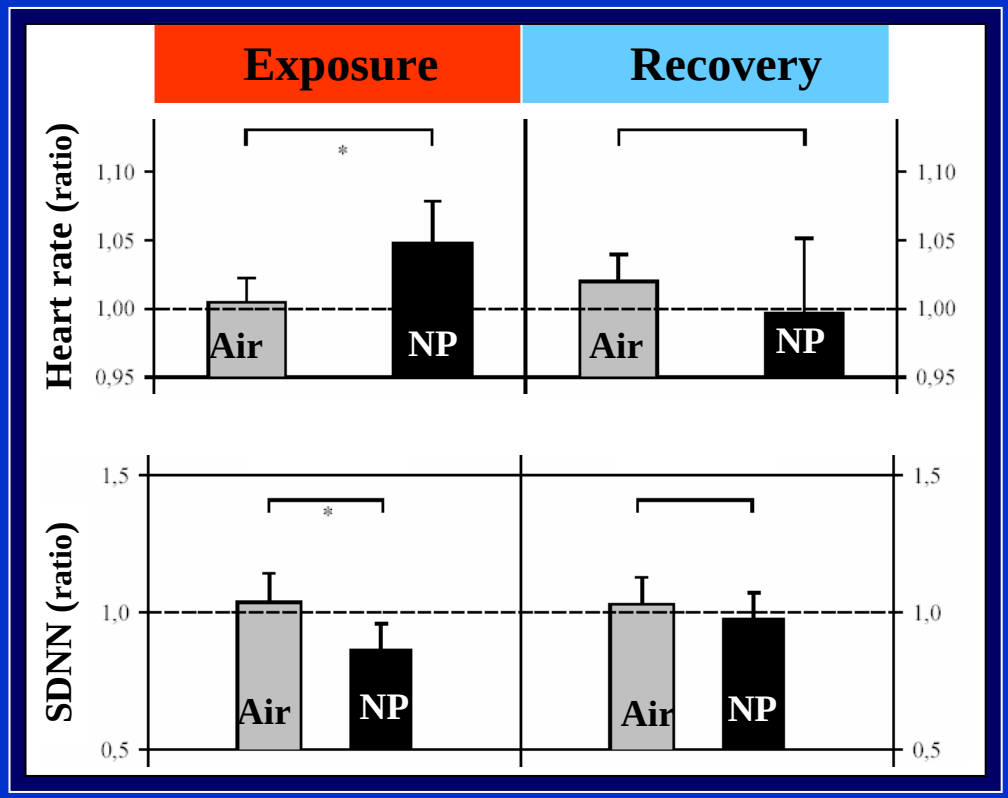
Heart rate: RR-Intervall

Heart rate variability:

