BBA - Molecular Basis of Disease xxx (xxxx) xxxx

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Neurological diseases at the blood-brain barrier: Stemming new scientific paradigms using patient-derived induced pluripotent cells☆

Shyanne Page[1], Ronak Patel[1], Snehal Raut, Abraham Al-Ahmad*

Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, School of Pharmacy, Amarillo, TX, United States of America

ARTICLE INFO

Keywords:
Blood-brain barrier
Stem cells
Diseases modeling
Endothelial cells
Neurological diseases
Induced pluripotent stem cells

ABSTRACT

The blood-brain barrier (BBB) is a component of the neurovascular unit formed by specialized brain microvascular endothelial cells (BMECs) surrounded by a specific basement membrane interacting with astrocytes, neurons, and pericytes. The BBB plays an essential function in the maintenance of brain homeostasis, by providing a physical and chemical barrier against pathogens and xenobiotics. Although the disruption of the BBB occurs with several neurological disorders, the scarcity of patient material source and lack of reliability of current *in vitro* models hindered our ability to model the BBB during such neurological conditions. The development of novel *in vitro* models based on patient-derived stem cells opened new venues in modeling the human BBB *in vitro*, by being more accurate than existing *in vitro* models, but also bringing such models closer to the *in vivo* setting. In addition, patient-derived models of the BBB opens the avenue to address the contribution of genetic factors commonly associated with certain neurological diseases on the BBB pathophysiology. This review provides a comprehensive understanding of the BBB, the current development of stem cell-based models in the field, the current challenges and limitations of such models.

1. Introduction

1.1. The blood-brain barrier: the gatekeeper of the central nervous system

The blood-brain barrier (BBB) constitutes a unique feature of the brain vasculature present in the vertebrate central nervous system [1]. Although initially observed by Paul Ehrlich in 1885 [2] as the absence of the central nervous system to absorb histological dyes (Trypan Blue), it took another 30 years to have the presence of such barrier experimentally demonstrated by the work of Edwin Goldmann [3,4]. Initially described by Reese and Karnovsky as a ultrastructural feature (tight junction complexes) present in brain microvascular endothelial cells lining the brain vasculature [5], the current description of the BBB is defined as a multi-cellular component of the neurovascular unit.

Brain microvascular endothelial cells (BMECs) lining the brain microvasculature constitutes the most important cell type of such unit (Fig. 1), providing a physical and chemical barrier between the circulating blood flow and the brain parenchyma. BMECs are ensheathed by a specialized basement membrane enriched in collagen type IV, fibronectin, laminin (411 and 511 isoforms), and perlecan [6]. Such basement membrane is shared with brain pericytes capable of directly interacting with BMECs through peg-and-socket interactions [7]. Although the lining of endothelium by pericytes is common, endothelial-pericyte coverage is the highest at the CNS vasculature. The presence of a perivascular space between the basement membrane and the glia limitans allows the circulation of CSF *via* bulk flow [8]. Astrocytes interact with the blood-brain barrier by the presence of end-feet processes juxtaposed on the abluminal side. Although neurons during development play a significant role in brain angiogenesis and the onset of the BBB during development [9,10], their contribution at the adult BBB remains unclear [11,12]. We distinguish two types of barriers at the BBB: a physical barrier (formed by tight junction complexes) and a chemical barrier (represented by an array of drug transporters). The presence of tight junction complexes, coupled with the presence of low pinocytosis [13,14], limits the diffusion of ions and solutes between the two compartments. Such restrictive diffusion contributes to a defined microenvironment in the CNS, as marked by a distinct electrolyte composition between the cerebral spinal fluid (CSF) and blood plasma. In addition to such physical barrier, the presence of a chemical barrier provides a tightly regulated diffusion of nutrients and hormones inside

☆ This article is part of a Special Issue entitled: Stem Cells and Their Applications to Human Diseases edited by Hemachandra Reddy

* Corresponding author at: Texas Tech University Health Sciences Center, School of Pharmacy, Department of Pharmaceutical Sciences, 1300 South Coulter Street, Amarillo, TX 79106, United States of America.
E-mail address: abraham.al-ahmad@ttuhsc.edu (A. Al-Ahmad).

[1] Authors have contributed equally to this work.

https://doi.org/10.1016/j.bbadis.2018.12.009
Received 19 October 2018; Received in revised form 15 November 2018; Accepted 5 December 2018

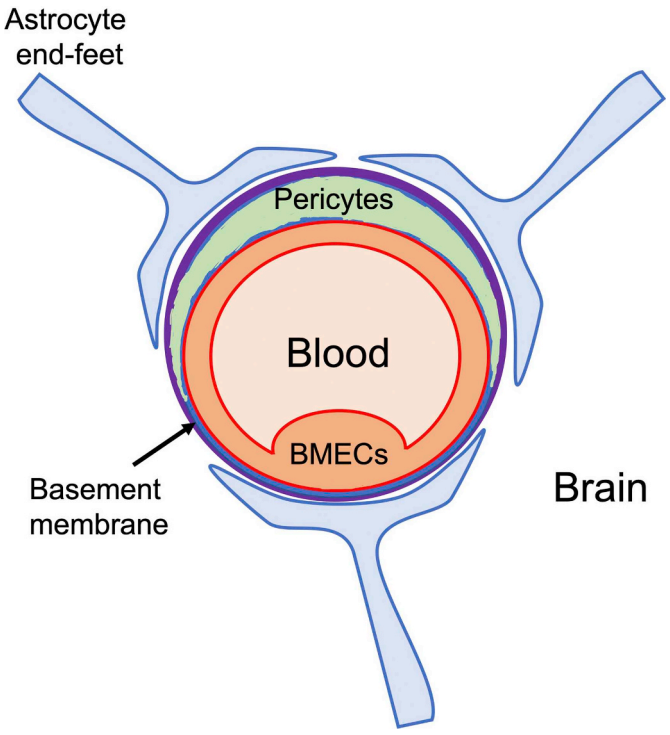

Fig. 1. Schematic representation of the blood-brain barrier.

the BBB. Diffusion of nutrients across the BBB is ensured by the presence of an array of different solute carriers (SLCs) and organic anion transporter proteins (OATPs) responsible for the diffusion of nutrients such as glucose (GLUT1, GLUT3) [15,16], amino-acids (L-system, y-system amino acids) [17,18], thyroid hormones (MCT8, MCT10) [19–22], or small organic anions (MCT1, OATP1A4) [23,24]. Larger molecules, such as transferrin (Tf) or insulin (Ins) are mediated *via* transcytosis *via* receptor-mediated transcytosis (RMT) [25,26], which allows the delivering proteins of across the BBB *via* an endosomal-lysosomal system.

On the other hand, the presence of drug efflux pumps belonging to the ATP-binding cassette (ABC) transporters super-family, significantly impacts the diffusion of lipophilic small molecules across the BBB. Several efflux pumps have been described at the human BBB and include breast cancer resistance protein (BCRP/ABCG2), P-glycoprotein (P-gp/ABCB1), and several multidrug resistance proteins (MRPs/ABCCs). The presence of such efflux pumps constitutes an important evolutionary feature primarily aimed to protect the CNS from the penetration of harmful xenobiotics and environmental toxins, but also a formidable challenge for small molecule drug delivery. It is estimated that at least 98% of small molecule drugs and virtually 100% of large molecule drugs cannot cross the BBB [27]. In summary, the presence of a functional BBB is essential for the maintenance of the CNS homeostasis, yet its dysfunction during neurological disorders remains mostly unclear or undefined.

1.2. The dysfunctional BBB: a key element of several neurological disorders

According to the World Health Organization [28], cerebrovascular diseases represent over 55% of disability-adjusted life years impacted by neurological disorders, and 85% of death attributed to neurological disorders. Although the contribution of a dysfunctional BBB during neurological disorders has been occulted for decades, recent studies highlight the critical role of the BBB in the pathophysiology of many neurological diseases including Alzheimer's disease [29], epilepsies [30,31], Huntington's disease [32,33], Multiple Sclerosis [34], Parkinson's disease [35], stroke [36–38], and vascular cognitive impairment [39–41]. Yet, our current understanding of the cellular and molecular mechanisms associating the BBB with such diseases are greatly hampered by our limited access to cells and tissues from patients (biopsies or post-mortem tissue), by *in vitro* models displaying poor barrier tightness, by our inability to fully integrate most of the different components of the neurovascular unit, and by the limitations inherent to the use of rodent-based animal models. In conclusion, a need for *in vitro* models of the BBB capable of recapitulating features observed *in vivo*, as well as providing a scalable and patient-specific source of cells is needed to improve our ability to understand the contribution of the BBB in neurological disorders.

2. Diseases modeling and human pluripotent stem cells

2.1. Use of patient-derived pluripotent stem cells for neurological diseases modeling: promises and challenges

According to the World Health Organization [28], neurological diseases combined represent over 6.5% total burden of diseases worldwide. Yet, most of these diseases remain incurable and most interventions focus on symptomatic treatment. Furthermore, the translation of novel drug candidates into clinically efficacious drugs is hampered by several pitfalls inherent to modeling the CNS *in vitro* and *in vivo*, *such as* the post-mitotic nature of neurons (greatly limiting access to primary cultures), the presence of a complex tissue cytoarchitecture (partially addressed by tissue culture slices), anatomical and molecular inter-species differences (between rodents and human brain), and ultimately limited access to human brain samples. Since the seminal work of Shinya Yanamaka establishing the derivation protocol of induced pluripotent stem cells (iPSCs) from somatic cells [42,43] and the neuronal differentiation protocol established by Zhang and colleagues on human embryonic stem cells [44], modeling neurological diseases *in vitro* using iPSCs has revolutionized the medical field. In the last decade, several neurological diseases based on iPSCs have been documented and include: X-linked adrenoleukodystrophy [45–47], Alzheimer's disease [48–54], autism spectrum disorders [55–58], Down's syndrome [59–61], epilepsies [62–68], Huntington's disease [69–71], and Parkinson's disease [72–75].

Although the initial derivation protocol has been an incremental step in obtaining a scalable source of patient cells, such protocol harbors several caveats: it relies on the random and permanent insertion of these transcription factors, remains a laborious process with very low yield (6 months for a yield of iPSCs ~0.01%), and a strict removal of epigenetic markers associated with age or environmental exposure. In addition, the use of a genome integrating method in the derivation process constitutes a major challenge in translating such findings into iPSC-based cell replacement therapies (CRT) [76].

To tackle these issues, several approaches have been developed [76]. Firstly, several non-genomes integrating reprogramming methods have been described including the use of episomal vectors that allow cell reprogramming without the insertion of genomic sequences [77]. In addition to genomic approaches, recent attempts have been made to identify small molecules capable to achieve similar outcomes and relieve the issue inherent to the insertion of foreign nucleic acids [78]. Yet, such approaches still suffer from the epigenetic reprogramming and the loss of patient-specific epigenetic signature.

