3D modelling of the Blood-Brain Barrier (BBB) for nanoparticle delivery screening – a quantitative approach

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Despite advancements in drug discovery, brain tumours remain the leading cause of cancer-related death in children. The delivery of therapeutics to the brain is often restricted by the blood-brain barrier (BBB). While current 2D *in vitro* models fail to capture the complexity of the BBB, *in vivo* models are associated with significant ethical and financial costs. Therefore, advanced models that better reflect the selectivity of the BBB are essential for screening brain-permeable drugs.

Building on the model by Cho *et al.* (2017)[1], we developed a 3D BBB multi-cell type spheroid model (consisting of human brain endothelial cells, astrocytes and pericytes) for high-throughput *in vitro* drug screening. Unlike other models, the cells have been immortalised for ease of use and modified to express GFP for visualisation. The cellular organisation was confirmed by confocal microscopy, expression of key BBB markers (*e.g.* ZO-1, PgP) was verified through immunofluorescence, and permeability was assessed by dextran and gadolinium-based contrast agents exclusion. Moreover, using lactoferrin-conjugated mesoporous silica nanoparticles, we have demonstrated the capability of this model to evaluate nanoparticle penetration and quantified uptake kinetics using advanced imaging and analysis techniques.

Our findings indicate that the 3D BBB model developed herein could be of high relevance in the evaluation of brain-penetrating therapeutics and BBB modulating agents *in vitro*.

References:

[1] Cho, CF., Wolfe J, Fadzen, C, *et al.*, *Nat Commun* **2017**, 8,15623.