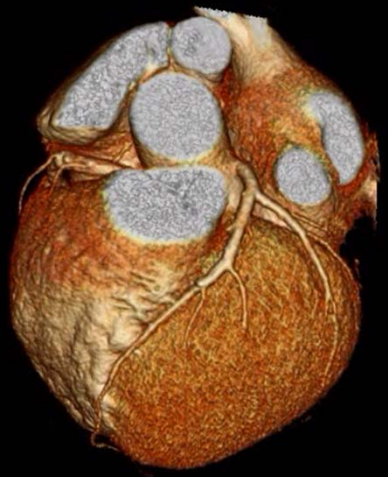
ELEGI
THE EDINBURGH LUNG AND THE ENVIRONMENT GROUP INIATIVE

The Health Effects Of Nanoparticles
What, Where, How?



Dr. Rodger Duffin
MRC Senior Fellow in Respiratory Medicine
The Queen's Medical Research Institute
University of Edinburgh

rodger.duffin@ed.ac.uk

Combustion-derived Nanoparticles

- WHO attributes 3 million premature deaths each year to air-pollution

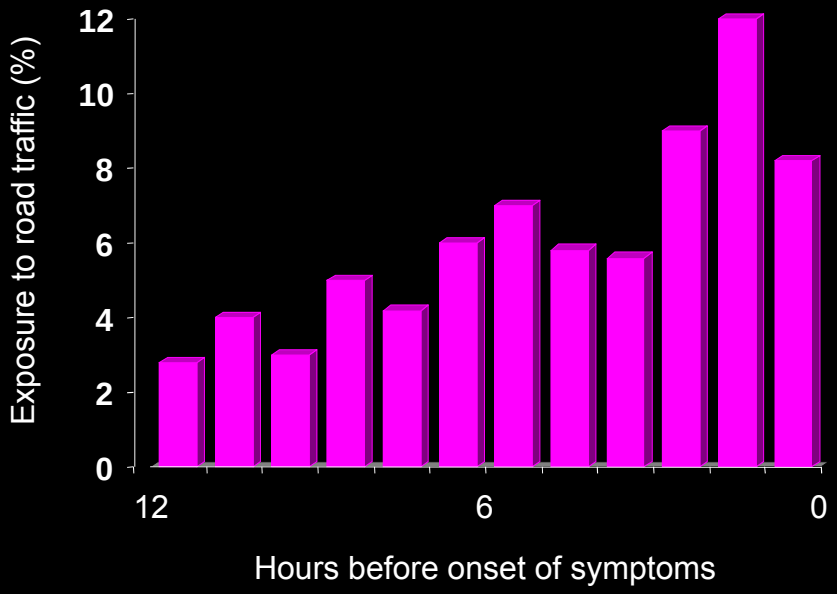
- AHA estimate 63% of women and 48% of men that die from a heart condition have no previous symptoms

- Well documented epidemiological evidence linking increased exposure to CDNP with cardiovascular disease

Road traffic and cardiovascular disease

Acute exposure

Association between exposure to traffic and the onset of acute myocardial infarction [Odds ratio 2.9; CI, 2.2 to 3.8][1]

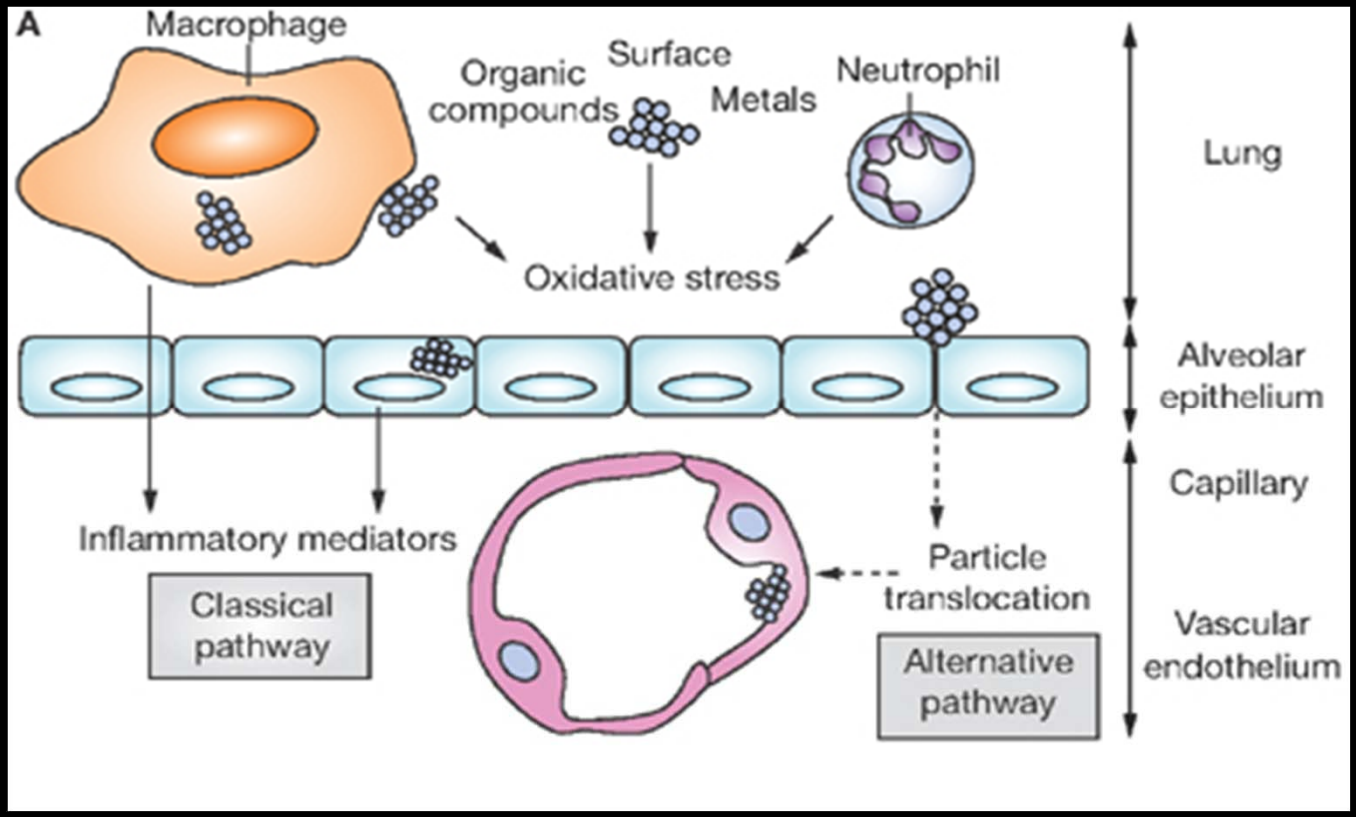


Chronic exposure

Living within 100 yards of a major road is associated with increased cardiopulmonary mortality [Relative risk 1.95; CI, 1.09 to 3.52] [2]