An alternative approach consists of differentiating somatic cells directly into neurons without generating iPSCs. This process has been showing some promising features: it appears successful in generating motor neurons from patients suffering from amyotrophic lateral sclerosis (ALS) [79], capable to occur *in vivo* by reprogramming astrocytes into neuroblasts [80], and maintain age-related epigenetic changes [81],

Finally, recent advances in gene editing techniques (*e.g.* CRISPR/Cas9) provides novel avenues in diseases modeling using iPSCs. A major challenge in diseases modeling using patient-derived iPSCs is marked

by the limited supply of patient-derived iPSC lines, the limited availability matched-controls (*e.g.* siblings, parents) and a possible inherent genetic drifting due to the derivation and the maintenance of such lines. The use of genome editing provides a fast and elegant approach by selectively being able to insert or correct a mutation in an iPSC line in a precise manner [82]. This approach allows investigators to obtain parental cell lines with precise mutations, allowing a direct comparison of the phenotype to the wild-type gene and removes variability inherent to clonal or individual differences. In addition, such approach also provides a "proof-of-concept" in assessing the viability of a gene therapy-based approach to determine if the correction of a defective gene result in the resolution of the phenotype observed.

In addition of challenges inherent to iPSCs, additional challenges are still hindering iPSC-based disease models. One of the major challenges is the absence of a 3-dimensional structures reflective of the cellular cytoarchitecture observed *in vivo*. An alternative to such issue is the description of brain "organoids" providing a primitive multilayer sphere. Yet, such structures suffer from multiple limitations including: the absence of an extracellular matrix scaffold reflecting of the *in vivo*, the absence of neuronal projections and tracts, and most importantly the presence of multicellular organoids integrative of glial cells.

In conclusion, the use of iPSCs as a tool to model human neurological diseases has gained an important momentum in the last decade in the field of neurosciences. Yet, iPSC-based models still suffer from important limitations inherent to culturing conditions and raise the need to address critical parameters (*e.g.* 3-dimensional cultures, multicellular models…) that can make these models reliable alternatives to existing *in vivo* models. Thus, a similar adoption of the same experimental paradigm within the blood-brain barrier scientific community may provide an influx of novelty and innovation, but also facing similar challenges.

2.2. Modeling the human blood-brain barrier using human pluripotent stem cells

Until recently, the use of *in vitro* models of the BBB for drug discovery and disease modeling was hindered by several challenges: limited amount of biological material (brain blood vessels account for only 1% of the total brain content) obtainable from patients, the challenge in the isolation and the scalability of primary BMEC cultures, the rapid loss of BMEC phenotype and barrier function following sub-culturing conditions [17,83], and notable differences in drug transporters between rodents and the human BBB. Such limitations dramatically reduced the interest and relevance of the use of *in vitro* BBB models as a tool for CNS drug discovery and diseases modeling. Since the seminal work of Lippmann and colleagues [84], over 30 peer-reviewed studies using *in vitro* models based on stem cells have been published. As of today, most studies utilizing human pluripotent stem cells (hPSCs) for the generation of BMECs are based on the original differentiation protocol defined by Lippman and colleagues [84] and schematized in Fig. 2. This protocol has been successfully used to differentiate human amniotic stem cells [85], cord blood endothelial progenitor stem cells [86], patient-derived induced pluripotent stem cells [69,87,88], and hematopoietic stem cells [89] into BMECs. These hPSC-derived BMECs, upon purification, display an immunological and barrier phenotype (Fig. 3) comparable to somatic human BMECs obtained from primary or immortalized cell cultures.

Although this protocol yields to differentiate stem cells into BMEC-like cells that share similar features than somatic human BMECs, However, this initial protocol suffers from several caveats: Firstly, the undirected differentiation that fails to address the cell lineages present during the first four days of differentiation and trace the origin of the endothelial cell lineage. Secondly, the outcome of a mixed cultures (endothelial cells/neuron progenitor cells) that may contribute to a batch-to-batch variability in yield. Thirdly, the uncontrolled initial cell density upon priming the differentiation that can influence the yield and quality of BMECs monolayers. Finally, the modest barrier tightness observed in BMECs monolayers upon purification.

To overcome such issues, several iterations of this protocol has been published to improve the quality of the differentiation (see Table 1 and Fig. 4). Addition of retinoic acid (RA) [90], a signaling pathway enriched at the BBB [91,92], during the differentiation yielded to the formation of tight monolayers, comparable to *in vivo* (TEER ~ 4000–5000 $\Omega \cdot cm^2$). The establishment of an optimal cell density seeding contributed to diminish batch-to-batch variability in terms of yield [93]. Transition from complex medium formulation to xeno-free and defined medium formulation [94,95] further contributed in the reduction in batch-to-batch variability and into the introduction of a xeno-free differentiation protocol.

Yet, the most significant advancement in BMECs differentiation protocol came from a new differentiation protocol by Qian and colleagues [96]. In this BMEC differentiation protocol, BMECs are originating from a mesoderm, the same germ layer giving rise to the vascular system (brain and non-brain vasculature) in vertebrates [97,98]. In particular, this protocol follows similarities with *in vivo* development: endothelial cells are generated from a mesoderm lineage followed by the induction of a BBB phenotype on endothelial cells using RA.

In summary, the use of hPSC as a source for blood-brain barrier modeling has gained a momentum in the literature during the last five years and have demonstrated clear signs of reproducibility. Yet, such method is still in its infancy stage with significant challenges that need to be addressed in order to have such models brought closer to preclinical (*in vivo*) and clinical models.

3. Induced pluripotent stem cells as a tool to model the diseased BBB

3.1. Adrenoleukodystrophy

Adrenoleukodystrophy (ALD) is a lethal metabolic, X-linked neurological disease affecting 1 in 18,000 patients in the US. ALD is caused by mutations in the ABCD1 gene, responsible for the efflux of very long chain fatty acids (VLCFAs) from the cytosol into the peroxisome for β-oxidation. Mutations in the ABCD1 gene leads to accumulation of VLCFAs, which can lead to different clinical outcomes. Adrenomyeloneuropathy (AMN) is the more common and less severe form of the disease and is characterized by progressive axonopathy and demyelination of the long tracts of the spinal cord. Onset of AMN occurs in adult patients during their 40s and 50s. Until now, most research on this disease has focused on VLCFAs accumulation, however, such accumulation has not been a reliable prediction of clinical phenotype. A recent publication by Lee and colleagues [99] showed that iPSC-derived BMECs isolated from patients suffering from childhood cerebral ALD (ccALD, the most severe form of ALD) exhibited an impaired barrier phenotype (decreased TEER, increased permeability) compared to controls. Furthermore, impaired barrier function was accompanied by frayed tight junction complexes. Moreover, a transcriptome analysis revealed an increased expression profile of genes associated with transforming growth factor-β1 (TGF-β1) pathway in ccALD-BMECs compared to controls. These observations were consistent with previous findings in post-mortem brain tissues from ALD patients, but also with a study by Musolino and colleagues in which silencing of ABCD1, but not ABCD2, led to an increased TGF-β1 activity and decreased claudin-5 expression [100]. Interestingly, addition of block copolymers not only reversed the impaired barrier function in ccALD BMECs, but also attenuated the accumulation of lipids. In conclusion, the use of ccALD patient-derived BMECs aided in understanding the effect that ccALD has on the barrier phenotype [99]. Yet, the pertinence of such findings to clinical settings remains to be demonstrated. A recent study highlighted the presence of white matter hyperintensity (possibly associated with a BBB disruption) in patients diagnosed as an ataxic variant of ALD (AVALD) and harboring mutations in the ABCD1 gene [101]. However,

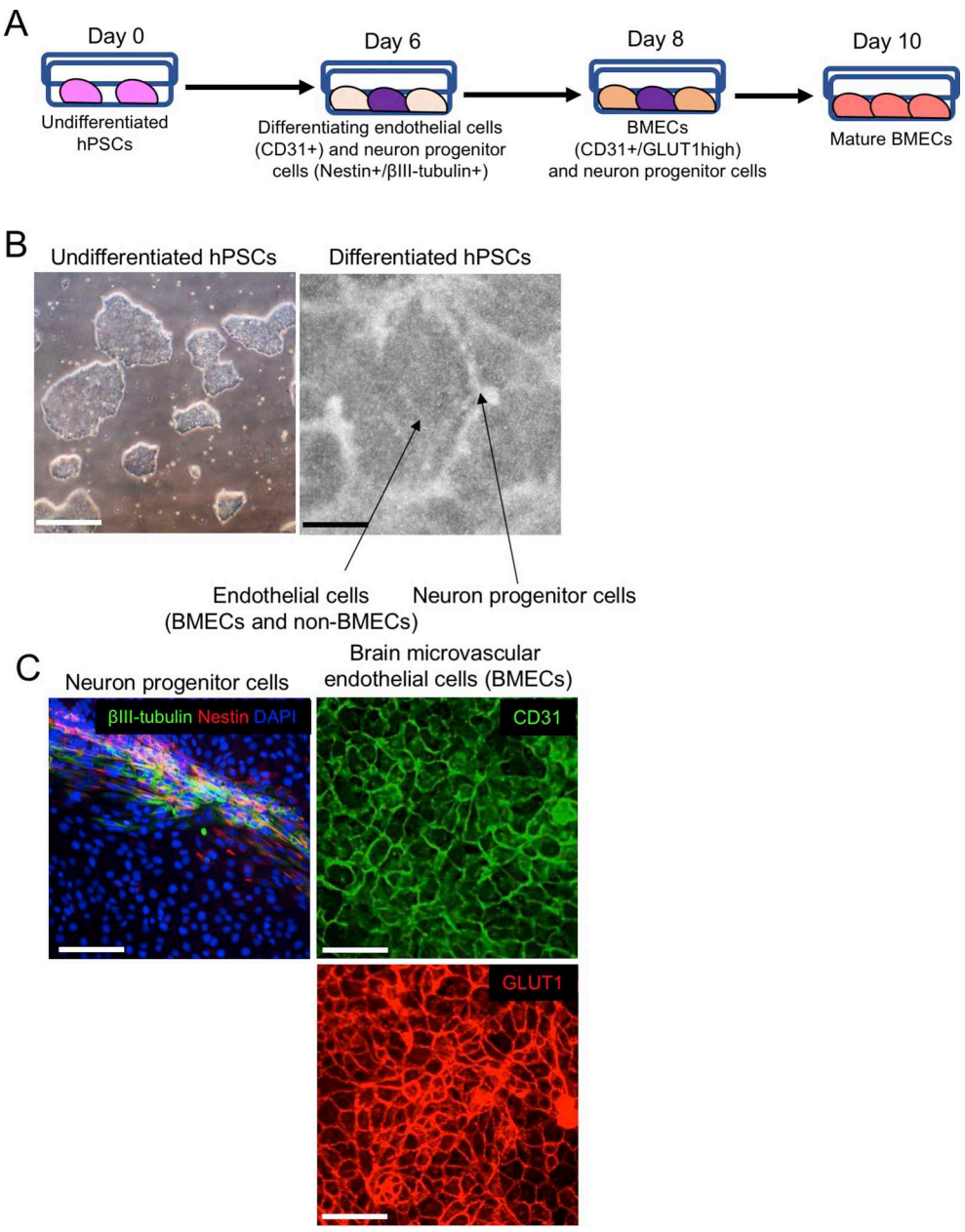

Fig. 2. Description of the differentiation protocol of hPSCs into BMECs monocultures. (A) Schematic representation of the differentiation protocol. (B) Brightfield pictures of hPSC cultures at day 0 and day 8 of differentiation. Note the presence of tube-like structures emanating from hPSC colonies ridges. Scale bar = 200 µm. (C) Representative micrograph pictures of day 8 hPSC colonies by immunofluorescence. Note the presence selective positive immunostaining of these tube-like structures, indicative of a neural cell lineage. The inner cell population of these colonies were positive for CD31 (endothelial cell marker) and for GLUT1 (Glucose transporter 1, selectively expressed in BMECs). Scale bar = 50 µm.

the presence of a disrupted BBB in patients suffering from other forms of ALD remains undocumented.

