



# The Cellular and Molecular Toxicity of Low Solubility Nanoparticles

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<http://www.lifesciences.napier.ac.uk/Research/CHE1.htm>

Safety of nanomaterials Interdisciplinary Research Centre  
<http://www.snirc.org/>

# PM<sub>10</sub>

Carbon based particles

- Traffic and industrial

- \*\*\* • Many ultrafine \*\*\*

- Transition metals

Sulphates/Nitrates

- Traffic/photochemical

- Mainly ultrafine

Wind blown dust

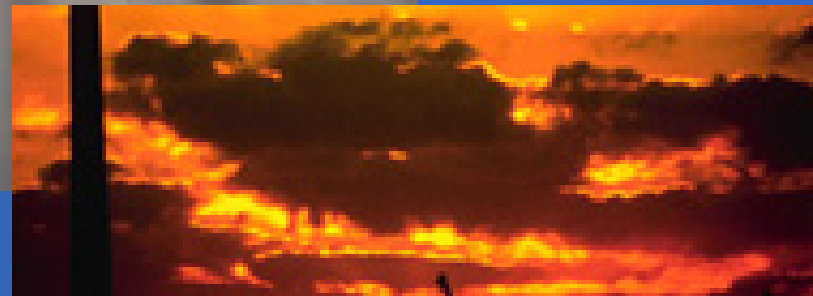
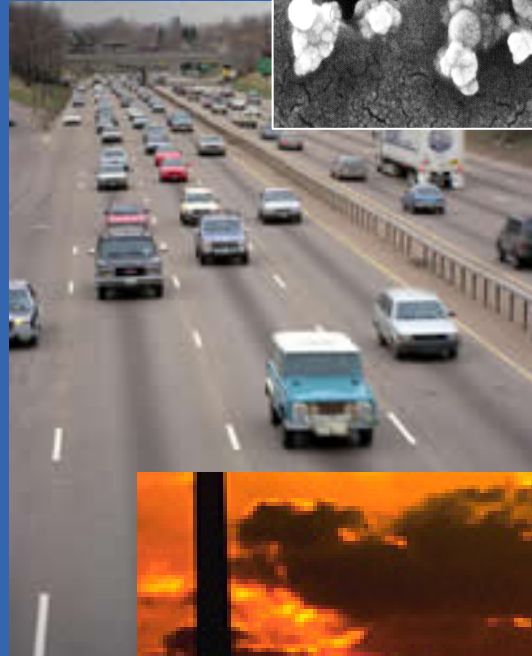
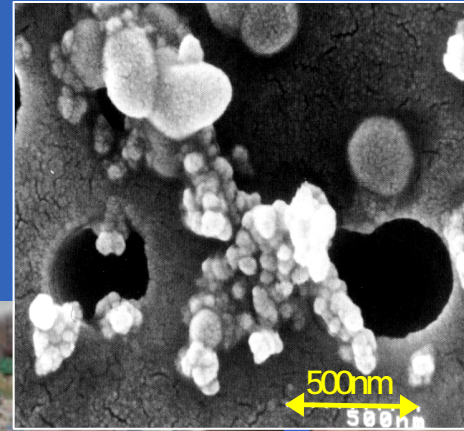
- Mainly coarse

Biological components

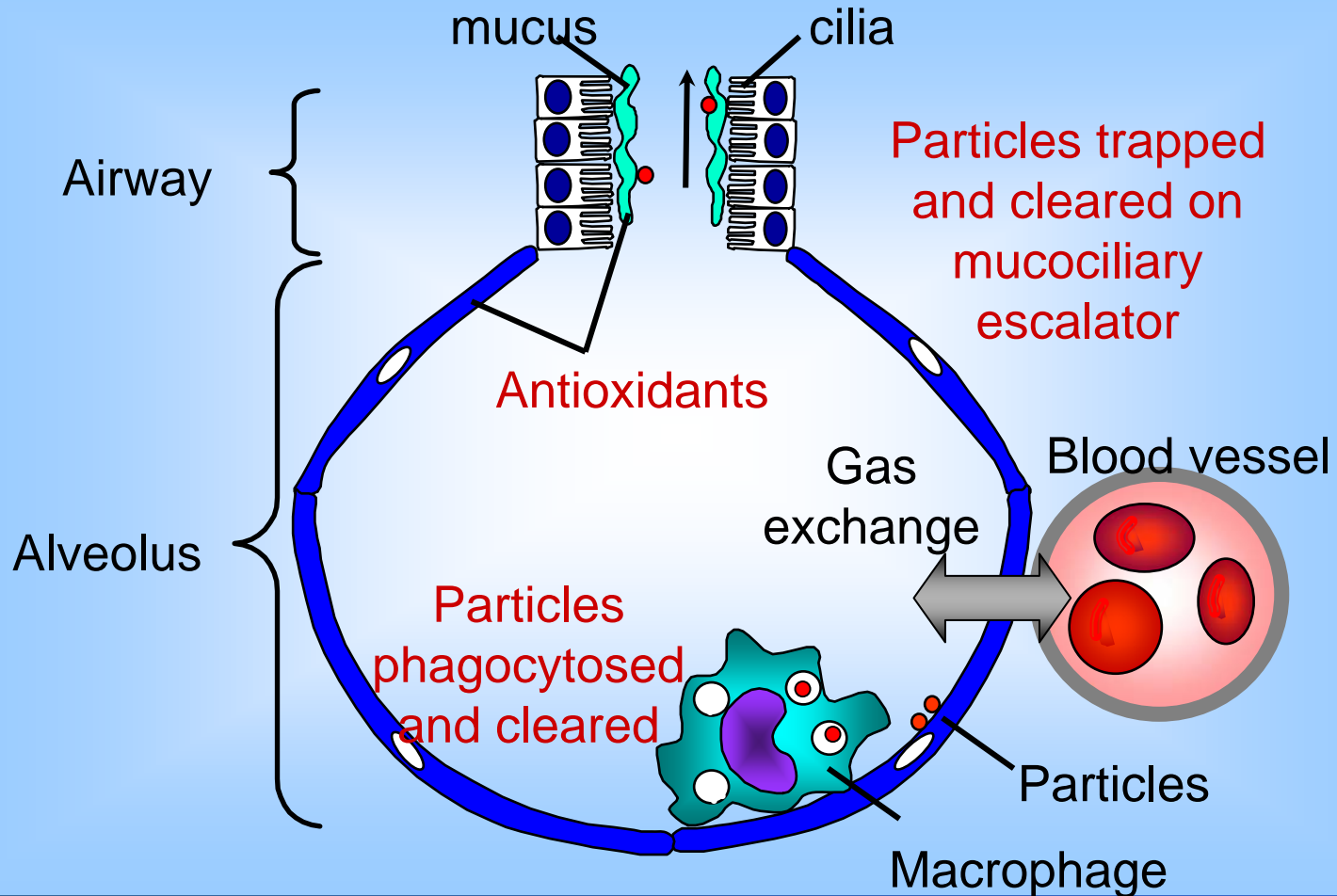
- Spores

- Pollen

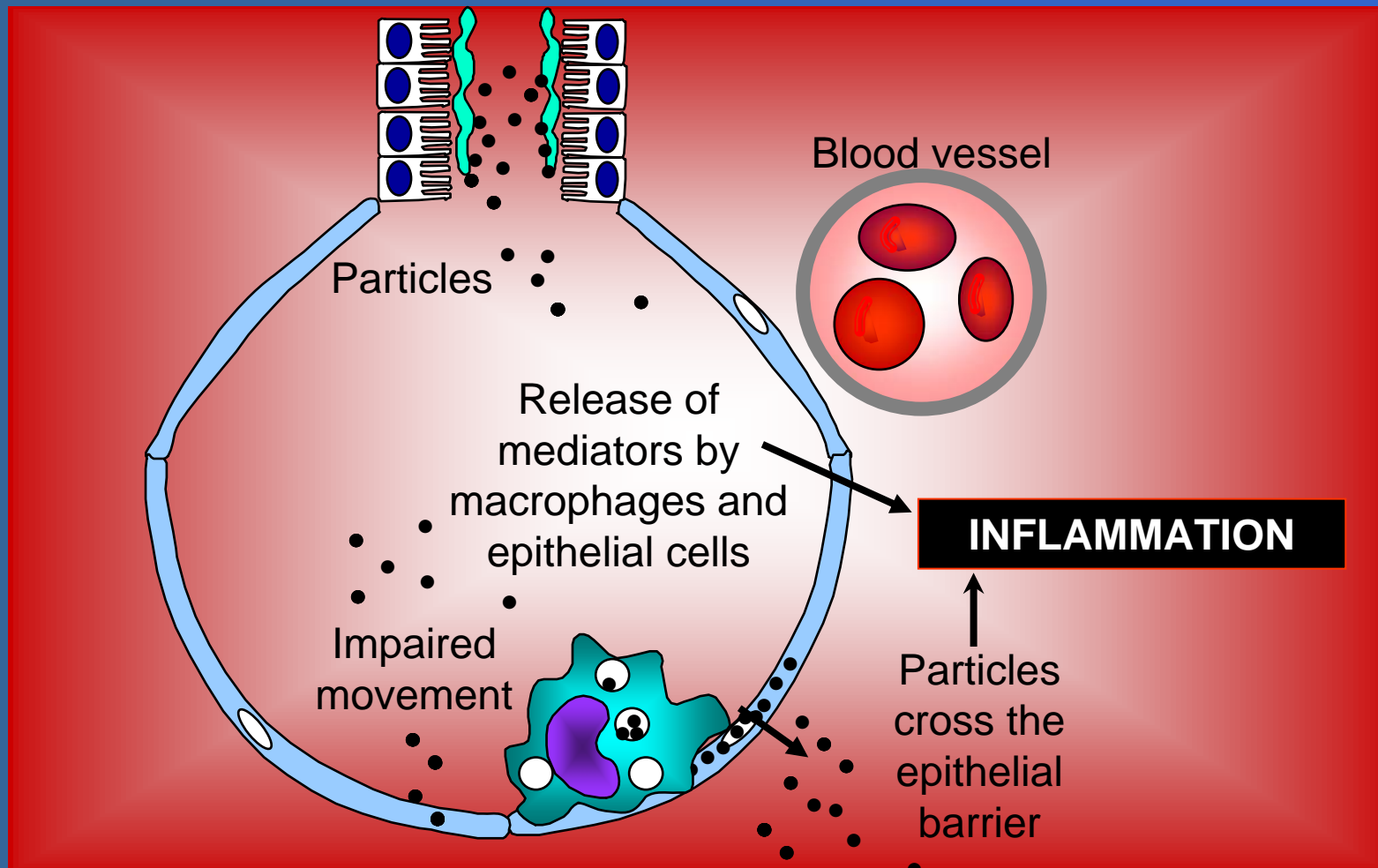
- Mainly fine and coarse



# Lung Defence Mechanisms



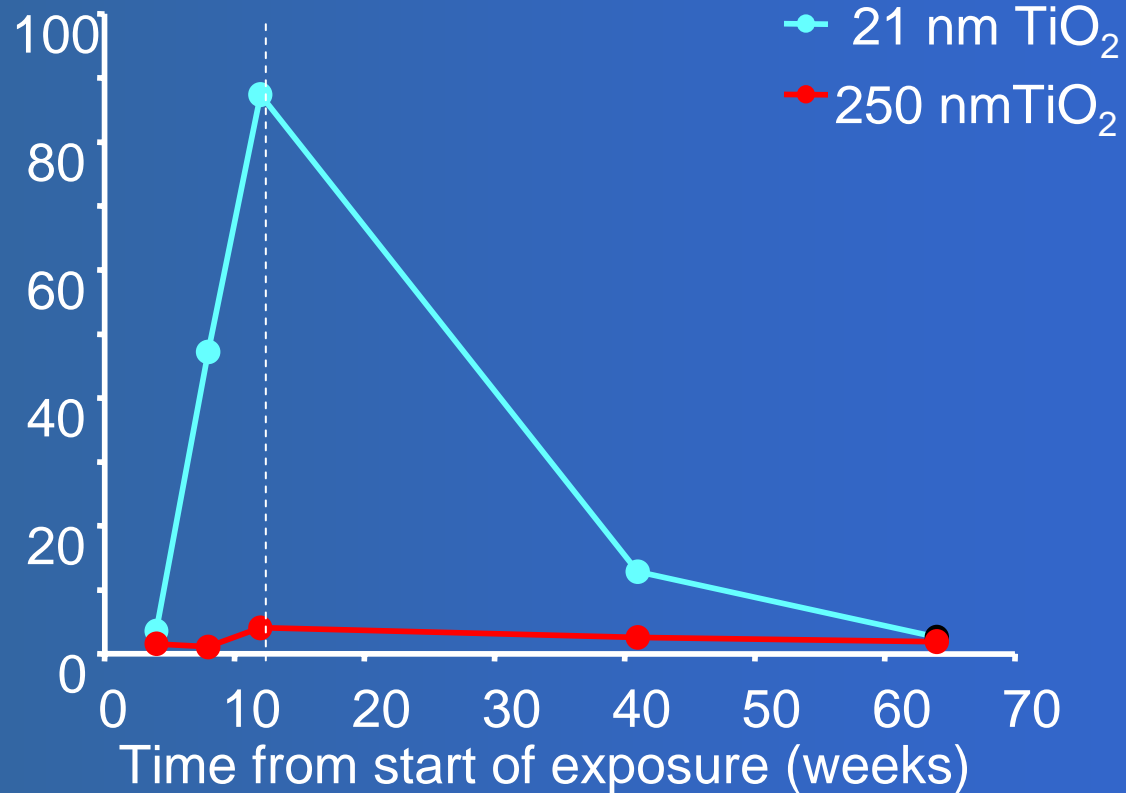
# The Ultrafine Particle Hypothesis



Seaton al. 1995 The Lancet 345: 176-178

# Ultrafine particle toxicology

Neutrophils in  
bronchoalveolar  
lavage fluid  
 $\times 10^5$



Ferin et al 1992

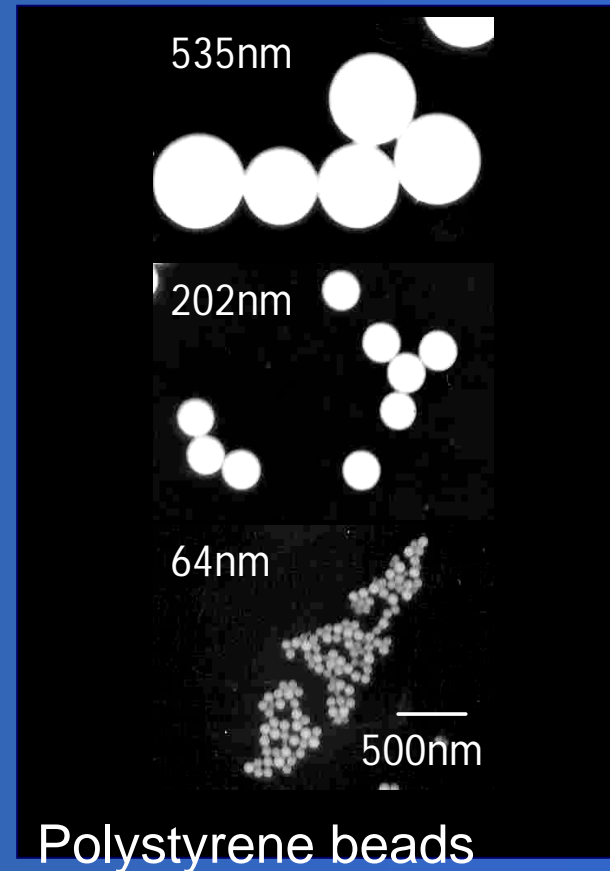
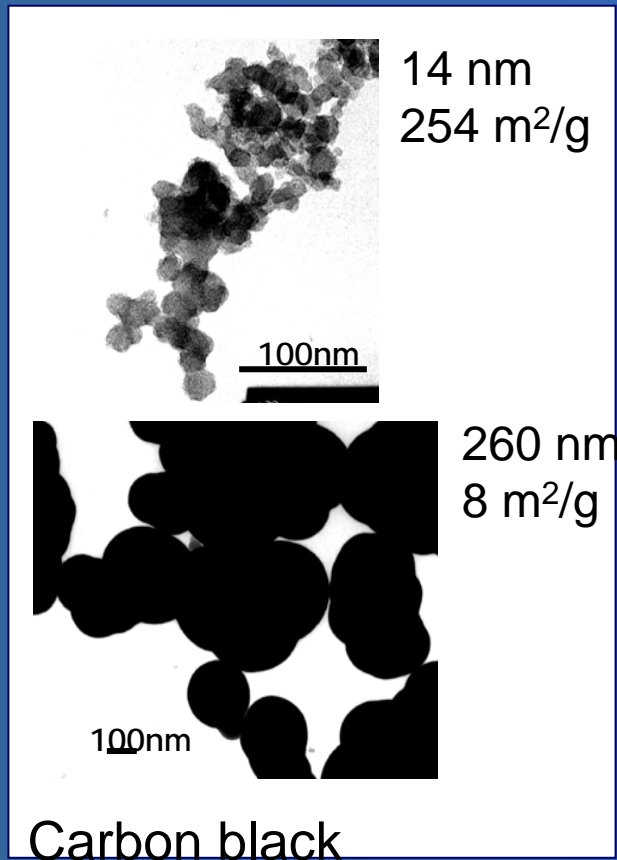
Am.J.Respir.Cell.Mol.Biol. 6: 535-542