[1]Peters A *et al.* N Engl J Med 2004 [2]Hoek G *et al.* Lancet 2002

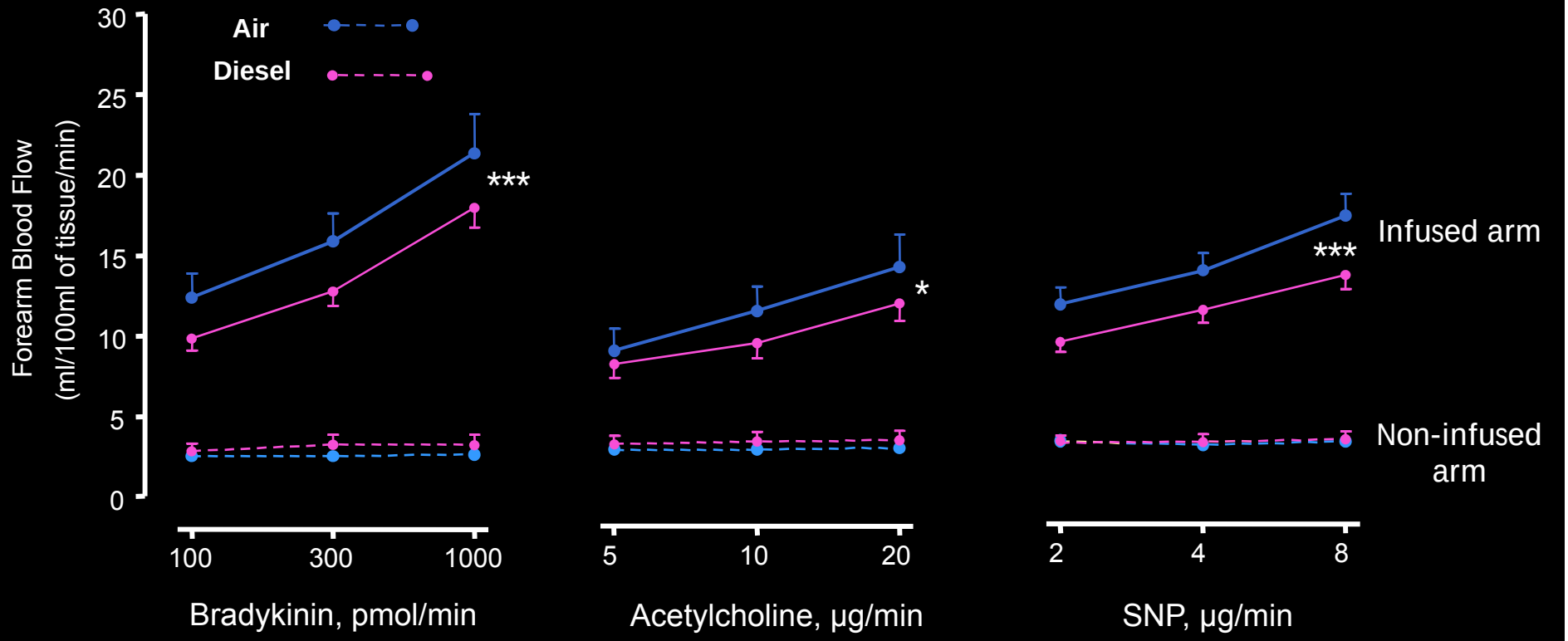
Proposed Mechanism



- Macrophage mediated pulmonary derived effects
- Direct translocation of nanoparticles into circulatory system
  - Thrombogenesis
  - Vasoconstriction
  - Plaque rupture

Mills NL *et al*. (2008) Adverse cardiovascular effects of air pollution *Nat Clin Pract Cardiovasc Med*

Exposure to dilute diesel exhaust for one hour impairs endothelium dependent and independent vasomotor function



Infused (solid line) and non-infused (dashed line) FBF following diesel (●) and air (●) during bradykinin (P=0.006), acetylcholine (P=0.07) and sodium nitroprusside (P=0.0002).

Mills *et al* Circulation 2005

The Paradox

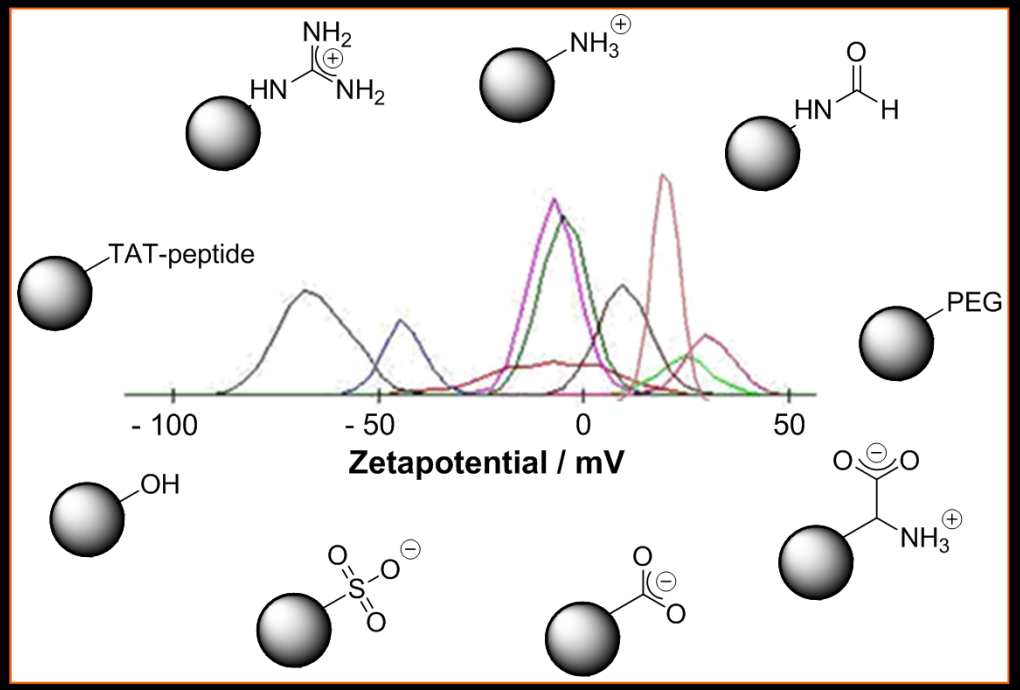
Engineered nanoparticles, being developed for medical applications, share certain structural properties with combustion derived nanoparticles; exposure to which is known to have adverse cardiovascular effects.

Aims

1. To assess the particokinetics of inhaled and intravenously administered engineered nanoparticles.
2. Determine whether circulating nanoparticles cause platelet activation, platelet-monocyte aggregation, and thrombus formation.
3. Understand the effect of size, surface area and surface chemistry on the pro-thrombotic effects of nanoparticles.

Bespoke nanoparticles made in-house

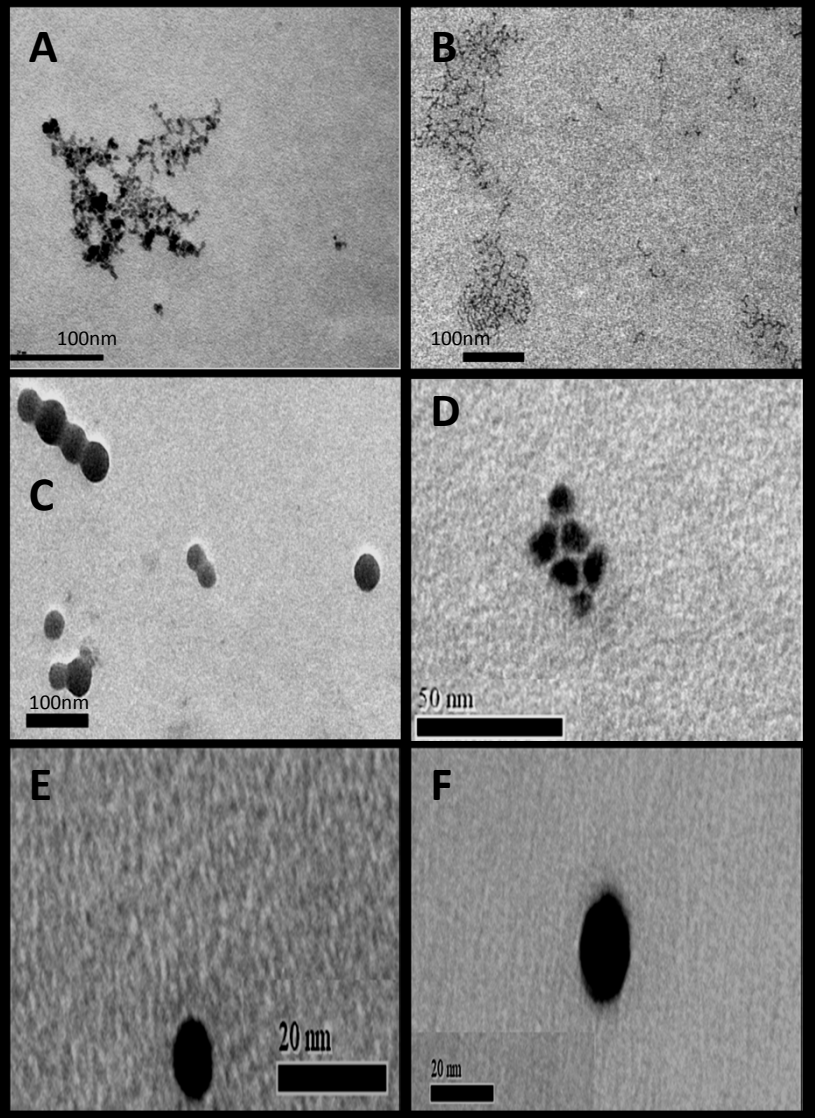
Particle	Size(nm)	Functionalisation
Polystyrene beads	50, 100, 200	OH
	50, 100, 200	COOH
	50, 100, 200	NH
	50, 100, 200	Dex
	50, 100, 200	PEG
	50, 100, 200	CY5.5



