In-vitro modelling of the lungs response to environmental nanoparticulates

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Introduction

Nanoparticles are particles in the range of 1-100nm having at least one dimension less than 100 nm and comprise a class of materials that exhibit unique physical, chemical, and biological properties, differing distinctly from their subsequent small molecules and bulk materials(1). Due to this they are widely used in a variety of applications and products due to their unique material properties thus human exposure is inevitable.

WHO recommendations on air pollution don't focus on the 1-100nm range for nanoparticles(2) and typically testing the toxicity of such particles is performed using animal models, which have been invaluable. However, regardless of the studies investigating predictability of animal studies for replicating within human clinical trials show only 37% were replicated in humans and 20% were contradicted when tested in humans, the FDA still recommend animal testing for toxicity(3).

Animal models remain the gold standard, with no standalone alternatives in representing human lung toxicity interactions.
Therefore, the use of High content imaging/analysis, cell culture, traditional viability assays and expression studies will be used in conjunction to bridge this gap.

Aims

- Develop a better understanding of nanoparticle toxicology
- Produce reliable predictive models – cost and time effective
- Reduce need of animal models that are not representative

Method

The use of traditional cell culture remains un-translational standalone, however with the addition of High content imaging and analysis in conjunction with other method may provide the solution.

Cell culture

NCI-H441 (ATCC) were cultured at 37 °C in 5% CO_2 atmosphere They were seeded on a 96 well plate and incubated for 24h before exposure.

Table-1: shows the range of nanoparticles being tested

Nanoparticles		
Copper Oxide	Cerium (iv) oxide	Wollastonite
Silicon dioxide	Polystyrene	AgCu
Zinc Oxide	A1 test dust	Gel

Cell Health

Cell were exposed to the various nanoparticles (Table-1) at decreasing concentrations from 100µg/ml and incubated at 37 °C in 5% CO_2 overnight. Then for morphology assessment (bridging the gap which traditional assays and cell culture do not provide in this level of detail), cells were stained with Hoechst, MitoTracker Red, Image-It Dead Green and Cell Mask Deep Red, proceeding to be fixed and imaged using a automated fluorescence imager Invitrogen's EVOS M7000.

Quantitative high content analysis

Automated image acquisition allows for the capture of multiple images per well, stitched together for the segmentation of cells and quantification of cell health.

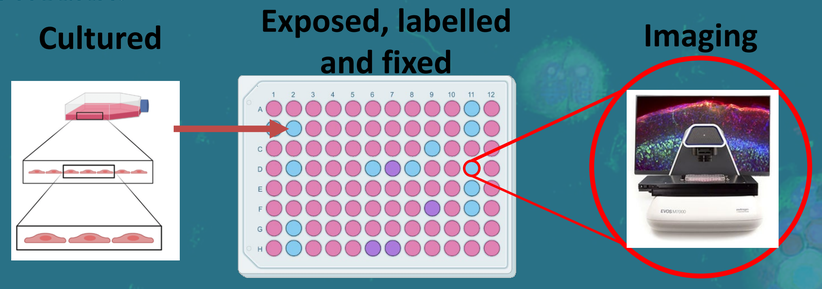


Figure-1: shows the procedure from cell culture, NP exposure and cell fixing (PFA-paraformaldehyde) to high content imaging

Thermofisher's Celleste image analysis software allows for the segmentation and subsequent analysis of the images. This uses the nuclear staining and cell mask for cell segmentation (Figure-2), allowing for the quantification of marker intensity, localisation and ultimately providing information on morphometric changes from nanoparticle exposure.

A In-house method of data analysis was utilised to analyse the morphometric changes from the raw Celleste image data after segmentation allowing for distribution and population analysis over the many different parameters such as membrane permeability of the cells.

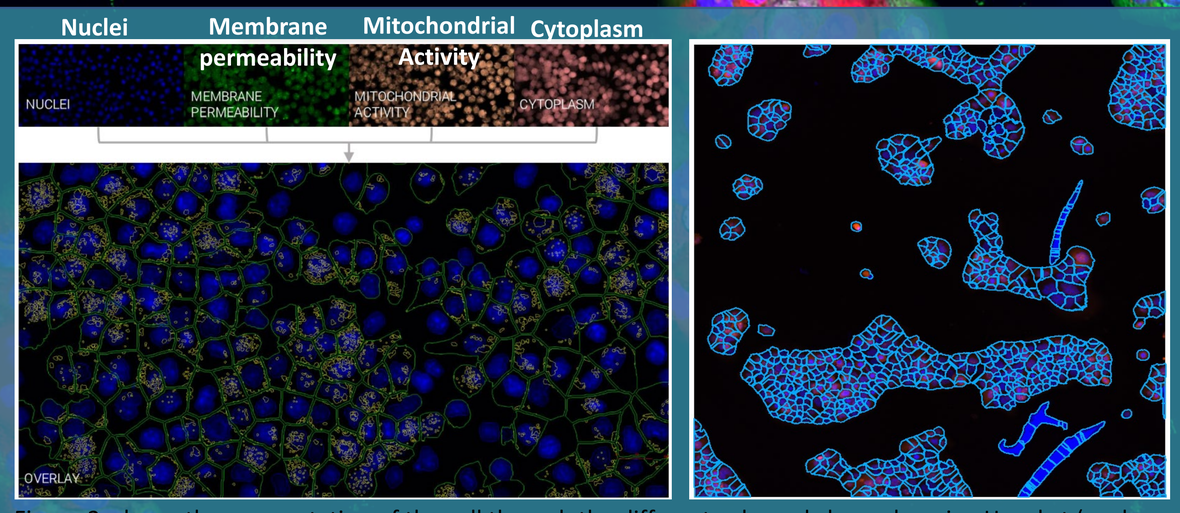
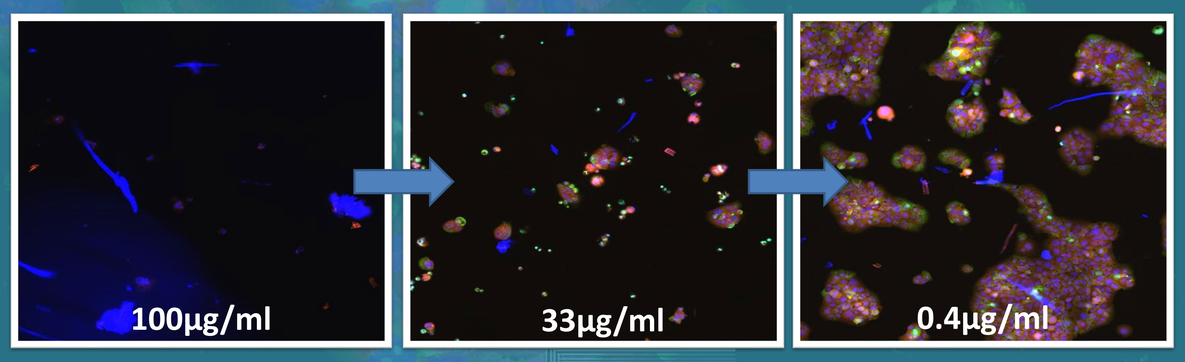


