Exposure to urban particulate matter has detrimental effects on cerebrovascular integrity associated with Alzheimer’s disease (AD) risk. Notably, particles in the finer <2.5 μm fraction (PM$_{2.5}$) possess xenobiotic properties, bioaccumulating at the neurovascular basement membrane, within brain cells and organelles. Due to the ease with which these particles cross biological barriers, their ubiquitous distribution may lead to early brain imbalances, exacerbating the path between oxidative stress, inflammation and misfolded protein accumulation observed in the early stages of AD.

Given the capacity of astrogliosis to undergo activation of molecular programs in response to pathological stimuli, initiation of reactive reprogramming induced by PM$_{2.5}$ may result in the elucidation of disease-specific reactive phenotypes.

The aim of this study is to assess the effects of PM$_{2.5}$-induced astrogliosis on a model of neurovascular communication as an early indicator of neurodegeneration and AD-like pathology using a human cell model.

### Methods

- **Methods 1:** Primary human cortical astrocytes were characterized for their functional and physiological properties including reactive capacity as measured through cytokine release. As a reference for reactive astrocyte behaviour, cultured primary human astrocytes were co-stimulated with proinflammatory cytokines interleukin-1β (IL-1β; 20 ng/ml) and tumour necrosis factor-α (TNF-α; 20ng/ml) for a 7-day period. Following cytokine treatment, media and cells were collected for cytokine assay and immunofluorescent staining, respectively.

- **Methods 2:** Primary astrocytes and iPSC-derived brain microvascular endothelial cells (BMECs) were seeded on the upper compartment of the transwell. Two conditions are considered: 1) A positive control consisting of a BMEC monoculture plated on top of the upper side of the insert in hESFM medium and 2) A BMEC/iPSC co-culture insert in 1:1 mixed medium, with astrocytes embedded inside the hydrogel while BMECs are seeded on top. Neurovascular intercellular communication between astrocytes and brain microvascular endothelial cells (BMECs) was evaluated by measuring transendothelial electrical resistance (TEER), indicating barrier functionality across time within a transwell-type neurovascular unit (NVU) model. Measurements were then taken every 24 hours until a clear decrease in resistance was continually observed.

### Results

**Results 1:** Short-term exposure to PM$_{2.5}$ led to an increased astrogliosis release of proinflammatory cytokine IL-6, similar to the acute response measured from prolonged treatment with cytokines IL-1β and TNF-α.

![Figure 1](image1.png)

**Results 2:** Co-culture of untreated primary astrocytes and iPSC-derived BMECs led to significantly higher TEER values and prolonged barrier functionality when compared with monocolones.

![Figure 2](image2.png)

### Conclusion

Current data suggests a distinct molecular response from primary astrocytes in relation to physiologically accurate concentrations of PM$_{2.5}$. Given the effect of astrocytes on endothelial barrier maintenance, expanding on these findings to address the effect of this reactive state on adjacent neurovascular cell types will procure a robust understanding on the wider cerebrovascular effects of environmental pollution.

### References