3.2. Allan-Herndon-Dudley Syndrome

Thyroid hormones play an important role during brain development [102].

Triiodothyronine (T3) constitutes the biologically active form of thyroid hormone, obtained from the deiodination of thyroxine (T4) in the 5′ position. At the human BBB, T3 hormone-exclusive uptake occurs mostly *via* MCT8 (SLC16A2), however, several reports have highlighted the contribution of other transporters in rodents, including MCT10, Oatp1c1 or LAT-1, which were capable of transporting both T3 and T4 hormones [20,22,103,104]. Upon entering the brain, T3's (and T4 following its conversion by glial cells) primary target is neurons, in which it acts as a nuclear factor and controls the expression of key genes involved in brain developmental processes such as cell migration and differentiation, myelination, and other signaling. Allan-Herndon-Dudley Syndrome (AHDS) is a rare, X-linked mental retardation disease associated with mutations in the *SLC16A2* gene [105]. Although the generation of $Mct8^{-/-}$ animals resulted in the reproduction of certain traits observed in AHDS patients, the presence of other transporters exclusively expressed at the rodent BBB (Mct10, Oatp1c1) hampered the relevance of such models [20,106,107]. Recently, a report by Vatine and colleagues [88] using iPSC-derived BMECs from AHDS patients demonstrated that with the exception of impaired T3 transport, AHDS-derived BMECs showed no differences in BMEC phenotype compared to parental controls. Similar outcomes were observed in iPSC-derived neurons, as AHDS-neurons displayed a lower T3 uptake compared to controls. Notably, such model has been used both as a screening tool for assessing the permeability of DIPTA (diiodothyropropionic acid, a T3 analog) and *SLC16A2* gene correction using CRISPR/Cas9. Taken together, this iPSC-derived BBB model provided a complementary tool capable to provide an approach that was limited in animal models (due to the presence of other TH transporters), and suggested the ability to use such model for screening potential drug candidates. However, this model still suffers from several caveats: it only addressed a limited number of mutations associated with MCT8, and it also failed to

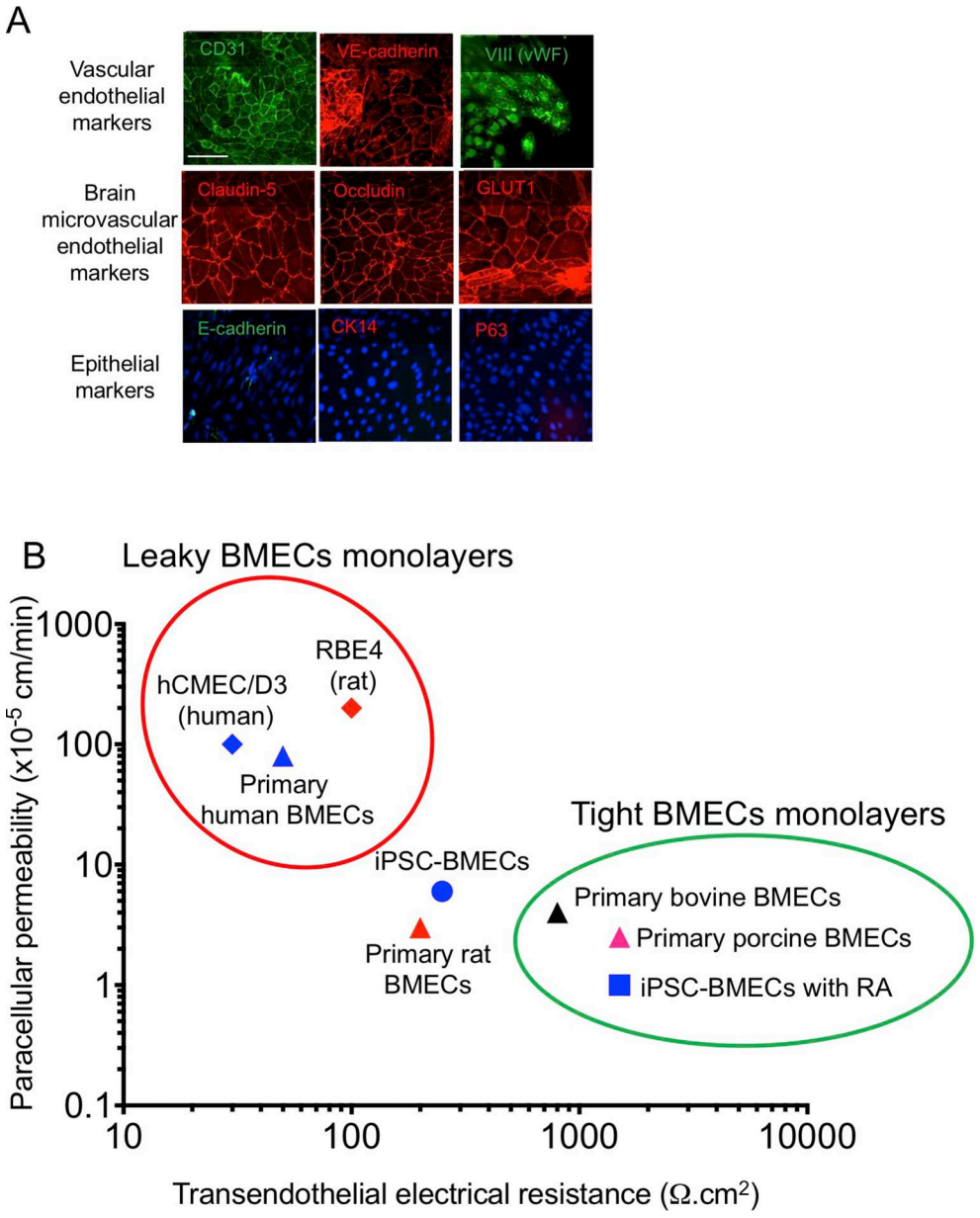

Fig. 3. Immunological and barrier phenotype of purified iPSC-derived BMECs.
(A) Representative micrograph picture of purified iPSC-derived BMECs monocultures at day 10 of differentiation. Note the absence of cellular markers indicative of epithelial cell lineage (E-cadherin, cytokeratin 14, P63). Scale bar = 50 µm. (B) Barrier properties of iPSC-derived BMECs (without or with retinoic acid treatment) compared to primary cultures and immortalized cells. Cells capable to harbor TEER values over 500 Ω·cm^2 are considered as tight monolayers.

provide an integrative model (inclusive of astrocytes and neurons) capable to better reflect the *in vivo* situation.

3.3. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia, marked by progressive loss of hippocampal neurons, characterized by the presence of amyloid senile plaques (formed by Aβ peptides aggregation) and neurofibrillary tangles (enriched in hyperphosphorylated tau proteins). Despite the important effort centered around two major hypotheses (Aβ and tau hypotheses), tangible outcomes in terms of clinically relevant drugs remains to be assessed [108]. More recently, several studies highlighted the presence of a dysfunctional BBB in AD patients and raised several hypotheses revolving around the BBB and the NVU. Notably, several studies highlighted an impaired cerebral blood flow (CBF) in an animal model of hypertension. Such models correlated with similar outcomes observed in patients diagnosed with mild cognitive impairment (MCI). Another hypothesis supported by experimental data is the "Aβ clearance" hypothesis. In this hypothesis, the BBB plays an important role as a site for Aβ peptide clearance. Such clearance occurs *via* a brain-to-blood efflux mechanism *via* an RMT-dependent mechanism. In this hypothesis, Aβ peptides bind to LDL receptor related protein (LRP1), a receptor involved in brain cholesterol homeostasis, internalize through the recruitment of PICALM, and are excreted on the luminal side by a P-glycoprotein dependent efflux [51,109–116]. This hypothesis has been reinforced identifying the APOE allelic variant (ε4) as an increased risk factor for early onset of AD [117–119] and the reduction of PICALM in primary BMEC cultures isolated from AD patients [51]. In addition to the Aβ clearance mechanism, a study by Winkler and colleagues using a GLUT-1 deficient/APP-PS1 overexpressing mouse strain, reported a negative association between decreased GLUT-1 expression and impaired Aβ

Table 1
hPSC-derived BMECs differentiation protocols.

Protocol type	Defined lineage	Differentiation length (days)	Additional steps	Cell populations	Xeno-free	Barrier properties	Inducible by co-cultures
Lippmann et al. [84]	No	14–16	–	BMECs, neuron progenitors	No	Modest	Yes
Lippmann et al. [90]	No	14–16	10 μM retinoic acid	BMECs, neuron progenitors	No	Tight	Yes
Wilson et al. [93]	No	14–16	Defined cell density	BMECs, neuron progenitors	No	Tight	N.D.
Patel et Alahmad [94]	No	14–16	Xeno-free medium	BMECs, neuron progenitors	Yes	Tight	N.D.
Hollmann et al. [95]	No	12–14	Xeno-free medium	Endothelial progenitors	Yes	Tight	N.D.
Qian et al. [91]	Mesoderm	14–16	10 μM CHIR99021	Endothelial progenitors	Yes	Tight	Yes

clearance [113]. The use of iPSC models to assess the presence of similar mechanisms at the BBB remains as today anecdotical. Using an *in vitro* model based on iPSC-derived monocultures, Zhao and colleagues demonstrated the contribution of PICALM in Aβ transcytosis and identified the single nucleotide polymorphism rs3851179 in PICALM gene (GG variant) as potentially a contributing risk factor [51]. Yet such model did not address the contribution of genes associated with the familial form of AD (FAD). Our group recently initiated the differentiation and the characterization of iPSC-derived BMECs from patients diagnosed as FAD and harboring mutations in PSEN1 and PSEN2 genes (unpublished data). However, the relevance of such PSEN mutations to clinically relevant signs of the BBB disruption remains to be asserted. Two studies highlighted the presence of cerebral microbleeds in PSEN1 transgenic mice [120,121], suggesting a similar outcome than observed in our model and suggest a potential *in vitro-in vivo* correlation. Yet, such model cannot answer the presence of a disrupted BBB in patients presenting a late-onset of the disease and may also have to consider other cofounding risk factors associated with AD (*e.g.* stroke, type 2 diabetes) known to induce a BBB disruption [36,37]. Taken together, iPSC-based model of FAD can provide an interesting insight on how mutations commonly documented on a neural phenotype may impact the BBB function. Yet, the pertinence of such models remains hindered by our limited understanding of the AD pathophysiology on the BBB function. Therefore, iPSC-based models of the BBB for studying AD can provide a complementary tool to existing animal models, yet such tool remains to be validated and demonstrate its ability to reflect phenomenon observed *in vivo*.