Ttime domain: SDNN

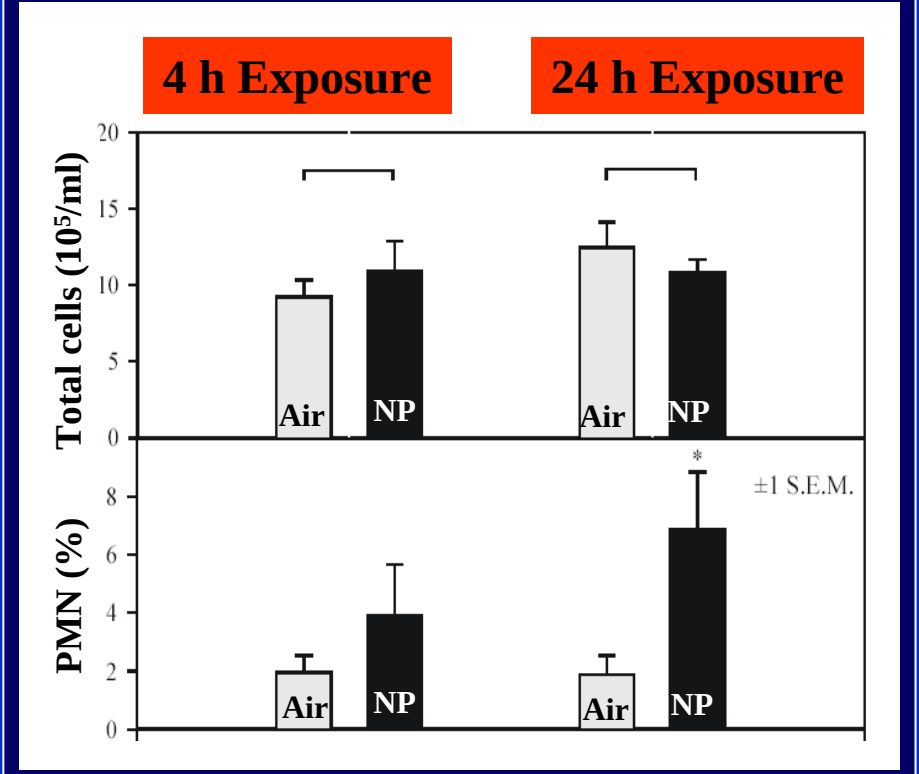
Effects on the Autonomic Nervous System

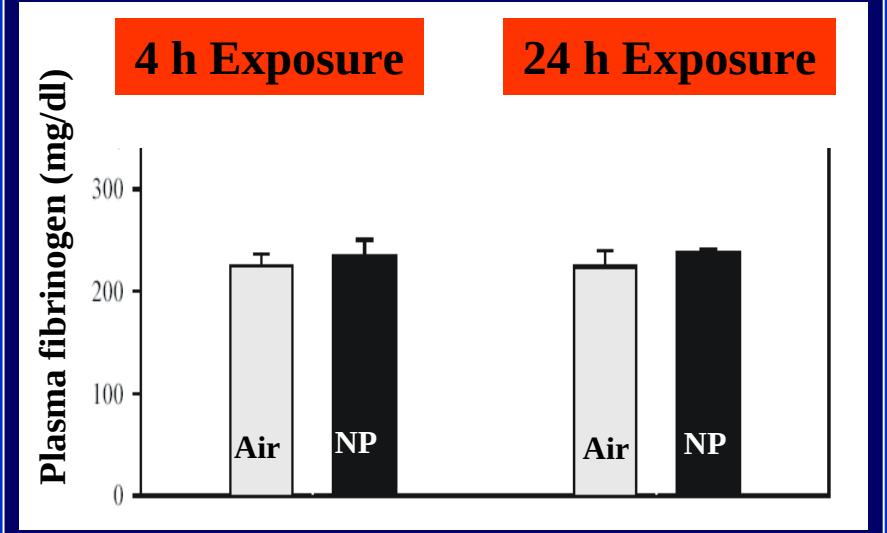




Increased heart rate and reduced heart rate variability during exposure suggest a sympathetic stress response.

Is the stress response associated with pulmonary inflammation?



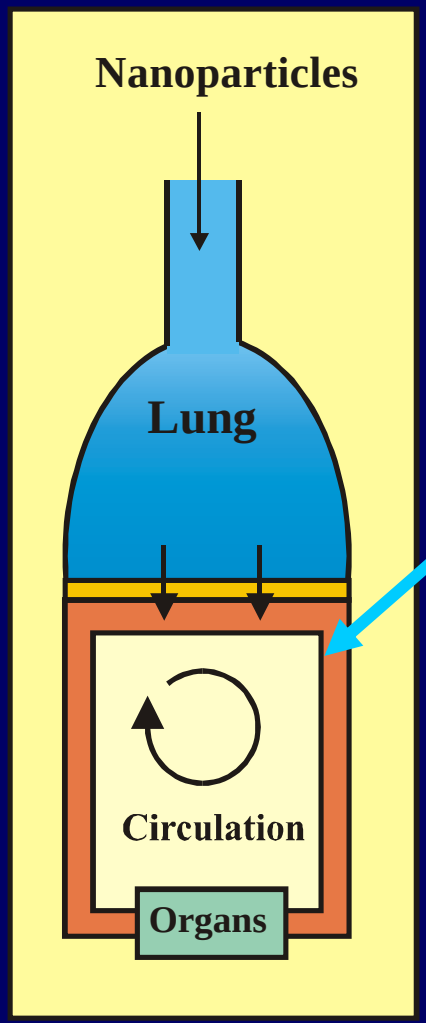


Acute phase reactants and coagulation parameters are not altered.

The dissociation between cardiac and inflammatory responses and the lack of a systemic acute phase reaction

- **suggest a neural pathway for cardiac stress response via activation of receptors in the lungs.**

