In-vitro modelling of the lungs response to nanoparticulates

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Introduction

Nanoparticles are particles in the range of 1-100 nm 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). Human pulmonary exposure is inevitable with the abundance of everyday consumer products and the environmental release of nanoparticles. What is evident, is the poor understanding and investigation of the health risks nanoparticles may pose both in acute and chronic exposure(1).

Regarding inhaled compound toxicology, historically the translation into human subjects from animal models is particularly unpredictable. Thus, recently there are ethical concerns and more importantly scientific questioning the clinical application and validity of animal testing (2). Nevertheless, for toxicological studies, animal models remain the gold standard, and there are no standalone alternatives in representing human lung toxicity interactions.

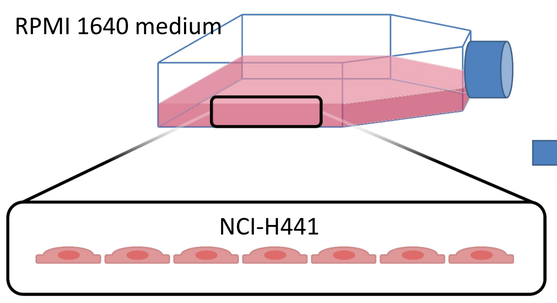
Cell culture can provide many benefits such as being inexpensive and time efficient in comparison to animal models but have their own limitations for translation to humans. With the aim to bridge this gap this project investigates the development of a standalone model which encompasses the use of 2D cell culture, high content imaging and analysis to give us a more detailed account and understanding of the morphological changes of individual cells exposed to known toxic nanoparticles.

Aims

- Develop a better understanding of nanoparticle toxicology
- Produce time and cost effective predictive models
- Reduce need for animal models

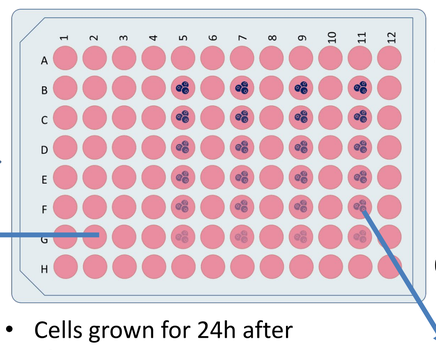
Method

Culturing of H441 alveolar epithelial line



ImageItDead Green – permeability marker
Hoechst – nuclear stain
Mitotracker red – mitochondrial stain
Cell mask deep red – cell stain

Plating, exposure with CuO and addition of cell health fluorescent markers

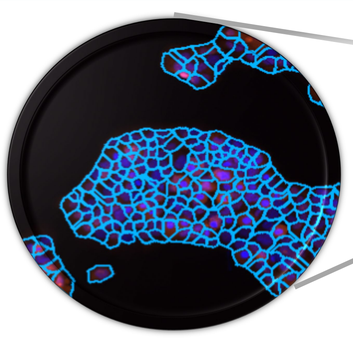


- Cells grown for 24h after plating
- Then exposed for 24h before cell health assay
- (37°C, 5% CO_2)

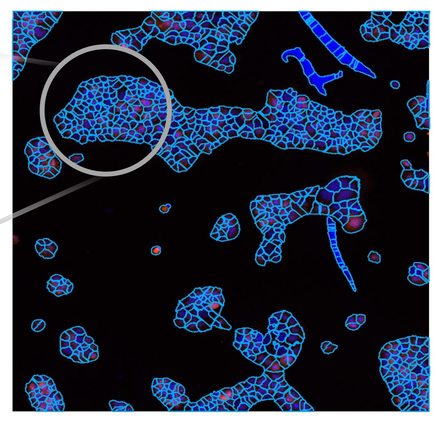
CuO exposed cells varying concentrations

Fluorescence imaging

Automated fluorescence imaging of plate (EVOS M7000)



- Morphometric parameters were quantified based on nuclear, membrane permeability, mitochondrial activity and cytoplasm staining using Celleste image analysis software.
- Segmentation was performed using nuclear and cell stains
- Segmented date were z-scored normalized in Python to allow for comparisons between plates.