3.4. Amyotrophic lateral sclerosis

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease characterized by a progressive and lethal loss of motor neurons. Until now, there are no treatments available that are capable of reducing or reversing this condition. Two forms of ALS are described in the literature: a sporadic form representing the vast majority of total cases of ALS and a familial form linked to mutations in several genes (C9ORF72, FUS, SOD1, TDP-43) [122,123]. Although familial forms of ALS represent a fraction of total cases of ALS (< 15%), the presence of a genetic component has allowed researchers to identify putative gene mutations associated with ALS. Several studies have highlighted features associated with a dysfunctional BBB including a disruption of the BBB [124], an activation of the BBB allowing the migration of immune cells [124], an impaired response to oxidative stress [123], and an abnormal increase in ABC transporter activity [125,126]. As of today, with the exception of preliminary results based on iPSC-derived BMECs co-cultured with motoneurons (Sances and colleagues, personal communication), the publication of an iPSC-derived model of the BBB based on ALS patients remains undocumented. As other models, the pertinence of such *in vitro* models remains limited to genetic forms of ALS, which still represent only a fraction (< 15%) of the total cases of ALS described in clinic.

3.5. GLUT1 deficiency syndrome

Despite its small size (~2% total body weight), the brain consumes about 25% of the daily amount of glucose. Glucose transport across the BBB is occurring *via* the presence of glucose transporters. At the human BBB, various GLUT isoforms have been identified and include GLUT1 (considered as the major glucose transporter at the BBB), GLUT3, and to a lesser extent, GLUT4 [15,16,113]. Although distribution of GLUT1 is considered mostly on the luminal surface, a recent study by Devraj and colleagues highlighted the presence of an abluminal distribution of GLUT1 at the BBB [127]. Paradoxically, BMEC glucose utilization relies on an anaerobic utilization despite being exposed to elevated O_2 levels [128], suggesting an important dependence of BMECs on glucose. GLUT1 deficiency syndrome (G1D) is a rare, autosomal dominant

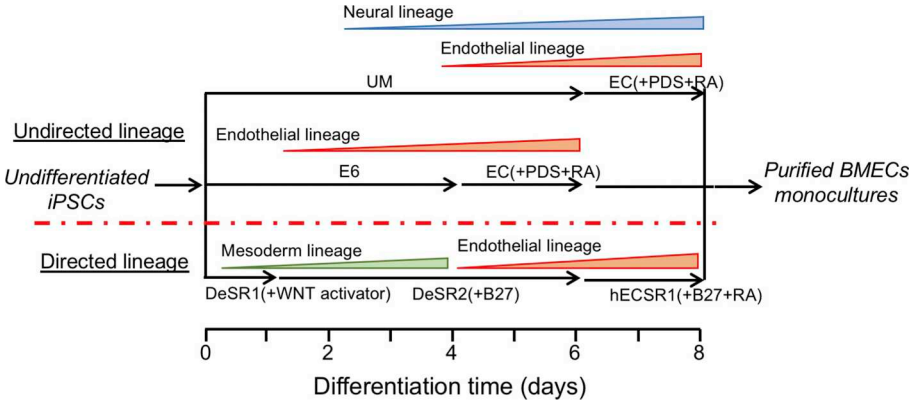

Fig. 4. Comparative flowchart of current iPSC-derived BMECs differentiation protocols.
Descriptive overview of the differentiation timeline and described cell lineage of the different BMECs differentiation protocols reported in the literature. The detailed composition of media were as the following: B27 (1× B-27 supplement); bFGF (basic fibroblast growth factor, 20 ng/mL), DeSR1 (DMEM/Ham's F-12, 1× non-essential aminoacids, 1× Glutamax, 0.1 mM β-mercaptoethanol, 6 μM CHIR99021), DeSR2 (DMEM/Ham's-F12, 1× non-essential aminoacids, 1× Glutamax, 0.1 mM β-mercaptoethanol), E6 (Essential-6® Medium), EC (human endothelial cell serum-free medium), hECSR1 (EC + bFGF + RA + B27), RA (10 μM retinoic acid), UM (DMEM/Ham's-F12, 20% knockout serum replacement, 1× non-essential aminoacids, 1× Glutamax, 0.1 mM β-mercaptoethanol).

disease characterized by the presence of epileptic-like seizure during infancy, a developmental delay, and a movement disorder [129]. A major pathophysiological aspect of the disease is marked by a decrease CSF/blood ratio for both glucose and lactate levels in G1D patients compared to controls. Mutations in the GLUT1 transporter have been primarily reported by De Vivo and colleagues in 1991 [130], with several mutations identified so far. Yet, the association between GLUT1 mutations and glucose uptake remains unclear. The current treatment for G1D is the submission of these patients to a restrictive ketogenic diet at an early age, with a progressive transition into a Modified Atkins Diet [129,131–134]. Yet, such diet remains highly challenging in terms of adherence and nutritional balance, as well as long-term issues of a lipid-rich diet on the cardiovascular outcomes in patient. Although the availability of G1D iPSCs remains virtually inexistent today, our group has characterized the use of patient-derived iPSCs (isolated from asymptomatic controls) as a screening tool to better understand glucose transport at the BBB [15], as well as the impact of a ketogenic diet at the BBB (unpublished data). Ultimately, our aim is to generate G1D iPSCs from patients or *via* genetic editing to develop a screening platform for identifying small molecules capable of partially restoring functional glucose uptake at the BBB in G1D patients.

3.6. Huntington's disease

Huntington's disease (HD) is a lethal autosomal dominant disorder affecting about 200,000 patients in the US per year. It is characterized by a progressive degeneration of cortical and striatal neurons [135]. Individuals suffering from HD display several symptoms including anxiety, depression, and progressive and irreversible cognitive and motor impairment. The main pathological event of HD is marked by a gain-of-function mutation in the huntingtin gene (HTT). Although the function of HTT gene product remains unclear, HD patients display a high sequence of CAG repeats in the HTT gene, resulting in the presence of a polyglutamine (PolyQ) domain, allowing mutated HTT gene product (mHTT) to aggregate and form oligomers. mHTT oligomers appear microscopically as nuclear inclusions in striatal neurons. The incidence of HD at the BBB remains mostly unclear, but recent studies noted aberrant neurovascular features and neurovascular uncoupling in HD mice models [32,136,137]. In particular, Drouin-Ouellet and colleagues noted the presence of mHTT aggregates in several cerebrovascular regions of R6/2 mice as well in HD patients brain tissue samples suggesting an impaired BBB function *in vivo* [32]. Notably, this model highlighted the presence of a BBB leakage in the striatum suggesting the possible detrimental effect of mHTT at the BBB. Yet, a better understanding of the molecular mechanisms underlying such observation was obtained by a recent study from Lim and colleagues [69]. In this study, the authors have differentiated iPSCs obtained from HD patients into BMECs. Notably, HD-BMECs showed a higher migratory and angiogenic phenotype compared to control-BMECs. In addition, HD-BMECs showed lower barrier function compared to controls as marked by lower TEER values, higher permeability to paracellular tracers, down-regulation of tight junction protein expression, and reduced P-glycoprotein activity. Further, impaired barrier function was inversely correlated with the number of CAG repeats. A transcriptomic analysis highlighted the possible delay in maturation of HD-BMECs compared to control BMECs, and this delay appeared to be associated with an overactive canonical WNT (WNT/β-catenin) pathway, a signaling pathway associated with CNS angiogenesis and development of the BBB [138,139]. Taken together, HD-derived BBB model provides a potential insight of the contribution of a dysfunctional BBB function in HD patients. Yet, the correlation between such disruption and clinical features reflecting such disruption remains to be documented.

3.7. Ischemic stroke

Stroke constitutes the fifth leading cause of death in men and the third leading cause of death women in the US, according to the most recent statistic of the American Heart Association (AHA) [140]. Amongst the different types of stroke, ischemic stroke still represents the most common type of stroke (85%) and remains a major cause of disability. Ischemic stroke onset is characterized by the detachment of a thrombus from atherosclerotic plaques present in one of afferent arteries (often at the carotid bifurcation). Such thrombus ultimately results in the occlusion of small caliber blood vessels, resulting in a severe hypoperfusion, thereby depriving the tissue from adequate oxygen and necessary nutrients. The BBB plays a critical role during stroke, as such injury leads to the loss of tight junction complexes, leading to a unregulated water and solute diffusion inside the brain parenchyma, and eventually neuronal cell death by excitotoxicity and edema [141]. Despite an important investment in finding treatments capable of stopping or reversing ischemic injury, only one therapeutic (tissue plasminogen activator) has been approved by the Food and Drug administration [142–146]. Until now, *in vitro* models modestly contributed to our global understanding of the disease at the human BBB due to poor barrier properties. In addition, animal models of stroke injury (MCAo) provide only a limited representation of the morbidity and cardiovascular risk factors associated with stroke in patients [147]. Recently, our lab has assessed and characterized the cellular response of iPSC-derived BMEC monolayers to ischemic stroke *in vitro* [148]. Using oxygen-glucose deprivation (OGD), our group demonstrated the ability to display a significant disruption of the BBB, as marked by a decrease in TEER and an increased permeability. Furthermore, preliminary data (unpublished results) indicates that such barrier disruption is not restored during reoxygenation and involves the activation of the hypoxia-inducible factor-1 (HIF-1) pathway, as well as the up-regulation of vascular endothelial growth factor (VEGF). These two factors are well

known hypoxia-driven pathways involved in the BBB disruption [149]. Furthermore, the use of an isogeneic model allowed us handful insights on how other cells of the neurovascular unit (astrocytes, neurons) modulate iPSC-derived BMECs response to OGD/reoxygenation injury (unpublished data). Although iPSC-derived model appears as an adequate tool to elucidate the mechanisms behind ischemia-induced barrier disruption at the BBB and eventually maybe suitable as a screening tool for identifying novel neuroprotective pathways, the absence of perfusion-based *in vitro* models capable to reproduce the hypoperfusion/reperfusion as seen *in vivo* still represents an important limiting factor.

3.8. Pathogen-host interactions

Although the BBB naturally protects the CNS from infectious agents, it can be impaired and disrupted by certain biological entities such as viruses, bacteria, fungi, and parasites. Until now, our comprehension of the pathophysiology underlying pathogen-host interactions at the BBB remains unclear and poorly characterized. Several studies have been investigating the cellular mechanisms by which these pathogens interact with the BBB using iPSC-based models. For instance, a recent study by Alimonti and colleagues investigated the interaction of Zika virus (ZIKV) with iPSC-derived BMEC monocultures [85]. They observed that ZIKV was able to diffuse across the BBB *via* a transcellular route, without affecting tight junction complexes. Another study published by Kim and colleagues characterized the ability of Group B *Streptococcus* (a Gram+ cocci associated with neonatal bacterial meningitis) to impair the barrier function in iPSC-derived BMEC monolayers [150]. In particular, expression of virulence factors on the bacteria surface of this bacterium induced the disruption of the BBB by disruption of the tight junction complexes *via* activation of Snail-1. More recently, our group characterized the ability of 2,5-diketopiperazine gliotoxin, a class of mycotoxin produced by *Aspergillus* to breach the BBB. Gliotoxin was able to impair the barrier function in iPSC-derived BMECs without altering tight junction complexes [151]. As these particular pathogens only account for a handful of neurological disorders associated with pathogen-host interaction, more research needs to be done to investigate how other pathogens may breach the BBB to cause neurological dysfunction.

4. Current opportunities, challenges and future directions of iPSC-based models of the blood-brain barrier

The blood-brain barrier (BBB) constitutes an important cellular interface between the circulatory system and the central nervous system, providing an essential physical and chemical barrier aimed to provide a stable and protected microenvironment to neurons.

