SAQN Awards End of Project Report

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Project Title	
Impacts of inhaled nanoparticles on the brain	
Project Team	
Name	Role (PI / Co-I)
Dr. Chang Guo	*PI*
Dr. Andy Ward	*Co-I*
Dr. Sanghamitra Mukhopadhyay	*Co-I*
Dr. Nwabueze Emekwuru	*Co-I*
Proposed activities (copy from your project proposal)	
1. *Nanoparticle exposure (PHE, 4 weeks): In vitro model of blood-brain barrier (BBB) using human brain endothelial cells (e.g. HBEC-5i) will be maintained in the Transwell inserts, with abluminal (brain-facing) compartments and luminal (blood-facing) compartment with nanoparticle agglomerates.* 2. *Observation of the nanoparticles in the BBB (STFC, 2 weeks) applying high-resolution microscope, focus on the tight junctions of BBB, and intracellular trafficking-involved, as well as the disruption on the BBB membrane permeability.* 3. *Use neutron spectroscopy (STFC, 2 weeks) to identify any bioactivity of nanoparticles before and after associated with the BBB.* 4. *Identify the biological toxicity effects of nanoparticles in the BBB (PHE, 12 weeks), including neuroinflammatory effects, release of cytokine/chemokine (e.g. TNF-α, IL-1β, IL-6, CCL11 etc), oxidative stress, as well as effects on barrier integrity and the effects on junction adhesion proteins.*	

5. *Explore more on the liquid transport characteristics of inhaled nanoparticles between blood and brain (COV, 4 weeks).*

Please report on the activities completed in the project

1. *The test particles are particulate matter (PM) samples (PM2.5 and PM10) collected from London roadsides and diesel exhaust particles (DEP). Particle samples of PM2.5 and PM10 were kindly supplied by Dr Ian Mudway (Imperial College London).*

2. *Particle exposure: In vitro model of blood-brain barrier (BBB) using human brain endothelial cells (hCMEC/D3 cells) were exposed to particles (PM2.5, PM10, DEP) at different concentrations (0-100 µg/mL) for different lengths. Cells were observed under light microscopy. Toxicity assessment was performed later. Immunofluorescence experiment was performed for the tight junction staining and lysosome identification. The above would contribute to identify the parameters of particle exposure for samples later.*

