Rationale and Aims:
Engineered nanomaterials (ENM) pose an inevitable health risk to humans through long-term, repetitive, low-dose exposures. We aim to develop a system that allows prediction of the long-term impact of ENM exposure, achieved by an advanced in vitro system using short-term exposures via optimising the alveolar lung model with in vitro extrapolated dosing strategies, resembling realistic ENM lung exposures, combined with specific biological endpoint measurements.

Characterisation of epithelial layer, addition of macrophage-like cells and implementation of established dosing strategy to enable both single and repeated ENM exposures

An alveolar airway co-culture model to determine the potential (pro-)inflammatory impact of ENMs.

**Figure 1.** Characterisation of A549 cells. Membrane integrity measured by transmembrane electrical resistance (GΩ) and live cells (FACS) - measured the transfection of the live cell from the apical to the basal side of the monolayer in a control culture method. Higher fold values against the negative control represents lower membrane integrity, (B) Membrane integrity of the culture (B) Cell count and viability of the cell culture, (C) - all values are expressed in triplicate. The data is presented as the mean ± SEM. Significance is denoted as the following: compared to either Sub or ALI day 1 p<0.05 (*);

**Figure 2.** Characterisation of epithelial layer, addition of macrophage-like cells with specific biological endpoint measurements.

**Figure 3.** Particles and exposures used: (A) DQ12 used in this work. Concentrations used were extrapolated from characterisation and historical in vivo data (PNN method); range is taken from the (Cancer Research UK) database. **Figure 4.** Optimal exposure conditions to achieve the maximum uptake and duration. Cells within the Quasi-ALI system (Endes, Thex) were exposed to the material twice over 48 hours (repeated dose) before the co-culture was used. Cells within the VitroCell Cloud2 system were exposed and analysed 6, 24 and 72 hours post-exposure. DQ12 cytometry work has been completed using both the Quasi-ALI exposure method and the VitroCell Cloud2, with the Quasi-ALI method. Whole blood exposure data also been demonstrated.

**Figure 5.** Repeated exposure over 48 hours. DQ12, TiO2, ZnO and BaSO4 were exposed at a Quasi-ALI repeated exposure over 48 hours. These exposures indicated that the co-culture was more sensitive to the effects of the ENM (increase in IL-6 and IL-8) except the response to BaSO4, which caused an increased IL-6 response in the monocoulture compared to the co-culture. Within the co-culture models, DQ12 elicited the biggest IL-6 response, with ZnO causing the highest IL-8 response when compared to the negative control (p<0.01).

**Figure 6.** Changes in gene expression as analysed by PCR (A549+dTHP-1). RT-PCR was completed at each time point (6, 24 and 72 hours post-exposure) and for each ENM concentration used. Concentrations were measured using the same exposure method.

**Figure 7.** Changes in gene expression as analysed by PCR (A549+DQ12). RT-PCR was completed at each time point (6, 24 and 72 hours post-exposure) and for each ENM concentration used. Concentrations were measured using the same exposure method.