For decades, the contribution of the cerebrovascular system in the development and progression of neurological diseases has been occulted. However, in the recent years, a growing number of studies have highlighted the contribution of a dysfunctional BBB in many neurological diseases. Yet, the use of *in vitro* and *in vivo* models of the diseased BBB has been hindered by several obstacles including the poor barrier properties of current *in vitro* models, the lack of scalability of primary cell cultures, and most importantly, the limited access to tissue samples from patients suffering from such diseases. Since the initial differentiation protocol of human embryonic stem cells into neurons [44] and the derivation of somatic cells into induced pluripotent stem cells (iPSCs) [42,43], significant advances have been made in the use of iPSCs to model neurological disorders and to better understand the cellular and molecular mechanisms underlying such conditions. Since the original publication of the differentiation protocol by Lippmann and colleagues [84], over 30 studies using stem cell derived models of the BBB have been published, suggesting the rising popularity of this model amongst the community. Stem cell-based models of the BBB provide key advantages compared to existing *in vitro* models as such models provide a human, scalable, and reproducible source of cells capable of achieving barrier properties similar to *in vivo*. In addition, the use of iPSCs provides a modular model allowing the use of isogeneic co-cultures (astrocytes and/or neurons) and therefore bring such models closer to the complexity observed *in vivo*. More recently, iPSC-derived models of the BBB have been documented to model the barrier during neurological diseases triggered by genetic or environmental factors. The use of patient-specific *in vitro* models of the BBB opens up novel avenues to investigate how genetic disorders associated with neurological symptoms impair the barrier function, thus eventually leading us to the identification of novel therapeutic targets. Yet, as promising as such models are, they still suffer from several limitations, including several limitations inherent to the use of iPSC-based models.

Firstly, the presence of limited availability iPSC lines from stem cell repository makes it difficult to sort phenotype outcomes inherently due to a gene mutation from the genetic variability due to the derivation and maintenance of iPSCs.

Secondly, there is still a skepticism on whether these iPSC-derived BMECs are fully representative of human BMECs, both primary and immortalized sources. Until now, we and others assume that such iPSC-derived BMECs are representative of human BMECs based on the phenotyping using limited criteria (protein expression of cell markers, barrier function…). Thus, a comprehensive and comparative multi-omics approach (*e.g.* RNA-seq, proteomics, lipidomics) directly comparing such iPSC-derived BMECs to somatic BMECs is still needed. Vatine and colleagues [88] has provided a limited insight by comparing their iPSC-derived BMECs to primary human BMECs and reported about 90% gene expression overlap between their iPSC-derived BMECs and primary BMECs. Yet, such comparison focused on a limited set of genes (504 genes) and did not assess differences in terms of relative gene expression.

Thirdly, a recurrent issue that was observed with iPSC-based models of the BBB from diseased patients is the limited or absent *in vitro-to-in vivo* correlation (IVIVC) and the pertinence of a disrupted BBB in regards of the clinical pathology. Although such models show a disrupted barrier function compared to controls, the pertinence of such disruption appears limited to genetic forms that represent only a fraction of the total number of cases for such diseases. Therefore, clinical studies supporting changes in the BBB integrity *via* the documentation of changes in biomarkers and neuroimaging can help bolster the translational aspects of iPSC-based models.

Finally, an important caveat of such model remains the limited reflectivity of the *in vivo* situation: these models often are based on BMECs monocultures grown in a static microenvironment. The integration of the different cell types in cultures as described with “isogeneic models of the BBB” [87,152] are aimed to provide an integrative *in vitro* model closer to *in vivo* settings (Fig. 5). In such models, astrocytes and neurons are obtained from the same iPSC line using distinct protocols and reunited as co-cultures upon complete differentiation of these cell types. The advantage of such models allows a modularity while maintaining a patient-specific source of cells, but also bring such models closer to *in vivo*. Although current iPSC-based models have several advantages compared to other models, the absence of a shear stress at the luminal side of BMEC monolayers still constitutes an important issue that cannot be ignored. However, recent attempts have been made to overcome such limitations by adopting the use of microfluidic devices (Fig. 5) [153–155]. Earlier studies have used primary and/or immortalized BMECs grown in monocultures or as co-cultures [155–158] More recent studies have used BMECs derived from iPSCs to mimic the NVU using microfluidic technology. Some incentives of microfluidics technology combined with iPSC-derived BMECs are flow based conditions with continuous maintenance of high barrier properties (TEER $> 3000\,\Omega{\cdot}cm^2$) for more than a week [159]. Microfluidic models combined with iPSC-derived BMECs and spinal neural progenitors have been used to understand developmental perspectives of spinal cord development in the presence of the BBB [160]. More research into iPSC-

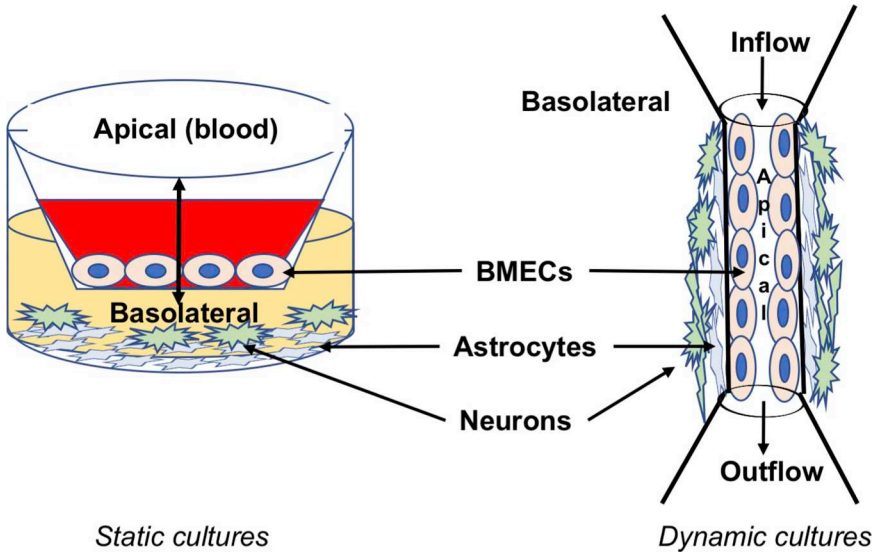

Fig. 5. Comparative experimental setup between static and dynamic *in vitro* models of the BBB.

derived BMECs used with microfluidics devices would provide a more *in vivo*-like NVU for disease modeling and therapeutic screening, but also bring such models closer to pre-clinical and clinical models.

In conclusion, stem-cell based models of the blood-brain barrier show promising potentials, yet there are several challenges ahead to consider such models as viable alternatives to *in vivo* models. Potential avenues of improvement of current iPSC-based models may include: 1) the development of differentiation protocols allowing a direct differentiation of somatic cells into BMECs, 2) the inclusion of iPSCs from patients with sporadic forms of the neurological diseases, 3) the integration of the different components of the neurovascular unit into a hydrodynamic cell culture.

We can hypothesize in a foreseeable future that patient-derived *in vitro* models of the blood-brain barrier may contribute as a valuable tool for personalized and precision medicine.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

S.P. and R.P. contributed equally to the bibliographical research and the writing of this manuscript. S.R. contributed in the illustrations presented in this review. A.A. prepared the figures and micrograph pictures presented in the review, supervised, designed and contributed in the writing of this manuscript. The authors have no competitive interests to declare. This work was funded by institutional funds to A.A.

References

[1] N.J. Abbott, Blood-brain barrier structure and function and the challenges for CNS drug delivery, J. Inherit. Metab. Dis. 36 (2013) 437–449.
[2] P. Ehrlich, Das sauerstufbudurfnis des organismus, Eine Farbenanalytische Studie, (1885).
[3] E.E. Goldmann, Vitalfärbung am Zentralnervensyatem. Beitrag zur Physio-Pathologie des plexus chorioideus und der Hirnhäute., Abh preuss, Akad. Wiss. Phys-Math Kl 1 (1913) 1–60.
[4] E.E. Goldmann, Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der 'vitalen Färbung, Beitr. Klin. Chir. 64 (1909) 192–265.
[5] T.S. Reese, M.J. Karnovsky, Fine structural localization of a blood-brain barrier to exogenous peroxidase, J. Cell Biol. 34 (1967) 207–217.
[6] M.S. Thomsen, L.J. Routhe, T. Moos, The vascular basement membrane in the healthy and pathological brain, J. Cereb. Blood Flow Metab. 37 (2017) 3300–3317.
[7] P. Dore-Duffy, K. Cleary, Morphology and properties of pericytes, Methods Mol. Biol. 686 (2011) 49–68.
[8] N.J. Abbott, M.E. Pizzo, J.E. Preston, D. Janigro, R.G. Thorne, The role of brain barriers in fluid movement in the CNS: is there a 'glymphatic' system? Acta Neuropathol. 135 (2018) 387–407.
[9] S. Ma, H.J. Kwon, H. Johng, K. Zang, Z. Huang, Radial glial neural progenitors regulate nascent brain vascular network stabilization via inhibition of Wnt signaling, PLoS Biol. 11 (2013) e1001469.
[10] E.S. Lippmann, C. Weidenfeller, C.N. Svendsen, E.V. Shusta, Blood-brain barrier modeling with co-cultured neural progenitor cell-derived astrocytes and neurons, J. Neurochem. 119 (2011) 507–520.
[11] G. Schiera, E. Bono, M.P. Raffa, A. Gallo, G.L. Pitarresi, I. Di Liegro, G. Savettieri, Synergistic effects of neurons and astrocytes on the differentiation of brain capillary endothelial cells in culture, J. Cell. Mol. Med. 7 (2003) 165–170.
[12] G. Savettieri, I. Di Liegro, C. Catania, L. Licata, G.L. Pitarresi, S. D'Agostino, G. Schiera, V. De Caro, G. Giandalia, L.I. Giannola, A. Cestelli, Neurons and ECM regulate occludin localization in brain endothelial cells, Neuroreport 11 (2000) 1081–1084.
[13] R. Daneman, L. Zhou, A.A. Kebede, B.A. Barres, Pericytes are required for blood-brain barrier integrity during embryogenesis, Nature 468 (2010) 562–566.
[14] F. Sohet, C. Lin, R.N. Munji, S.Y. Lee, N. Ruderisch, A. Soung, T.D. Arnold, N. Derugin, Z.S. Vexler, F.T. Yen, R. Daneman, LSR/angulin-1 is a tricellular tight junction protein involved in blood-brain barrier formation, J. Cell Biol. 208 (2015) 703–711.
[15] A.J. Al-Ahmad, Comparative study of expression and activity of glucose transporters between stem cell-derived brain microvascular endothelial cells and hCMEC/D3 cells, Am. J. Physiol. Cell Physiol. 313 (2017) C421–C429.
[16] M. Meireles, F. Martel, J. Araujo, C. Santos-Buelga, S. Gonzalez-Manzano, M. Duenas, V. de Freitas, N. Mateus, C. Calhau, A. Faria, Characterization and modulation of glucose uptake in a human blood-brain barrier model, J. Membr. Biol. 246 (2013) 669–677.
[17] R. Lyck, N. Ruderisch, A.G. Moll, O. Steiner, C.D. Cohen, B. Engelhardt, V. Makrides, F. Verrey, Culture-induced changes in blood-brain barrier transcriptome: implications for amino-acid transporters in vivo, J. Cereb. Blood Flow Metab. 29 (2009) 1491–1502.
[18] E. Dolgodilina, S. Imobersteg, E. Laczko, T. Welt, F. Verrey, V. Makrides, Brain interstitial fluid glutamine homeostasis is controlled by blood-brain barrier SLC7A5/LAT1 amino acid transporter, J. Cereb. Blood Flow Metab. 36 (2016) 1929–1941.
[19] J. Fu, A.M. Dumitrescu, Inherited defects in thyroid hormone cell-membrane transport and metabolism, Best Pract. Res. Clin. Endocrinol. Metab. 28 (2014) 189–201.
[20] S. Mayerl, J. Muller, R. Bauer, S. Richert, C.M. Kassmann, V.M. Darras, K. Buder, A. Boelen, T.J. Visser, H. Heuer, Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis, J. Clin. Invest. 124 (2014) 1987–1999.
[21] E.K. Wirth, U. Schweizer, J. Kohrle, Transport of thyroid hormone in brain, Front. Endocrinol. 5 (2014) 98.
[22] A.M. Muzzio, P.D. Noyes, H.M. Stapleton, S.C. Lema, Tissue distribution and thyroid hormone effects on mRNA abundance for membrane transporters Mct8, Mct10, and organic anion-transporting polypeptides (Oatps) in a teleost fish, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 167 (2014) 77–89.
[23] P.T. Ronaldson, Targeting transporters for CNS drug delivery, Curr. Pharm. Des. 20 (2014) 1419–1421.
[24] L. Sanchez-Covarrubias, L.M. Slosky, B.J. Thompson, T.P. Davis, P.T. Ronaldson, Transporters at CNS barrier sites: obstacles or opportunities for drug delivery?