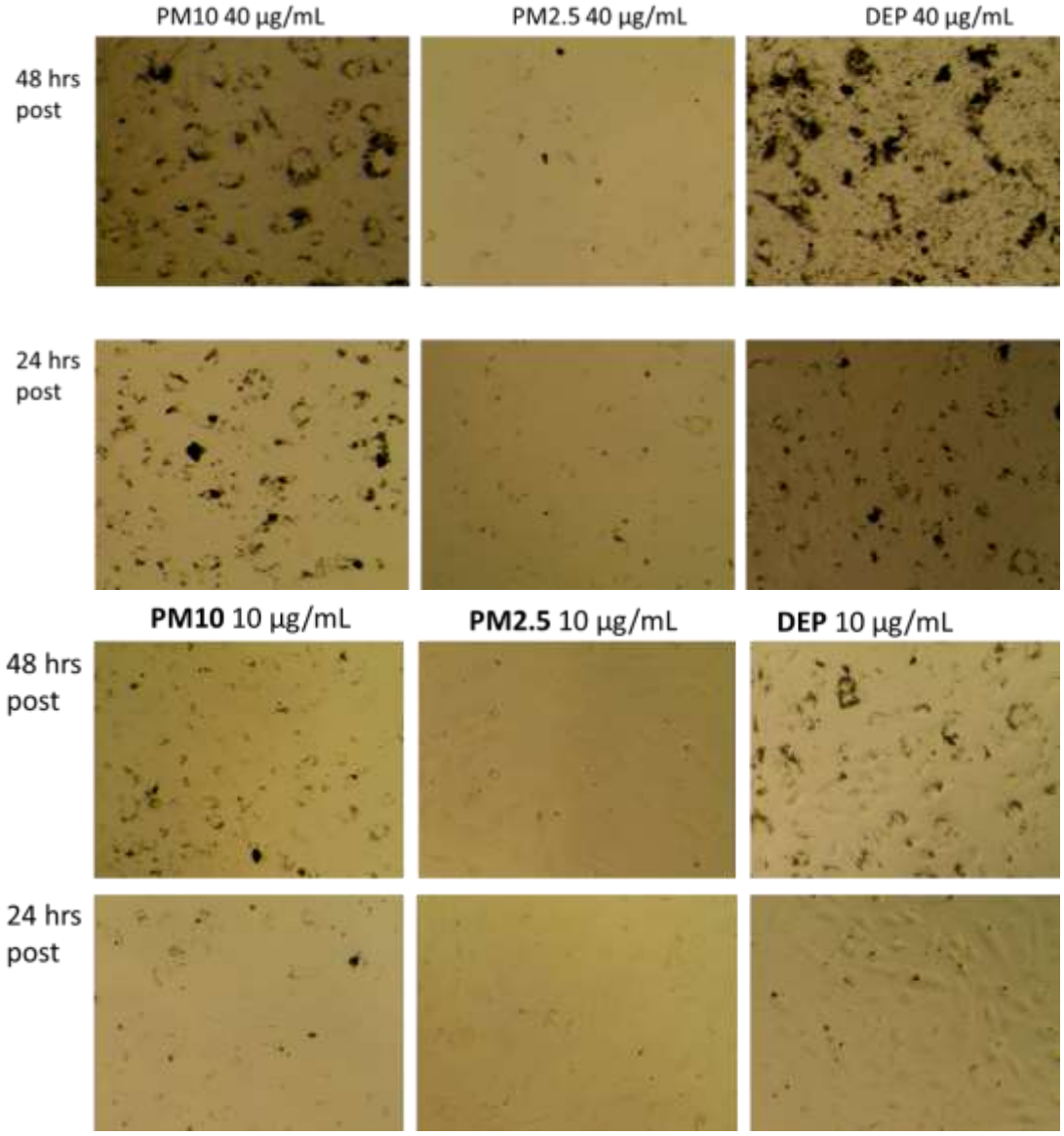

Figure 1. Representative images of hCMEC/D3 cells exposed to particles.

3. *Identify the biological toxicity effects of nanoparticles in the BBB.*
   A modified version of the lactate dehydrogenase (LDH) assay was used to assess cytotoxicity.

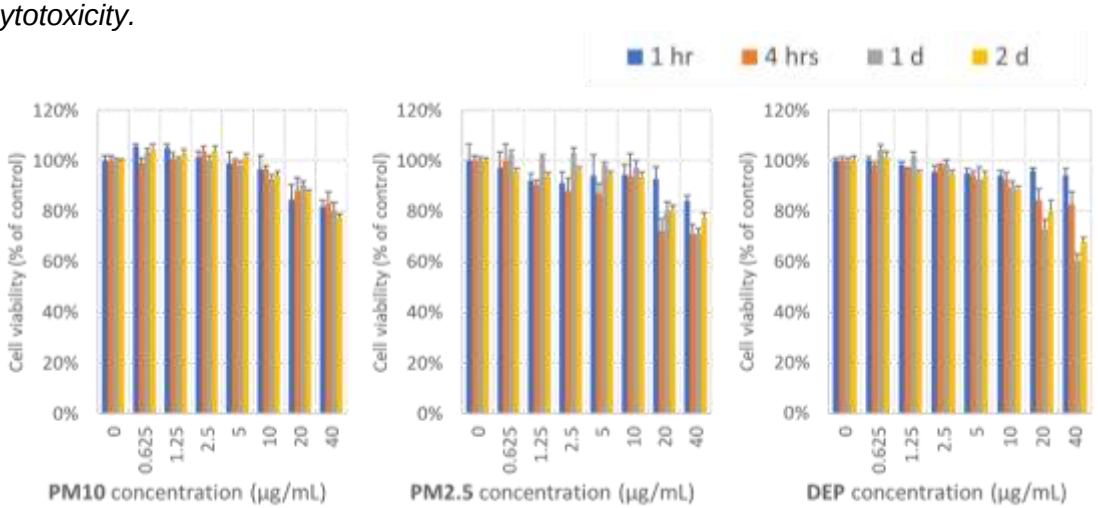

Figure 2. Cell viability of hCMEX/D3 cells exposed to particles.