# 'Accidental' versus 'engineered' nanoparticles

Nanoparticles	Source	Exposure	Toxicology
Accidental	Fossil fuel combustion E.g. Traffic, cooking.	Low exposure to everyone	Lots e.g. diesel, CB, welding fume etc
Purposeful	Bulk use of nanoparticles in industry e.g. carbon black  Nanotechnology	High exposure to workers followed by low exposure to everyone	Virtually none

Adapted from Ken Donaldson

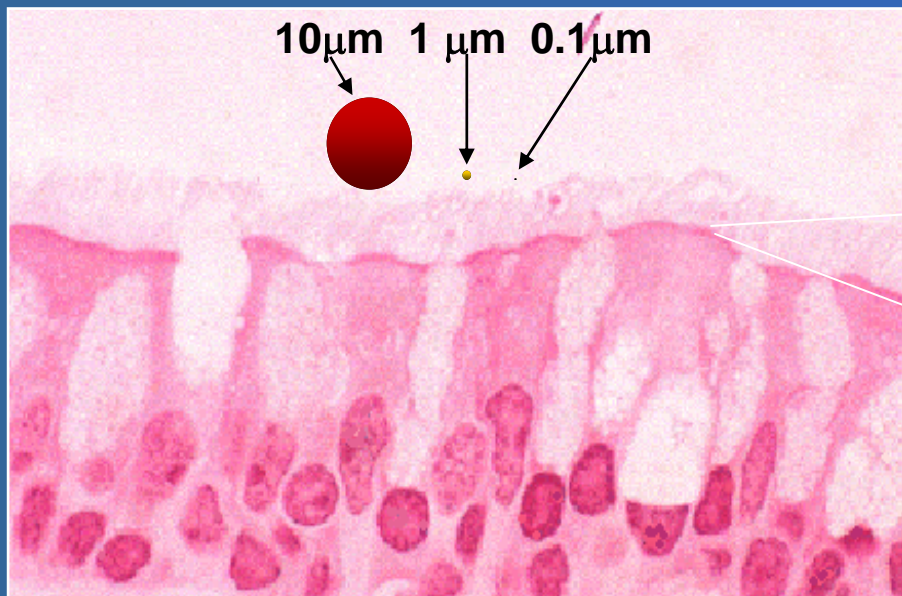
# Model nanoparticles



Low solubility, low toxicity materials.

# Factors involved in NP toxicity

## 1. SIZE

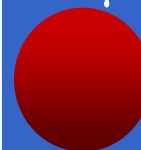


Bronchial epithelium

Cilia  $0.25\mu\text{m}$  diameter



$1.0\mu\text{m}$



$0.1\mu\text{m}$





# Factors involved in NP toxicity

## 2. PARTICLE NUMBER

There are thousands more ultrafine particles / mg of dust than larger, respirable particles.

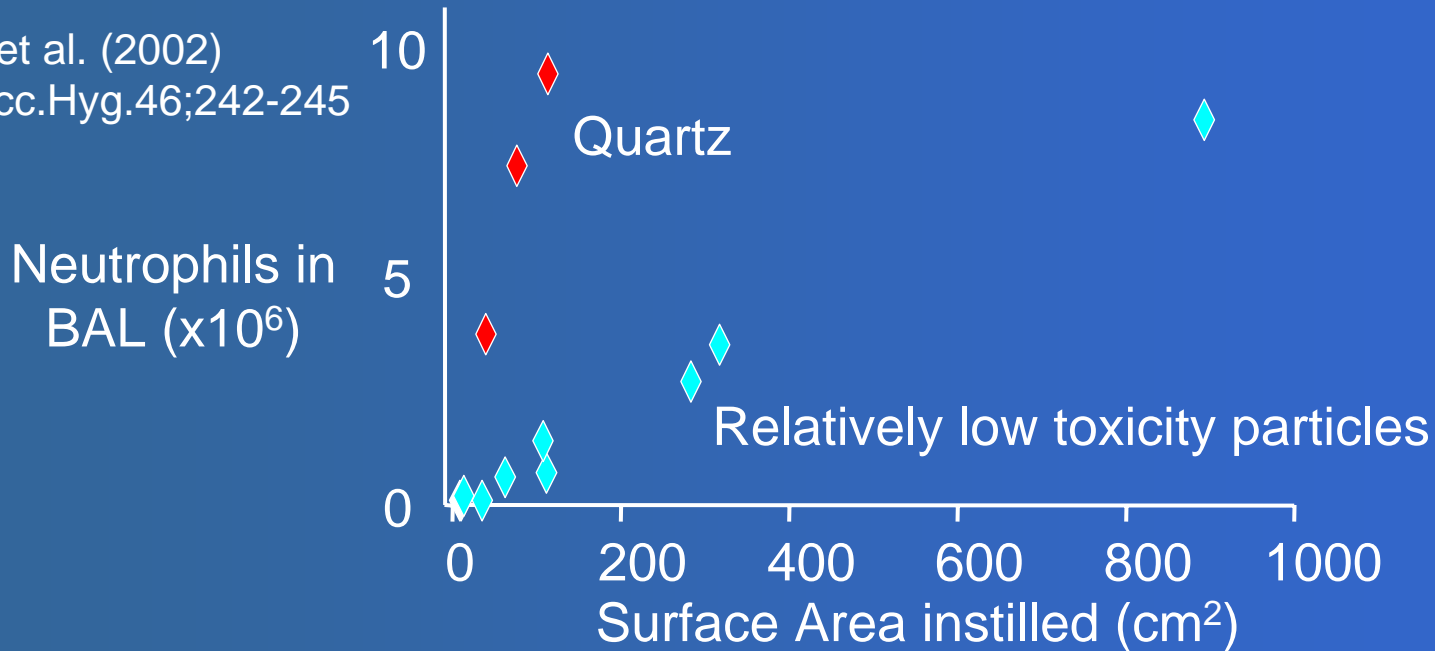
## 3. SURFACE AREA

Particle surface area / mg is much greater for ultrafine particles than for larger, respirable particles.

Fine carbon black	7.9 m <sup>2</sup> /g
Ultrafine carbon black	253.9 m <sup>2</sup> /g

# Particle surface area and surface reactivity

Duffin et al. (2002)  
Ann.Occ.Hyg.46;242-245



Low toxicity low solubility particles

- straight line relationship between SA and neutrophils.

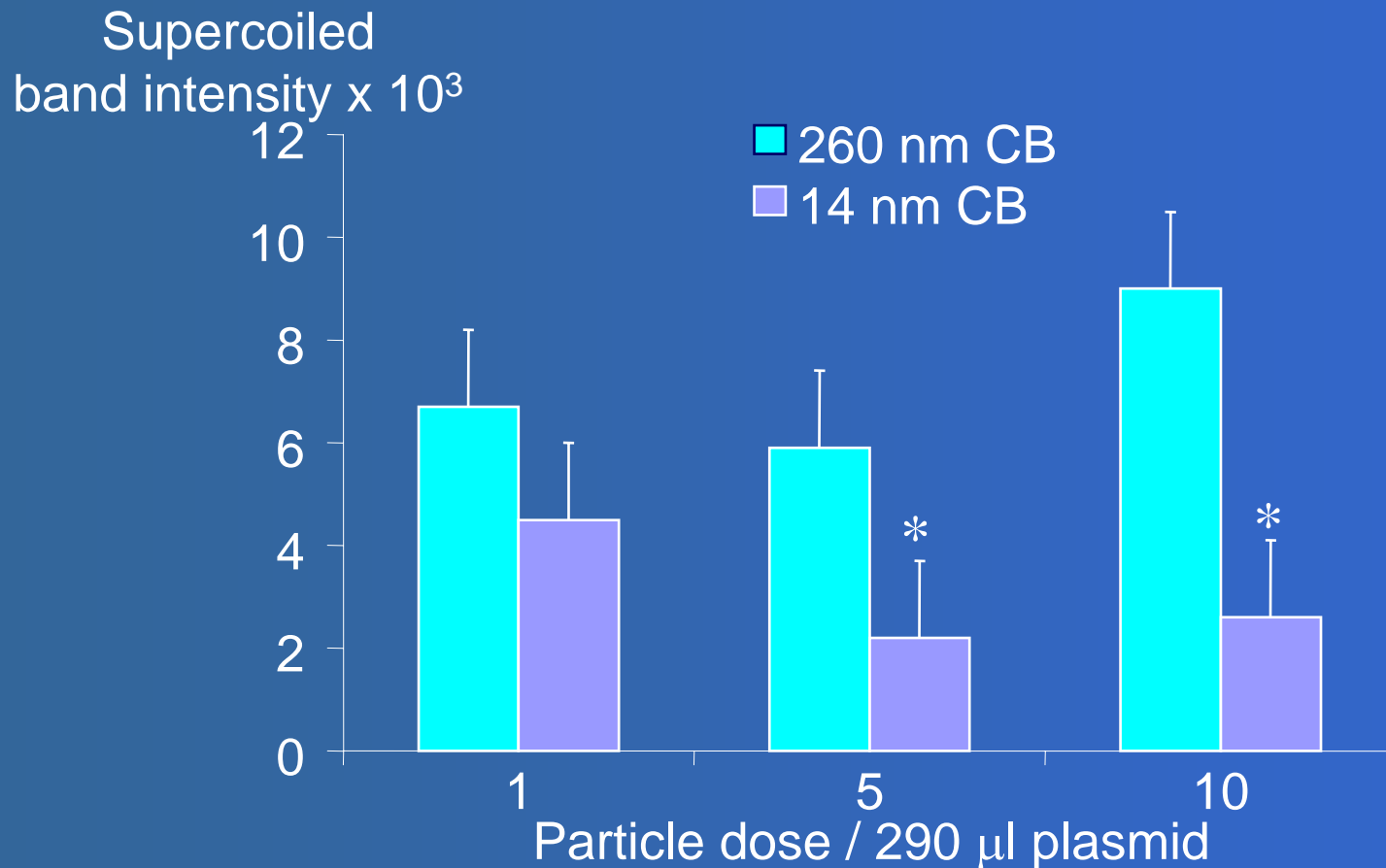
Highly pathogenic particles

- highly reactive surface (eg quartz).
- do not sit on same line as low toxicity particles.



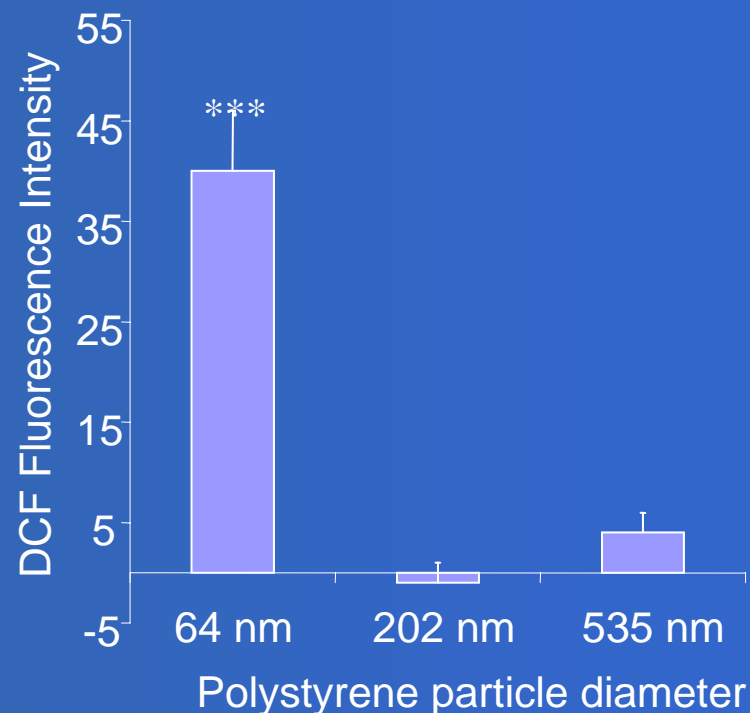
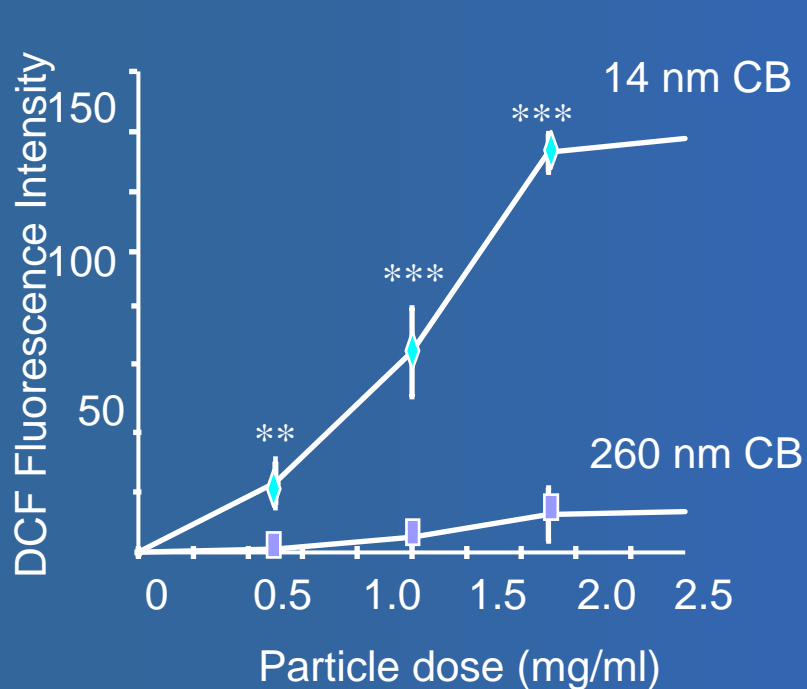
Nanoparticles, ROS and oxidative stress.

# Reactive oxygen species production by carbon particles



Stone *et al.* 1998 *Toxicol In Vitro* 12: 649-659.

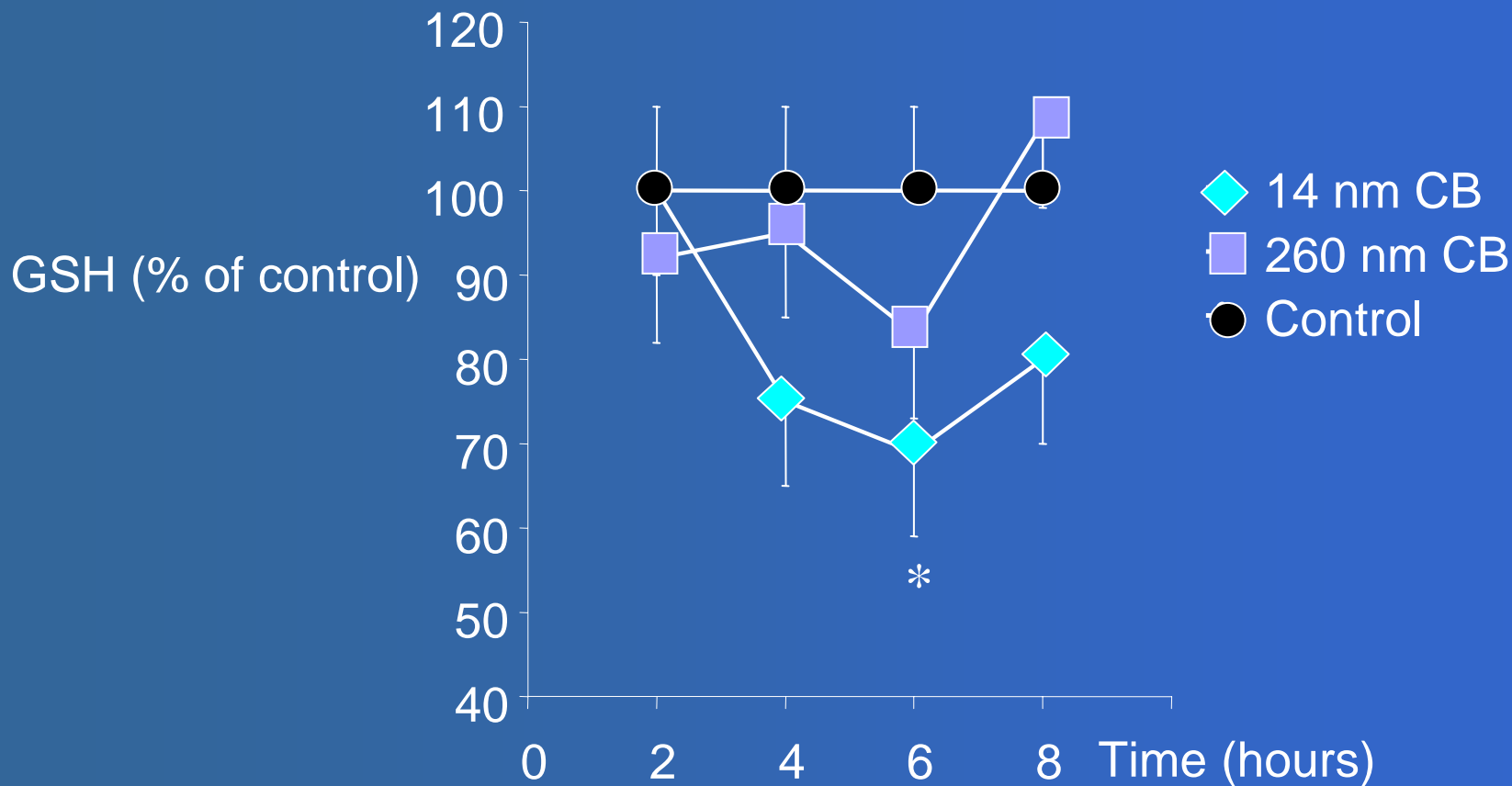
# Reactive oxygen species produced by nanoparticles



Wilson *et al.* 2002 TAP 184: 172-179.


