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

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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 effect of uptake by the different corona models.
- Determine the changes of gene expression and secretions.

Experimental

THP1 Monocyte → (50ng/ml PMA) → THP1-d Macrophage MO → (10ug/ml LPS) → THP1-d Macrophage M1

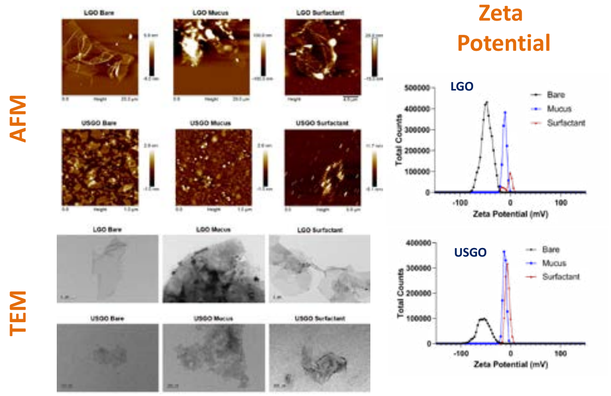
Model	Components	GO conc	Mixing Time
Lung Surfactant	DPPC (900µg/ml) BSA (100µg/ml)	100µg/ml	2 hours
Mucus	Mucin (1mg/ml)	100µg/ml	2 hours

Material	Lateral Dimensions	Thickness	C:O ratio (XPS)
L-GO	1 µm – 30 µm	1 – 5 nm	2.2
US-GO	10 nm – 300 nm	1 – 4 nm	2.2

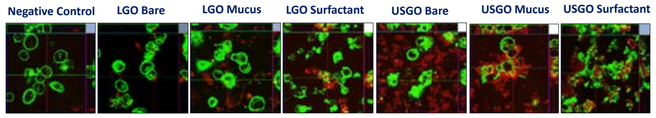
- **Time points**: 2hr and 24hr
- **Concentration**: 0.1-50µg/ml
- **Media**: RPMI-1640, 10%FBS (2h w/o)

Results

Material Characterization



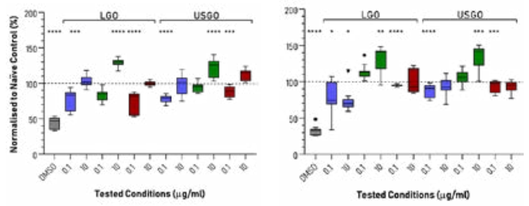
Material Uptake & Interaction — Confocal microscopy



- Limited uptake was observed for the tested conditions.
- This was regardless of lateral size or surface properties.

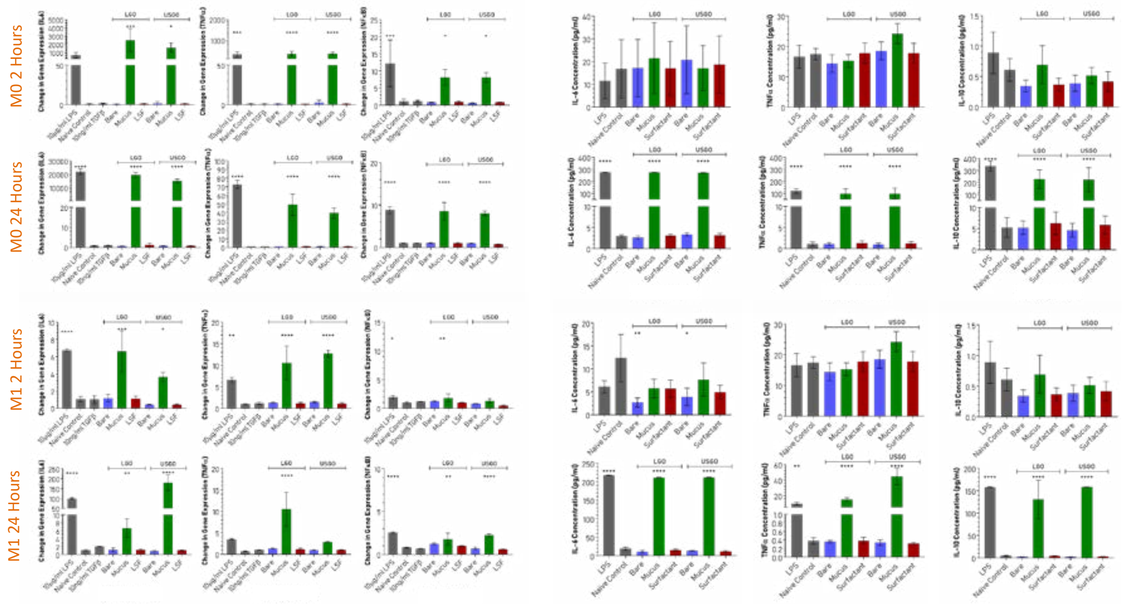
Macrophage Viability — Alamar blue

- 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).



2 Hours | 24 Hours

Gene Expression/Secretion — PCR / ELISA



- Mucus promotes expression of pro-inflammatory genes.
- Surfactant and bare flakes do not induce a significant change.
- Limited increase in secretion at 2 h.
- At 24 h, only mucus caused large increase in secretion.

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.**

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