Microvascular Effects of Translocated Nanoparticles

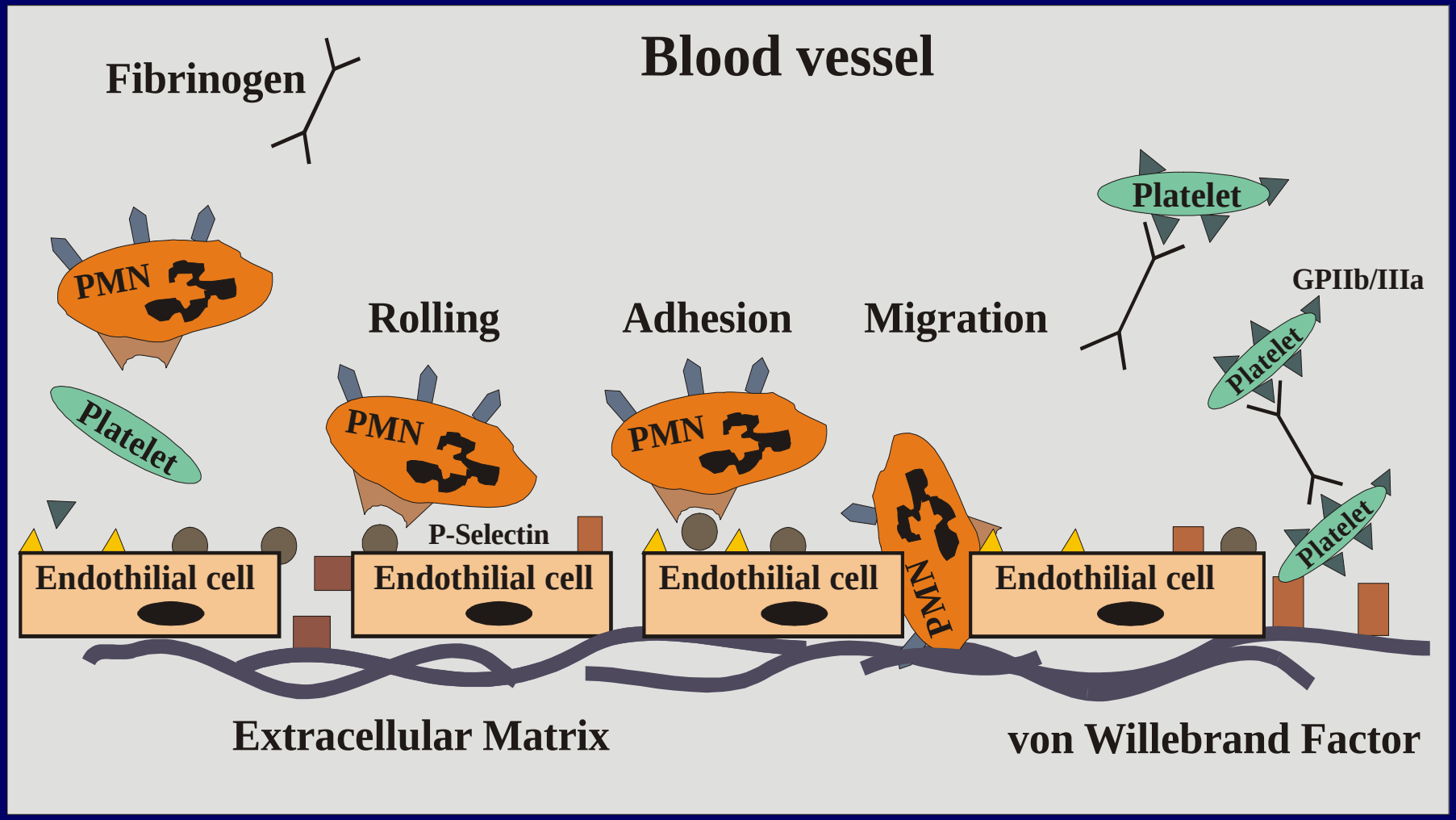


Do systemically available nanoparticles exert inflammatory and/or prothrombotic effects in the vascular system?