Brown *et al.* 2001 TAP 175: 191-199.

# Effect of carbon particles on intracellular antioxidant content of A549 cells



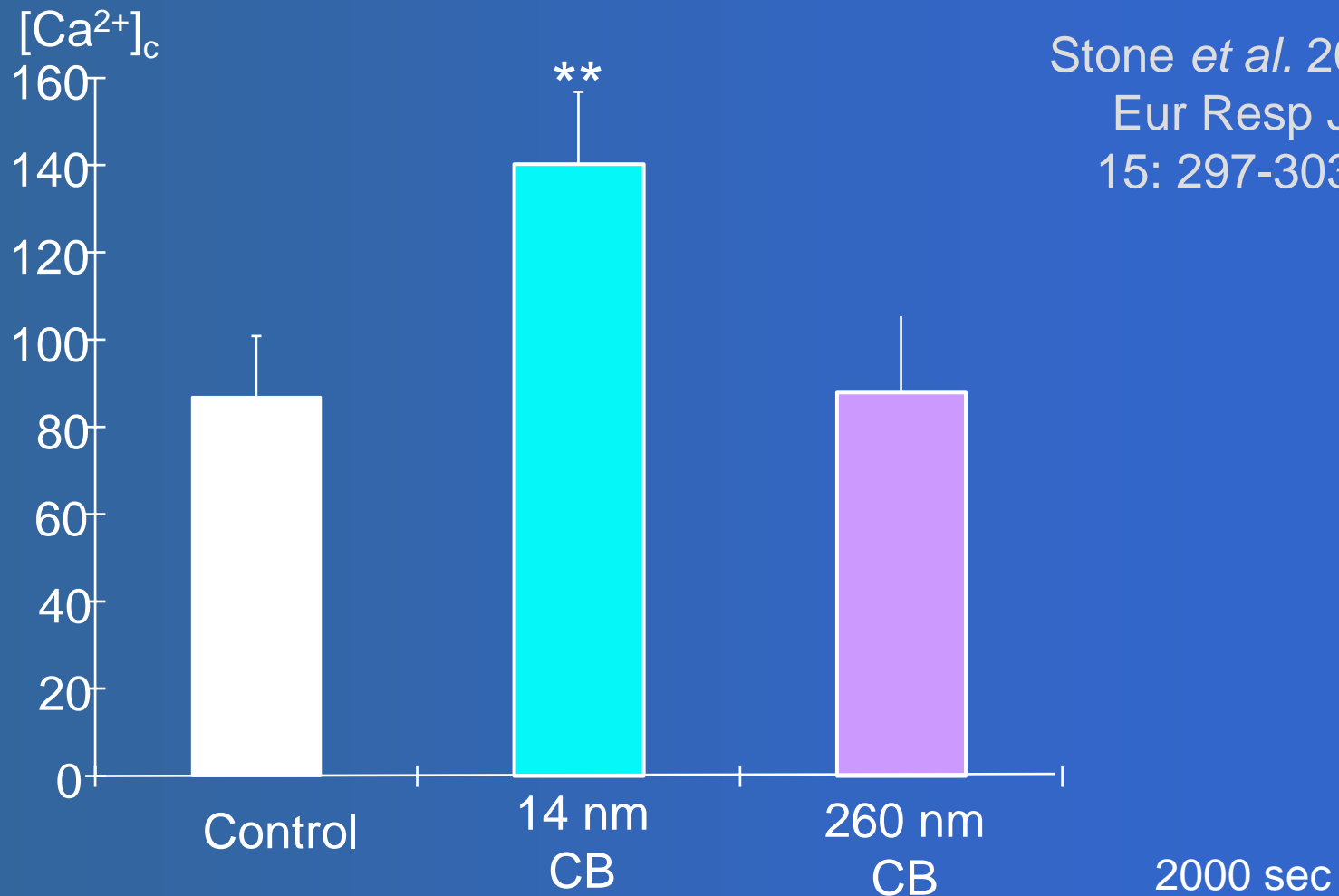
0.78  $\mu\text{g}/\text{mm}^2$

Stone *et al.* 1998 TIV 12: 649-659.



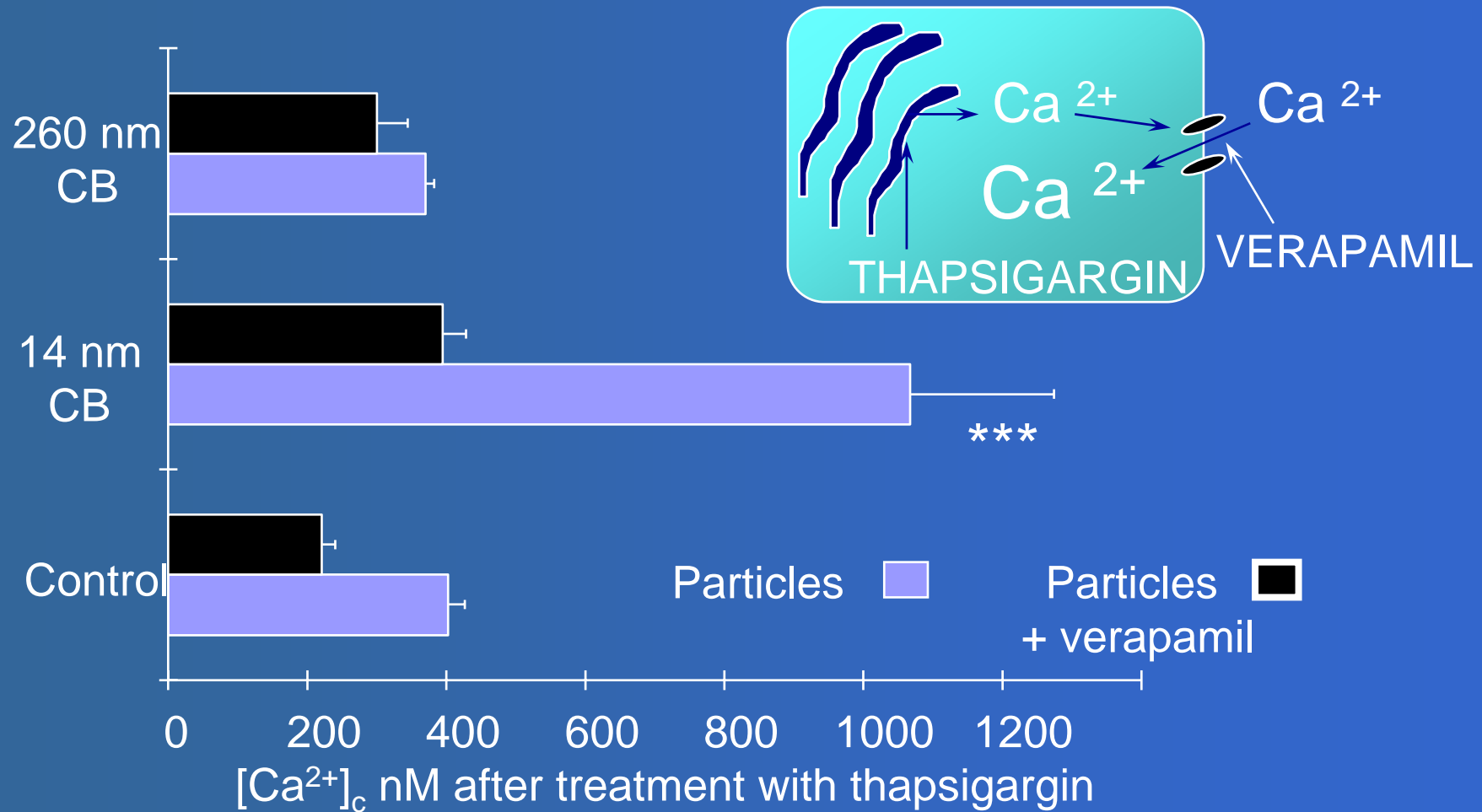
# Effects of Nanoparticles and PM<sub>10</sub> on Intracellular Signalling Pathways

# Effect of particles on $\text{Ca}^{2+}$ signalling in human MonoMac 6 cells



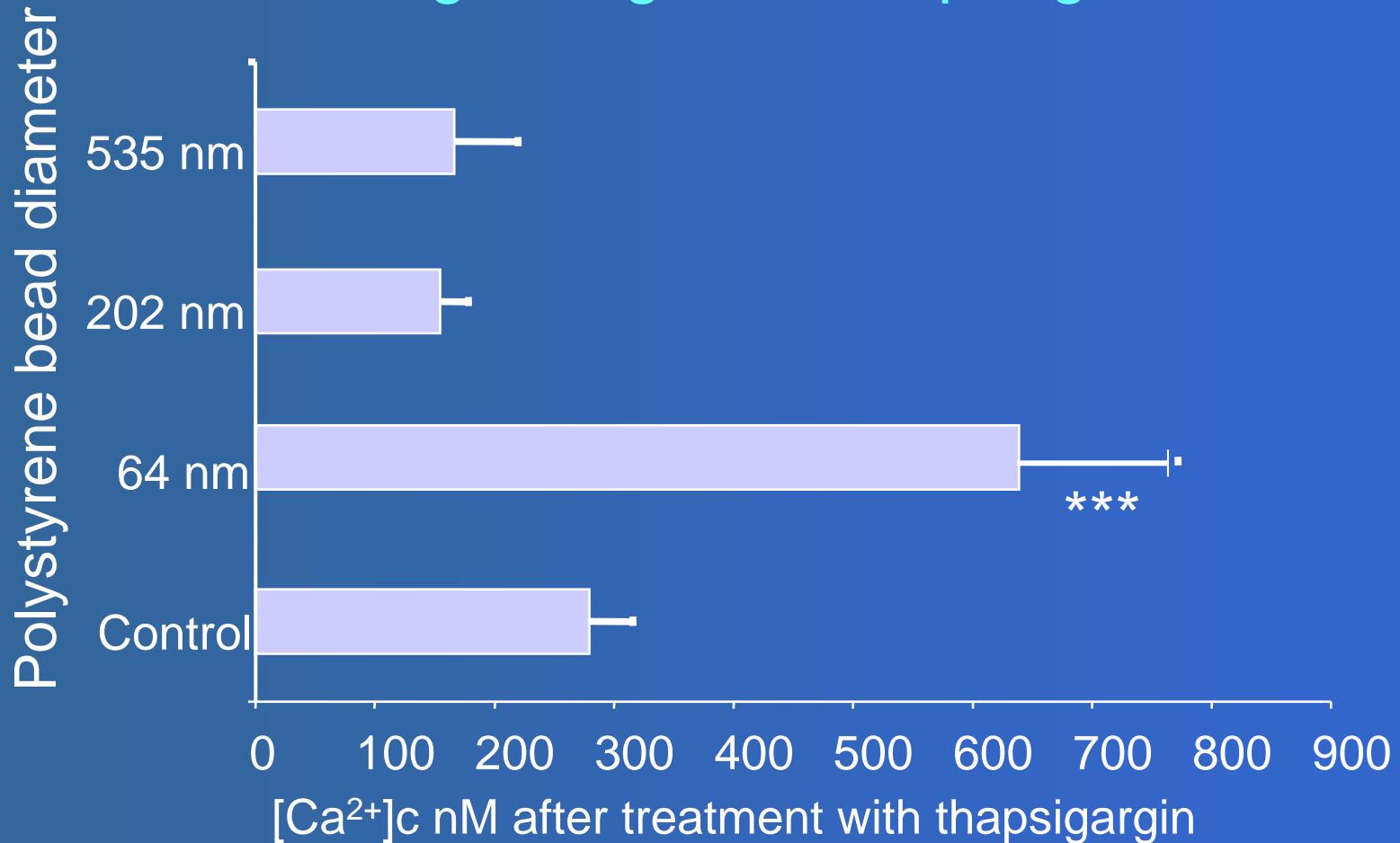


# The response to thapsigargin in human MM6 cells in the presence of CB and verapamil



Stone *et al.* 2000 *Eur Resp J* 15: 297-303.

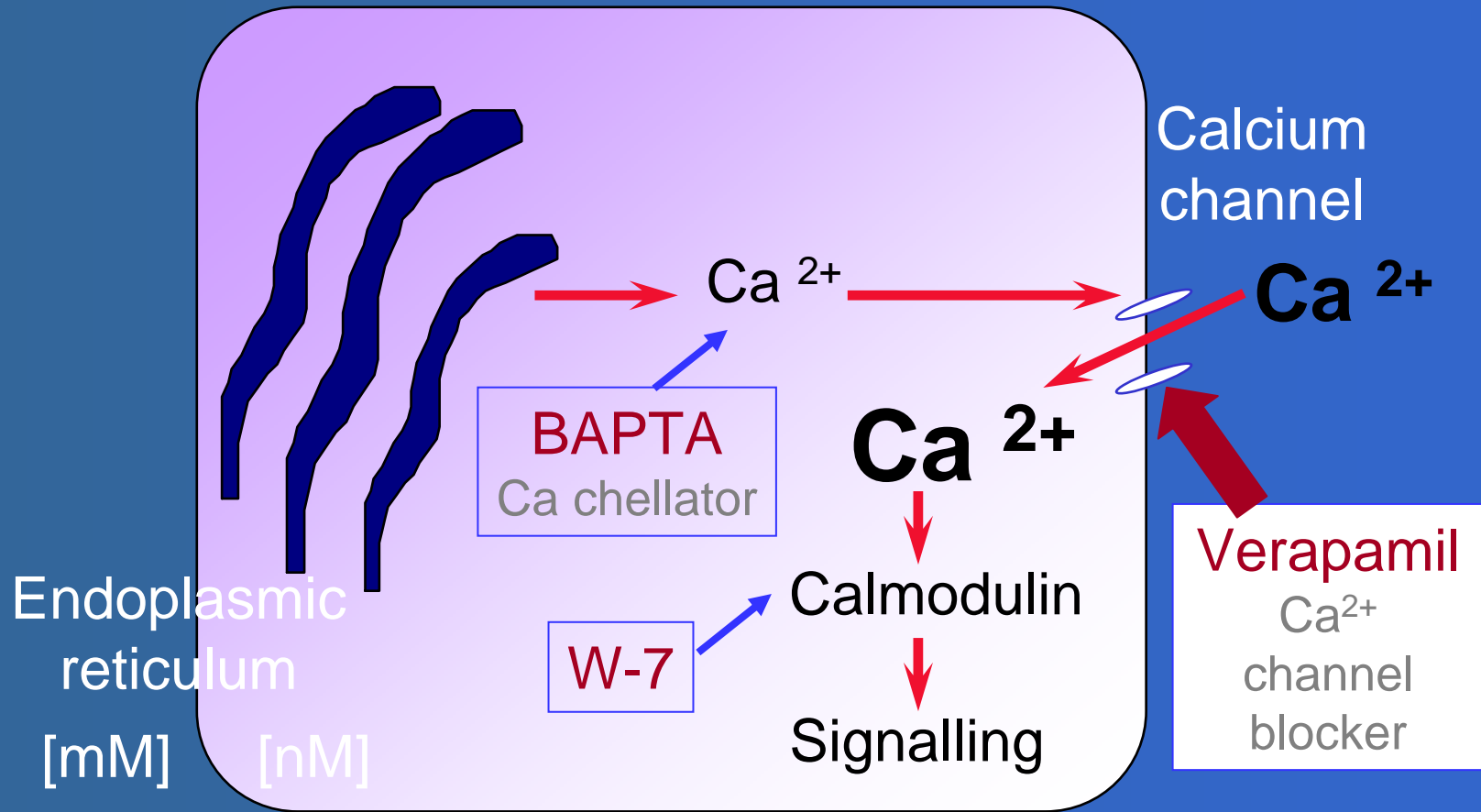
# The effect of polystyrene nanoparticles on calcium signalling in macrophages