Curr. Pharm. Des. 20 (2014) 1422–1449.
[25] I. Mager, A.H. Meyer, J. Li, M. Lenter, T. Hildebrandt, G. Leparc, M.J.A. Wood, Targeting blood-brain-barrier transcytosis - perspectives for drug delivery, Neuropharmacology 120 (2017) 4–7.
[26] W.M. Pardridge, Targeted delivery of protein and gene medicines through the blood-brain barrier, Clin. Pharmacol. Ther. 97 (2015) 347–361.
[27] W.M. Pardridge, The blood-brain barrier: bottleneck in brain drug development, NeuroRx 2 (2005) 3–14.
[28] W.H. Organization, Neurological Disorders: Public Health Challenges, WHO Press, 2006 (Place Published).
[29] A. Montagne, Z. Zhao, B.V. Zlokovic, Alzheimer's disease: A matter of blood-brain barrier dysfunction? J. Exp. Med. 214 (2017) 3151–3169.
[30] E.A. van Vliet, E. Aronica, J.A. Gorter, Blood-brain barrier dysfunction, seizures and epilepsy, Semin. Cell Dev. Biol. 38 (2015) 26–34.
[31] E.A. van Vliet, E. Aronica, J.A. Gorter, Role of blood-brain barrier in temporal lobe epilepsy and pharmacoresistance, Neuroscience 277 (2014) 455–473.
[32] J. Drouin-Ouellet, S.J. Sawiak, G. Cisbani, M. Lagace, W.L. Kuan, M. Saint-Pierre, R.J. Dury, W. Alata, I. St-Amour, S.L. Mason, F. Calon, S. Lacroix, P.A. Gowland, S.T. Francis, R.A. Barker, F. Cicchetti, Cerebrovascular and blood-brain barrier impairments in Huntington's disease: potential implications for its pathophysiology, Ann. Neurol. 78 (2015) 160–177.
[33] H.J. Waldvogel, M. Dragunow, R.L. Faull, Disrupted vasculature and blood-brain barrier in Huntington disease, Ann. Neurol. 78 (2015) 158–159.
[34] M.A. Lopes Pinheiro, G. Kooij, M.R. Mizee, A. Kamermans, G. Enzmann, R. Lyck, M. Schwaninger, B. Engelhardt, H.E. de Vries, Immune cell trafficking across the barriers of the central nervous system in multiple sclerosis and stroke, Biochim. Biophys. Acta 1862 (2016) 461–471.
[35] M.T. Gray, J.M. Woulfe, Striatal blood-brain barrier permeability in Parkinson's disease, J. Cereb. Blood Flow Metab. 35 (2015) 747–750.
[36] M.J. Haley, C.B. Lawrence, The blood-brain barrier after stroke: structural studies and the role of transcytotic vesicles, J. Cereb. Blood Flow Metab. 37 (2017) 456–470.
[37] Z. Merali, K. Huang, D. Mikulis, F. Silver, A. Kassner, Evolution of blood-brain-barrier permeability after acute ischemic stroke, PLoS One 12 (2017) e0171558.
[38] Y. Yang, G.A. Rosenberg, Blood-brain barrier breakdown in acute and chronic cerebrovascular disease, Stroke 42 (2011) 3323–3328.
[39] R. Raja, G.A. Rosenberg, A. Caprihan, MRI measurements of blood-brain barrier function in dementia: A review of recent studies, Neuropharmacology 134 (2018) 259–271.
[40] G.A. Rosenberg, Extracellular matrix inflammation in vascular cognitive impairment and dementia, Clin. Sci. (Lond.) 131 (2017) 425–437.
[41] G.A. Rosenberg, Neurological diseases in relation to the blood-brain barrier, J. Cereb. Blood Flow Metab. 32 (2012) 1139–1151.
[42] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[43] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.
[44] S.C. Zhang, M. Wernig, I.D. Duncan, O. Brüstle, J.A. Thomson, In vitro differentiation of transplantable neural precursors from human embryonic stem cells, Nat. Biotechnol. (2001) 1129–1133.
[45] D. Son, Z. Quan, P.J. Kang, G. Park, H.C. Kang, S. You, Generation of two induced pluripotent stem cell (iPSC) lines from X-linked adrenoleukodystrophy (X-ALD) patients with adrenomyeloneuropathy (AMN), Stem Cell Res. 25 (2017) 46–49.
[46] Y.R. You, D. Son, P.J. Kang, S. You, D.S. Kim, Generation of induced pluripotent stem cell (iPSC) line from a 21-year-old X-linked adrenoleukodystrophy (X-ALD) patient, Stem Cell Res. 25 (2017) 136–138.
[47] M. Baarine, M. Khan, A. Singh, I. Singh, Functional characterization of IPSC-derived brain cells as a model for X-linked adrenoleukodystrophy, PLoS One 10 (2015) e0143238.
[48] C.M. Karch, D. Hernandez, J.C. Wang, J. Marsh, A.W. Hewitt, S. Hsu, J. Norton, D. Levitch, T. Donahue, W. Sigurdson, B. Ghetti, M. Farlow, J. Chhatwal, S. Berman, C. Cruchaga, J.C. Morris, R.J. Bateman, N. Dominantly Inherited Alzheimer, A. Pebay, A.M. Goate, Human fibroblast and stem cell resource from the Dominantly Inherited Alzheimer Network, Alzheimers Res. Ther. 10 (2018) 69.
[49] J.A. Garcia-Leon, A. Cabrera-Socorro, K. Eggermont, A. Swijsen, J. Terryn, R. Fazal, F. Nami, L. Ordovas, A. Quiles, F. Lluis, L. Serneels, K. Wierda, A. Sierksma, M. Kreir, F. Pestana, P. Van Damme, B. De Strooper, L. Thorrez, A. Ebneth, C.M. Verfaillie, Generation of a human induced pluripotent stem cell-based model for tauopathies combining three microtubule-associated protein TAU mutations which displays several phenotypes linked to neurodegeneration, Alzheimers Dement. 14 (2018) 1261–1280.
[50] P. Martin-Maestro, R. Gargini, A.S. A, E. Garcia, L.C. Anton, S. Noggle, O. Arancio, J. Avila, V. Garcia-Escudero, Mitophagy failure in fibroblasts and iPSC-derived neurons of Alzheimer's Disease-associated presenilin 1 mutation, Front. Mol. Neurosci. 10 (2017) 291.
[51] Z. Zhao, A.P. Sagare, Q. Ma, M.R. Halliday, P. Kong, K. Kisler, E.A. Winkler, A. Ramanathan, T. Kanekiyo, G. Bu, N.C. Owens, S.V. Rege, G. Si, A. Ahuja, D. Zhu, C.A. Miller, J.A. Schneider, M. Maeda, T. Maeda, T. Sugawara, J.K. Ichida, B.V. Zlokovic, Central role for PICALM in amyloid-beta blood-brain barrier transcytosis and clearance, Nat. Neurosci. 18 (2015) 978–987.
[52] L. Mohamet, N.J. Miazga, C.M. Ward, Familial Alzheimer's disease modelling using induced pluripotent stem cell technology, World J. Stem Cells 6 (2014) 239–247.
[53] M.A. Israel, S.H. Yuan, C. Bardy, S.M. Reyna, Y. Mu, C. Herrera, M.P. Hefferan, S. Van Gorp, K.L. Nazor, F.S. Boscolo, C.T. Carson, L.C. Laurent, M. Marsala, F.H. Gage, A.M. Remes, E.H. Koo, L.S. Goldstein, Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells, Nature 482 (2012) 216–220.
[54] T. Yagi, D. Ito, Y. Okada, W. Akamatsu, Y. Nihei, T. Yoshizaki, S. Yamanaka, H. Okano, N. Suzuki, Modeling familial Alzheimer's disease with induced pluripotent stem cells, Hum. Mol. Genet. 20 (2011) 4530–4539.
[55] A. Vitrac, I. Cloez-Tayarani, Induced pluripotent stem cells as a tool to study brain circuits in autism-related disorders, Stem Cell Res Ther 9 (2018) 226.
[56] J. Barnes, F. Salas, R. Mokhtari, H. Dolstra, E. Pedrosa, H.M. Lachman, Modeling the neuropsychiatric manifestations of Lowe syndrome using induced pluripotent stem cells: defective F-actin polymerization and WAVE-1 expression in neuronal cells, Mol. Autism 9 (2018) 44.
[57] J.M. Crook, G. Wallace, E. Tomaskovic-Crook, The potential of induced pluripotent stem cells in models of neurological disorders: implications on future therapy, Expert. Rev. Neurother. 15 (2015) 295–304.
[58] M.E. Doers, M.T. Musser, R. Nichol, E.R. Berndt, M. Baker, T.M. Gomez, S.C. Zhang, L. Abbeduto, A. Bhattacharyya, iPSC-derived forebrain neurons from FXS individuals show defects in initial neurite outgrowth, Stem Cells Dev. 23 (2014) 1777–1787.
[59] A.L. Brigida, D. Siniscalco, Induced pluripotent stem cells as a cellular model for studying Down Syndrome, J. Stem Cells Regen. Med. 12 (2016) 54–60.
[60] Y. Hibaoui, I. Grad, A. Letourneau, M.R. Sailani, S. Dahoun, F.A. Santoni, S. Gimelli, M. Guipponi, M.F. Pelte, F. Bena, S.E. Antonarakis, A. Feki, Modelling and rescuing neurodevelopmental defect of Down syndrome using induced pluripotent stem cells from monozygotic twins discordant for trisomy 21, EMBO Mol. Med. 6 (2014) 259–277.
[61] X. Mou, Y. Wu, H. Cao, Q. Meng, Q. Wang, C. Sun, S. Hu, Y. Ma, H. Zhang, Generation of disease-specific induced pluripotent stem cells from patients with different karyotypes of Down syndrome, Stem Cell Res Ther 3 (2012) 14.
[62] R. Zhou, G. Jiang, X. Tian, X. Wang, Progress in the molecular mechanisms of genetic epilepsies using patient-induced pluripotent stem cells, Epilepsia Open 3 (2018) 331–339.
[63] L. van Diepen, F.F.R. Buettner, D. Hoffmann, C.T. Thiesler, O. von Bohlen Und Halbach, V. von Bohlen Und Halbach, L.R. Jensen, D. Steinemann, S. Edvardson, O. Elpeleg, A. Schambach, R. Gerardy-Schahn, A.W. Kuss, A patient-specific induced pluripotent stem cell model for West syndrome caused by ST3GAL3 deficiency, Eur. J. Hum. Genet. 26 (12) (2018) 1773–1783 https://www.nature.com/articles/s41431-018-0220-5.
[64] Y. Tanaka, N. Higurashi, N. Shirasu, S. Yasunaga, K.M. Moreira, H. Okano, S. Hirose, Establishment of a human induced stem cell line (FUi002-A) from Dravet syndrome patient carrying heterozygous R1525X mutation in SCN1A gene, Stem Cell Res. 31 (2018) 11–15.
[65] Y.T. Wu, Y.H. Hsu, C.Y. Huang, M.C. Ho, Y.C. Cheng, C.H. Wen, H.W. Ko, H.E. Lu, Y.C. Chen, C.L. Tsai, Y.C. Hsu, Y.H. Wei, P.C.H. Hsieh, Generation of an induced pluripotent stem cell (iPSC) line from a 40-year-old patient with the A8344G mutation of mitochondrial DNA and MERRF (myoclonic epilepsy with ragged red fibers) syndrome, Stem Cell Res. 27 (2018) 10–14.
[66] G.W. Tan, T. Kondo, N. Murakami, K. Imamura, T. Enami, K. Tsukita, R. Shibukawa, M. Funayama, R. Matsumoto, A. Ikeda, R. Takahashi, H. Inoue, Induced pluripotent stem cells derived from an autosomal dominant lateral temporal epilepsy (ADLTE) patient carrying S473L mutation in leucine-rich glioma inactivated 1 (LGI1), Stem Cell Res. 24 (2017) 12–15.
[67] J.M. Parent, S.A. Anderson, Reprogramming patient-derived cells to study the epilepsies, Nat. Neurosci. 18 (2015) 360–366.
[68] Y. Liu, L.F. Lopez-Santiago, Y. Yuan, J.M. Jones, H. Zhang, H.A. O'Malley, G.A. Patino, J.E. O'Brien, R. Rusconi, A. Gupta, R.C. Thompson, M.R. Natowicz, M.H. Meisler, L.L. Isom, J.M. Parent, Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism, Ann. Neurol. 74 (2013) 128–139.
[69] R.G. Lim, C. Quan, A.M. Reyes-Ortiz, S.E. Lutz, A.J. Kedaigle, T.A. Gipson, J. Wu, G.D. Vatine, J. Stocksdale, M.S. Casale, C.N. Svendsen, E. Fraenkel, D.E. Housman, D. Agalliu, L.M. Thompson, Huntington's Disease iPSC-derived brain microvascular endothelial cells reveal WNT-mediated Angiogenic and blood-brain barrier deficits, Cell Rep. 19 (2017) 1365–1377.
[70] G. Hargus, M. Ehrlich, A.L. Hallmann, T. Kuhlmann, Human stem cell models of neurodegeneration: a novel approach to study mechanisms of disease development, Acta Neuropathol. 127 (2014) 151–173.
[71] H.D.i. Consortium, Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes, Cell Stem Cell 11 (2012) 264–278.
[72] R. Fernandez-Santiago, I. Carballo-Carbajal, G. Castellano, R. Torrent, Y. Richaud, A. Sanchez-Danes, R. Vilarrasa-Blasi, A. Sanchez-Pla, J.L. Mosquera, J. Soriano, J. Lopez-Barneo, J.M. Canals, J. Alberch, A. Raya, M. Vila, A. Consiglio, J.I. Martin-Subero, M. Ezquerra, E. Tolosa, Aberrant epigenome in iPSC-derived dopaminergic neurons from Parkinson's disease patients, EMBO Mol. Med. 7 (2015) 1529–1546.
[73] J.E. Beevers, T.M. Caffrey, R. Wade-Martins, Induced pluripotent stem cell (iPSC)-derived dopaminergic models of Parkinson's disease, Biochem. Soc. Trans. 41 (2013) 1503–1508.
[74] Y. Imaizumi, Y. Okada, W. Akamatsu, M. Koike, N. Kuzumaki, H. Hayakawa, T. Nihira, T. Kobayashi, M. Ohyama, S. Sato, M. Takanashi, M. Funayama, A. Hirayama, T. Soga, T. Hishiki, M. Suematsu, T. Yagi, D. Ito, A. Kosakai, K. Hayashi, M. Shouji, A. Nakanishi, N. Suzuki, Y. Mizuno, N. Mizushima, M. Amagai, Y. Uchiyama, H. Mochizuki, N. Hattori, H. Okano, Mitochondrial dysfunction associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue, Mol. Brain

