Development of a human stem cell-derived, hydrogel-based blood-brain barrier model to investigate the limited brain delivery of immunoglobulin G

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Immunoglobulin G (IgG)-based immunotherapies hold tremendous promise as the first disease-modifying treatment for Alzheimer's disease and related dementias. However, the poor brain delivery of IgG (~0.01% of the administered dose) necessitates unprecedented dosing regimens that raise concerns about treatment cost and accessibility. Efforts to improve the penetrance of bloodborne IgG into the brain have attempted to identify the intracellular processes governing IgG transport across the restrictive vasculature of the brain (termed the blood-brain barrier (BBB)). Despite numerous studies, the difficulty of performing cellular-level characterizations on the BBB *in vivo* have led to conflicting findings. To this end, the objective of this thesis is to investigate the processing and transport of IgG at the BBB by using cellular-level *in vitro* characterizations.

Initially, an *in vitro* approach capable of simultaneously visualizing and quantifying IgG transport across the brain endothelial cells (BECs) that form the BBB was developed. Previous work from our group demonstrated the utility of induced pluripotent stem cell-derived BEC-like cells (termed iBECs) to study IgG transport, however experimental limitations of the cell culture inserts traditionally used to construct *in vitro* BBB models hindered further investigation. Here, a microvolume (4 μL) collagen type I (COL1) hydrogel was coupled with iBECs to establish an *in vitro* BBB model amendable with live-cell fluorescence microscopy. The resultant hydrogel-based BBB model and microscopy-based transport quantification method were validated with proteomic and functional comparisons to benchmark data previously reported for iBECs on cell

culture inserts. The easy-to-construct COL1-based BBB model presented here is the first to enable the visual and quantitative assessment of molecular transport across BECs at nanomolar concentrations.

Evidence suggests that IgG-specific processing occurs within BECs, but any influence on transport remains unclear. Here, the involvement of the neonatal Fc receptor (FcRn), which can salvage internalized IgG away from lysosomes and back to the luminal cell surface (i.e. recycling) or shuttle internalized IgG to the abluminal cell surface (i.e. transcytosis), was investigated by comparing the transport and processing of IgGs that are recognized or unrecognized by FcRn. Using super-resolution fluorescence microscopy, the two IgGs demonstrated differences in lysosomal accumulation consistent with FcRn-mediated recycling. Yet, IgG transport rates were independent of FcRn engagement or concentration, indicating that while FcRn can recycle IgG at the BBB, it does not influence its transcytosis. Complementary studies with macromolecules ranging in molecular weight (12-155 kDa) demonstrated comparable transport rates to IgG (150 kDa), suggesting concentration/size-independent fluid-phase endocytosis and nonspecific transcytosis events as the basis for IgG and most macromolecular transport at the BBB. Indeed, only macromolecules leveraging receptor-mediated or adsorptive endocytosis mechanisms exhibited faster transport rates in a concentration or charge-dependent manner, respectively. The ability of adsorptive endocytosis to improve IgG transport was demonstrated via the conjugation of negatively-charged fluorescent probes, however alterations in FcRn-mediated recycling were detected and were attributed to indirect inhibition of FcRn engagement within endosomes as conjugated/unconjugated IgG exhibited comparable FcRn-IgG binding in solution.

Collectively, these findings highlight the utility of *in vitro* BBB models to characterize and screen therapeutics, shed light on the factors influencing the transport of IgG across the BBB, and provide possible routes to engineer improved IgG variants with enhanced BBB penetrance.