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Modelling the human blood-brain-barrier microvasculature and nanocarrier transport on a microfluidic chip

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The blood-brain-barrier (BBB) is a highly selective barrier that help to maintain brain homeostasis; however, it also represents a near-impenetrable hurdle against the delivery of therapeutics to the central nervous system. Since only small compounds can cross the BBB, this hinders most neuropharmaceuticals from eliciting a desired dose and effect, reducing treatments available for neurodegenerative diseases and cancer [1]. Polymer nanoparticles (NPs) have emerged as a potential solution for delivering therapeutics across the BBB to brain targets. One of the problems that has slowed down the development and approval after clinical trials of new drug candidates for brain diseases is the lack of preclinical models that accurately reproduce the human BBB. Indeed, current models such as *in vitro* transwells or mouse models fail to reproduce the anatomical complexity of the human BBB [2]. For these reasons, an innovative and reliable *in vitro* BBB model that adequately reflects the human *in vivo* morphologies and characteristics is required. Moreover, as transport across the BBB represents the first evidence of NP delivery capabilities, the development of an *in vitro* testing platform and method for quantifying NP transport behavior provides an invaluable tool to assess therapeutic efficacy. To address these limitations, a microfluidic *in vitro* microvascular model of the human BBB was developed, containing human induced pluripotent stem cell-derived endothelial cells, brain pericytes, and astrocytes supported in 3D fibrin gel matrix as self-organized microvasculature. The microvascular BBB model was developed via vasculogenesis to accurately replicate the *in vivo* neurovascular organization. The microvasculature of the BBB model was perfusable within 5-7 days, showing permeability coefficient comparable to previous *in vitro* models and similar to *in vivo* measurements in rat brain [3]. Gene expression of tight and adherent junctions (ZO-1, occludin, and claudin-5), extra-cellular matrix proteins (Laminin and Collagen IV), and membrane transporters (PG-P, LAT1, LRP1) was higher in tri-culture condition consistently with quantitative immunocytochemistry analysis indicating maturation and differentiation into more BBB-like structure. Microvessel-pericytes/astrocytes contact-interactions were validated using laser confocal microscopy. When pericytes and astrocytes were included to form microvasculature, the vascular parameters such as vascular diameter, branches length and vascular network area coverage became lower compared to mono-culture of endothelial cells. This revealed that morphological changes were induced by not only the secretion of pro-angiogenic and vasculogenic growth factors but also contact signaling between cells. After characterization, this established 3D *in vitro* model of the human BBB was preliminarily exploited to evaluate nanocarrier permeability such as nanoparticles. Indeed, ongoing experiments are showing that the 3D BBB model might be capable to elucidating differences in 3D transport between Polymeric NPs compared to Transwell assays. This robust and translational BBB microvascular model could be potentially applied to patient-specific and neurodegenerative diseases modelling [4], offering a novel platform to study both drug candidates transport as well as neurovascular functions within a physiologically-relevant BBB microvasculature. This is the first version of an innovative BBB model that potentially might change how therapeutic compounds are designed and transported across the human BBB, reducing and refining the use of animal models.

References

[1] Pardridge, W. M. (2016). *Expert Opin Drug Deliv 5247*, 1-13.
[2] Crawford, L. et al. (2016). *J Control Release 240*, 251-266.
[3] Campisi, M. et al. (2018). *Biomaterials 180*, 117-129.
[4] Osaki, T. et al. (2018). *Sci Rep, 8*, 5168.

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