Role of Respiratory Biofluids in the in vitro Interactions of Graphene Oxide Sheets with Macrophages.

Alexander Fordham¹,² and Cyrill Bussy ¹
alexander.fordham@postgrad.manchester.ac.uk ; cyrill.bussy@manchester.ac.uk

Introduction
- Prevalence of Graphene Oxide (GO) is increasing rapidly.
- Inhalation route of exposure will be a common exposure pathway for GO.
- Macrophage predominant immune component within lungs.
- Study the interactions of GO with macrophages.
- Observe how mucus and lung surfactant affect these interactions.

Hypothesis & Aims
Hypothesis: Lung biofluids will affect GO interactions with macrophages.
Aims:
- Determine the extent of GO coronation from mucus and Lung surfactant (LSF).
- Determine effect on viability by the different corona models.
- Determine the changes of gene expression and secretions.
- Determine extent of GO coronation from mucus and Lung biofluids will affect GO interactions with macrophages.
- Minor improvement in viability from bare GO flakes compared to mucus & surfactant.
- Lower concentrations appear to have a greater decrease of viability (may be mitochondrial interference rather than true viability).

Experimental
- THP1: Macrophage M0
- THP1-d: Macrophage M1
- 10ug/ml LPS
- 100ug/ml LPS
- 0.1-50g/ml RPMI-1640, 10%FBS (2h w/o) Media
- DPPC (900μg/ml) 100μg/ml BSA (50ng/ml PMA)
- Mixing Time 2 hours
- Time points: 2hr and 24hr
- Concentration:
  - GO conc
  - Concentrations: 0.1-50μg/ml
- Media:
  - RPMI-1640, 10%FBS (2h w/o)

Material Characterization
- Zeta Potential

Material Uptake & Interaction
- Confocal microscopy
- Mucus promotes expression of pro-inflammatory genes.
- Surfactant and bare flakes do not induce a significant change.

Results
- Gene Expression/Secretion
- PCR
- ELISA

Conclusion
- Mucus more readily adheres to the GO surface, but surfactant causes the greatest positive shift in zeta potential.
- Limited uptake of the material was observed regardless of surface properties.
- Coronation with LSF models improves viability at sub-lethal doses and does not change the expression/secretion profile.

Macrophage Viability
- Alamar blue
- Limited increase in secretion at 2 h.
- At 24 h, only mucus caused large increase in secretion.