Figure-2: shows the segmentation of the cell through the different coloured channels, using Hoechst (nuclear stain) and Cell mask (cell stain)

Results and discussion

Initial observations of the high content imaging shows dose dependent toxicity with 100µg/ml (of silicon dioxide example) having the greatest amount of cell death in comparison to lower doses of nanoparticles.



This is somewhat mirrored in the distributions of parameters of interest for which comparing different concentration exposure, were able to show discernible changes to the cells against each other and untreated cells. For example, (Figure-3) does not contain the distribution of the highest concentration of Copper oxide nanoparticles, because as like silicon dioxide no cells are viable at that concentration.

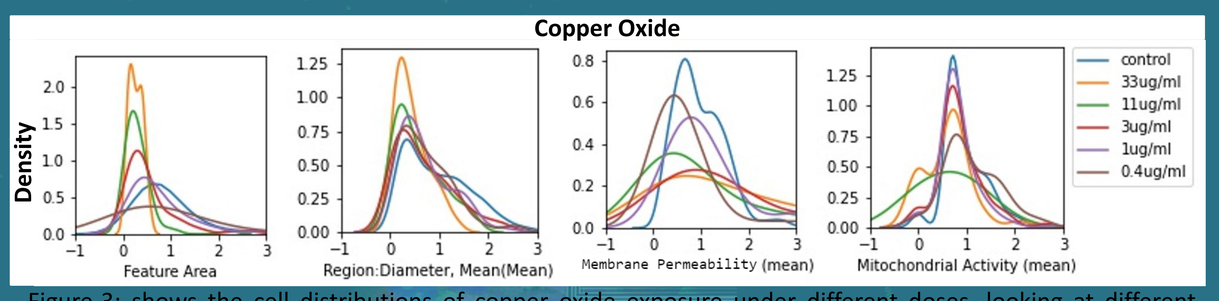
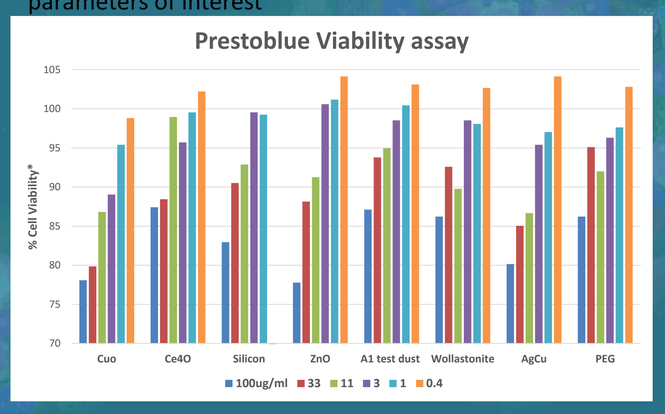


Figure-3: shows the cell distributions of copper oxide exposure under different doses, looking at different parameters of interest



Initial results show promising correlations to what we are seeing within the distributions with the traditional viability assay Prestoblue, which shows a dose dependent nature when normalised to the max of the control (untreated cells).

Figure-4: shows the Prestoblue viability assay absorption results for every NP of interest

Statistical analysis of the distributions are yet to be finalised, however the distributions do show promise in detecting minute differences with the cells after exposure. Additionally traditional assay are at this point ongoing.

Ongoing and Future work

Traditional viability assays: Prestoblue, LDH, DCFDA
- As a comparison to the cell health/high content imaging and an additional layer of toxicity profiling
Protein and gene expression studies: inflammation etc
- Develop an understanding on potential modes of action of the selected nanoparticles
Air – liquid interface models – increasing the complexity of the model as a comparison to traditional cell culture and in vivo
Exposure chamber toxicity models – most relevant in vitro model, allowing for psychologically relevant exposure

References

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3. Hackam DG, Hackam@ices D, Redelmeier DA, Koren G, Poussier P, Ray J, et al. Translation of Research Evidence From Animals to Humans. JAMA. [Online] American Medical Association; 2006;296(14): 1727–1732. Available from: doi:10.1001/JAMA.296.14.1731 [Accessed: 11th June 2022]