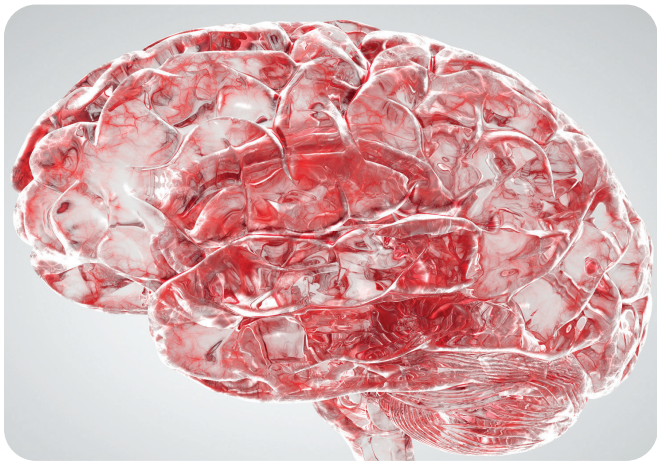
EXPERT INTERVIEW

Recapitulating the blood-brain barrier using *in vitro* hiPSC models in drug discovery

Introduction

Drug delivery to the Central Nervous System (CNS) is a substantial hurdle in the development of efficacious drug therapeutics for the treatment of neurodegenerative diseases. The Blood-Brain Barrier (BBB) limits the ability of drugs to easily reach its intended nervous system tissue at sufficient concentrations to exert their effects. Active research is ongoing to further understand the mechanisms and pathways involved, with the intention of supporting drug discovery programs tackling CNS disorders, and some researchers are turning to *in vitro* Human Induced Pluripotent Stem Cell (hiPSC) models as possible solutions to aid in the recapitulation of BBB complexity to not only increase physiological relevance, but impact the success of therapeutic programs in a truly predictive manner.

We were fortunate to speak with Dr. Ole Pless of the Fraunhofer Institute for Translational Medicine and Pharmacology ITMP ScreeningPort, who is a project leader for biomarker and translational research. Among his many responsibilities, Dr. Pless uses hiPSC technology to better understand the BBB for translational research and Drug Discovery (DD) efforts in neurodegenerative diseases.



Revvity: Thank you for joining us today Dr. Pless. Your group had recently published a great overview on this topic of hiPSC-derived BBB models as tools for preclinical DD and development, where you bring up some important insights into what's not being benchmarked rigorously enough in most CNS DD workflows. I'd like to kick off our discussion by asking for your thoughts on what the main challenges are in developing central nervous system therapies.

Dr. Ole Pless: One of the key topics in drug discovery is whether a compound has the capability to reach the Central Nervous System (CNS). There are quite a few models to assess whether a drug is fit-for-purpose to target CNS diseases. However, there are only a few that are predictive as it were to faithfully recapitulate human physiology. Overcoming this challenge has created a bottleneck in drug discovery, particularly in CNS drug discovery. The biggest hurdle is to mimic the Blood-Brain Barrier (BBB) to assess whether an agent can be developed to successfully cross over and reach the target of interest at sufficient concentrations to be effective.

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Revvity: How do these challenges contribute to a high failure rate classic of CNS drug development?

Dr. Ole Pless: CNS therapies have a notorious failure rate due to a lack of good data that illustrates that the drug can reach its intended target. Currently, we rely on artificial lipid bi-layer membranes, rodent *in vivo* models, and immortalized cell lines used to mimic barriers. These models only recapitulate certain aspects of the BBB while other important features are not successfully captured; therefore, there is an unmet need that needs to be addressed and incorporated into modern drug discovery approaches. In order to mimic the BBB, a fit-for-purpose model must be applied in order to discern relevant conclusions from meaningful and reproducible data. This is the prerequisite to engage with specific targets in the CNS.

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Revvity: What novel approaches have emerged that offer promise for the future of CNS drug development?