4. *Optimization of BBB cellular models*
   - *hCMEC/D3 cells were maintained in the Transwell inserts, with abluminal (brain-facing) compartments and luminal (blood-facing) compartment with nanoparticle agglomerates.*
   - *Cytotoxicity on hCMEC/D3 cells on Transwell culture: Toxicity assessment based on the LDH release in the apical and basal medium were assessed.*

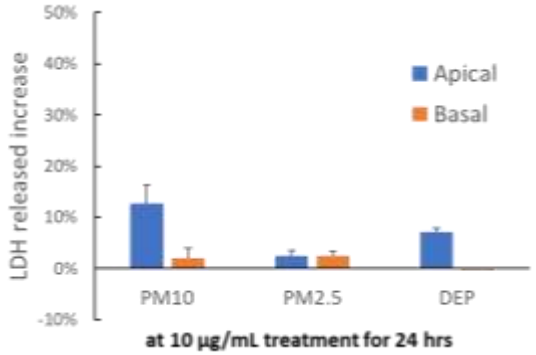

Figure 3. Changes in LDH release in the apical and basal medium of hCEMC/D3 cells cultured on Transwell inserts.

   - *Barrier integrity and surface tension: TEER measurement was performed at 10 μg/mL treatment. The surface intension was relatively low than expected for BBB barrier.*

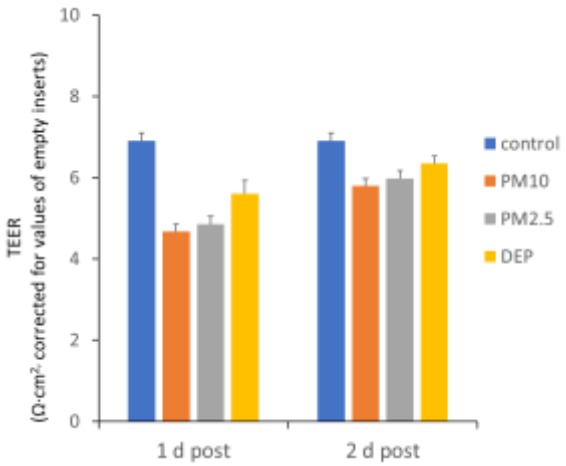

Figure 4*. TEER measurement of cellular barriers formed by hCMEC/D3 cells.*

- *Optimisation of staining of tight junction using several antibodies targeting specific tight junction proteins. Some intracellular compartments such as nuclei and lysosomes were co-stained using suitable markers. This was performed on both normal cell culture condition and Transwell culture condition.*

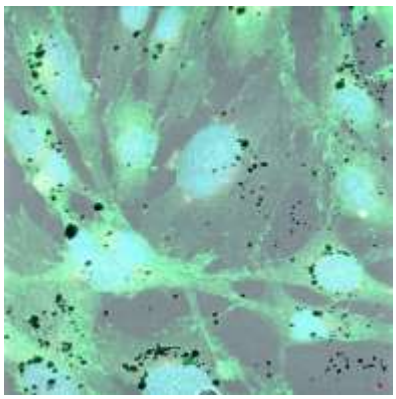

***Figure 5**. Representative confocal microscopy images of hCMEC/D3 cells (normal cell culture condition) exposed to PM2.5. Tight junctions are stained by immunofluorescence shown in green whereas lysosomes are shown in purple, and nuclei are shown in blue.*

It is concluded that Transwell culture condition is not feasible for fluorescence imaging under microscopy. Other approaches will be sought for improving this. For the above reason, experiments for observation of particles in BBB applying advanced microscopy used normal cell culture condition.

5. *Image/access the interaction of the particles with BBB cellular barriers by accessing FIB-SEM in combination with Confocal microscopy.*
   Application for direct access to Octopus system at CLF. STFC was successful. This experiment at Octopus was able to answer questions about the effects of PM exposure to BBB (e.g. toxicity, uptake, accumulation) on the cellular level:
   i) FIB-SEM can be used to explore the test particles allowing direct observation of the electron-dense carbon-based particles in biological samples (Figure 6).