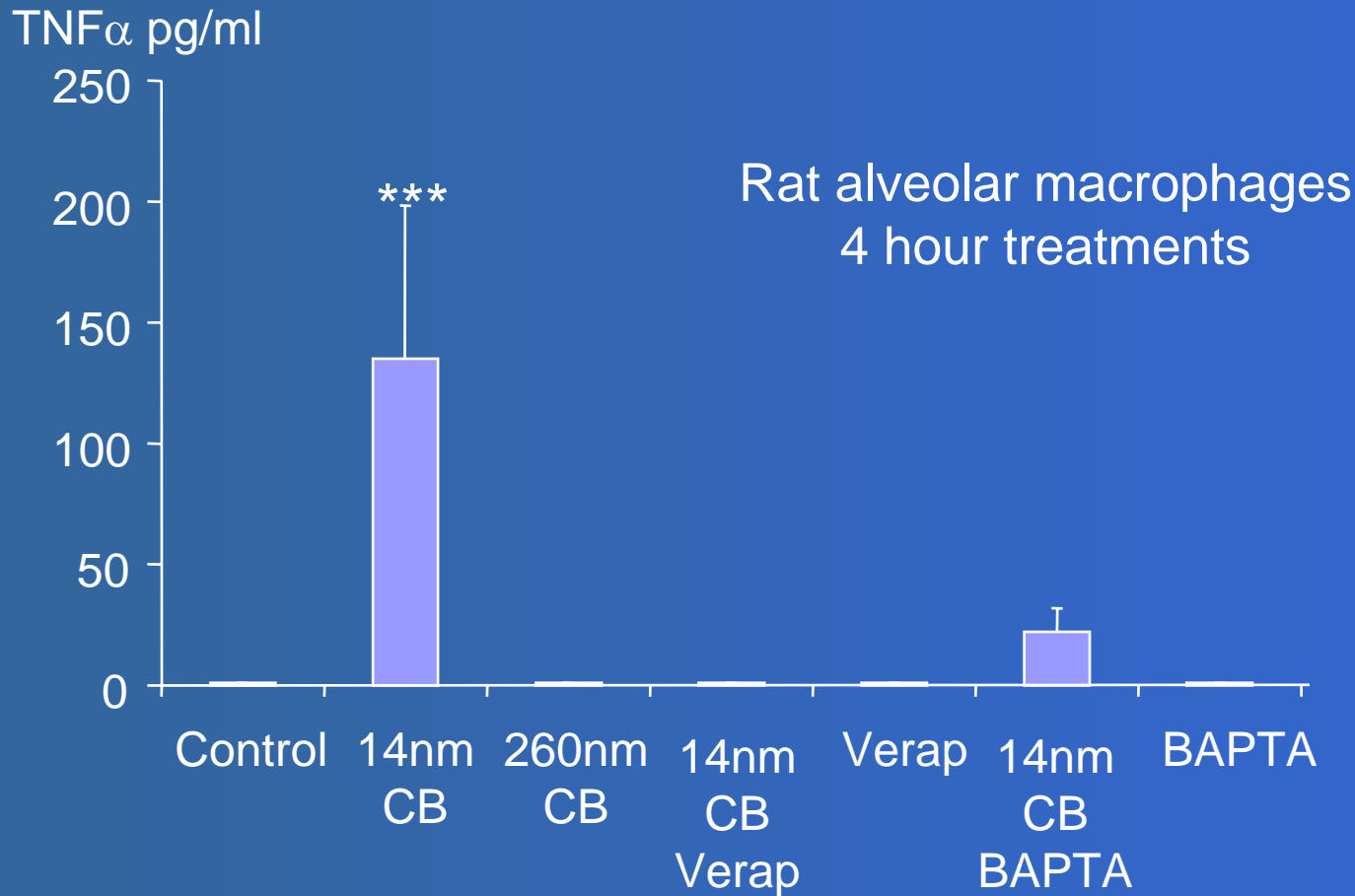
1500 sec

Stone *et al.* 2000 *Inhal Tox* 12 (suppl 3): 345-351.

# Calcium Signalling – Inhibitors

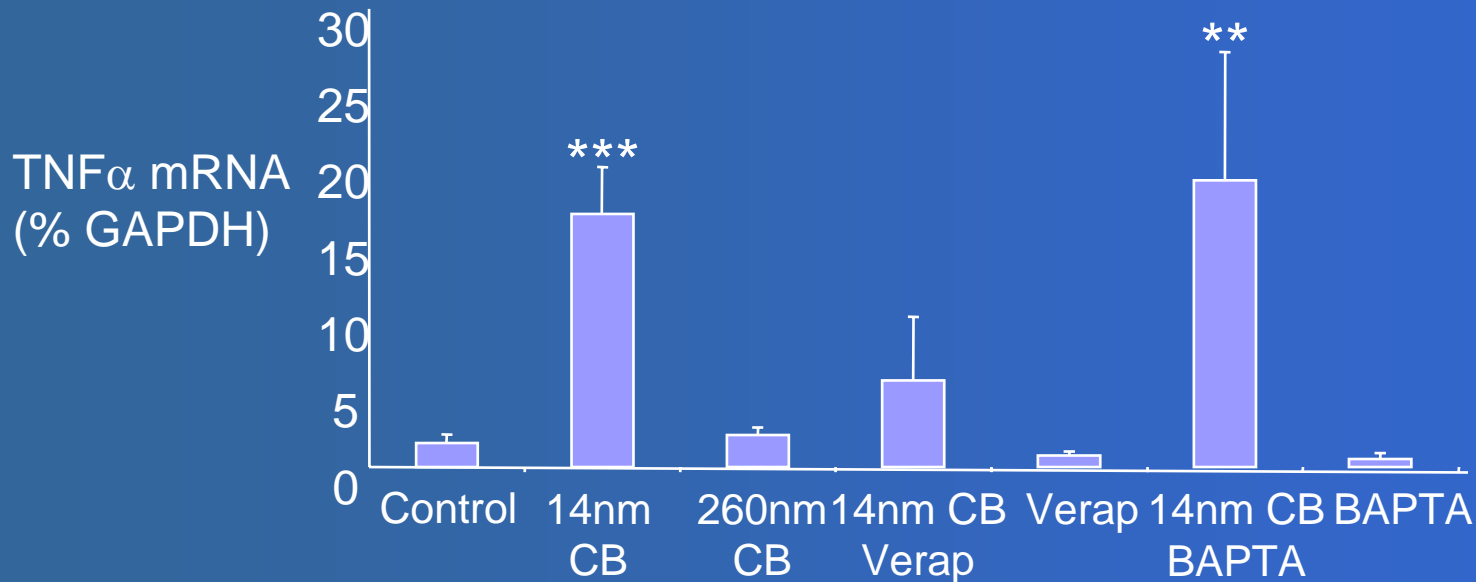
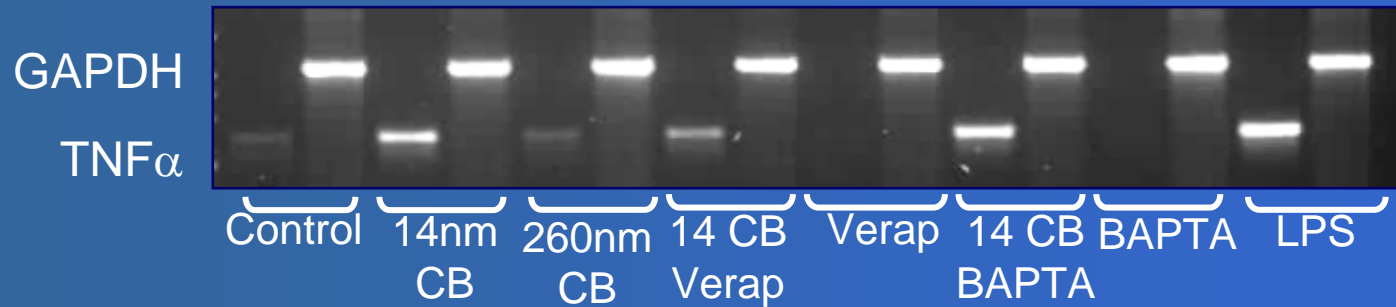


# The role of $\text{Ca}^{2+}$ in the induction of $\text{TNF}\alpha$ expression by carbon nanoparticles



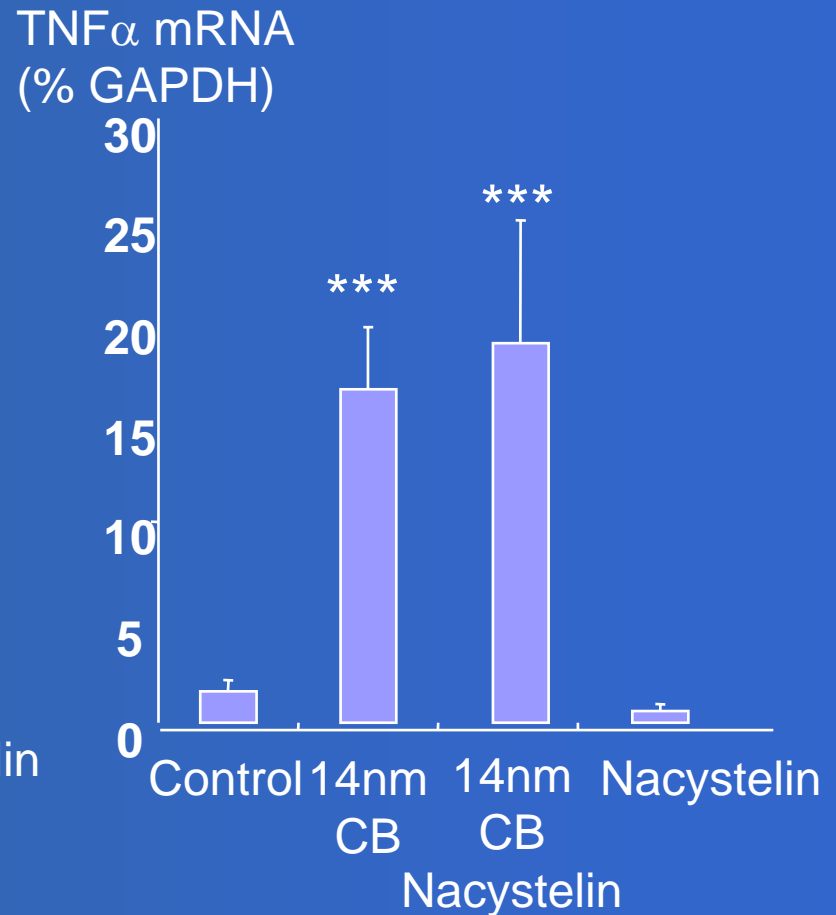
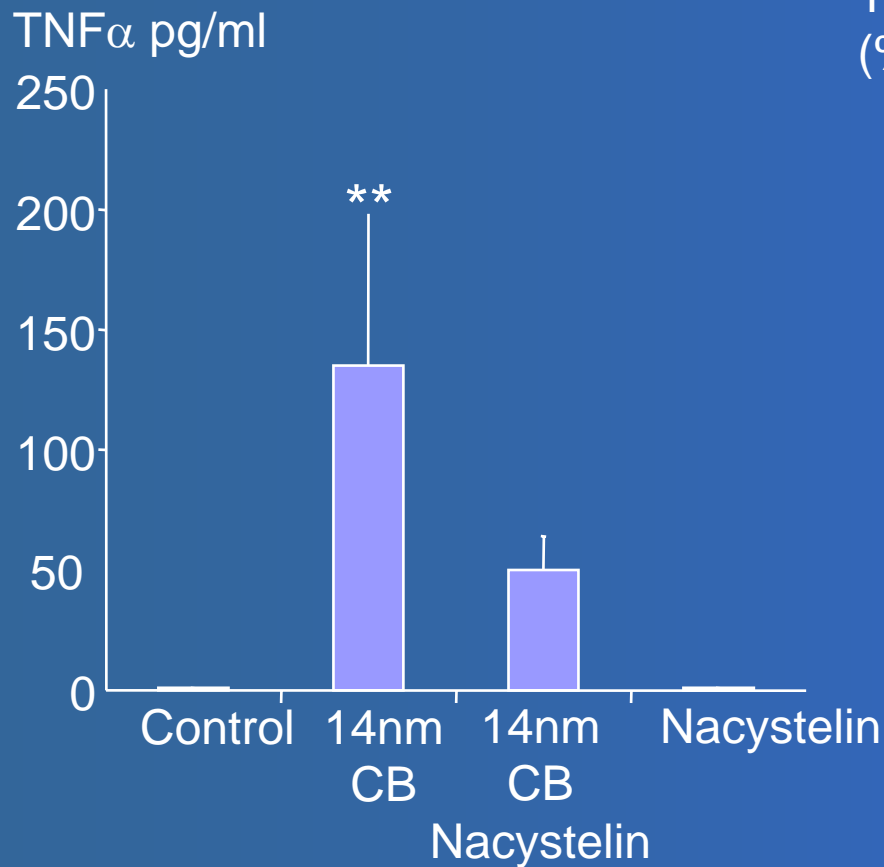
Brown *et al.* 2004 AJP 286; L344-L353