Dr. Ole Pless: The invention/discovery of Induced Pluripotent Stem Cells (iPSCs) and resulting cells that can be derived from these stem cells, revolutionized the field of applied life sciences for drug discovery. With this technology, initially described in 2006/2007 and subsequently garnering the Nobel Prize in 2012, large numbers of somatic cells of the human body could be generated, faithfully recapitulating features of the adult human tissue studied. By applying this technology, brain capillary endothelial cells or cells of the neurovascular unit including astrocytes or pericytes can be generated to help assemble constructs like the BBB *in vitro* that better mimicked human physiology for use in drug development testing than was possible with previous models or animal studies. Importantly, these cells are karyotypically defined like somatic cells of the human body and do not house aberrant growth characteristics or karyotypic alterations, which are common in tumor cells or some commonplace immortalized cell lines.

The emergence of human iPSCs (hiPSCs) in these translational applications is very exciting as it opens up a space in DD programs where you can utilize them to tackle CNS-specific challenges such as overcoming the BBB, as well as actually model certain organ systems where you want to see efficacy or safety for the therapeutics you want to develop. Of course, we need to be cautiously optimistic. Organoids, 3D organ-like microstructures, for example, often times still suffer from a lack of batch-to-batch reproducibility and maturity. There are, of course, challenges that still need to be tackled prior to routine application of these models. These models are yet to be used in pharma on a routine basis, but then again, they have to follow a certain statute of guidelines to bring drug candidates to a certain level of maturity. There is a lot of groundwork to do in order to increase the widespread acceptance and level of confidence in incorporating these complex models into DD workflows.

Revvity: Can you further discuss the utility of hiPSCs in regard to CNS disease and BBB modeling that could potentially enable better success of future CNS DD pipelines?

Dr. Ole Pless: Well, first, modelling of monogenic diseases was an early area of application for iPSC technology. There are a lot of examples where monogenic diseases have been studied and hallmarks of the disease have been recapitulated faithfully in a dish as point mutations in key genes are sometimes sufficient to cause drastic phenotypes in patient-derived iPSCs. This enables disease modelling in a dish and DD strategies to ameliorate respective phenotypes resulting from specific mutations. From an experimental standpoint, it's great to be able to generate an ideal control with isogenic cell lines: by applying modern genome engineering technologies, like CRISPR/Cas or other, genomic changes can be introduced on a single base level, thereby altering the affected DNA-sequence into the "healthy" configuration (or vice versa).

Nowadays though, more and more complex diseases are also being studies using hiPSC-derived models. We are involved in a program, for example, where we use patient-derived Alzheimer's disease iPSCs – carrying high-risk haplotypes predisposing for the disease – to disease-associated phenotypes of the BBB. It is thought that every CNS disease has a disease-specific component at the BBB, but until recently, these phenomena could not be studied in molecular detail. The BBB in an Alzheimer's patient could be differently diseased when compared to a Parkinson's patient, for example, and this can be dissected by applying hiPSCderived BBB models. Also, they can be used to study therapeutics acting on these BBBs.

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Revvity: In a recent publication[1], you discuss some fascinating details into all the biological complexity that exists in a functional BBB. Could you go ahead and describe the current state of hiPSC-based BBB models and the model your group has developed and/or are currently optimizing?

Dr. Ole Pless: Our most straightforward model is a transwell set-up, consisting of an apical and a basolateral compartment. hiPSC-derived brain capillary endothelial-like cells are seeded from the apical compartment. During a ten-day differentiation protocol, they form a tight monolayer in which the transcriptional profile and marker protein levels correlate with data from *in vivo* human brain capillaries. In addition, functional properties including Trans-Endothelial Electrical Resistance (TEER) and paracellular transport of tracer molecules such as fluorescein or size markers like FITC-labelled dextrans, etc. can be assessed to ideally show recapitulation of the situation *in vivo*. Applying this model, we can now determine by HPLC-MS/MS analytics how much of a compound of interest which was applied from the apical side reaches the basolateral compartment over time.