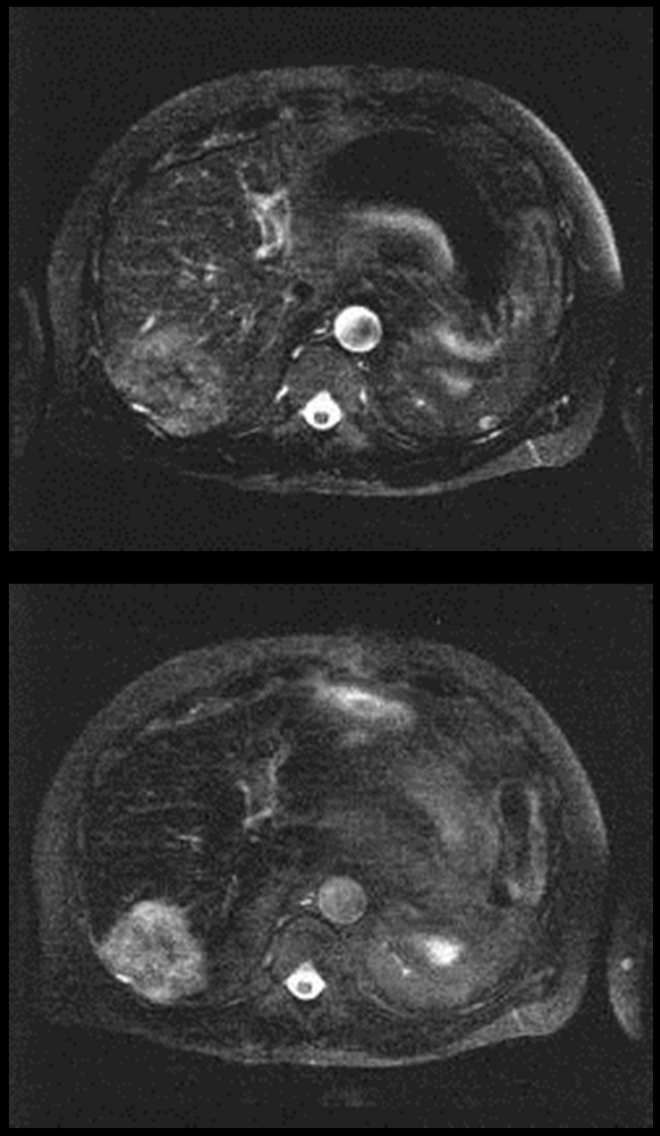
Made in collaboration with Professor Mark Bradley's group, University of Edinburgh.

Commercially available nanoparticles

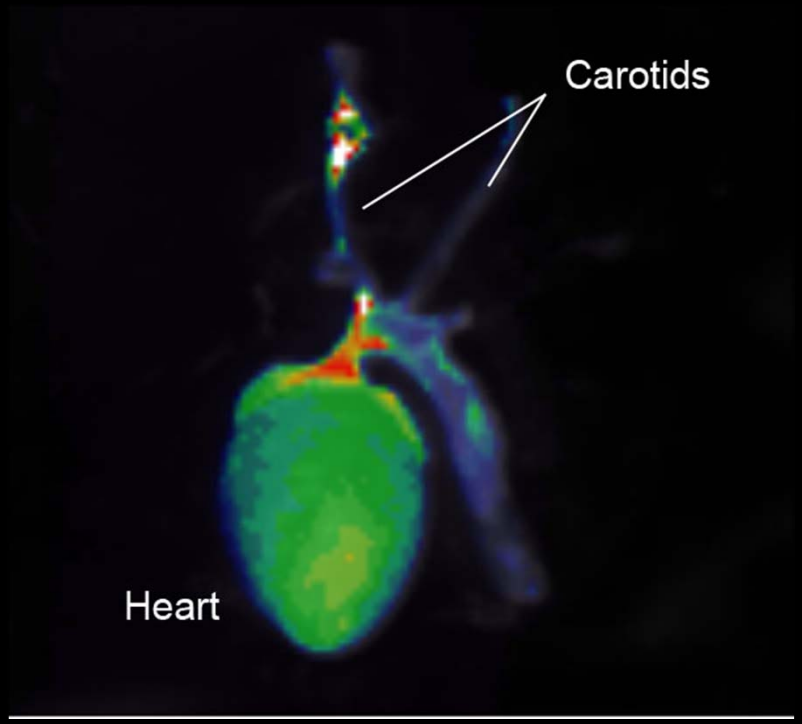
Particle	Size (nm)	Functionalisation	Company
Endorem **(A)**	50-150	Dextran coated	Guebert
AngioSPARK **(B)**	20-50	Biocompatible	VisEN
Polystyrene beads **(C)**	50	COOH	
	50	NH	
Quantum Dots (CdS-CdTe) **(D)**	1-10	polyacrylate sodium	ViveNano
Au **(E)**	5, 20, 50, 100, 200	N/A	Nanocs
	20	Pegylated	Nanocs
	20	Dextran coated	Nanocs



MRI of human liver using Endorem



Imaging vascular inflammation in mouse carotids using Angiospark



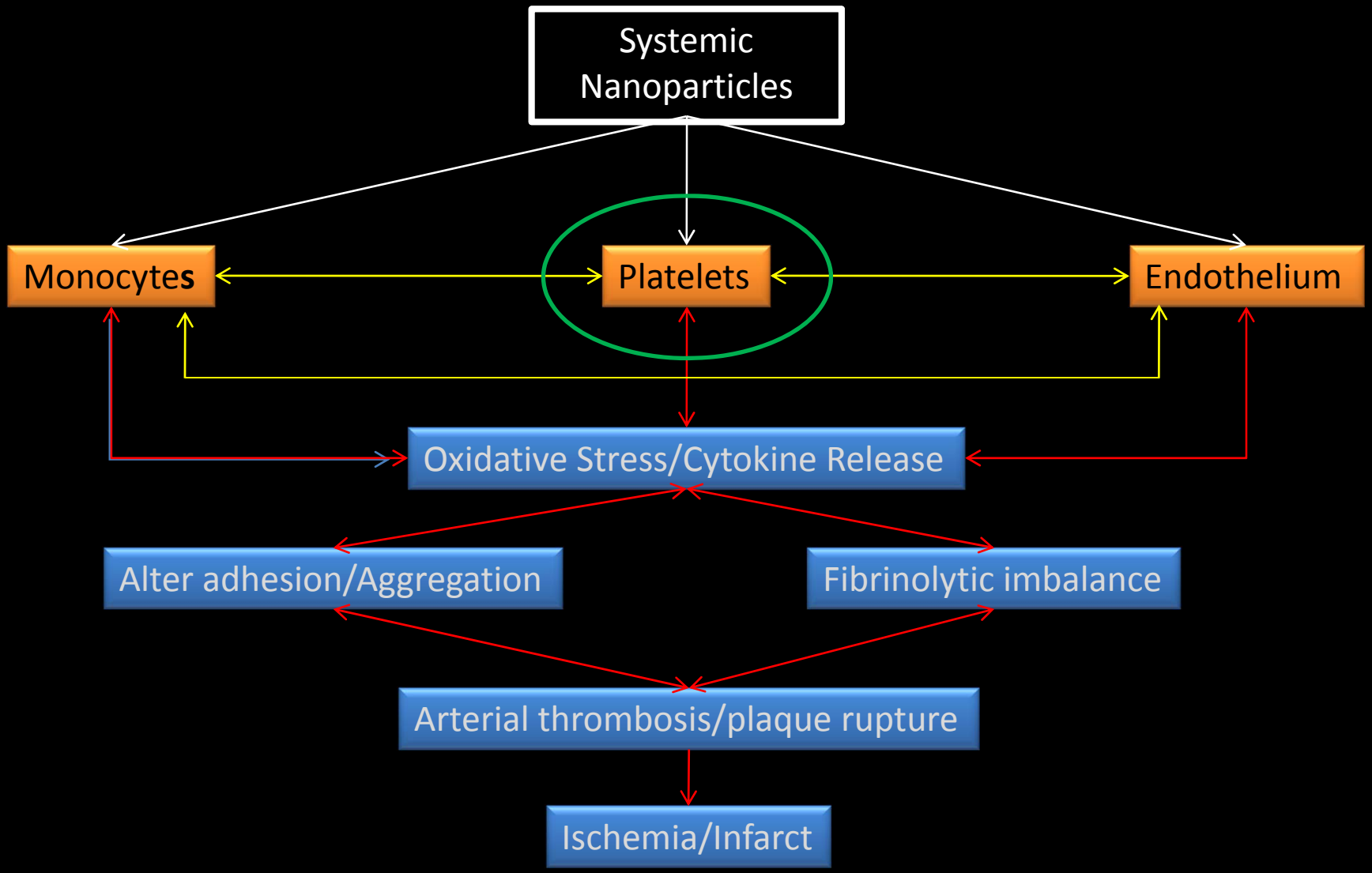
What do we hope to achieve?