# The role of $\text{Ca}^{2+}$ in the induction of $\text{TNF}\alpha$ mRNA expression by CB nanoparticles



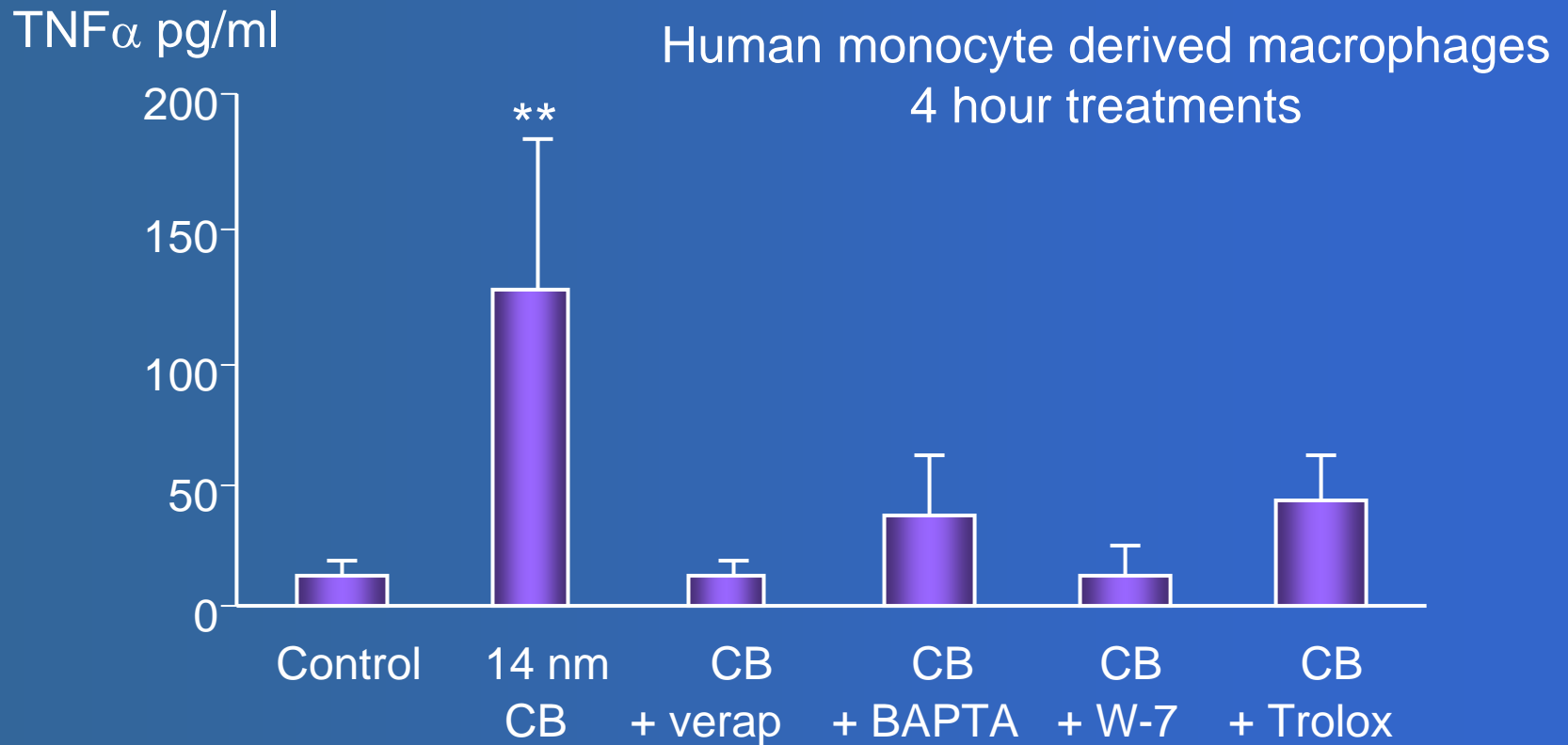
Brown *et al.* 2004 AJP 286; L344-L353

# The effect of antioxidants on CB induced $\text{TNF}\alpha$ expression



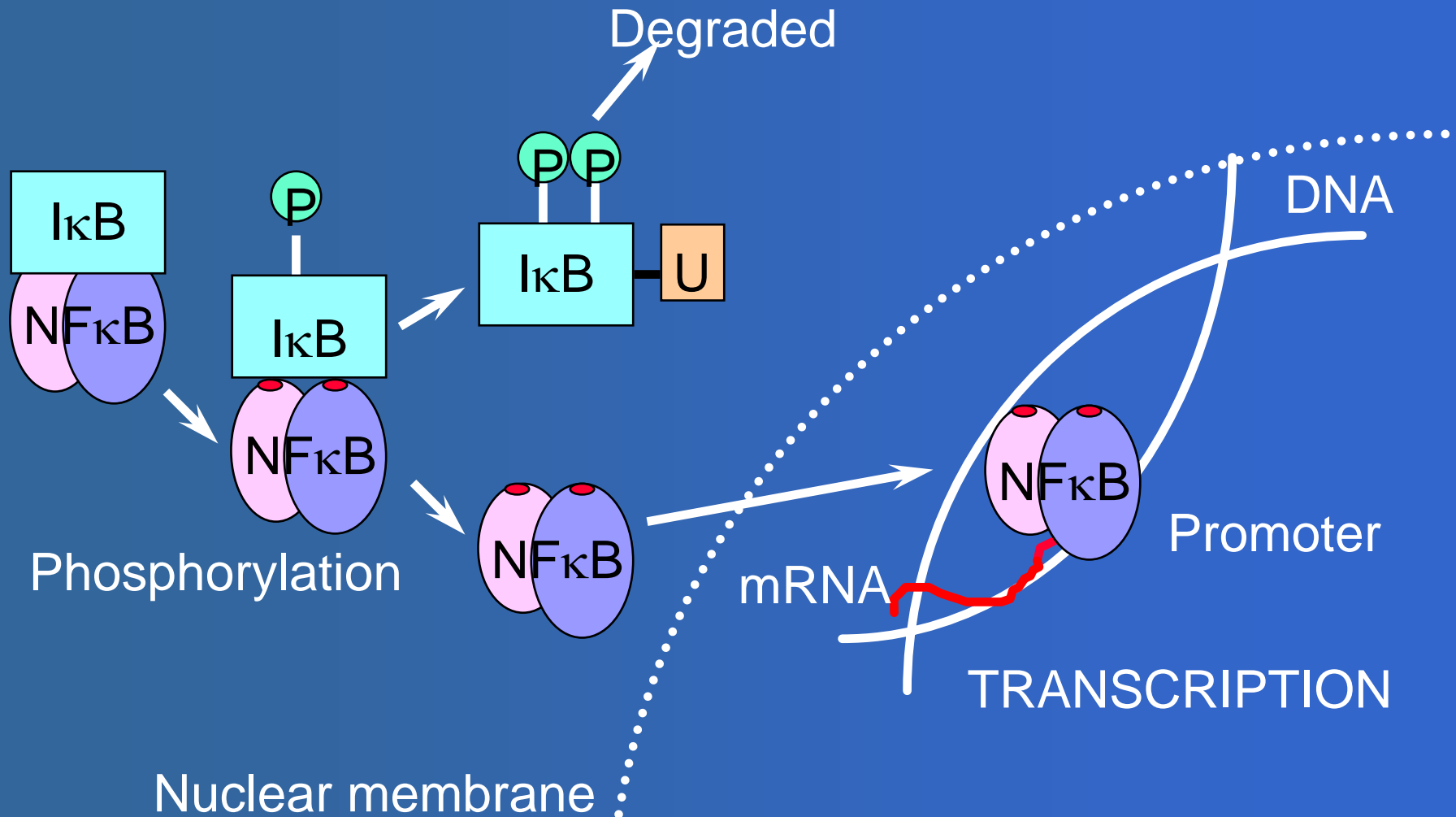
Brown *et al.* 2004 AJP 286; L344-L353

# Particle induced TNF $\alpha$ expression – role of calcium and oxidants



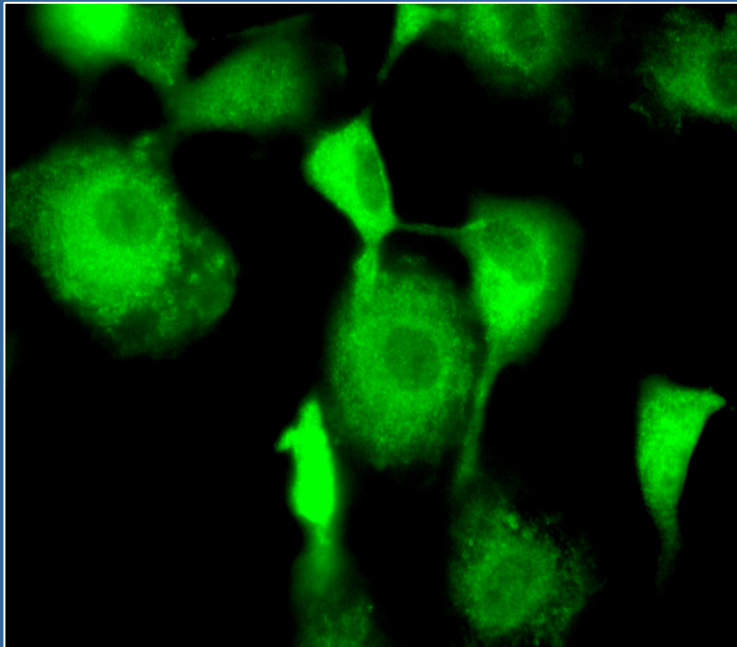
Brown *et al.* 2004 AJP 286; L344-L353

# Activation of NF $\kappa$ B and promoter binding



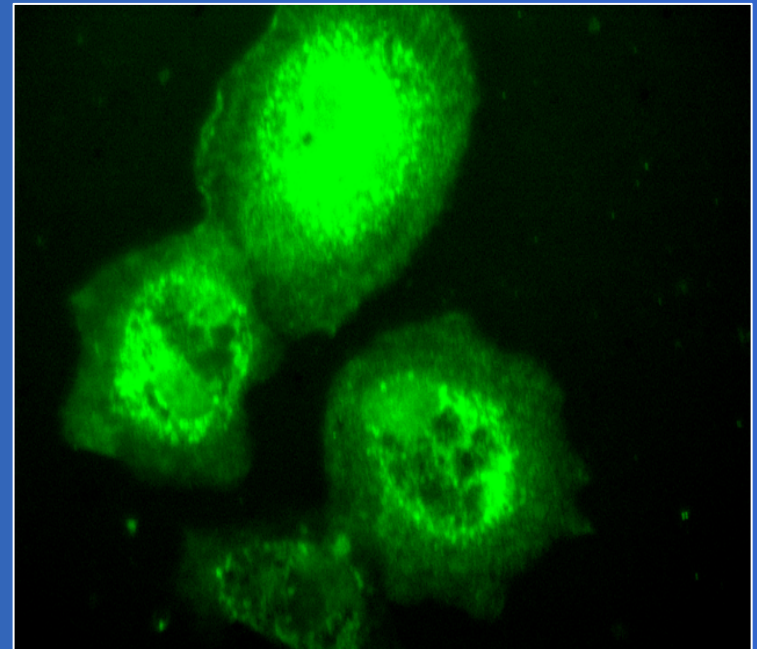


# Immunofluorescent staining of the p65 subunit of NF $\kappa$ B in human monocyte/macrophages



Control

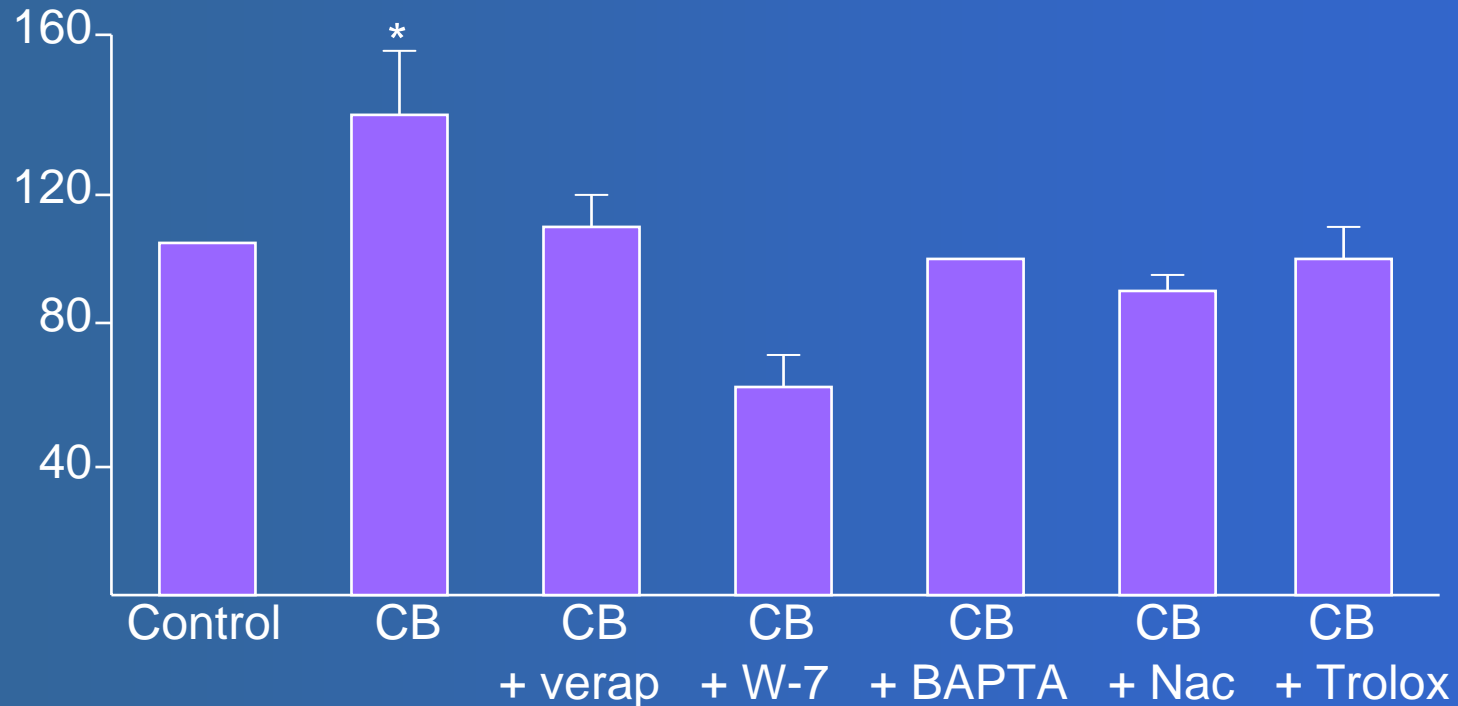
14 nm CB



Brown *et al.* 2004 AJP 286; L344-L353

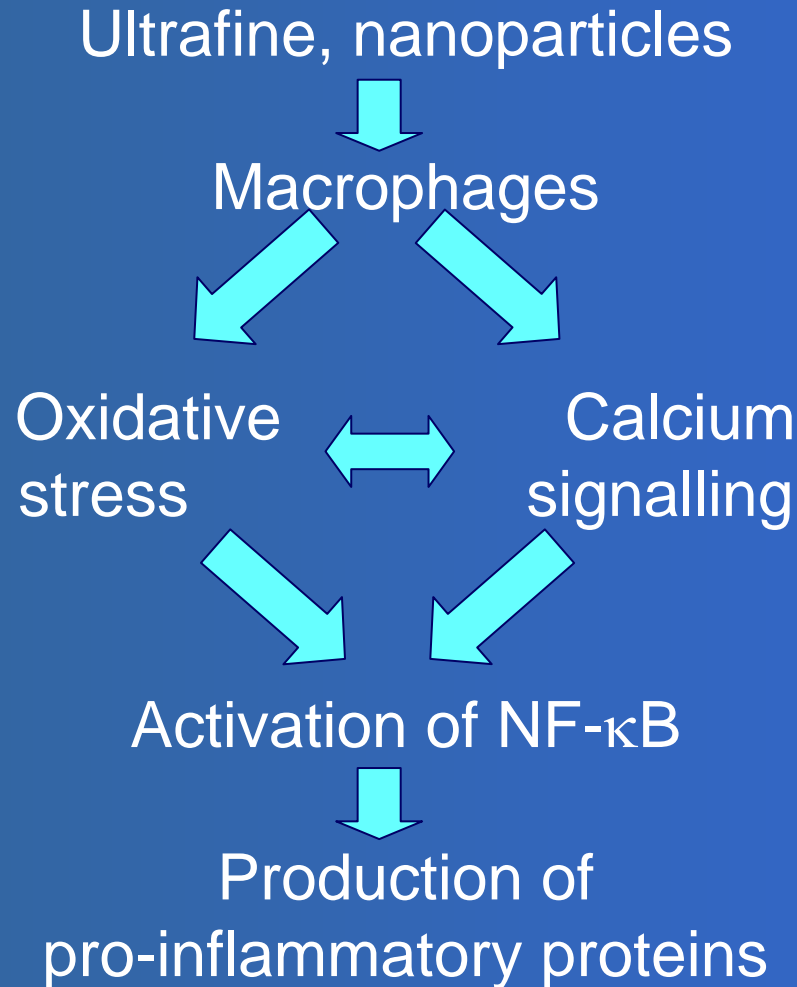
# Nuclear localisation of the p65 subunit of NF $\kappa$ B in human monocytes

Nuclear p65 intensity  
(% control)

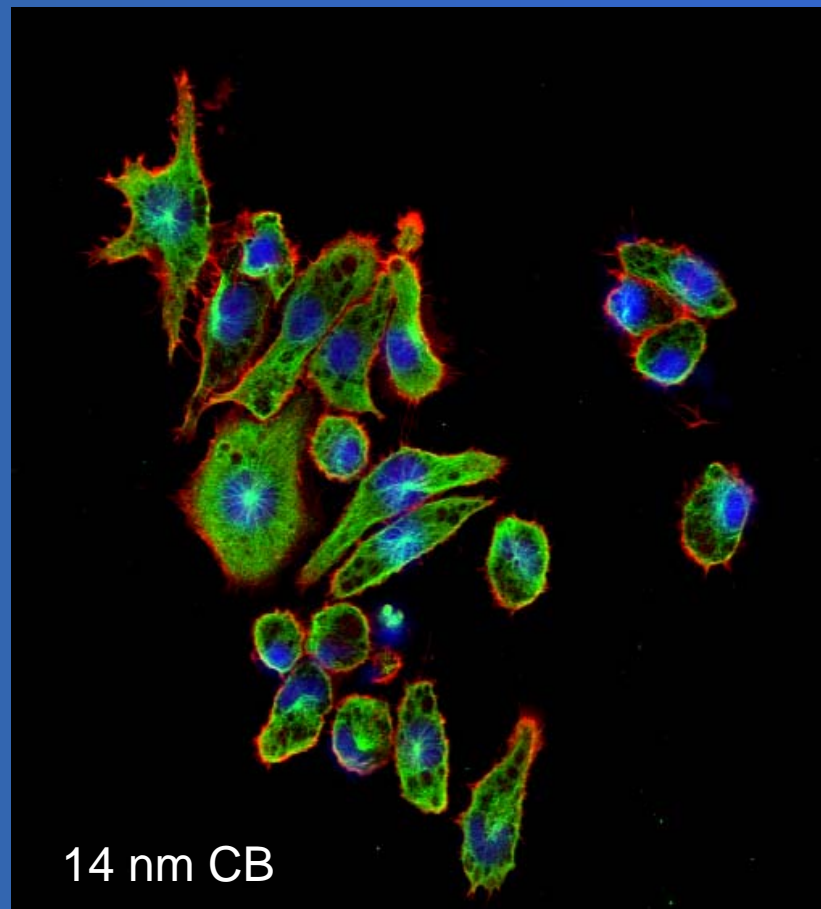
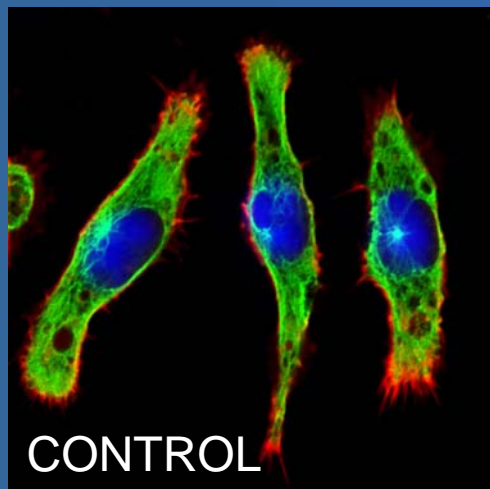