Parameter quantification using Celleste image analysis software

Results and discussion

Initial visual observations of the high content imaging showed a dose dependent toxicity of the copper oxide (CuO) treated cells. Cell number decreased with increasing CuO concentration and was comparable for untreated cells and the lowest nanoparticle concentration both between 500-600 cells with imaging 5% of the well.

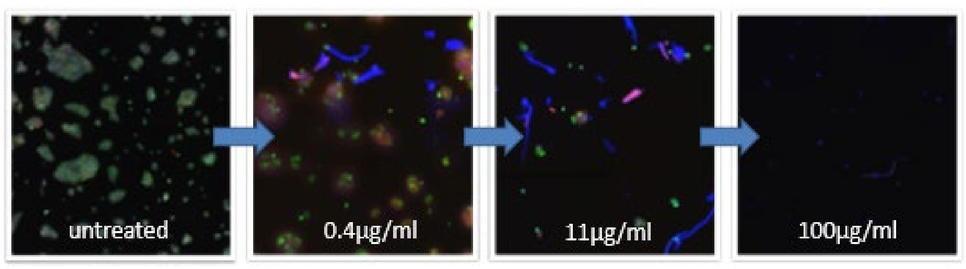


Figure-1: shows cells treated with different concentrations of Copper oxide (CuO)

With the use of Celleste, distributions of several parameters were represented for Cell area, cell mean diameter etc, focusing on the shape, size and morphometric changes of the cells rather than cell number. This allows for a more detailed view of cell toxicity which may not be observable with traditional viability assays.

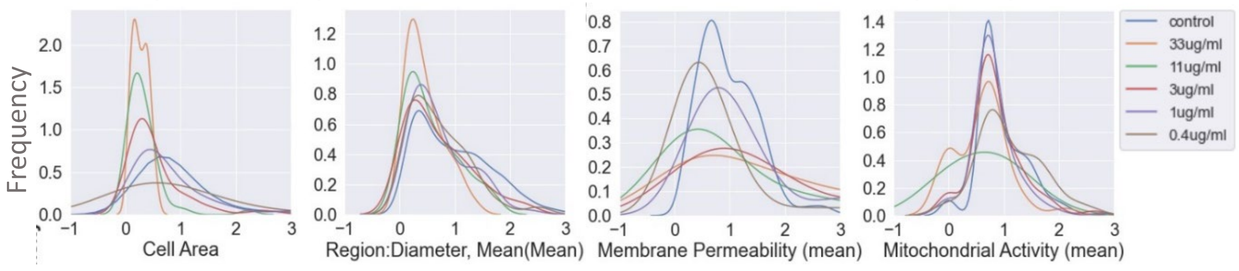


Figure-2: shows the cell distributions of copper oxide exposure under different doses, looking at different parameters of interest

The distributions presented in Figure-2 cell area showed a reduction in the size of the cell with an increasing dose of nanoparticle. At the maximum exposed dose of 100 µg/mL, no distribution was present due to absence of cell images for segmentation and quantification, following cell death as observed from the image in Figure-1. In regards to the membrane permeability, a larger proportion of cells had a greater intensity of permeability for the highest concentration (33 µg/mL, distribution range 2 to 3) compared to lower concentrations (0.4 µg/mL, distribution range -1 to 2). This is juxtaposed to mitochondrial activity, which presented a bimodal distribution at the high concentrations (a mode between -1 to 0.5), indicating a population with low mitochondrial activity. Furthermore, low concentrations led to a population of cells with high mitochondrial activity suggesting a population of viable cells. However the amount of raw data led to the use of dimensionality reduction for reduce the model's complexity.