- Development of safer engineered nanoparticles for medical applications

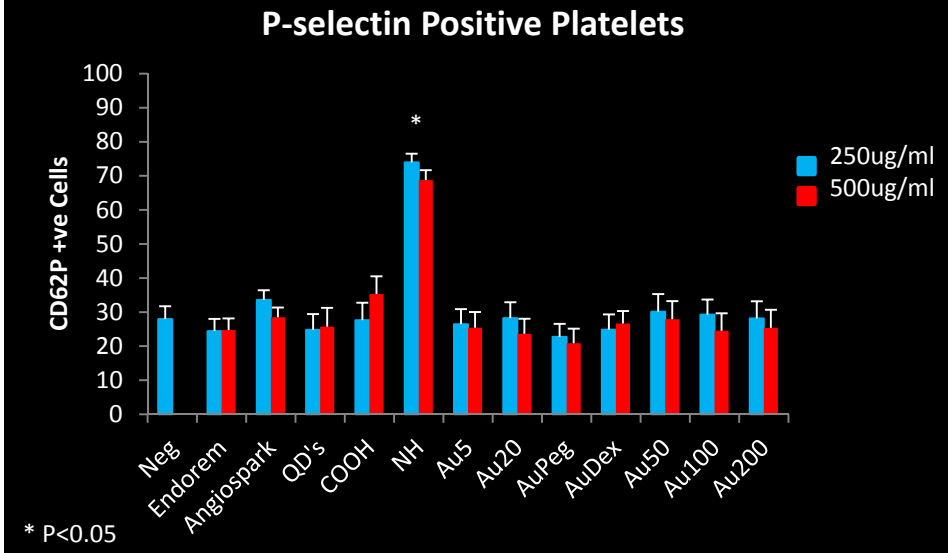
- Resolve a potentially important paradox in medical imaging and therapeutics: environmental nanoparticulate has been shown to have detrimental effects on the cardiovascular system, even at relatively low doses, yet in a quest to enhance cardiovascular imaging and therapy, direct intravascular infusion of nanoparticles (of potentially unknown toxicity and at high concentrations) into patients with unstable atheromatous disease is being proposed

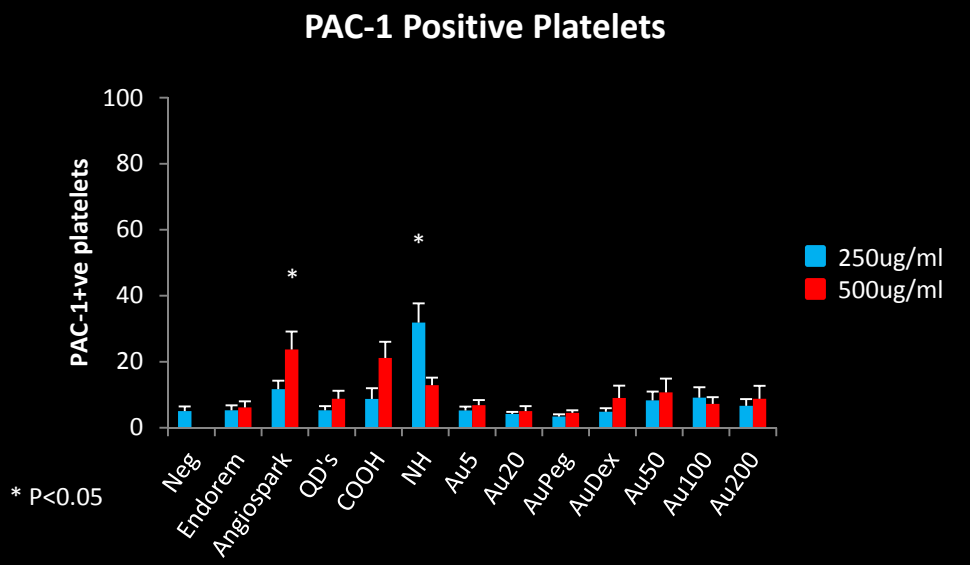
- Gain a better understanding of the mechanisms through which inhaled nanoparticulate can contribute to adverse cardiovascular outcomes

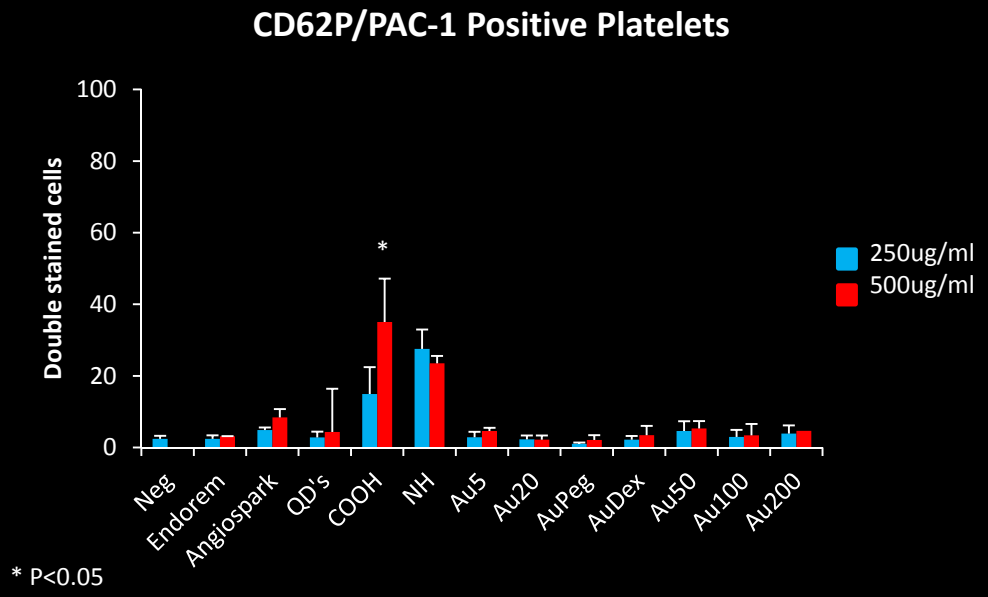
Potential fate of medical nanoparticles



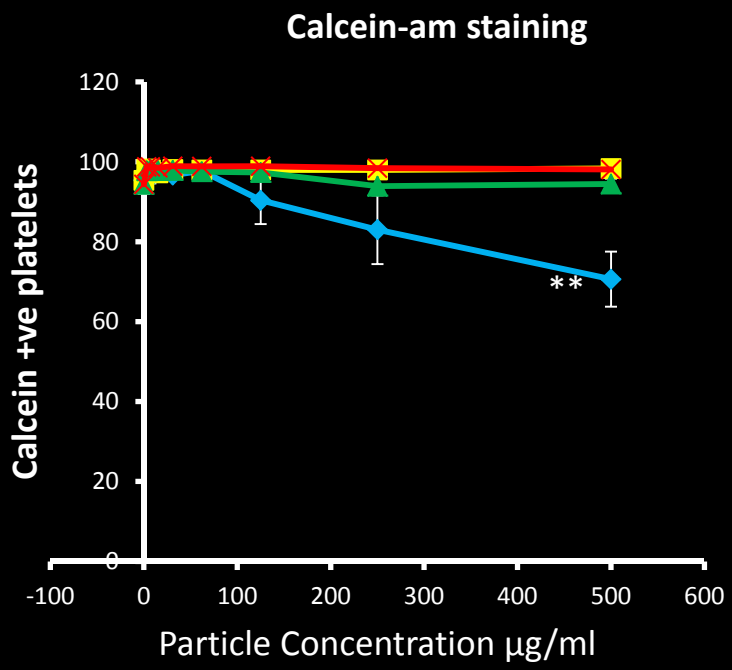
Flow cytometric analysis of platelet activation markers