Brown *et al.* 2004 AJP 286; L344-L353

# Nanoparticles, calcium and oxidants

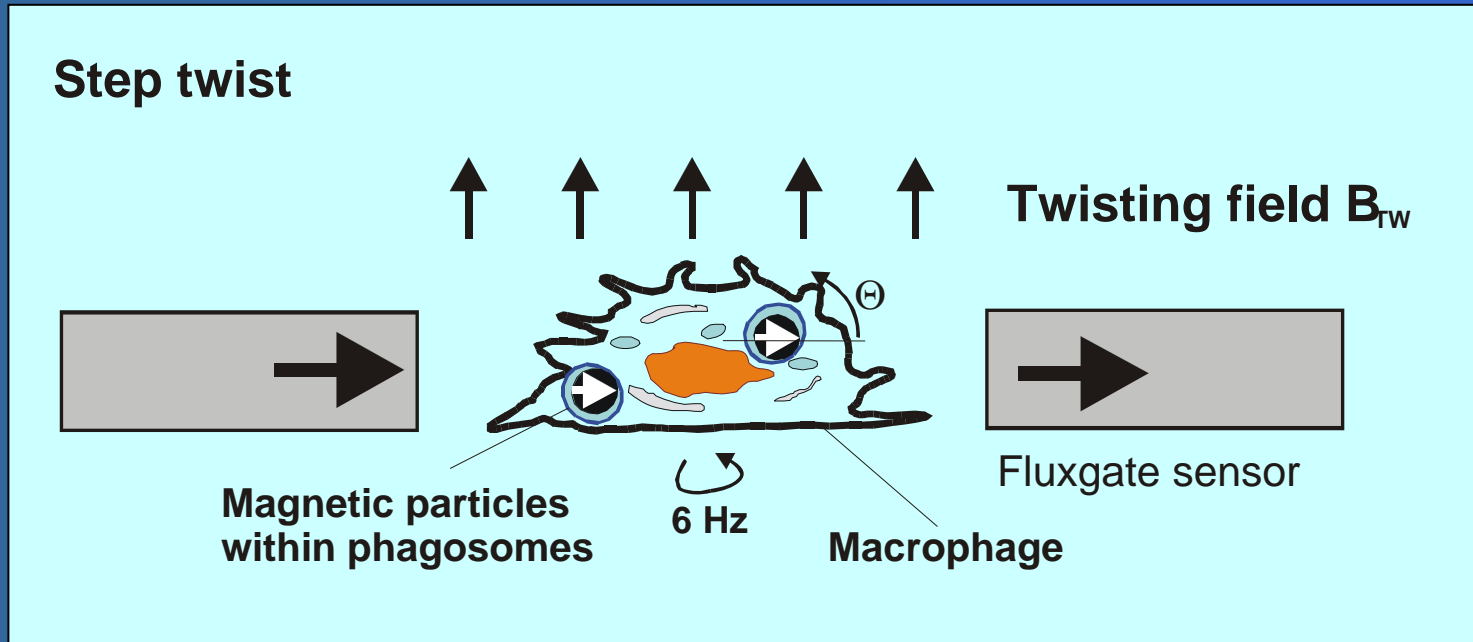


# J774 macrophages exposed to 14nm CB



Wilson et al., manuscript in prep

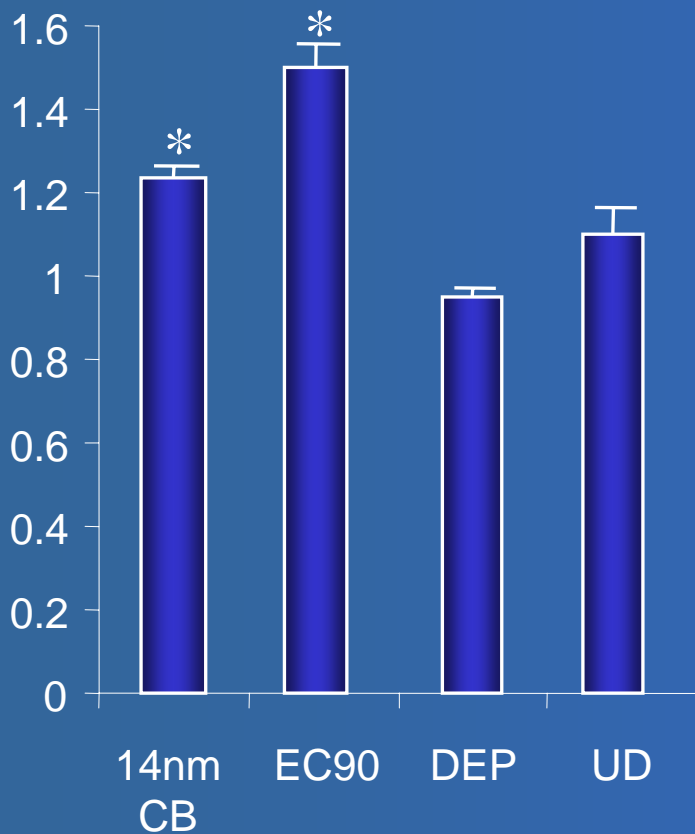
# Measurement of cytoskeletal stiffness using ferromagnetic particles



- Magnetic twisting device
- Aligned ferromagnetic microparticles ingested by macrophages,
- Detection by an array of magnetic fluxgate sensors (Förster device).

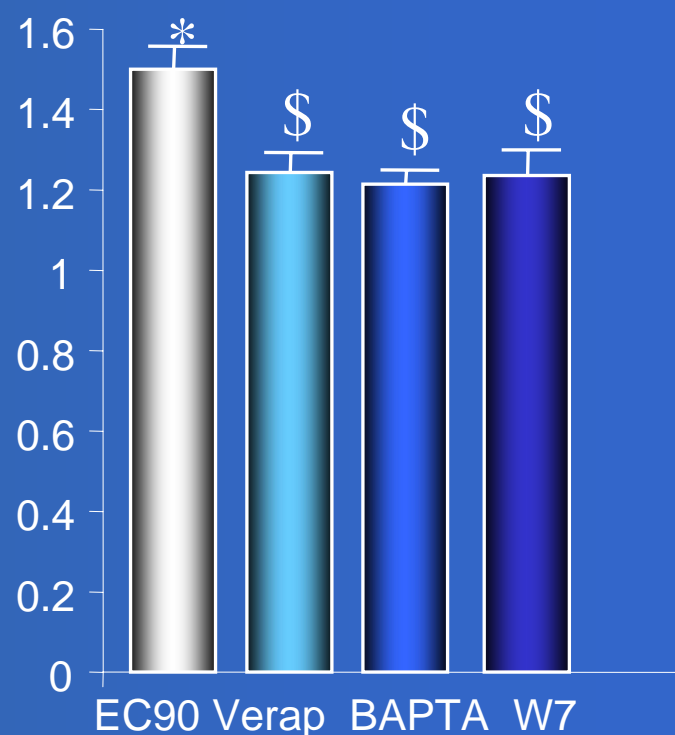
# Impact of particles on the macrophage phagosome transport

Relaxation b5 (drug/control)



320  $\mu$ g/million cells EC90

Relaxation b5 (drug/control)