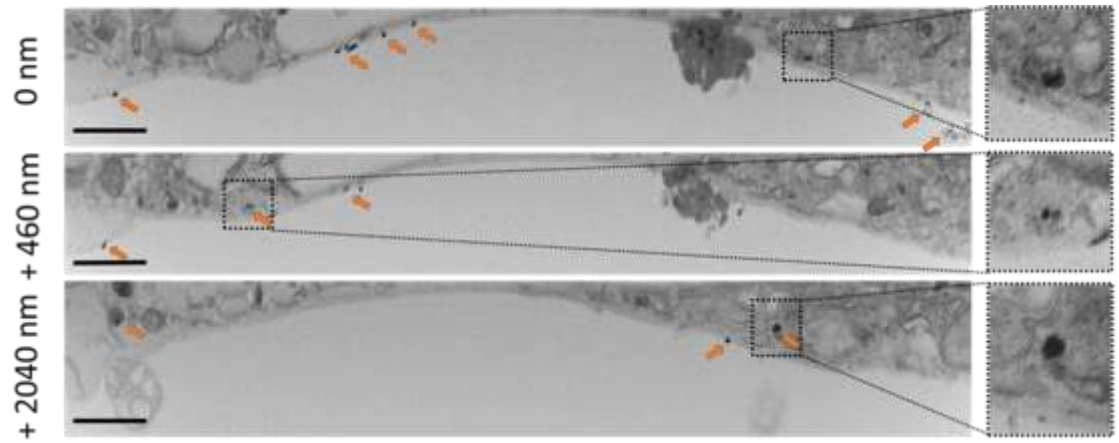

***Figure 6**. Selected FIB-SEM images of hCMEC/D3 cells exposed to fine particles PM2.5. Arrows indicate PM particles. Each slice is 20 nm. Scale bars, 1 µm.*

   ii) Collected images show that particle agglomerates could be attached to or internalised into brain endothelial cells, especially those small sized particles, indicating deagglomeration may happen during particles interacting with the plasma membrane to facilitate particles transporting through the BBB barriers.

iii) *By observing 3D ultrastructural images, some particles are found in the intracellular vesicles, suggesting endocytosis-involved internalisation, whereas some particles are found in cytoplasm without associating with any specific subcellular compartments, suggesting endocytosis-independent internalisation. The above suggests that transcytosis across BBB could contribute the particle translocation from blood circulation into the brain parenchyma.*

6. *Use neutron spectroscopy to identify any change in lipid dynamic after exposed to particles. Application to direct access of ISIS neutron beam time was successful, however, due to maintenance work on the beamline, this experiment is delayed by six months and currently has been scheduled during July 2023. Preliminary data based on Xpress access is shown below.*

   The Quasielastic neutron spectroscopy (QENS) experiments were carried out on the LET direct geometry time of flight spectrometer at the ISIS Neutron and Muon Source, STFC. This allows to probe lipid dynamics at picosecond time scale and at atomistic length scale providing microscopic insight on the effect of PM particles on lipids.