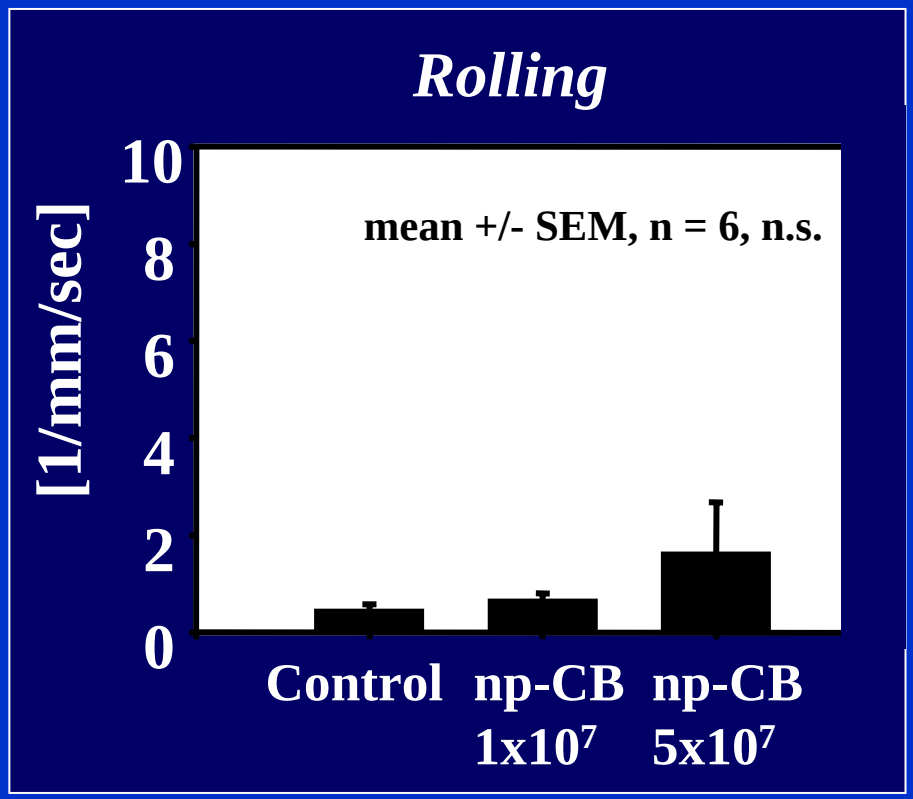
Experimental approach - C57BL/6J-mice

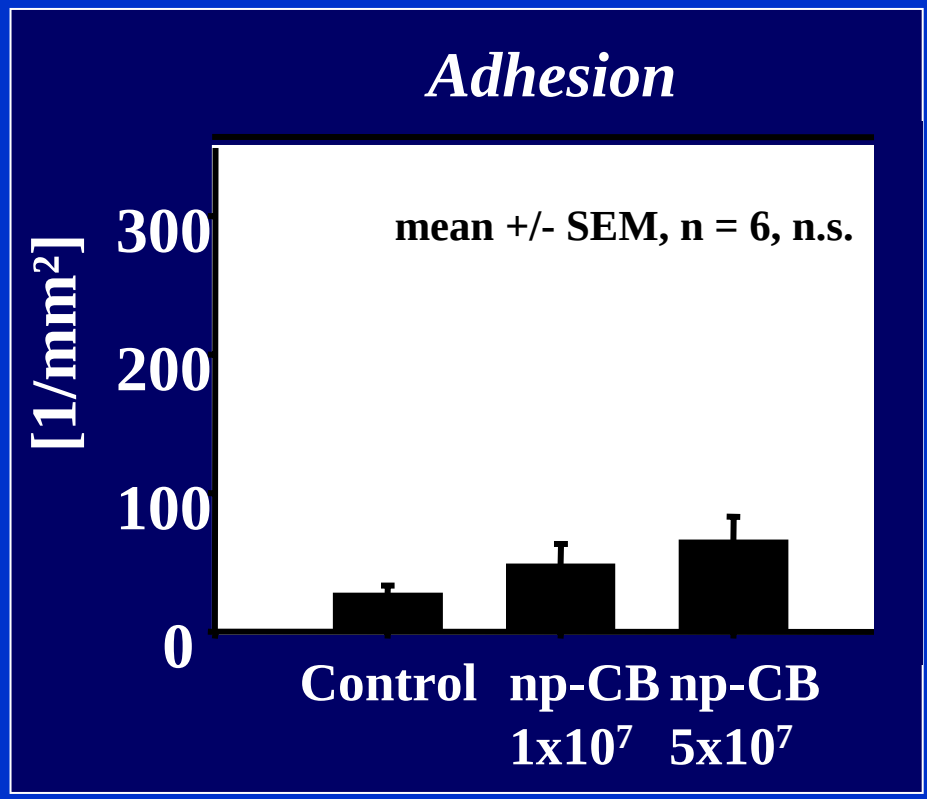
- Intraarterial application of nanoparticles (Printex 90)
- Solely mimicking effects of translocated particles
- Excluding effects of inflammatory signals from the lungs
- Intravital video-fluorescence microscopy of
  hepatic microcirculation:
  Leucocyte-platelet-endothelial cell interactions