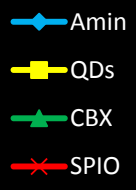


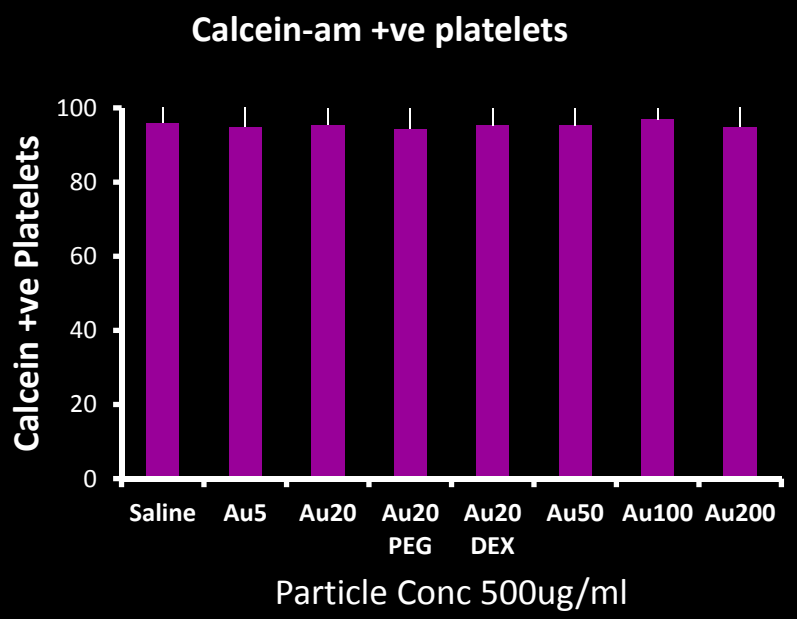




Assessment of platelet viability following exposure to nanoparticles

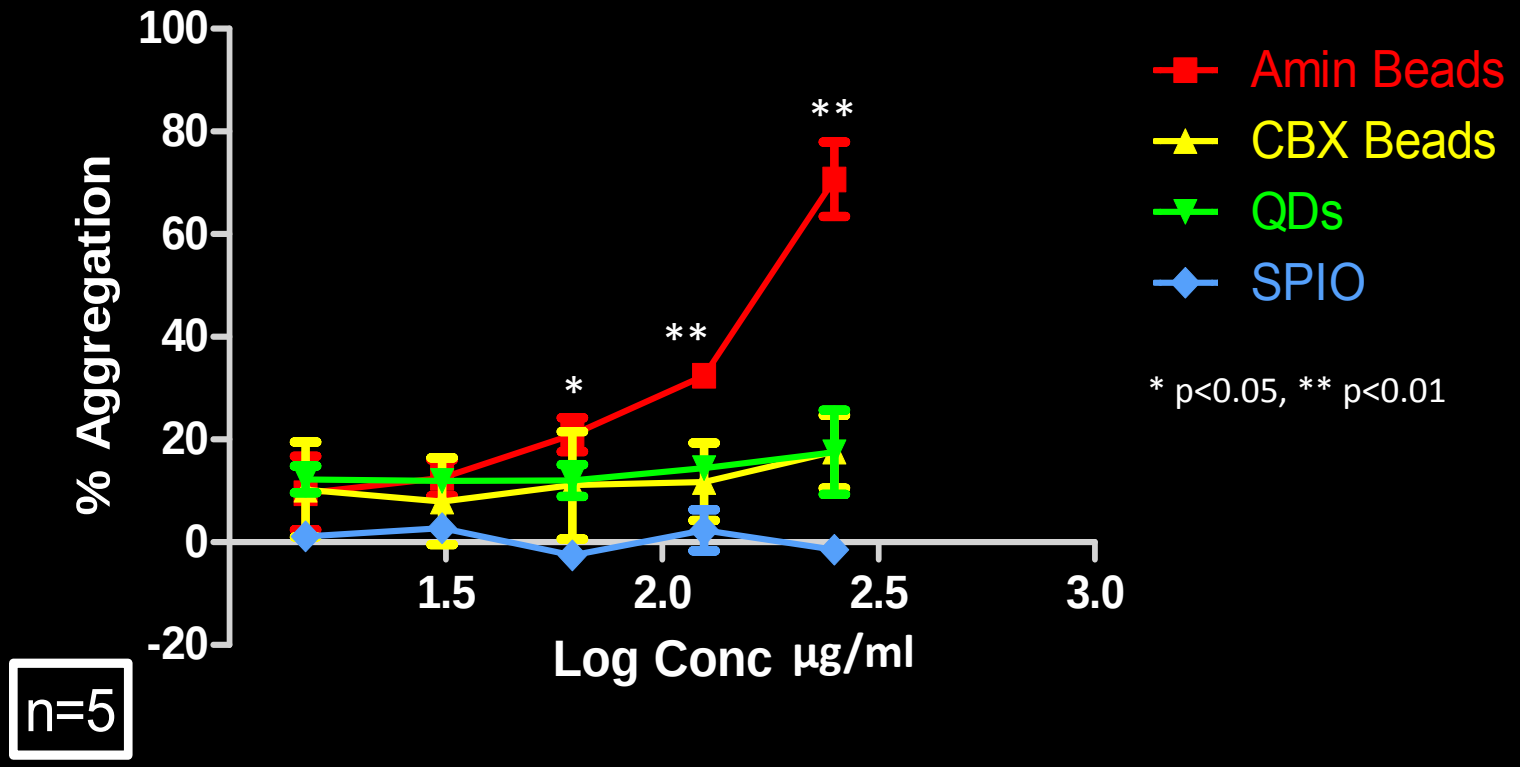






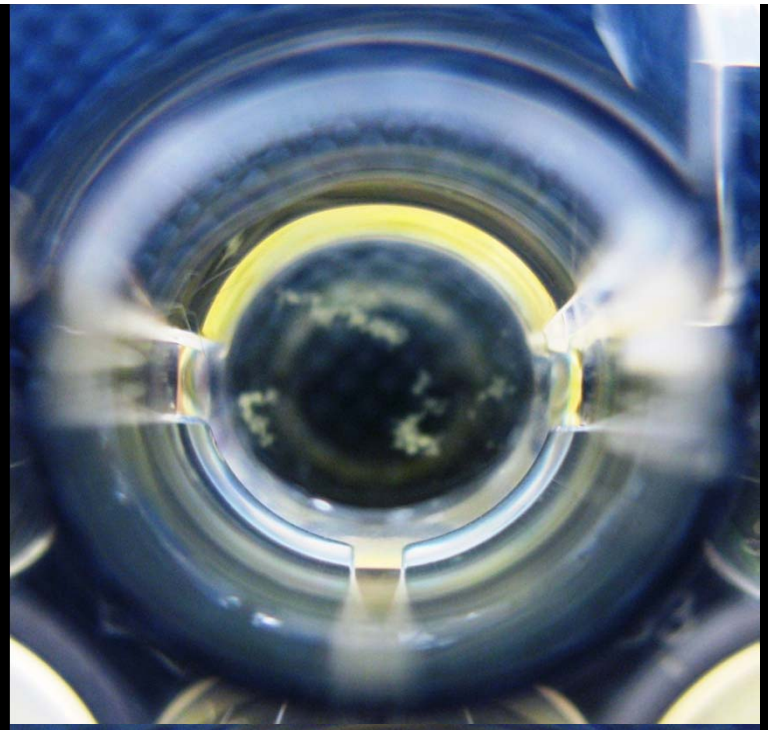
30 min incubation with particles n=4, **p<0.01

Platelet Aggregation

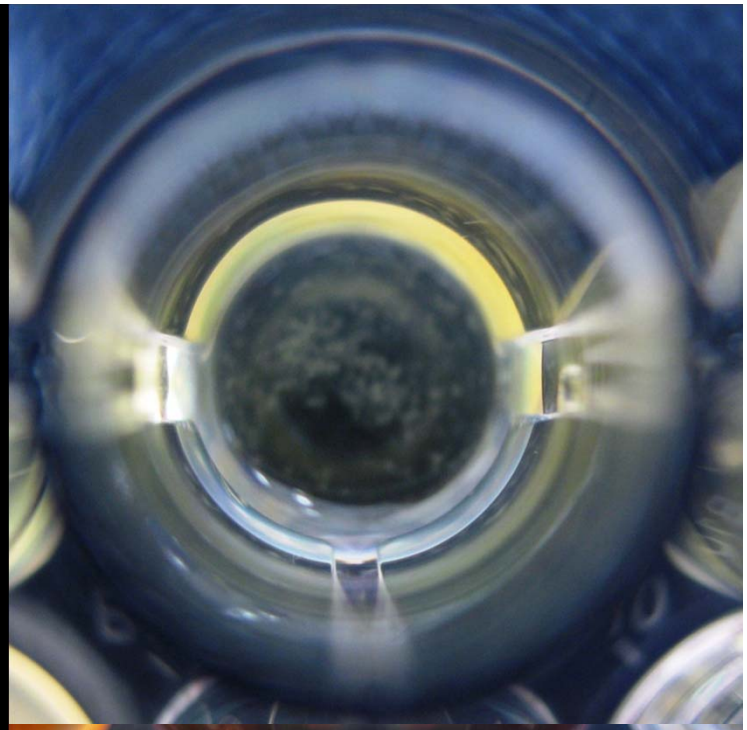


Exposure to amine beads caused a significant increase in platelet aggregation at relatively low doses