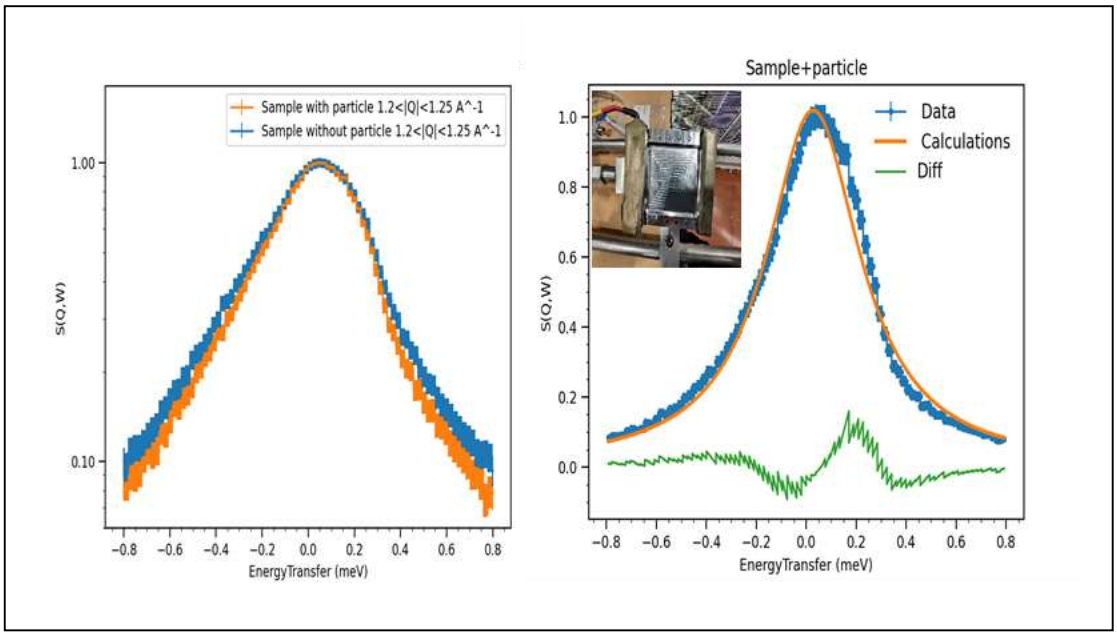

Figure 7*. (Left) QENS spectra obtained from LET Xpress run showing decrease in lipid dynamics due to presence of particles.(Right) Fitting of the spectra of samples with particle. The aluminium container loaded with lipid samples used in QENS experiments is shown as inset.*

Figure 7 shows the QENS spectra measured at 1.2 $Å^{-1}$< |Q| < 1.2 $Å^{-1}$. Q is the measure of momentum transfer having value 2π/L where L represents special dimension or length. Thus this figure represents the dynamics at the length scale of 3.56 Å. As the broadening (FWHM) of the spectra corresponds to inverse of relaxation time, Figure 7 tells us that the presence of the particle increases relaxation time of the lipid, that means the lipid becomes more rigid. A preliminary fitting with Lorentzian (Fig.7 –left) reveals that due to presence of particulates in the lipid its relaxation time decreases by about 8 picoseconds. More detailed analysis and experiments will be done on OSIRIS instrument in July, 2023.

7. *Liquid transport characteristics of particles between blood and brain*
   - *Kin and PS product are the two main parameters used for determining the rate of uptake through the brain. Kin is the unidirectional influx constant from the blood to the brain, whereas the PS product is the permeability-surface area product (that volume of plasma that gives up its content of the particular solute to interstitial fluid per unit time). The unit for both Kin and PS product is ml/min/g brain.*
   - *A number of analytical models for the study of transport mechanisms and fluid flow occurring across blood-rain-barrier were identified and, in summary,*
     1) *Trans Endothelial Electrical Resistance (TEER) model. The electrical resistance of cells can provide an indication of the ability of ions to transport through the cell membrane. Thus the permeability of the cell membrane can be measured using the voltage drop through the membrane in the TEER method.*
     2) *Frick's First Law of Diffusion. This can be used to measure the permeability of the barrier with regards to the balance (steady state) of the diffusion of the solutes through the barrier.*
     3) *Hydraulic Conductivity. With this the permeability through the membrane is measured as a function of the surface area of the membrane and the specific permeability for water through it.*
     4) *Shear Stress model. If the vessels in the BBB are treated as cylindrical vessels, then the shear stress for an ideal Newtonian fluid travelling down the vessel can be calculated, with an increase in shear rate values indicating an increase in the volumetric flow rate. This model does not account for the porosity through the vessel, however.*

What are the next steps for this research? Will you be applying for further funding? What will you need to continue researching this topic?

- *Access to facilities at ISIS Neuron and Muon Source has been delayed due to the maintenance work at the beamline. Further access to the neuron spectroscopy has been arranged in July 2023 followed with data analysis.*
- *Further access to facilities at CLF has been sought for looking for regions of particle ingress in brain tissue using advanced models (in vivo exposure models), using a workflow which has been established during previous access to the Octopus facility.*
- *Development and Improvement on the BBB cellular models.*
- *Further fundings have been sought (details in the plan part).*

Please outline the role of STFC in this project

Central Laser Facility (CLF) and ISIS Neuron and Muon Facilities at STFC provided the world class research capabilities with the unique combination of instrumentation and knowledge. This scoping project enables us to access the facilities at STFC and to work alongside STFC researchers. The expertise of researchers at both facilities have been very supportive throughout and collaboration built with this project would enable lasting collaborations in the future.