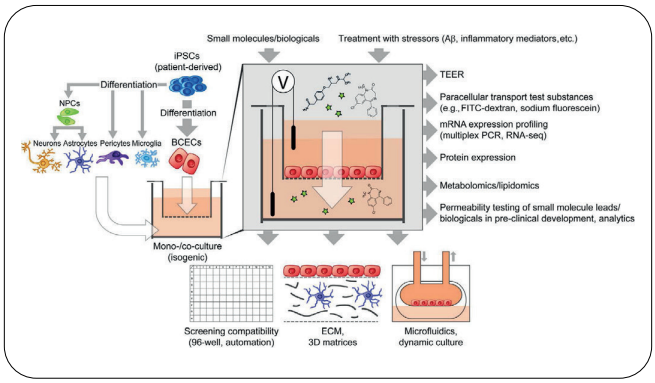

Figure 1. Design and features of hiPSC-derived BBB model systems. Directly taken from Figure 1, and additional details can be found in Appelt-Menzel, A., Oerter, S., Mathew, S., Haferkamp, U., Hartmann, C., Jung, M., Neuhaus, W., & Pless, O. (2020). Human iPSC-Derived Blood-Brain Barrier Models: Valuable Tools for Preclinical Drug Discovery and Development?. Current protocols in stem cell biology, 55(1), e122. https://doi.org/10.1002/cpsc.122.

When discussing current trends in biologics, we see a lot of therapeutic antibodies targeting the CNS that actually reach clinical testing; a prominent recent example being Aducanumab. In my eyes, it´s very relevant to rigorously test how well antibodies and other biologics cross the BBB prior to clinical development as there's a size exclusion phenomenon on these barriers not often appreciated. Similarly, you need to better consider the role of active transport mechanisms across the BBB, so a clear application for these hiPSC barrier models is in the study of relevant transporters at the BBB (ABC transporters, solute carriers, transferrin receptors, etc.). Most of these systems are not expressed in your standard cell line of interest, at least not to the same extent of these in human BBBs *in vivo*, so a lot of these active transport mechanisms might be amenable to analysis in hiPSC models of the BBB and could be part of a future biologics DD pipeline.

Functional assessment and quality control are also factors that need to be put into place when developing these models. Several drugs are well characterized and FDA and/or EMA approved, and we know exactly how they act in the CNS. For example, diazepam moves very quickly through the BBB, whereas atenolol cannot cross the BBB. These known characteristics can act as references to test the functionality of these models as well, and our feeling is that these types of *in vitro* hiPSC BBB models are superior to all the simpler system models that were historically applied in pharma. With enough optimization, they could be more predictive than the test systems that have been used until now.

Revvity: You had brought up the credo "as easy as possible, as complex as necessary" since the purpose of the study/question being asked is indeed the decisive criterion for BBB model selection. What are your thoughts on some of the current trends that focus on building atop past models to add complexity for better physiological relevance in generating more complex *in vitro* models?

Dr. Ole Pless: More complex models can also be established in the transwell setup described above, with brain capillary endothelial-like cells on the apical side of the membrane, and other co-cultured cells in the basolateral compartment. Typically, other cells of the neurovascular unit in addition to brain capillary endothelial-like cells, lead to an even more pronounced barrier functionality. Today, they can also be produced by applying hiPSC technology.

Another trend that is very important to consider for its applicability in many diseases is the role of immune cells migrating across the BBB into the CNS. Activated immune cells can lead to chronic disease and inflammation in the CNS. We are currently looking into this for translational multiple sclerosis research. In theory, our model would enable us to study the immune compartment, moving across the BBB into the CNS. We're thinking about clever *in vitro* set-ups to actually monitor T cells in the context of the BBB, how they would interact with a barrier, and how they would migrate across it. We know that T cells migrate across the paracellular space into the CNS, which could be a way to get a therapeutic molecule into the brain. Big pharma has embarked on several programs that take advantage of this phenomenon. One concept is to load T cells in the periphery with a therapeutic agent, and because they migrate into the CNS, you can deliver therapeutics with high specificity to a CNS target. That, I find quite intriguing.

Practically speaking, however, in our discussion of monocultures vs co-culture models, there are several cell types in the neurovascular unit, so it would be much more complex to establish a culture of say 4 cell types. At this time, this high level of complexity results in possibly less standardization, so pharma

may be more hesitant to adopt said models, especially if these organoid/cultures take several months to develop. For routine applications, there's a trade-off in efficiency and reproducibility vs requirements in complexity and physiological relevance. Our normal one cell type transwell model is a relevant yet a good trade-off that is attractive to pharma since it takes, say, 10 days to establish the barrier and 1 day to carry out the kinetic experiments as well as measure transmigration of the compound, and then you're done.