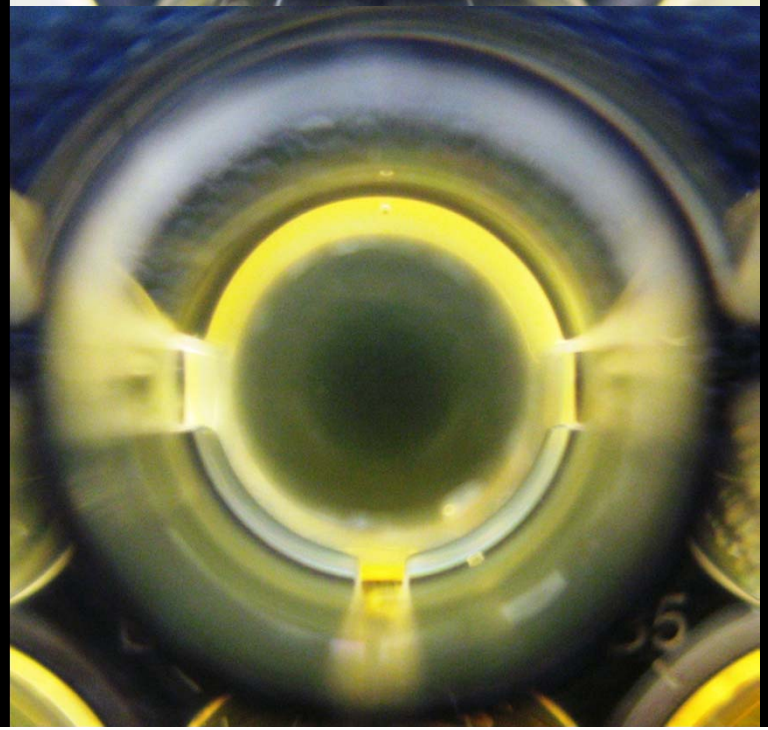
TRAP
50uM



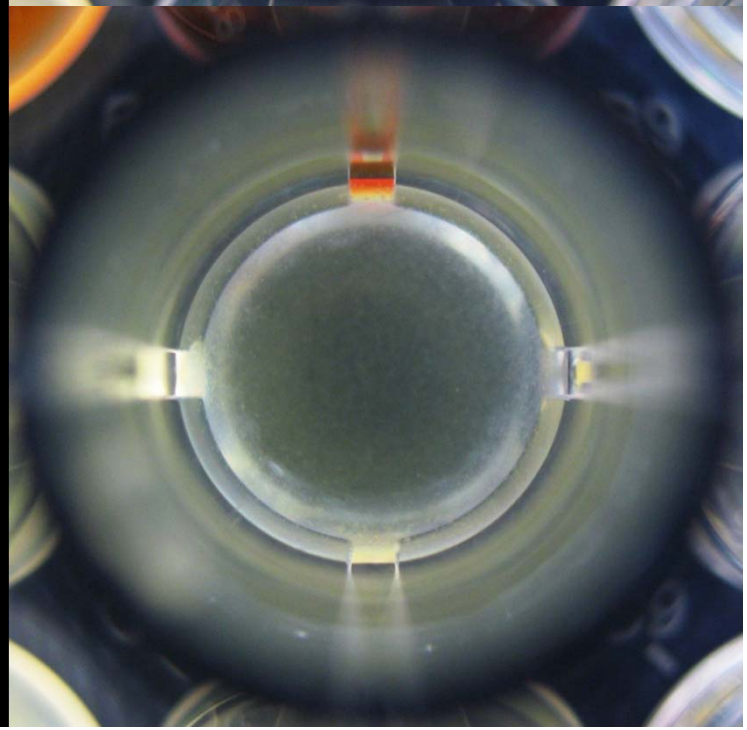
Aminated
beads



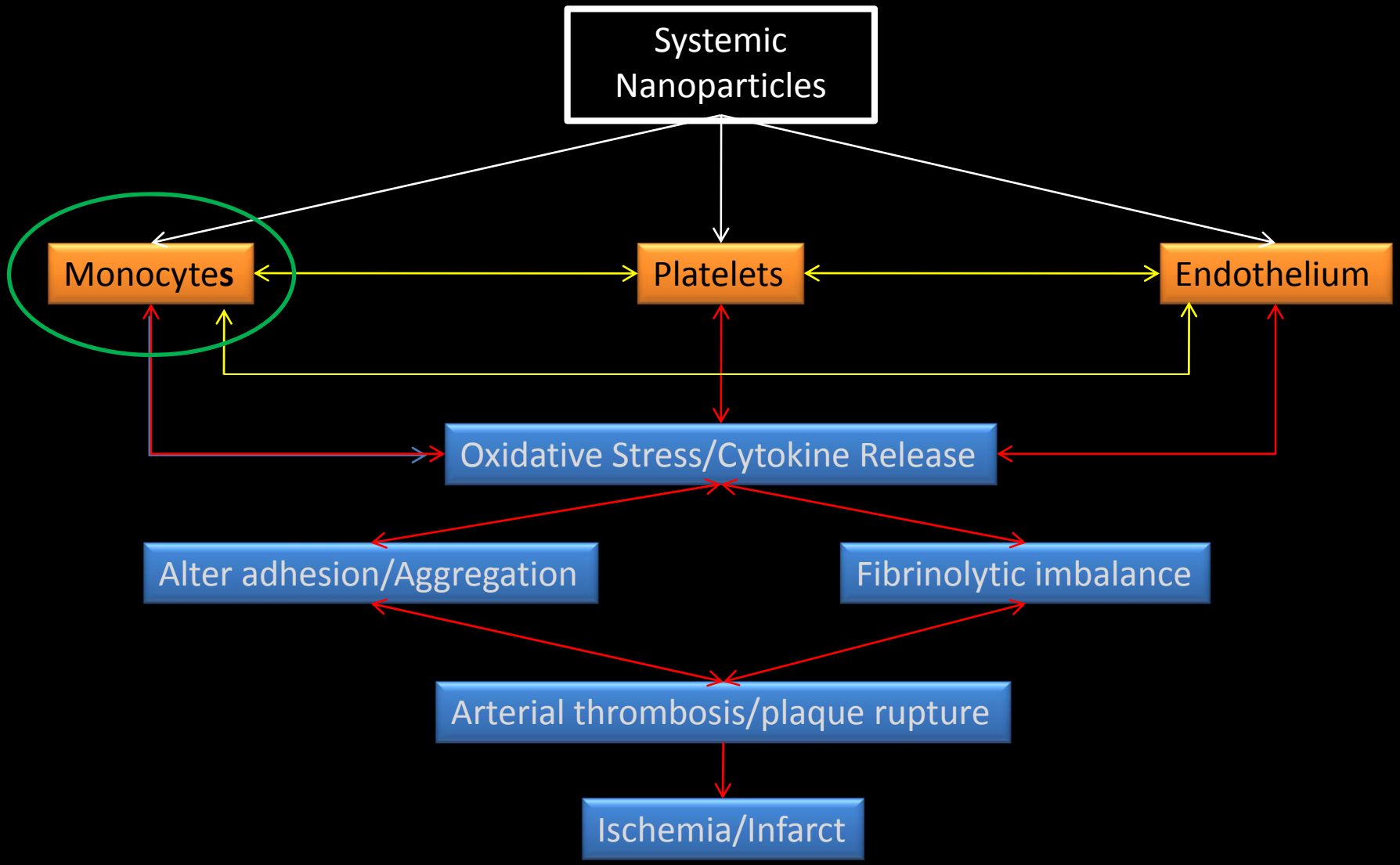
Saline



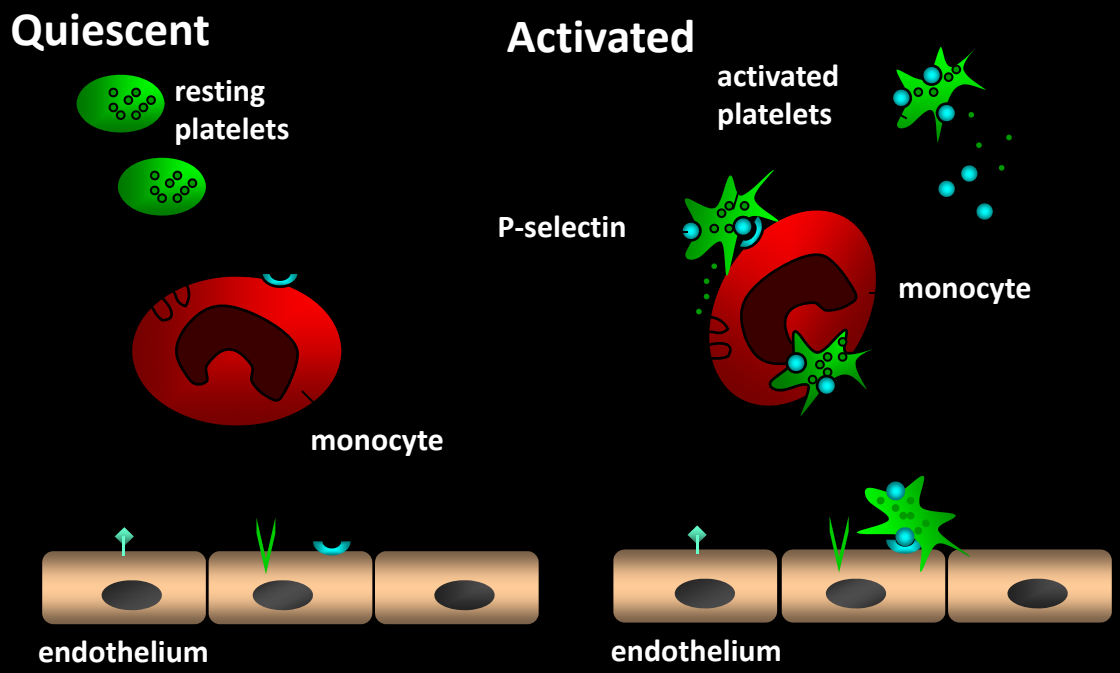
Carboxy
beads

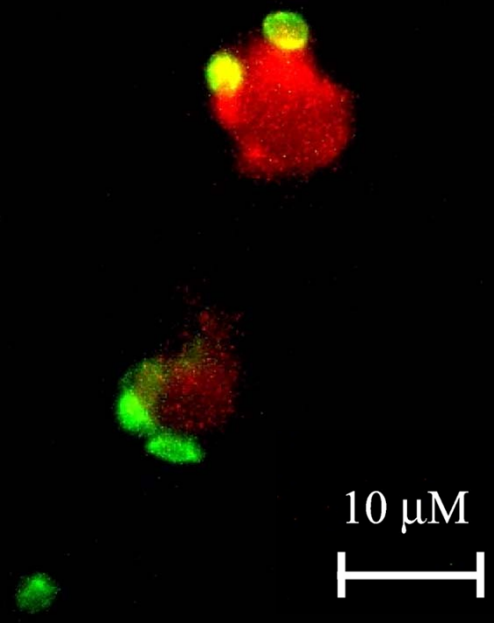