Leucocyte-Platelet-Endothelial Cell Interactions



Leucocyte-Endothelial Cell Interactions in Hepatic Microvessels



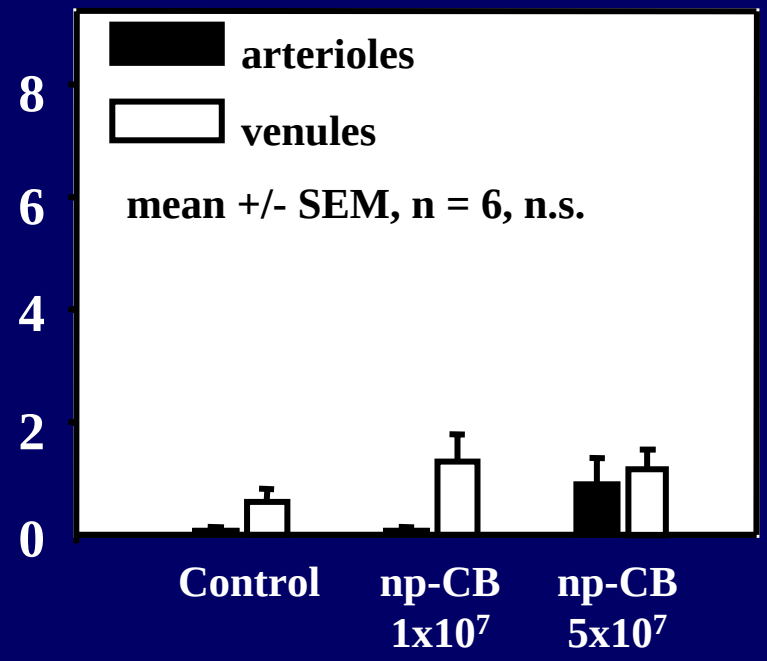


No effects on rolling and adhesion

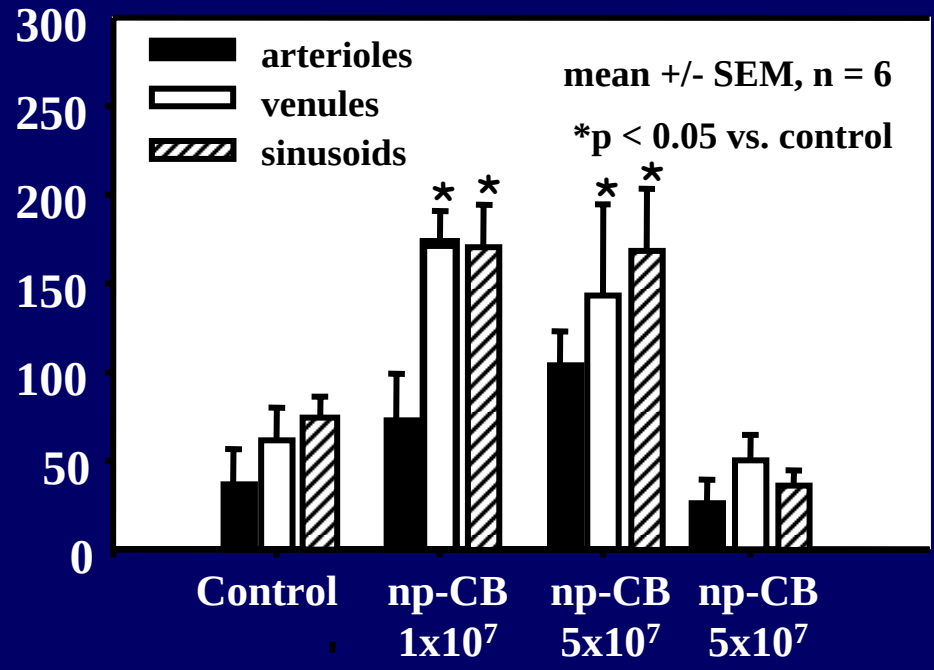
of leucocytes in postsinusoidal venules

Platelet-Endothelial Cell Interactions in Hepatic Microvessels

Rolling Platelets [1/mm/sec]



Adherent Platelets [1/mm²]



Glycoprotein IIb/IIIa inhibition by Tirofiban

Increased numbers of adherent platelets in microvessels

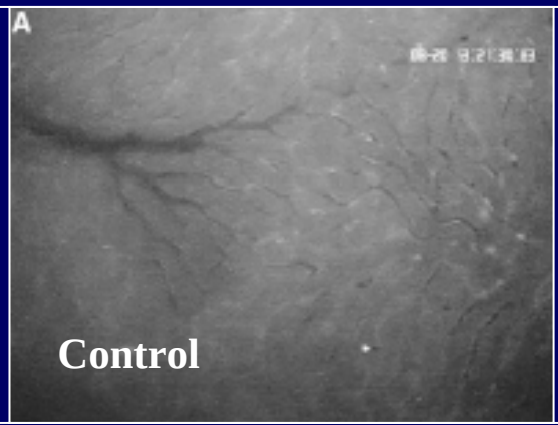
Prothrombotic Effects in Hepatic Mircovessels

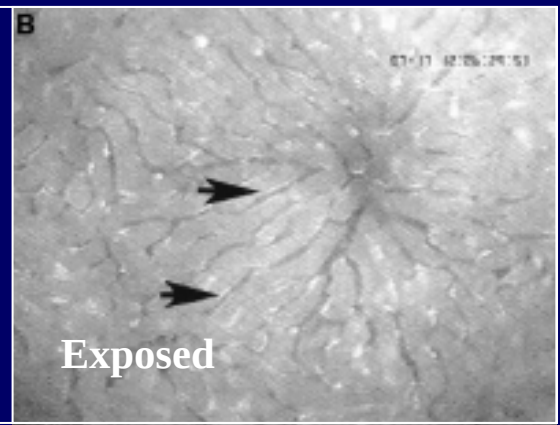
Deposition of Alexa 488 conjugated fibrin(ogen)