5 (2012) 35.
[75] B. Byers, B. Cord, H.N. Nguyen, B. Schule, L. Fenno, P.C. Lee, K. Deisseroth, J.W. Langston, R.R. Pera, T.D. Palmer, SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate alpha-synuclein and are susceptible to oxidative stress, PLoS One 6 (2011) e26159.
[76] A. Chhabra, Derivation of human induced pluripotent stem cell (iPSC) lines and mechanism of pluripotency: historical perspective and recent advances, Stem Cell Rev. 13 (2017) 757–773.
[77] F. Jia, K.D. Wilson, N. Sun, D.M. Gupta, M. Huang, Z. Li, N.J. Panetta, Z.Y. Chen, R.C. Robbins, M.A. Kay, M.T. Longaker, J.C. Wu, A nonviral minicircle vector for deriving human iPS cells, Nat. Methods 7 (2010) 197–199.
[78] X. Ma, L. Kong, S. Zhu, Reprogramming cell fates by small molecules, Protein Cell 8 (2017) 328–348.
[79] M.L. Liu, T. Zang, C.L. Zhang, Direct lineage reprogramming reveals disease-specific phenotypes of motor neurons from human ALS patients, Cell Rep. 14 (2016) 115–128.
[80] W. Niu, T. Zang, Y. Zou, S. Fang, D.K. Smith, R. Bachoo, C.L. Zhang, In vivo reprogramming of astrocytes to neuroblasts in the adult brain, Nat. Cell Biol. 15 (2013) 1164–1175.
[81] Y. Tang, M.L. Liu, T. Zang, C.L. Zhang, Direct reprogramming rather than iPSC-based reprogramming maintains aging hallmarks in human motor neurons, Front. Mol. Neurosci. 10 (2017) 359.
[82] R. Ben Jehuda, Y. Shemer, O. Binah, Genome editing in induced pluripotent stem cells using CRISPR/Cas9, Stem Cell Rev. 14 (2018) 323–336.
[83] E. Urich, S.E. Lazic, J. Molnos, I. Wells, P.O. Freskgard, Transcriptional profiling of human brain endothelial cells reveals key properties crucial for predictive in vitro blood-brain barrier models, PLoS One 7 (2012) e38149.
[84] E.S. Lippmann, S.M. Azarin, J.E. Kay, R.A. Nessler, H.K. Wilson, A. Al-Ahmad, S.P. Palecek, E.V. Shusta, Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells, Nat. Biotechnol. 30 (2012) 783–791.
[85] J.B. Alimonti, M. Ribecco-Lutkiewicz, C. Sodja, A. Jezierski, D.B. Stanimirovic, Q. Liu, A.S. Haqqani, W. Conlan, M. Bani-Yaghoub, Zika virus crosses an in vitro human blood brain barrier model, Fluids Barriers CNS 15 (2018) 15.
[86] R. Cecchelli, S. Aday, E. Sevin, C. Almeida, M. Culot, L. Dehouck, C. Coisne, B. Engelhardt, M.P. Dehouck, L. Ferreira, A stable and reproducible human blood-brain barrier model derived from hematopoietic stem cells, PLoS One 9 (2014) e99733.
[87] R. Patel, S. Page, A.J. Al-Ahmad, Isogenic blood-brain barrier models based on patient-derived stem cells display inter-individual differences in cell maturation and functionality, J. Neurochem. 142 (2017) 74–88.
[88] G.D. Vatine, A. Al-Ahmad, B.K. Barriga, S. Svendsen, A. Salim, L. Garcia, V.J. Garcia, R. Ho, N. Yucer, T. Qian, R.G. Lim, J. Wu, L.M. Thompson, W.R. Spivia, Z. Chen, J. Van Eyk, S.P. Palecek, S. Refetoff, E.V. Shusta, C.N. Svendsen, Modeling psychomotor retardation using iPSCs from MCT8-deficient patients indicates a prominent role for the blood-brain barrier, Cell Stem Cell 20 (2017) 831–843 (e835).
[89] M.E. Katt, Z.S. Xu, S. Gerecht, P.C. Searson, Human brain microvascular endothelial cells derived from the BC1 iPS cell line exhibit a blood-brain barrier phenotype, PLoS One 11 (2016) e0152105.
[90] E.S. Lippmann, A. Al-Ahmad, S.M. Azarin, S.P. Palecek, E.V. Shusta, A retinoic acid-enhanced, multicellular human blood-brain barrier model derived from stem cell sources, Sci. Rep. 4 (2014) 4160.
[91] R. Daneman, L. Zhou, D. Agalliu, J.D. Cahoy, A. Kaushal, B.A. Barres, The mouse blood-brain barrier transcriptome: a new resource for understanding the development and function of brain endothelial cells, PLoS One 5 (2010) e13741.
[92] M.R. Mizee, D. Wooldrik, K.A. Lakeman, B. van het Hof, J.A. Drexhage, D. Geerts, M. Bugiani, E. Aronica, R.E. Mebius, A. Prat, H.E. de Vries, A. Reijerkerk, Retinoic acid induces blood-brain barrier development, J. Neurosci. 33 (2013) 1660–1671.
[93] H.K. Wilson, S.G. Canfield, M.K. Hjortness, S.P. Palecek, E.V. Shusta, Exploring the effects of cell seeding density on the differentiation of human pluripotent stem cells to brain microvascular endothelial cells, Fluids Barriers CNS 12 (2015) 13.
[94] R. Patel, A.J. Alahmad, Growth-factor reduced Matrigel source influences stem cell derived brain microvascular endothelial cell barrier properties, Fluids Barriers CNS 13 (2016) 6.
[95] E.K. Hollmann, A.K. Bailey, A.V. Potharazu, M.D. Neely, A.B. Bowman, E.S. Lippmann, Accelerated differentiation of human induced pluripotent stem cells to blood-brain barrier endothelial cells, Fluids Barriers CNS 14 (2017) 9.
[96] T. Qian, S.E. Maguire, S.G. Canfield, X. Bao, W.R. Olson, E.V. Shusta, S.P. Palecek, Directed differentiation of human pluripotent stem cells to blood-brain barrier endothelial cells, Sci. Adv. 3 (2017) e1701679.
[97] I. Flamme, T. Frolich, W. Risau, Molecular mechanisms of vasculogenesis and embryonic angiogenesis, J. Cell. Physiol. 173 (1997) 206–210.
[98] W. Risau, Mechanisms of angiogenesis, Nature 386 (1997) 671–674.
[99] C.A.A. Lee, H.S. Seo, A.G. Armien, F.S. Bates, J. Tolar, S.M. Azarin, Modeling and rescue of defective blood-brain barrier function of induced brain microvascular endothelial cells from childhood cerebral adrenoleukodystrophy patients, Fluids Barriers CNS 15 (2018) 9.
[100] P.L. Musolino, Y. Gong, J.M. Snyder, S. Jimenez, J. Lok, E.H. Lo, A.B. Moser, E.F. Grabowski, M.P. Frosch, F.S. Eichler, Brain endothelial dysfunction in cerebral adrenoleukodystrophy, Brain 138 (2015) 3206–3220.
[101] Y.H. Chen, Y.C. Lee, Y.S. Tsai, Y.C. Guo, C.T. Hsiao, P.C. Tsai, J.A. Huang, Y.C. Liao, B.W. Soong, Unmasking adrenoleukodystrophy in a cohort of cerebellar ataxia, PLoS One 12 (2017) e0177296.
[102] J. Bernal, Thyroid Hormones in Brain Development and Function, Thyroid Disease Manager, ENDOCRINE EDUCATION Inc., 2013 (Place Published).
[103] L.M. Roberts, K. Woodford, M. Zhou, D.S. Black, J.E. Haggerty, E.H. Tate, K.K. Grindstaff, W. Mengesha, C. Raman, N. Zerangue, Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier, Endocrinology 149 (2008) 6251–6261.
[104] J. Muller, S. Mayerl, T.J. Visser, V.M. Darras, A. Boelen, L. Frappart, L. Mariotta, F. Verrey, H. Heuer, Tissue-specific alterations in thyroid hormone homeostasis in combined Mct10 and Mct8 deficiency, Endocrinology 155 (2014) 315–325.
[105] C.E. Schwartz, R.E. Stevenson, The MCT8 thyroid hormone transporter and Allan-Herndon-Dudley syndrome, Best Pract. Res. Clin. Endocrinol. Metab. 21 (2007) 307–321.
[106] Y.N. Sun, Y.J. Liu, L. Zhang, Y. Ye, L.X. Lin, Y.M. Li, Y.Q. Yan, Z.P. Chen, Expression of organic anion transporting polypeptide 1c1 and monocarboxylate transporter 8 in the rat placental barrier and the compensatory response to thyroid dysfunction, PLoS One 9 (2014) e96047.
[107] G.L. Roef, E.R. Rietzschel, T. De Meyer, S. Bekaert, M.L. De Buyzere, C. Van daele, K. Toye, J.M. Kaufman, Y.E. Taes, Associations between single nucleotide polymorphisms in thyroid hormone transporter genes (MCT8, MCT10 and OATP1C1) and circulating thyroid hormones, Clin. Chim. Acta 425 (2013) 227–232.
[108] J.L. Cummings, T. Morstorf, K. Zhong, Alzheimer's disease drug-development pipeline: few candidates, frequent failures, Alzheimers Res. Ther. 6 (2014) 37.
[109] M. Zandl-Lang, E. Fanaee-Danesh, Y. Sun, N.M. Albrecher, C.C. Gali, I. Cancar, A. Kober, C. Tam-Amersdorfer, A. Stracke, S.M. Storck, A. Saeed, J. Stefulj, C.U. Pietrzik, M.R. Wilson, I. Bjorkhem, U. Panzenboeck, Regulatory effects of simvastatin and apoJ on APP processing and amyloid-beta clearance in blood-brain barrier endothelial cells, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1863 (2018) 40–60.
[110] A. Chakraborty, N.M. de Wit, W.M. van der Flier, H.E. de Vries, The blood brain barrier in Alzheimer's disease, Vasc. Pharmacol. 89 (2017) 12–18.
[111] A.M. Hartz, Y. Zhong, A. Wolf, H. LeVine 3rd, D.S. Miller, B. Bauer, Abeta40 reduces P-glycoprotein at the blood-brain barrier through the ubiquitin-proteasome pathway, J. Neurosci. 36 (2016) 1930–1941.
[112] Y.X. Guo, L.Y. He, M. Zhang, F. Wang, F. Liu, W.X. Peng, 1,25-Dihydroxyvitamin D3 regulates expression of LRP1 and RAGE in vitro and in vivo, enhancing Abeta1-40 brain-to-blood efflux and peripheral uptake transport, Neuroscience 322 (2016) 28–38.
[113] E.A. Winkler, Y. Nishida, A.P. Sagare, S.V. Rege, R.D. Bell, D. Perlmutter, J.D. Sengillo, S. Hillman, P. Kong, A.R. Nelson, J.S. Sullivan, Z. Zhao, H.J. Meiselman, R.B. Wendy, J. Soto, E.D. Abel, J. Makshanoff, E. Zuniga, D.C. De Vivo, B.V. Zlokovic, GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration, Nat. Neurosci. 18 (2015) 521–530.
[114] F.H. Duits, M. Hernandez-Guillamon, J. Montaner, J.D. Goos, A. Montanola, M.P. Wattjes, F. Barkhof, P. Scheltens, C.E. Teunissen, W.M. van der Flier, Matrix metalloproteinases in Alzheimer's Disease and concurrent cerebral microbleeds, J. Alzheimers Dis. 48 (2015) 711–720.
[115] J. Ghiso, S. Fossati, A. Rostagno, Amyloidosis associated with cerebral amyloid angiopathy: cell signaling pathways elicited in cerebral endothelial cells, J. Alzheimers Dis. 42 (Suppl. 3) (2014) S167–S176.
[116] L. Liu, W. Wan, S. Xia, B. Kalionis, Y. Li, Dysfunctional Wnt/beta-catenin signaling contributes to blood-brain barrier breakdown in Alzheimer's disease, Neurochem. Int. 75 (2014) 19–25.
[117] J. Dorszewska, M. Prendecki, A. Oczkowska, M. Dezor, W. Kozubski, Molecular basis of familial and sporadic Alzheimer's Disease, Curr. Alzheimer Res. 13 (2016) 952–963.
[118] D.M. Michaelson, APOE epsilon4: the most prevalent yet understudied risk factor for Alzheimer's disease, Alzheimers Dement. 10 (2014) 861–868.
[119] C.C. Liu, C.C. Liu, T. Kanekiyo, H. Xu, G. Bu, Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy, Nat. Rev. Neurol. 9 (2013) 106–118.
[120] P.H. Wen, R. De Gasperi, M.A. Sosa, A.B. Rocher, V.L. Friedrich Jr., P.R. Hof, G.A. Elder, Selective expression of presenilin 1 in neural progenitor cells rescues the cerebral hemorrhages and cortical lamination defects in presenilin 1-null mutant mice, Development 132 (2005) 3873–3883.
[121] M.A. Gama Sosa, R.D. Gasperi, A.B. Rocher, A.C. Wang, W.G. Janssen, T. Flores, G.M. Perez, J. Schmeidler, D.L. Dickstein, P.R. Hof, G.A. Elder, Age-related vascular pathology in transgenic mice expressing presenilin 1-associated familial Alzheimer's disease mutations, Am. J. Pathol. 176 (2010) 353–368.
[122] X. Paez-Colasante, C. Figueroa-Romero, S.A. Sakowski, S.A. Goutman, E.L. Feldman, Amyotrophic lateral sclerosis: mechanisms and therapeutics in the epigenomic era, Nat. Rev. Neurol. 11 (2015) 266–279.
[123] A. Anand, K. Thakur, P.K. Gupta, ALS and oxidative stress: the neurovascular scenario, Oxidative Med. Cell. Longev. 2013 (2013) 635831.
[124] D. Bataveljic, M. Milosevic, L. Radenovic, P. Andjus, Novel molecular biomarkers at the blood-brain barrier in ALS, Biomed. Res. Int. 2014 (2014) 907545.
[125] M. Jablonski, D.S. Miller, P. Pasinelli, D. Trotti, ABC transporter-driven pharmacoresistance in amyotrophic lateral sclerosis, Brain Res. 1607 (2015) 1–14.
[126] M.R. Jablonski, S.S. Markandaiah, D. Jacob, N.J. Meng, K. Li, V. Gennaro, A.C. Lepore, D. Trotti, P. Pasinelli, Inhibiting drug efflux transporters improves efficacy of ALS therapeutics, Ann. Clin. Transl. Neurol. 1 (2014) 996–1005.
[127] K. Devraj, M.E. Klinger, R.L. Myers, A. Mokashi, R.A. Hawkins, I.A. Simpson, GLUT-1 glucose transporters in the blood-brain barrier: differential phosphorylation, J. Neurosci. Res. 89 (2011) 1913–1925.
[128] S. Vandekeere, M. Dewerchin, P. Carmeliet, Angiogenesis revisited: an overlooked role of endothelial cell metabolism in vessel sprouting, Microcirculation 22 (2015) 509–517.
[129] D. Gras, E. Roze, S. Caillet, A. Meneret, D. Doummar, T. Billette de Villemeur, M. Vidailhet, F. Mochel, GLUT1 deficiency syndrome: an update, Rev. Neurol. (Paris) 170 (2014) 91–99.