Potential fate of medical nanoparticles



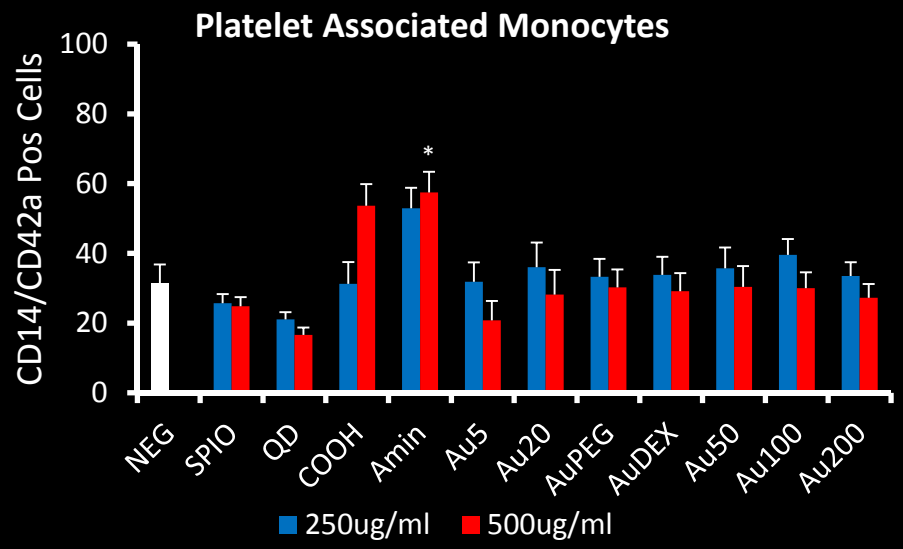
Upregulation of platelet-monocyte aggregates in unstable angina

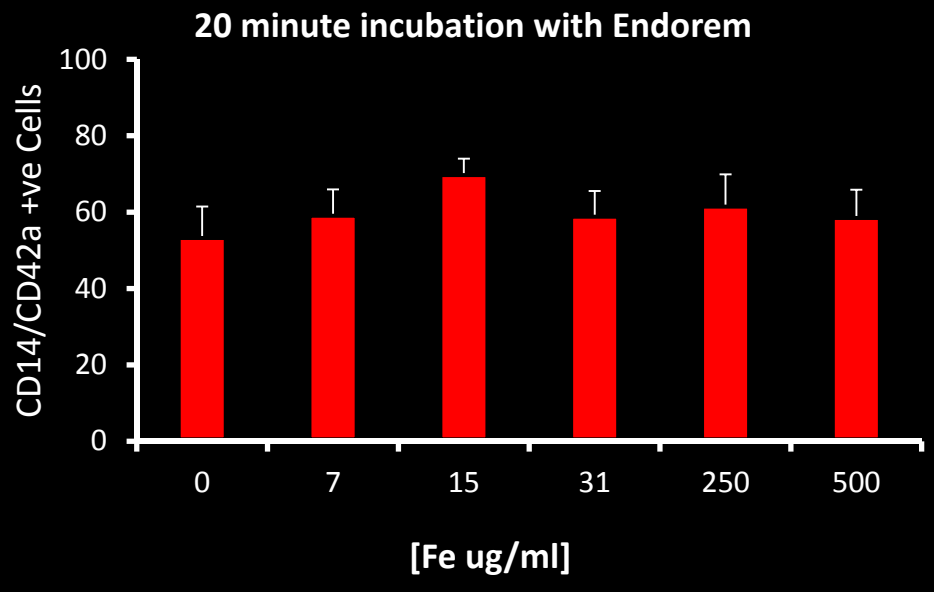




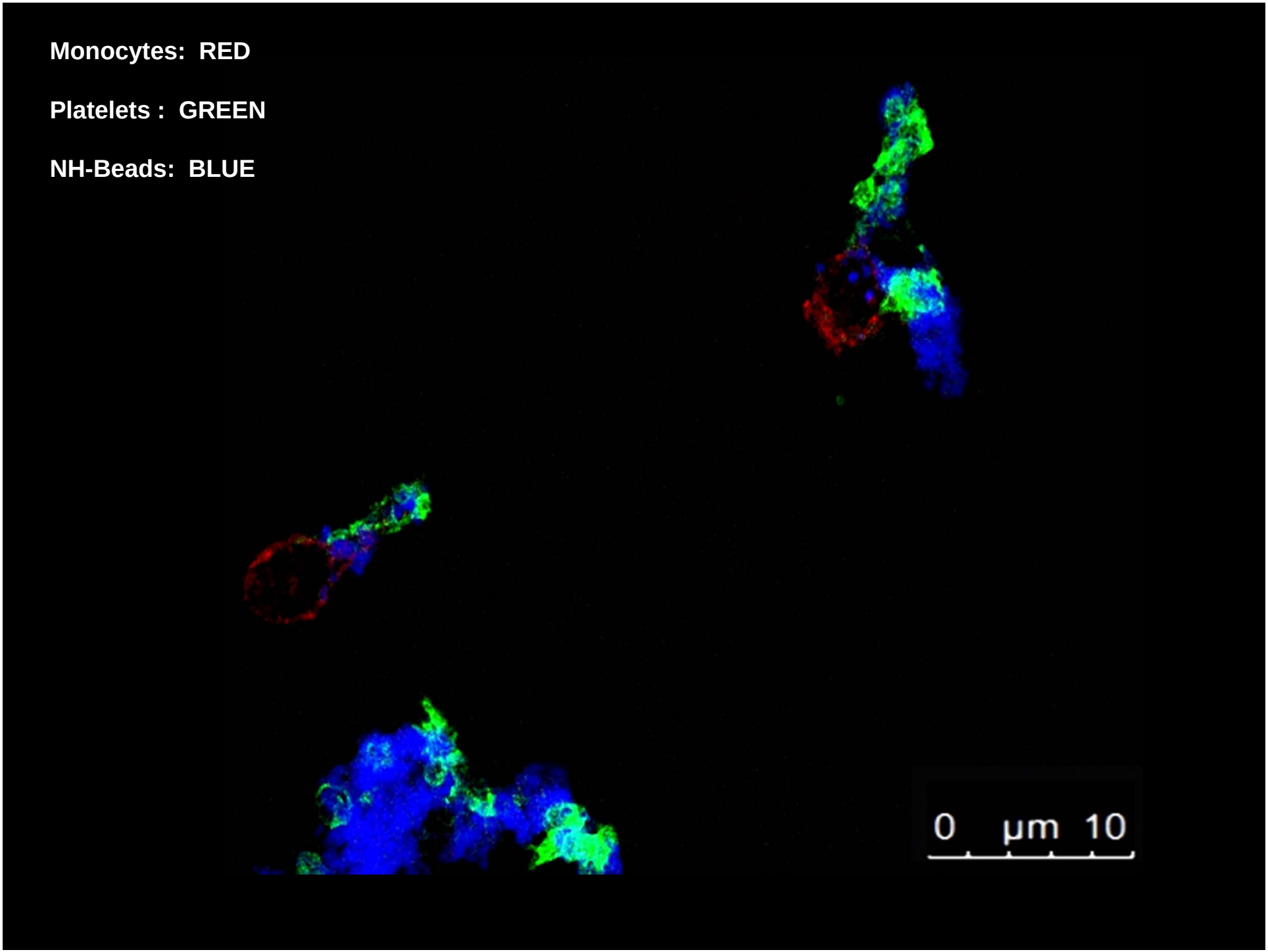
Sarma et al. Circulation 2002;105:2166-71

