The Health Effects Of Nanoparticles
What, Where, How?

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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
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

**Proposed Mechanism**

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

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
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

<table>
<thead>
<tr>
<th>Particle</th>
<th>Size (nm)</th>
<th>Functionalisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene beads</td>
<td>50, 100, 200</td>
<td>OH</td>
</tr>
<tr>
<td></td>
<td>50, 100, 200</td>
<td>COOH</td>
</tr>
<tr>
<td></td>
<td>50, 100, 200</td>
<td>NH</td>
</tr>
<tr>
<td></td>
<td>50, 100, 200</td>
<td>Dex</td>
</tr>
<tr>
<td></td>
<td>50, 100, 200</td>
<td>PEG</td>
</tr>
<tr>
<td></td>
<td>50, 100, 200</td>
<td>CY5.5</td>
</tr>
</tbody>
</table>

Made in collaboration with Professor Mark Bradley’s group, University of Edinburgh.
# Commercially available nanoparticles

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

*Commercially available nanoparticles*
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

Systemic Nanoparticles

Monocytes → Platelets → Endothelium

Oxidative Stress/Cytokine Release

Alter adhesion/Aggregation → Fibrinolytic imbalance

Arterial thrombosis/plaque rupture

Ischemia/Infarct
Flow cytometric analysis of platelet activation markers

**P-selectin Positive Platelets**

- 250ug/ml
- 500ug/ml

**PAC-1 Positive Platelets**

- 250ug/ml
- 500ug/ml

**CD62P/PAC-1 Positive Platelets**

- 250ug/ml
- 500ug/ml

* P<0.05
Assessment of platelet viability following exposure to nanoparticles

30 min incubation with particles  
n=4, **p<0.01
Exposure to amine beads caused a significant increase in platelet aggregation at relatively low doses.

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Oxidative Stress/Cytokine Release

Alter adhesion/Aggregation ➔ Fibrinolytic imbalance

Arterial thrombosis/plaque rupture

Ischemia/Infarct
Upregulation of platelet-monocyte aggregates in unstable angina

Quiescent

resting platelets

monocyte

Activated

activated platelets

P-selectin

monocyte

endothelium

Platelet monocyte binding after NP Exposure

N=5, * p<0.05
Monocytes: RED
Platelets: GREEN
NH-Beads: BLUE
The Badimon Chamber

*Ex Vivo* Model of Human Thrombosis

- Well established and validated method
- Advantages over other techniques
  - Human exposure system
  - 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
The Effect of Exogenous t-PA

12 Healthy Volunteers (4 Visits each)

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

Control (Saline)
Feraheme
Carboxylated Beads
Amine Beads
Acknowledgements

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