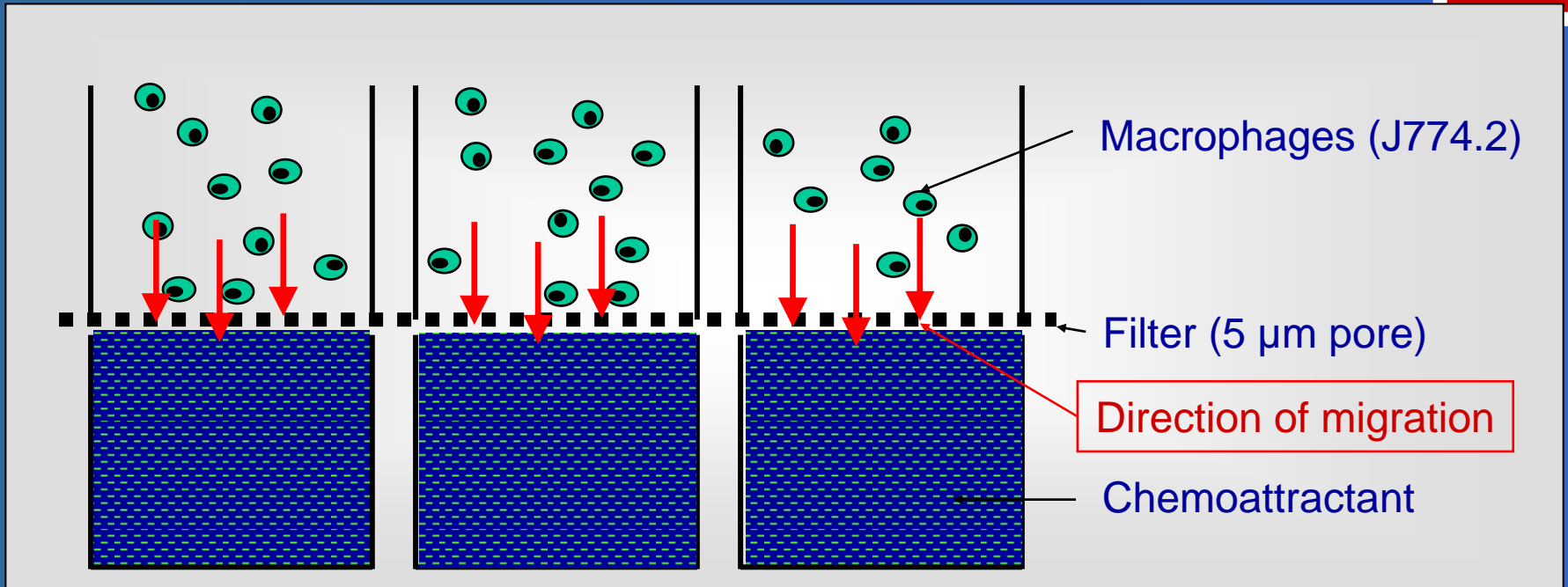


Moeller *et al.*, 2003 Manuscript submitted

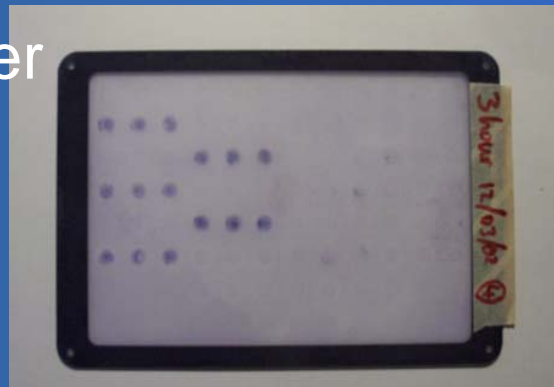


# Effects of nanoparticles and PM<sub>10</sub> on migration and phagocytosis

# Chemotaxis Chamber

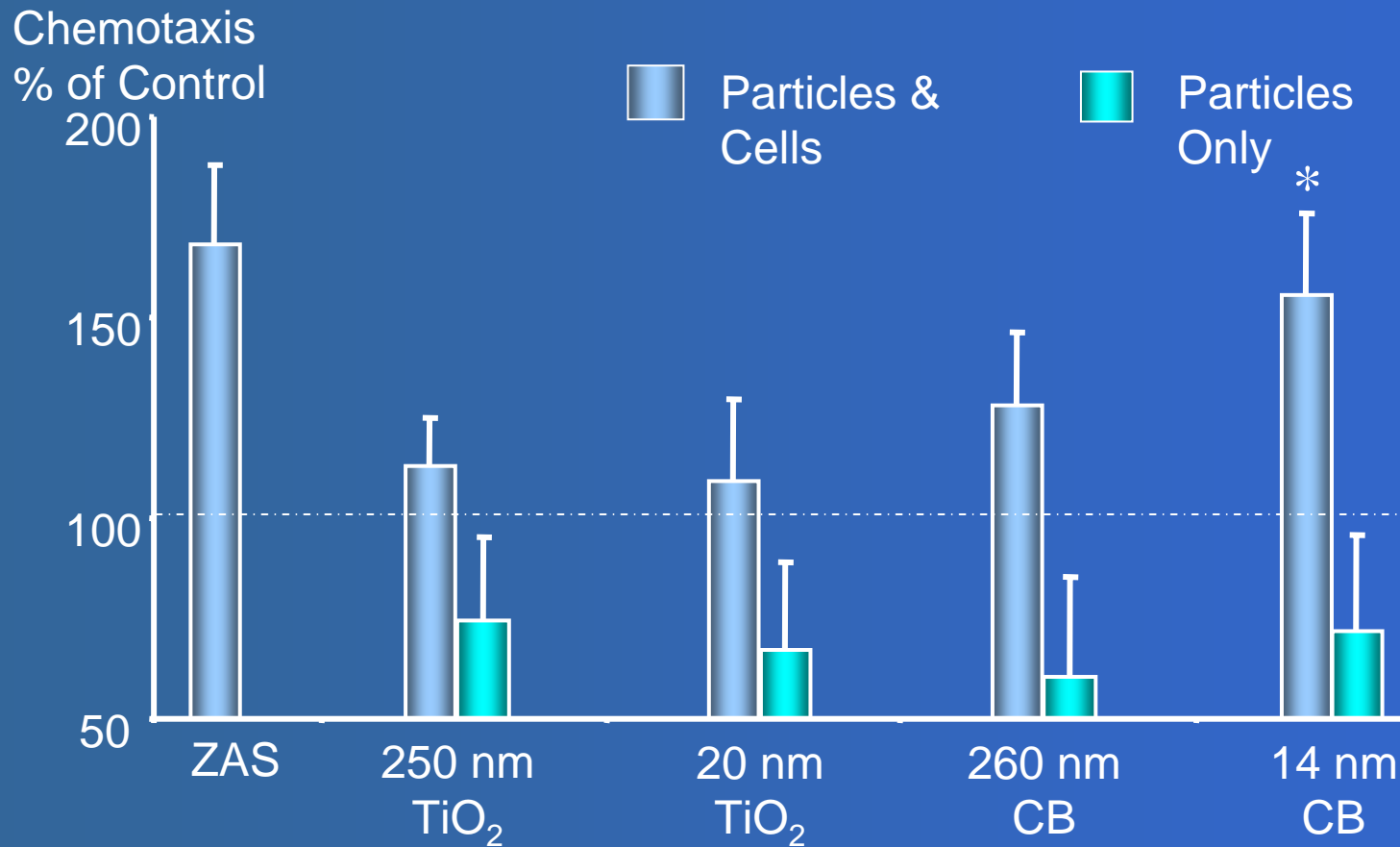


Actual Filter



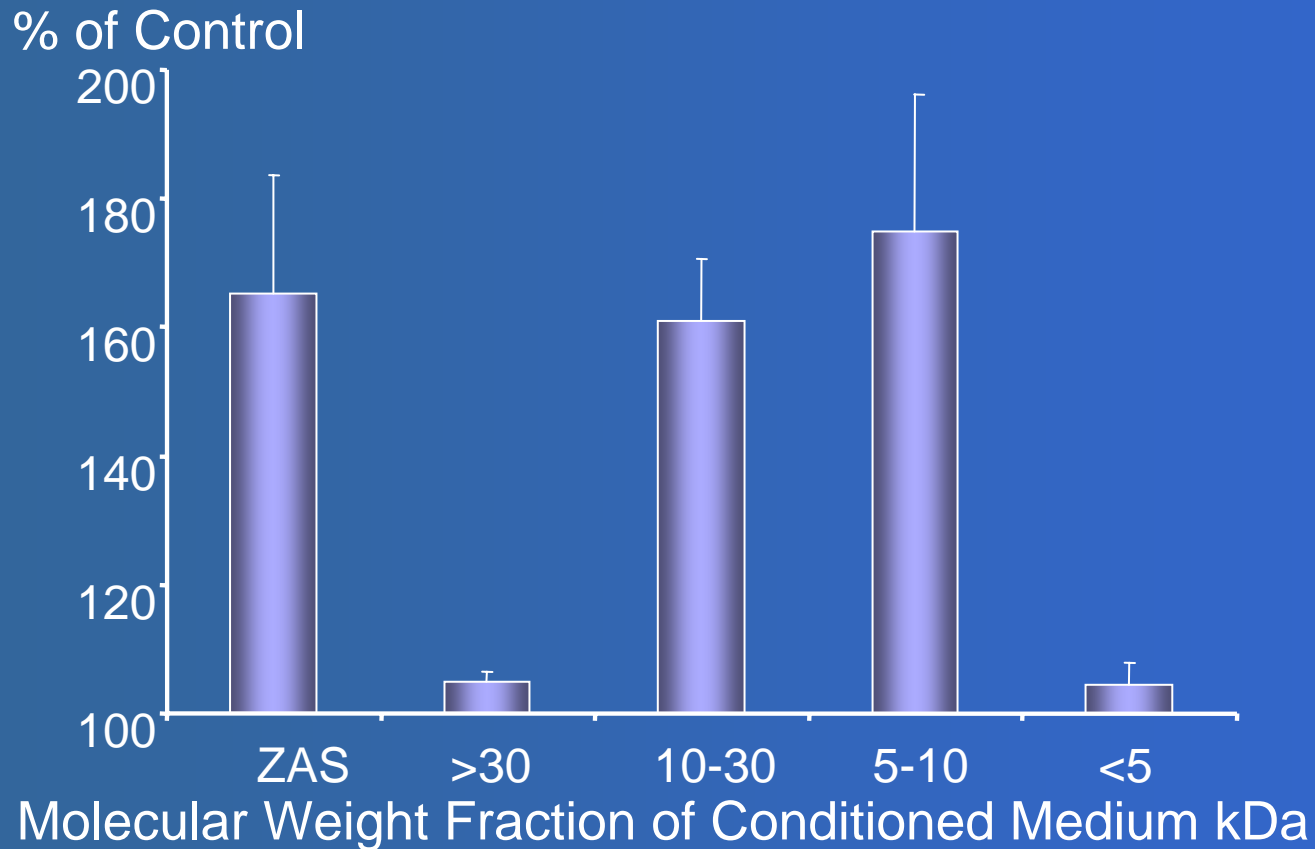


# Chemotaxis of J774 macrophages in response to epithelial cell conditioned medium



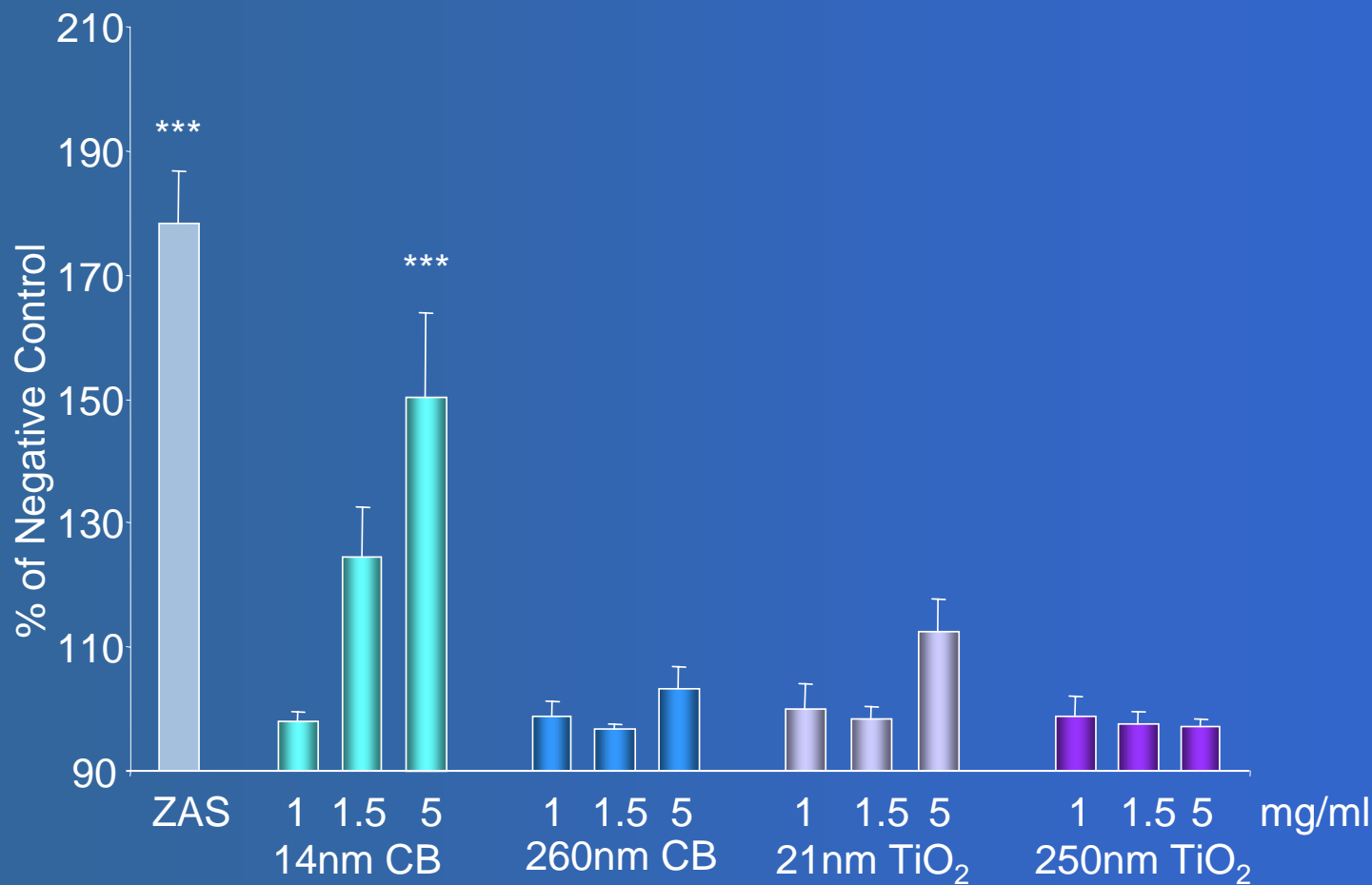
Barlow *et al.* 2005 Particle Fibre Toxicol 2:11.

# Chemotactic response of J774 macrophages to epithelial cell conditioned medium



Barlow *et al.* 2005 Particle Fibre Toxicol 2:11.

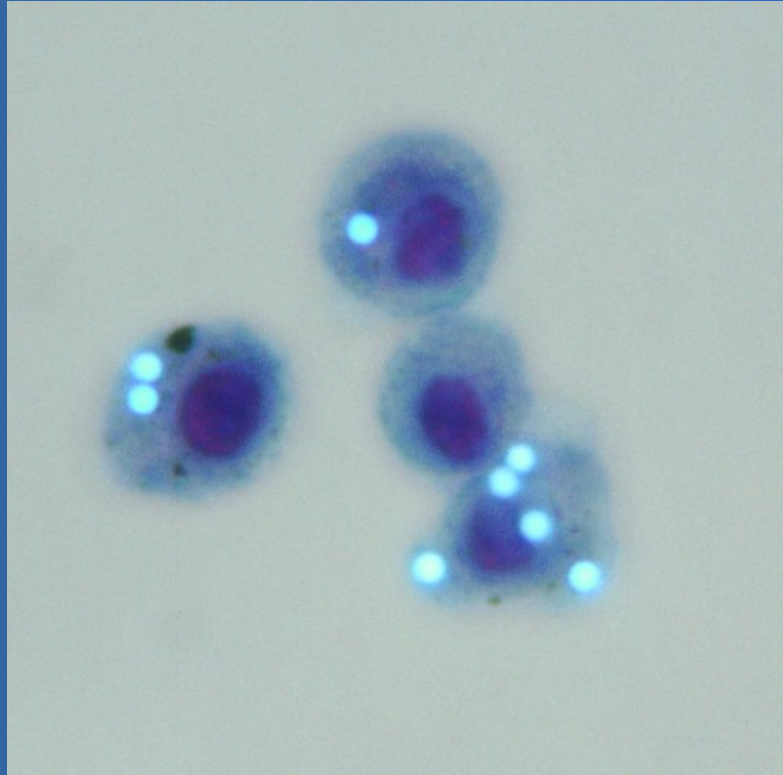
# Chemotactic response of J774 macrophages to serum treated with particles



Barlow *et al.* 2005 *Toxicol Lett* 155:397-401.

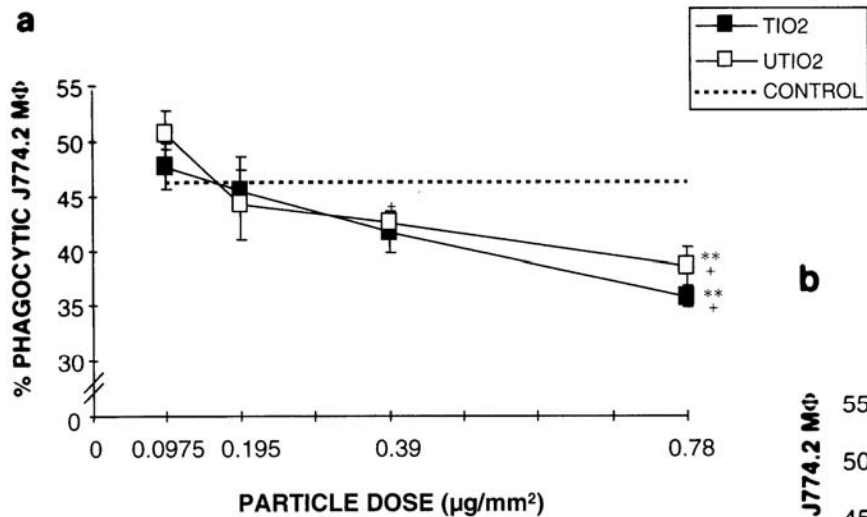
# Nanoparticle uptake by macrophages

# Phagocytosis

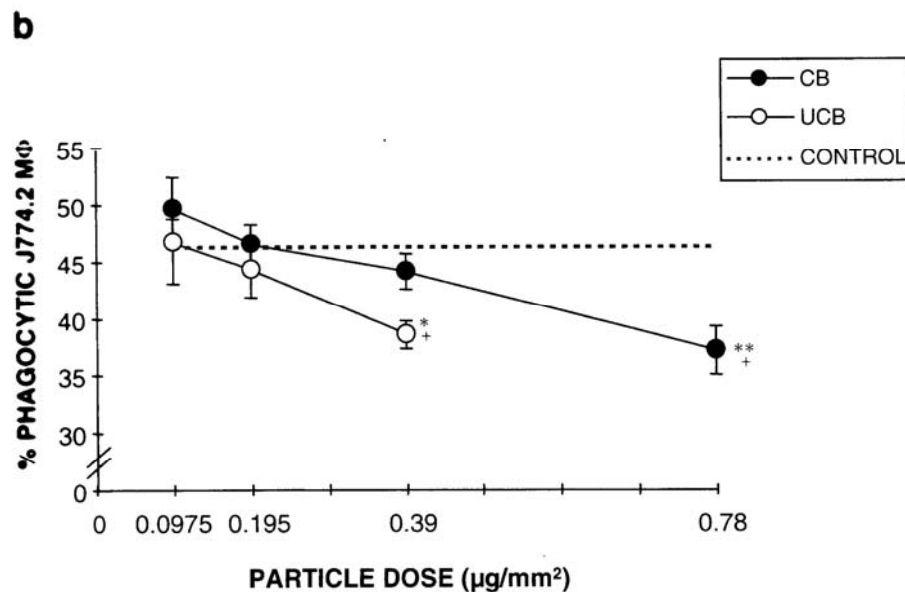


Barlow *et al.* 2006 Submitted.

# Effect of TiO<sub>2</sub> and CB on phagocytosis

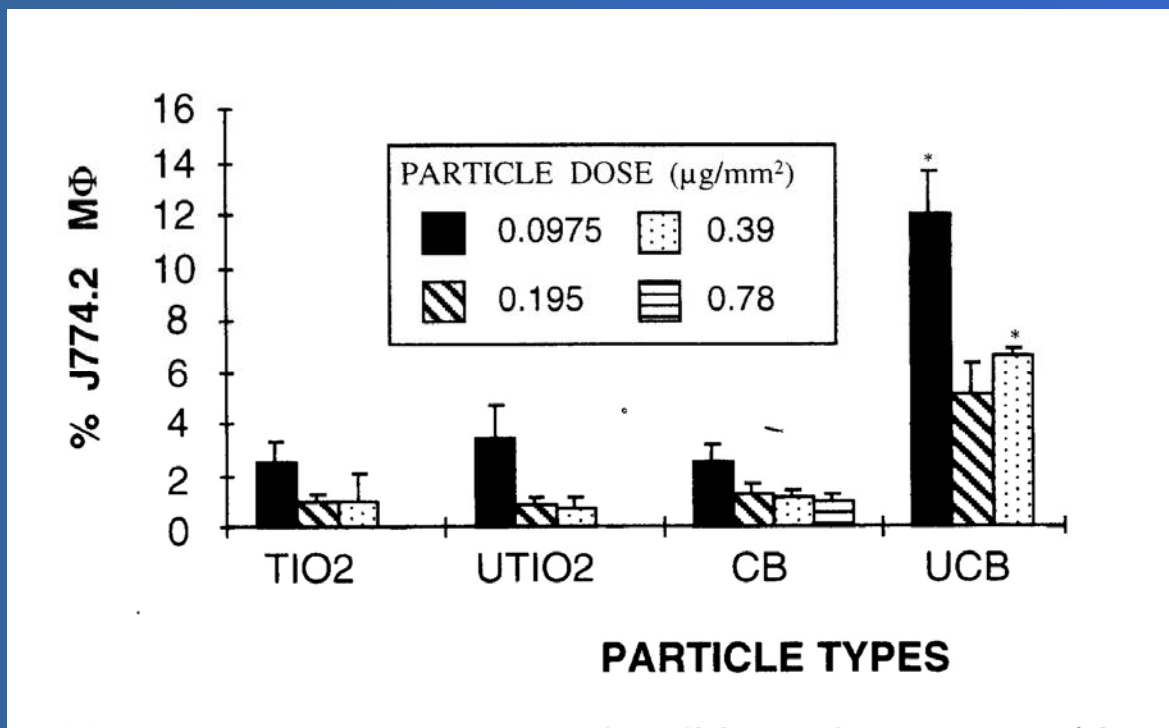


% of cells that phagocytose  $\geq 2$  beads  
8h exposure



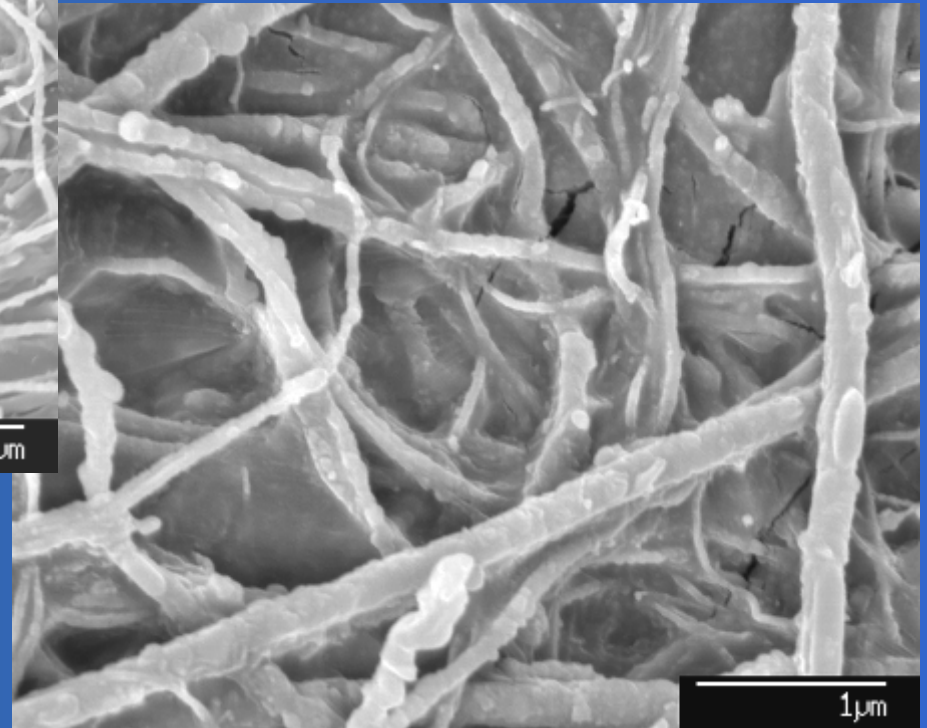
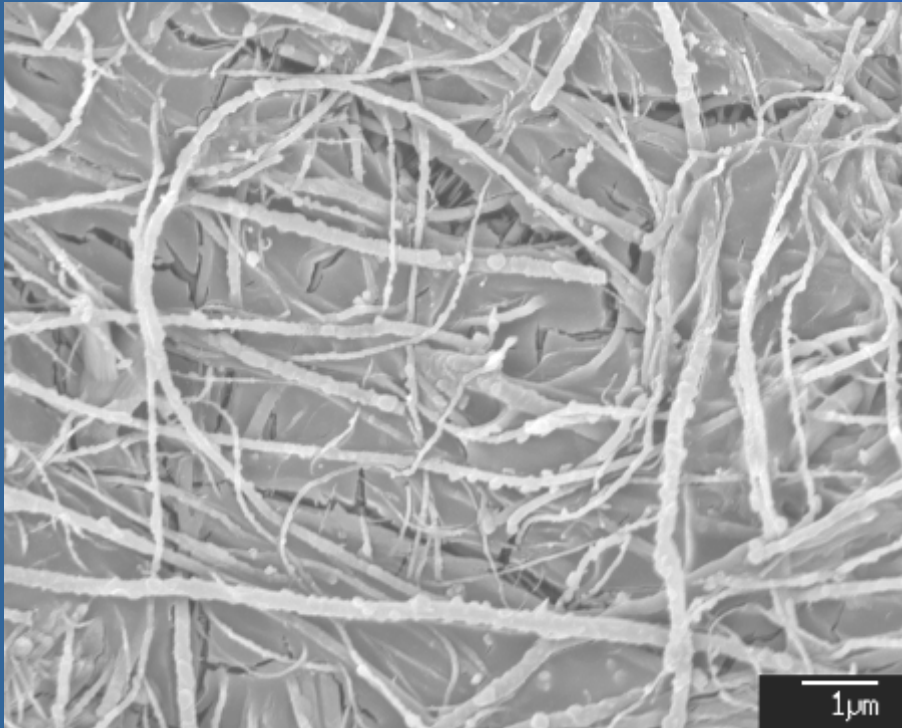
Renwick *et al.* 2001 *Toxicol Applied Pharmacol.* 172: 119-127