Please list a brief list of all outputs and impacts below. These may include papers, articles or blogs, presentations at events or conferences, meetings about future plans for the research. Please include links wherever possible

- *SAQN annual conference 2022, poster presentation.*
- *UKHSA annual conference 2022, oral presentation.*
- *UK Consortium of Particle Toxicology workshop 2023, poster presentation.*
- *This scoping study fund (£8,000) has in a way enabled us to access STFC facilities through peer reviewed application process.*
  - ✓ *Application for direct access to Octopus system at CLF, STFC was successful and experiment at Octopus system has been carried out in May - July 2022, with an equivalent grant of the beam time received at CLF around £40,000.*
  - ✓ *Application for direct access to neutron spectroscopy at ISIS was successful. Xpress run has been carried out in April 2022 and more experiments are to be run in July 2023. The equivalent grant of the beam time received at ISIS is about £95,000, including the time for Xpress run and experiments to be run in July 2023, and reimbursed consumables grant for sample preparations etc.*
- *A UKHSA PhD studentship, titled "How Might Air Pollution Hurt the Brain? – Understanding the Impacts of Inhaled Particulate Matter on Neurological Health", which has been granted (£119,625.82) for Oct 2022-Sep 2025. Further access to facilities at Central Laser Facility (CLF) at STFC have been made to explore if and how particles reach the brain parenchyma from blood circulation by utilising in vivo rodent models. The proposed experiment would facilitate the UKHSA PhD project by employing environmentally relevant particles combined with a workflow, which has been established previously at Octopus.*
- *A Coventry University BEng Mechanical Engineering thesis, titled "1D Analytical Model of Fluid Dynamics of the Blood-Brain Barrier." April, 2022.*

Were there any unexpected outcomes as part of the project?

The neuroinflammatory responses of the BBB cellular models was not further checked in details due to the poor surface tension of BBB barriers formed by single cell type. Also, it is challenging to do Confocal imaging on Transwell culturing condition therefore imaging correlation between FIB-SEM and Confocal microscopy would be very difficult. Moreover, collection of the samples for neutron spectroscopy would be challenging for Transwell culturing condition. For the above reasons, samples for some further analysis, including using the FIB-SEM in combination with Confocal microscopy at CLF and neuron spectroscopy at ISIS, used/will use cells from normal cell culture condition within this project.

What are your plans to share the outcomes of this research with others? (Give details of any future meetings, conferences, papers or other dissemination planned)

Further fundings have been sought elsewhere including an NC3Rs project funding scheme. An application for the funding scheme BBSRC-STFC facility access funding for bioscience partnerships has succeeded in the first stage and full proposal has been submitted in Feb 2023. An application for the UKRI cross research council responsive mode pilot scheme is currently under preparation.

Collaborations with related projects, e.g. a UKHSA PhD project focussing on the neurological effects of inhaled particles, a UKHSA PhD project focussing on "disentangling

the effects of nitrogen dioxide and particulates in air pollution on human health", a UKHSA-involved project to develop a microphysiological system interlinking gut-brain organ-on-a-chip for virus studies, and external projects e.g. NIHR HPRU project "Environmental Exposures and Health", would facilitate scientific dissemination.

Wider dissemination to the research community will be achieved by conferences and publications in peer-reviewed journals.

Project Impact: What is the most significant output/impact from this project?

This project provides us a great opportunity to build collaborations with researchers at STFC(CLF and ISIS) to pave the way for identifying the potential biological effects of inhaled particles by accessing the world class facilities at STFC.

The Committee on the Medical Effects of Air Pollutants (COMEAP) has recently published a report about air pollution and its effects on cognitive decline and dementia risk (https://www.gov.uk/government/publications/air-pollution-cognitive-decline-and-dementia). The outcomes from this project and further from the UKHSA PhD studentship project focussing on the neurological effects of inhaled particles will contribute to strengthening our understanding of the causal mechanisms driving the adverse associations reported in epidemiological studies. We hope to further strengthen the collaborative efforts in the future and to address COMEAP's recent request for further research around air pollution and neurological health, especially in human disease and susceptibility models.