Plasma concentrations

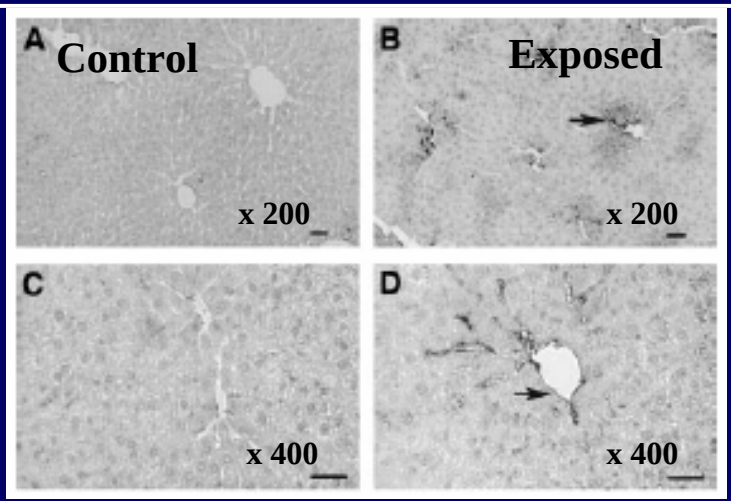
Sham 746 ± 219 µg/ml

nP-CB 1493 ± 256 µg/ml,
p = 0.093

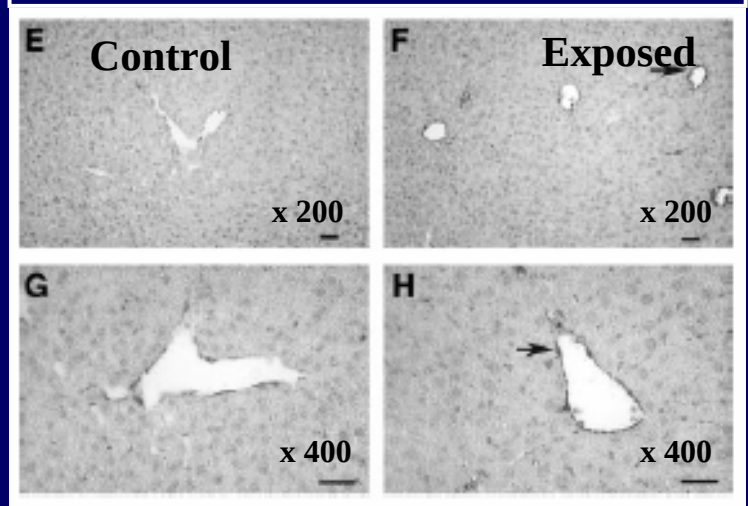




Immunostaining for fibrin(ogen)