Platelet monocyte binding after NP Exposure



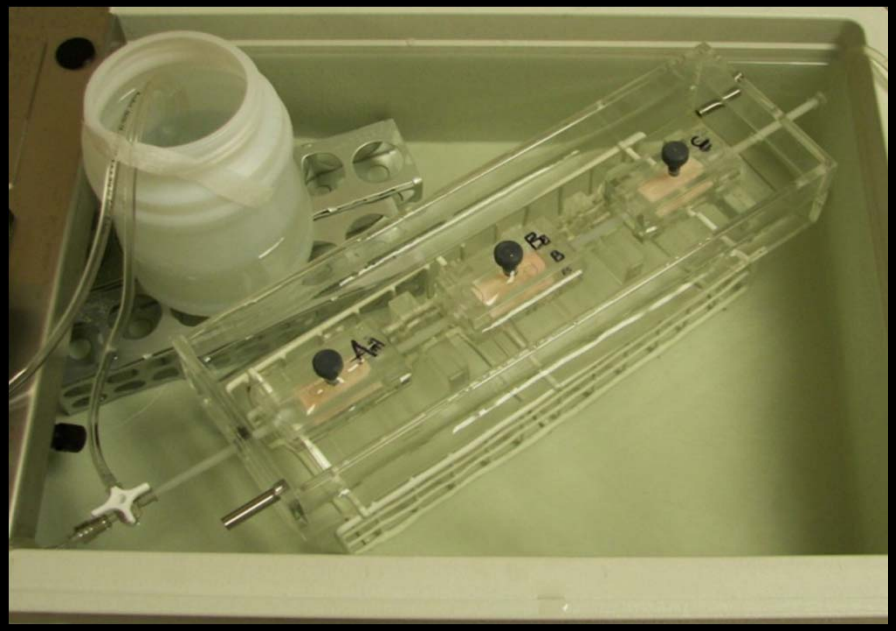


N=5, * p<0.05

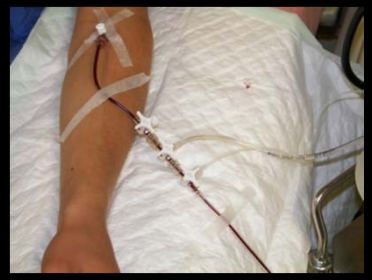

Monocytes: RED
Platelets : GREEN
NH-Beads: BLUE
0 µm 10

The Badimon Chamber
Ex Vivo Model of Human Thrombosis

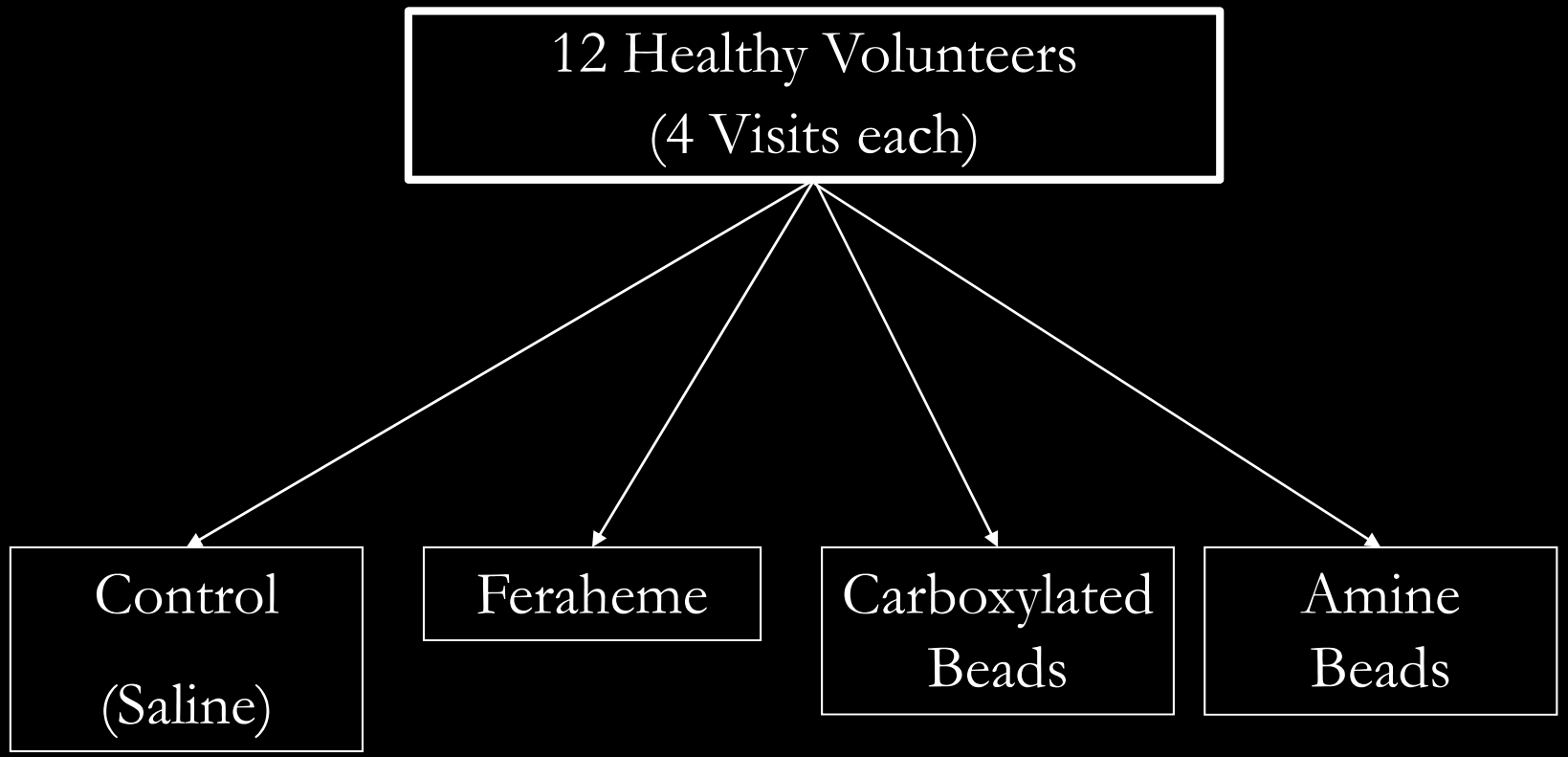
- Well established and validated method
- Advantages over other techniques
  - Human exposure sysytem
  - Thrombus forms under conditions of continuous flow
- Flow conditions and thrombogenic substrate well characterised and reproducible
  - Models deep arterial injury
- The provision to add compounds into the extra-corporeal circulation



Slides digitally acquired at ×10 magnification and stained with Combined Massons Elastin stain and with an anti-fibrin stain



- Randomised
- Double-Blind
- Controlled
- Cross-Over



Acknowledgements

Jennifer Raftis

Dr Nick Mills

Prof. Ken Donaldson
Prof. Dave Newby

Dr Andrew Lucking

Prof. Mark Bradley
Frank Thielbeer

Colleagues in the Centres for Inflammation Research
and Cardiovascular Sciences