# Non-phagocytic cells



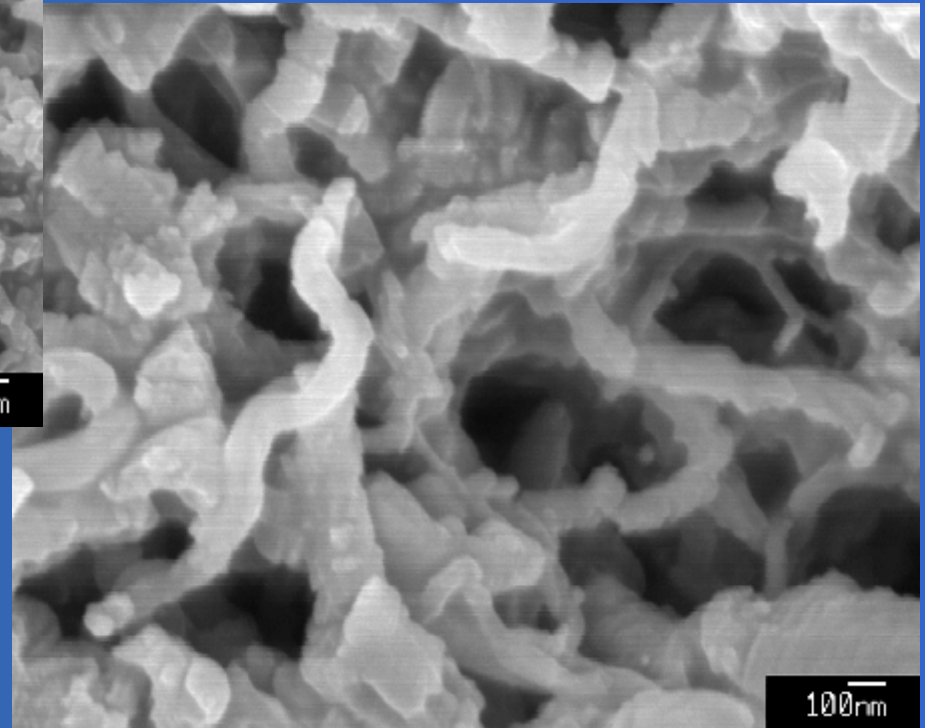
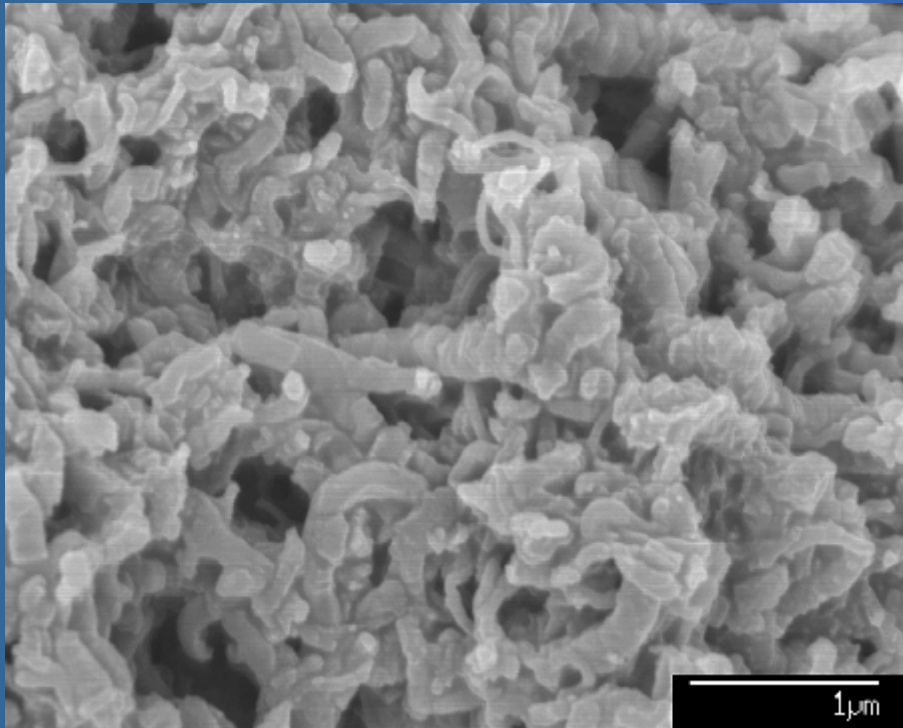
Renwick *et al.* 2001 *Toxicol Applied Pharmacol.* 172: 119-127

# Cambridge multiwalled carbon nanotube

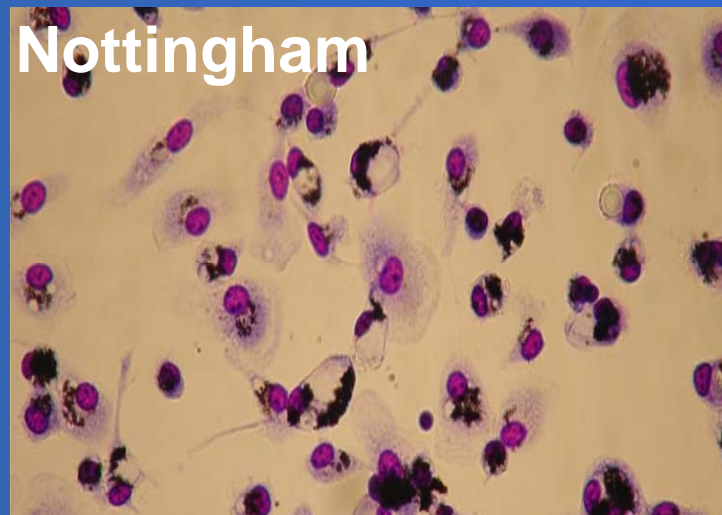
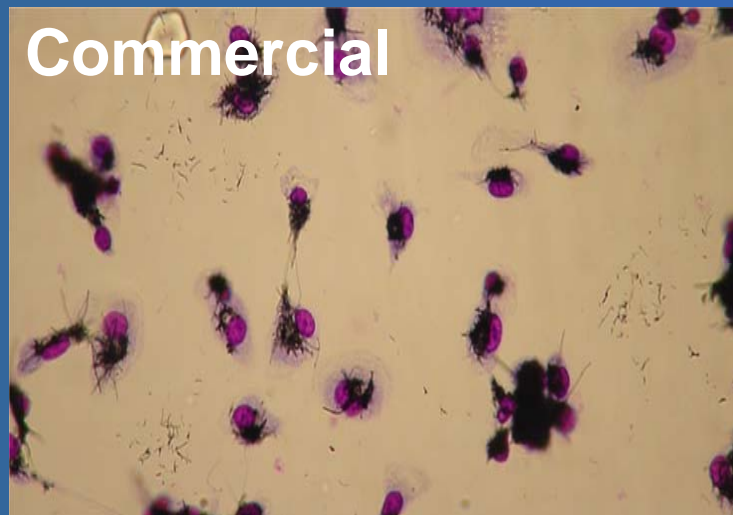
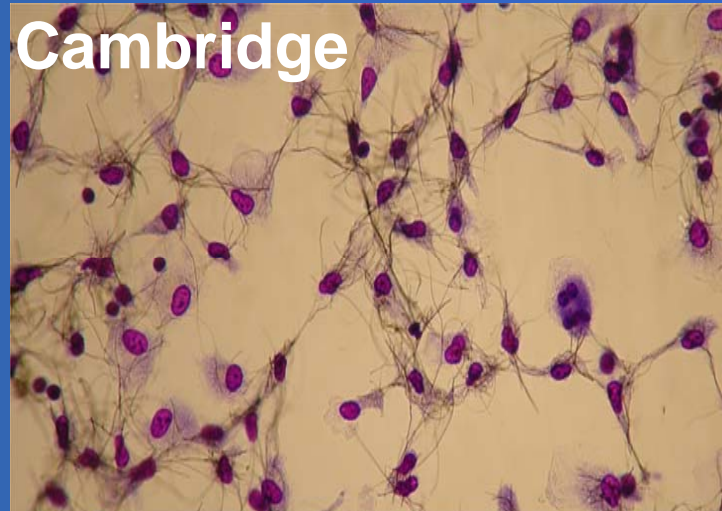
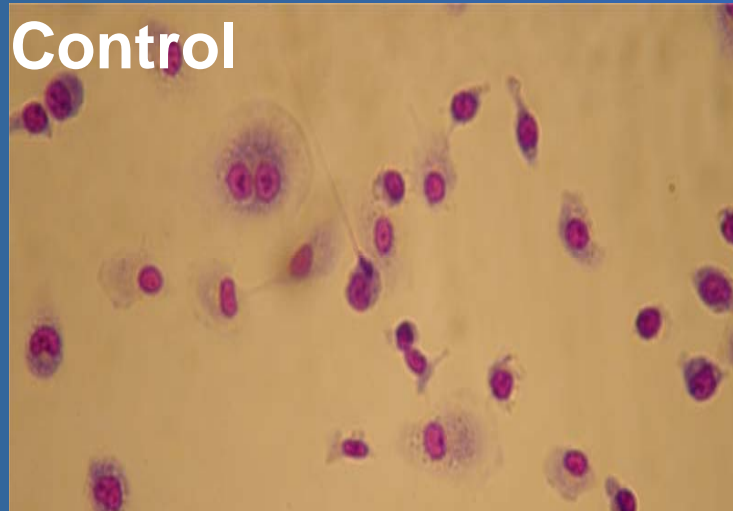




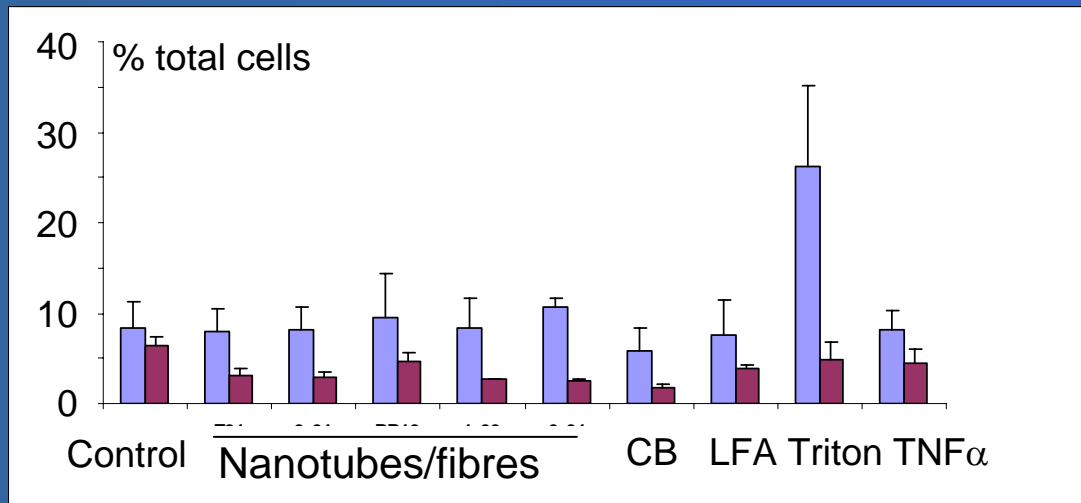
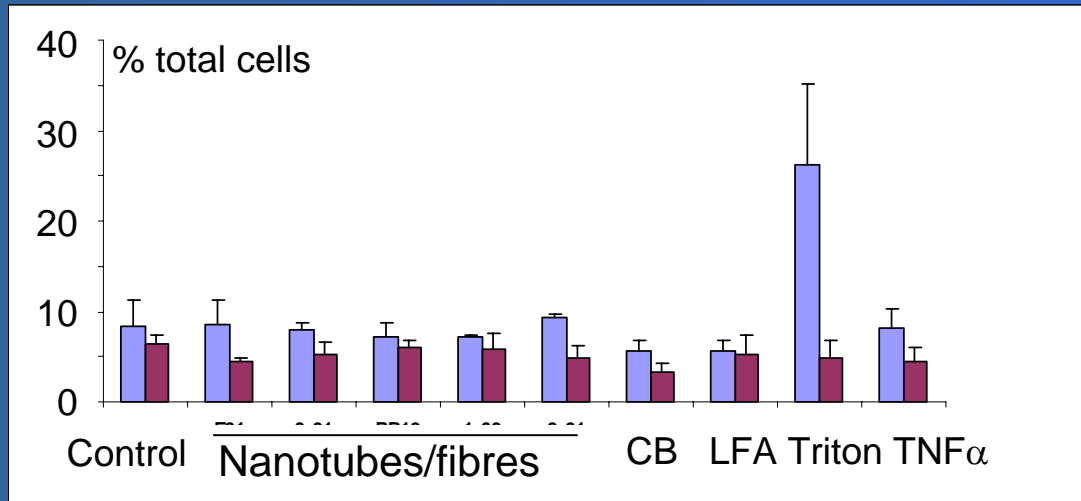
# Nottingham Multiwalled carbon nanotubes



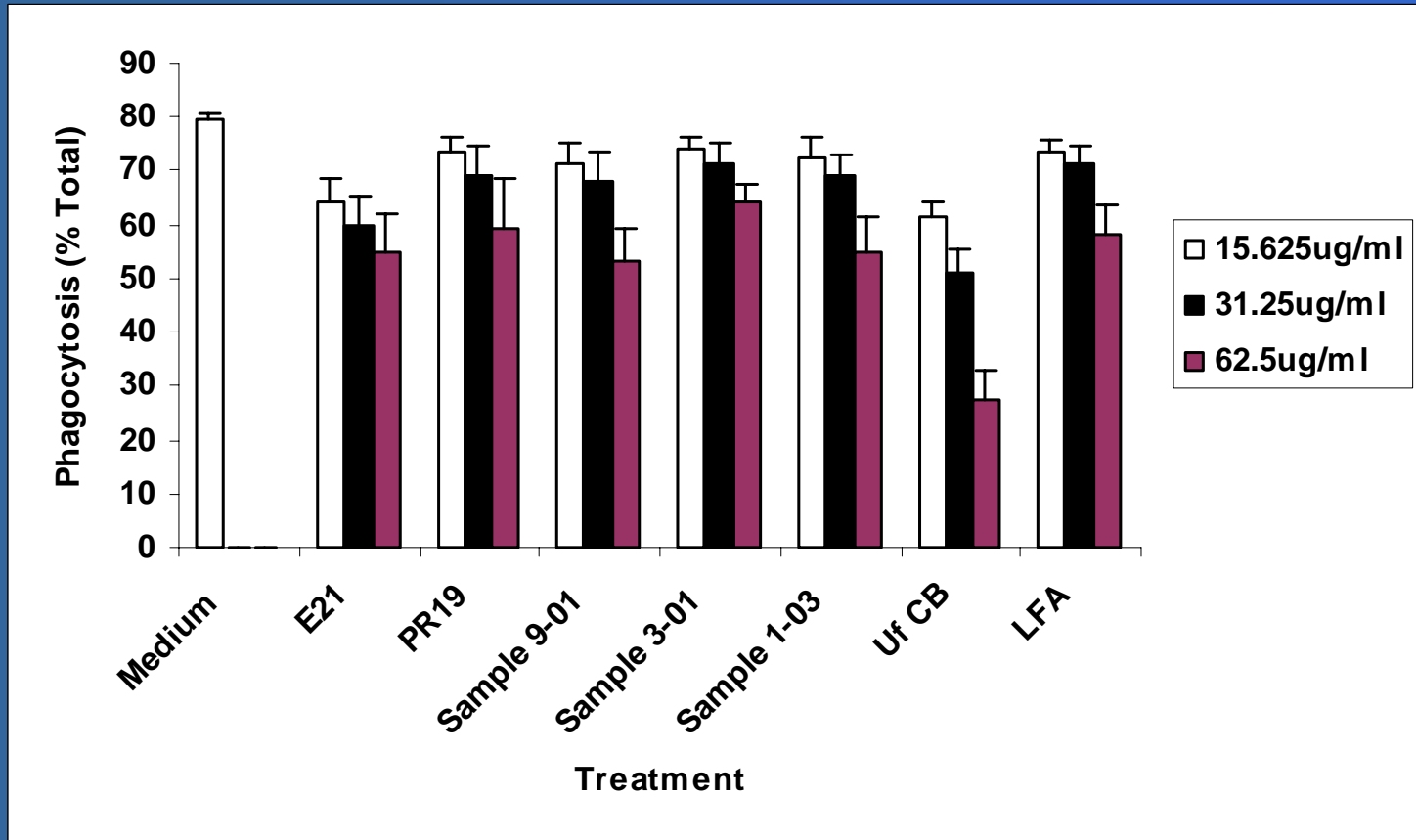
# Carbon nanotubes



# Effects of nanotubes on cell viability



# Effect of nanotubes and nanofibres on phagocytosis



All treatments induced a significant ( $p < 0.05$ ) inhibition

# Summary

Low toxicity, low solubility NP:

- Inflammation is related to surface area dose.
- Generate ROS leading to oxidative stress in epithelial and macrophage cells.
- Induce  $\text{Ca}^{2+}$  influx in macrophages *in vitro* resulting in;
  - activation of  $\text{NF}\kappa\text{B}$  and AP1
  - up-regulation of pro-inflammatory cytokine expression
  - Inhibition of phagosome transport

# Summary

Low toxicity, low solubility NP:

- Stimulate production of chemotaxins by epithelial cells.
- Rapidly taken up into macrophages.
- Inhibit subsequent phagocytosis of larger particles

MWCNT:

- Morphology determines uptake by macrophages.
- Inhibit subsequent phagocytosis of E.Coli.



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