Immunostaining for vWF

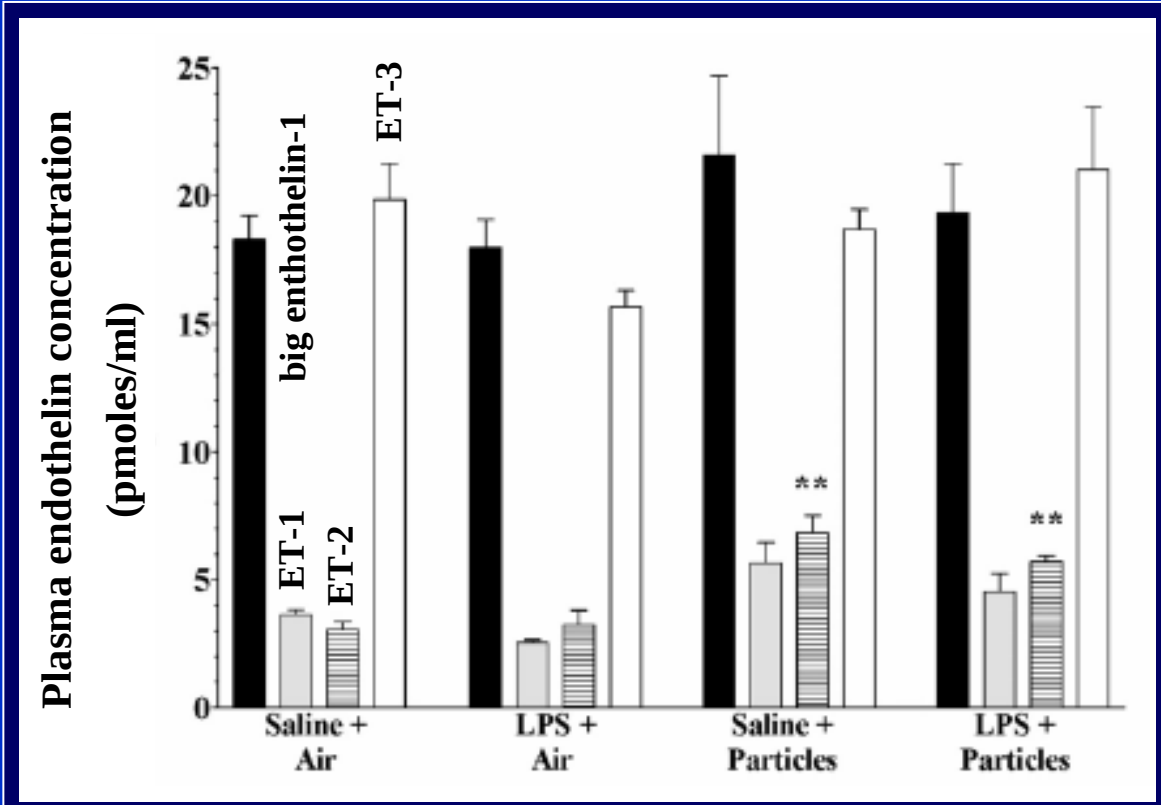


Microvascular Effects of „Translocated“ Nanoparticles

- **No effects on**
  - sinusoidal perfusion
  - leucocyte-endothelial cell interactions
  - microvascular permeability
  - (fibrinogen plasma level)
- **Induction of GPIIb/IIIa-mediated platelet adhesion**
- **Deposition of fibrin(ogen) and increased expression of vWF in microvessels**

➢ **Ultrafine particles exert prothrombotic but not inflammatory effects on the hepatic microcirculation *in vivo***

On-Road Exposure of Aged Rats to Highway Aerosols



Exposure

Highway aerosol

Truck - 6-h driving period

Mainly nanoparticles:

$1 – 3 \times 10^5$ cm^{-3}

Endothelins

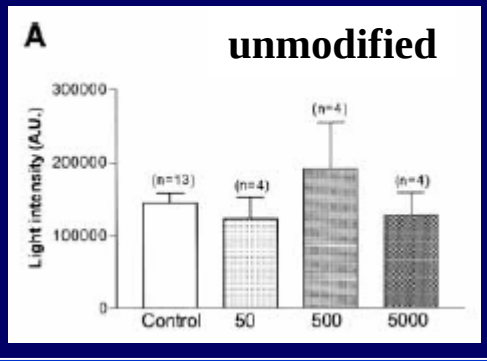
help to regulate normal cardiovascular homeostasis between vasoconstriction and vasodilation.

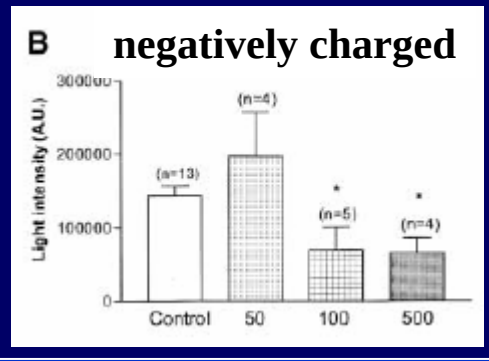
Increased plasma levels of ET-1 and ET-3 after nanoparticle exposure suggest endothelial dysfunction. Elevation of ET-3 was found to be associated with systemic vasoconstriction in cardiac patients.

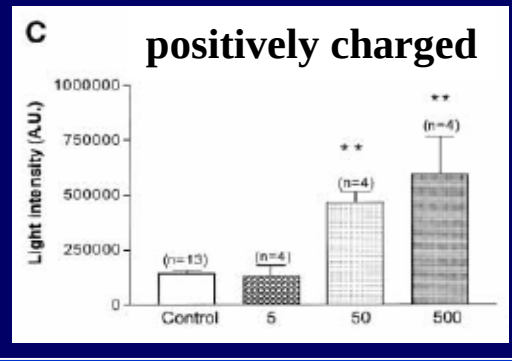
Ultrafine Particles Affect Experimental Peripheral Thrombosis in an In Vivo Hamster Model

Intravenous Application of Polystyrene Nanoparticles (60 nm)