Similarly, organ-on-a-chip systems are gaining huge momentum, and we have worked with collaborators in larger BBB consortia in that space. As you can imagine, however, this field is still relatively nascent, and the predominant thought at this time is that this field still has a long way to go before becoming part of routine pre-clinical applications. There are interesting strategies out there, but the level of complexity is still quite high and prohibitive for pharma to invest or commit to just yet.

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Revvity: To conclude, fostering collaborations are critical in accelerating the speed of discovery to face the urgent need to aid those afflicted by debilitating CNS disorders. Can you share some of the projects that are exploring your group's BBB model, as well as how your institution is generally working to help with these challenges?

Dr. Ole Pless: Part of what my organization does in the context of the BBB is participate in multi-center pre-clinical trials to establish the intra-lab and inter-lab variability of the hiPSC model. It is important to understand how the model performs in different labs when established by different operators and different hiPSC cultivation conditions. The robustness of the model needs to be established, with the intention of also convincing regulators of the superiority of the approach compared to the current standards. In this regard, the field has come a really long way over the past 15 years, but there is still work ahead.

Putting our work in the context of the current COVID-19 pandemic, we see that the virus can be found in the brain and read about the research being done to understand long-COVID and post-COVID symptoms as well as CNS pathologies. We are investigating with full force the if, when, and how of the virus' ability to cross the BBB using our model as well.

Another area in which our organization participates is in the examination of ways in which automation can improve our model by looking to scale-up from a 24-well to a 96-well format. Making space for development using liquid handling and automation-compatible solutions, combined with sophisticated readout technology with automated and online analysis, would allow things to move forward at the throughput and pace needed by pharma.

It's pretty amazing to think that the organization I'm at currently has been operational since 2007. Since then, there has been such a huge movement into new and improved ways of pharma and academia working together. We see ourselves as the facilitator of these interactions. Where pharma used to have everything set up internally, driven by their standard operating procedures, we are now seeing an emergence of

public-private partnerships to really test and interrogate new technologies and new ways of doing drug discovery. They really appreciate partners like us, where they can test out high-risk strategies – testing out new technologies or new ways of doing things – in a safe environment where they don't need to sacrifice huge internal budgets. Academia and pharma are very different worlds, and it requires mediators between both. We believe the more we see these types of interactions, it can only work favorably for drug discovery and CNS drug discovery in particular.

Revvity: Thank you for taking the time to talk about your research with us, Dr. Pless. We are excited about the great progress being made by groups like yourselves in this field and continue to look forward to hear about your group's work in the CNS community.

Reference

1. Appelt-Menzel, A., Oerter, S., Mathew, S., Haferkamp, U., Hartmann, C., Jung, M., Neuhaus, W., & Pless, O. (2020). Human iPSC-Derived Blood-Brain Barrier Models: ValuableTools for Preclinical Drug Discovery and Development? Current protocols in stem cell biology, 55(1), e122. https://doi.org/10.1002/cpsc.122 and references included within

About the scientist

Dr. Ole Pless is a senior scientist at Fraunhofer ITMP ScreeningPort in Hamburg, Germany, focusing on the application of pluripotent stem cell technology for efficacy and safety assessment of therapeutic candidate molecules.

Dr. Pless has leading roles in several pan-European research consortia focusing on neurodegenerative, metabolic, and infectious disease. Dr. Pless holds a PhD from the Max Delbrück Center for Molecular Medicine and the Humboldt University in Berlin.

About the institution

Fraunhofer is the largest application-oriented research organization in Europe with over 28,000 employees and operates more than 74 institutes and research facilities in Germany. ScreeningPort is a department of the Fraunhofer Institute for Translational Medicine and Pharmacology ITMP located in Frankfurt/Main and Hamburg. Fraunhofer ITMP conducts health research in line with the Fraunhofer 4D concept (Drugs, Diagnostics, Devices, Data), which aims to optimize inter- and transdisciplinary health research in order to improve patient treatment qualitatively and economically. One focus of ITMP ScreeningPort is on assay development for biochemical and cell-based formats, on development and application of efficacy and toxicity assays based on human pluripotent stem cells, on assay miniaturization, high-throughput and high-content screening, and on chemi- and bioinformatics. The indication focus is on infectious and immune-related diseases.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com