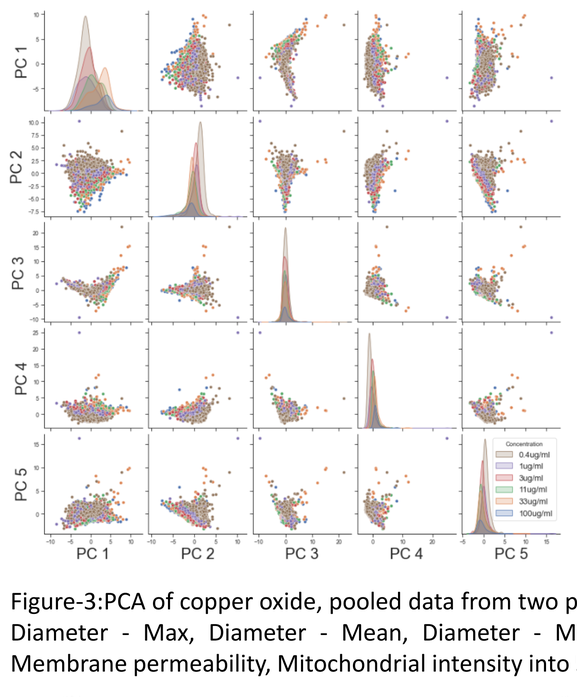


Figure-3:PCA of copper oxide, pooled data from two plates, grouping Cell Area, cell Intensity, Circularity, Clumpiness, Diameter - Max, Diameter - Mean, Diameter - Min, Heterogeneity, Hole Area, Hole Area Ratio, Roundness, Membrane permeability, Mitochondrial intensity into 5 principal components sorted by concentration.

Dimensionality reduction was performed on 13 features reducing them into 5 principal components via principal component analysis (PCA) (Figure-3) and the data were separated by concentration after plate data pooling. The principal components allow for visualisation of the data's multidimensionality showing a shift in the toxicity (PC1 vs PC2) as you increase the CuO concentration. The cumulative variance of this PCA was 74.75% (normalised data), disregarding 25.25% of the total dataset, suggesting more reproducible repeats are required to derive a discernible difference in the comparison of the components.

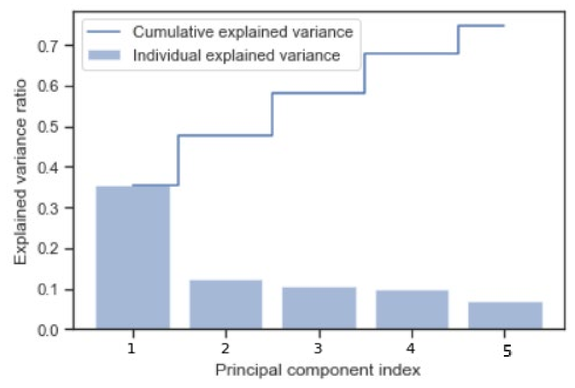


Figure-4: cumulative variance of the individual principal components

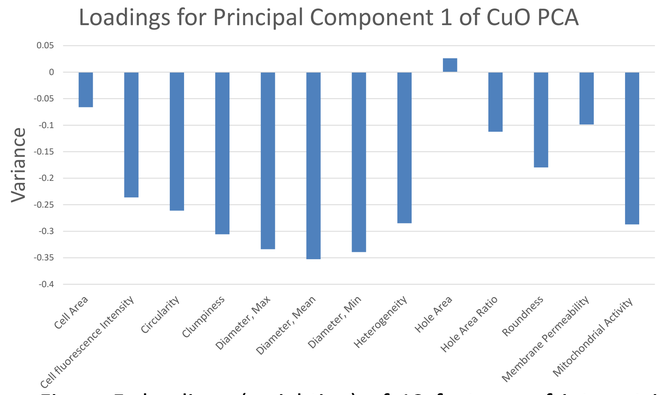


Figure-5: loadings (weighting) of 13 features of interest in the largest weighted variance principal component 1

The cumulative variance plot (Figure-4) demonstrated that the majority of the variation in the dataset was attributed to the initial principal component, however over 60% of variance was explained by PCs 2-5, approximately equally distributed. The loadings plot (Figure-5) shows the variance of all morphometric features for the principal component with the largest weighting in variance. The features most contributing to that weighting seem to be the cell diameters and mitochondrial activity of the cell.

Conclusion

In conclusion, the distributions and PCA show that there are apparent differences between different concentrations of CuO which may not be seen by viability assays such as Prestoblue. However to quantify this difference more reproducible replicates need to be performed to compute the toxic characteristics of CuO on cell health.

References

1. Yang W, Wang L, Mettenbrink EM, DeAngelis PL, Wilhelm S. Nanoparticle Toxicology. *Annual Review of Pharmacology and Toxicology*. [Online] Annual Reviews; 2021;61(1): 269–289. Available from: doi:10.1146/annurev-pharmtox-032320-110338
2. 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]