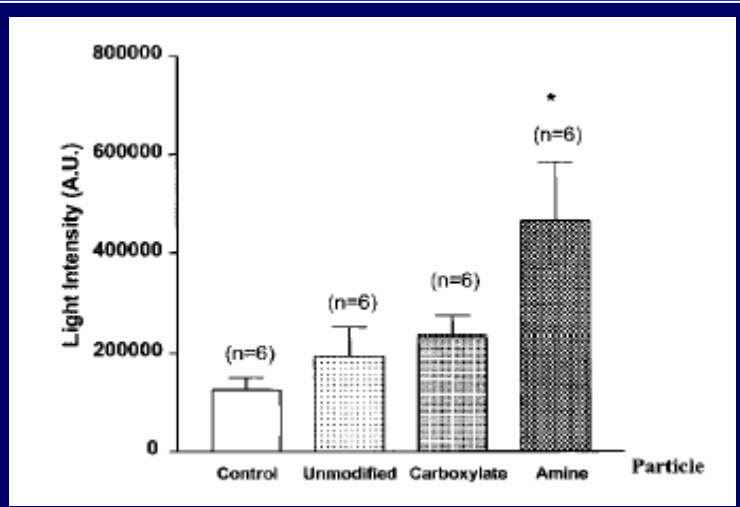
Size of thrombus





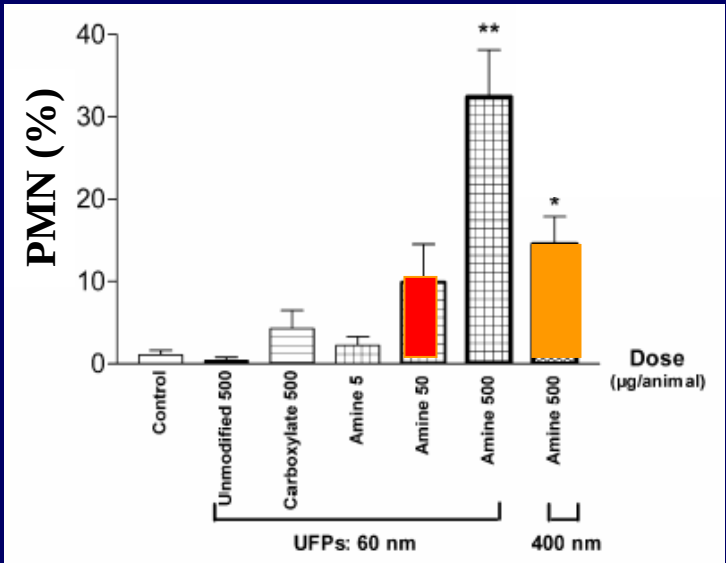


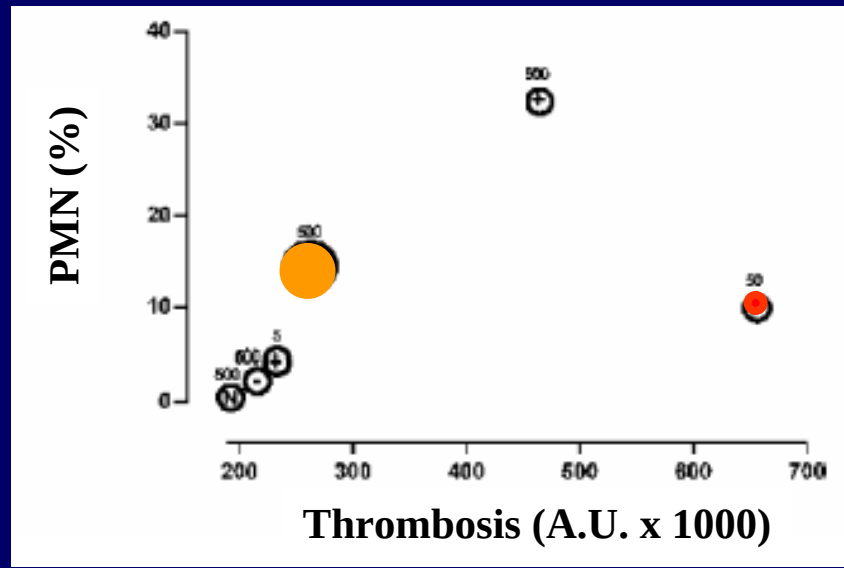
- Instillation of polystyrene NP
- unmodified
- negatively charged (carboxylate-modified)
- positively charged (amine-modified)



Association of Particle Induced Thrombosis and Lung Inflammation

PMN-Influx 1 h after Instillation of Polystyrene Particles





- The lack of a clear association between PMN influx and thrombosis suggests that pulmonary inflammation in itself is not the primary cause for the augmented peripheral thrombosis.
- Further studies show that platelets are also activated by translocated nanoparticles in this experimental setting.

Summary

- Current experimental evidence supports an association between nanoparticle exposure and adverse cardiovascular outcomes.
- Underlying pathophysiological pathways are:
  - alteration of the autonomic cardiac control, most likely mediated via neural reflexes from periperal lung receptors,
  - induction of a pro-thrombotic situation and endothelial dysfunction caused by translocated particles.
- Inflammatory responses in the lung appear not to be the primary cause for cardiovascular effects.
- More studies are clearly needed to substantiate our current understanding of the pathophysiological links between nanoparticle exposure and adverse cardiovascular outcomes.