[130] D.C. De Vivo, R.R. Trifiletti, R.I. Jacobson, G.M. Ronen, R.A. Behmand, S.I. Harik, Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay, N. Engl. J. Med. 325 (1991) 703–709.

[131] E. van der Louw, D. van den Hurk, E. Neal, B. Leiendecker, G. Fitzsimmon, L. Dority, L. Thompson, M. Marchio, M. Dudzinska, A. Dressler, J. Klepper, S. Auvin, J.H. Cross, Ketogenic diet guidelines for infants with refractory epilepsy, Eur. J. Paediatr. Neurol. 20 (2016) 798–809.

[132] F. Streijger, W.T. Plunet, J.H. Lee, J. Liu, C.K. Lam, S. Park, B.J. Hilton, B.L. Fransen, K.A. Matheson, P. Assinck, B.K. Kwon, W. Tetzlaff, Ketogenic diet improves forelimb motor function after spinal cord injury in rodents, PLoS One 8 (2013) e78765.

[133] A.A. Morris, Cerebral ketone body metabolism, J. Inherit. Metab. Dis. 28 (2005) 109–121.

[134] P.O. Kwiterovich Jr., E.P. Vining, P. Pyzik, R. Skolasky Jr., J.M. Freeman, Effect of a high-fat ketogenic diet on plasma levels of lipids, lipoproteins, and apolipoproteins in children, JAMA 290 (2003) 912–920.

[135] N.S. Caron, G.E.B. Wright, M.R. Hayden, Huntington disease, in: M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, K. Stephens, A. Amemiya (Eds.), GeneReviews((R)), 1993 (Place Published).

[136] H.Y. Hsiao, Y.C. Chen, C.H. Huang, C.C. Chen, Y.H. Hsu, H.M. Chen, F.L. Chiu, H.C. Kuo, C. Chang, Y. Chern, Aberrant astrocytes impair vascular reactivity in Huntington disease, Ann. Neurol. 78 (2015) 178–192.

[137] C.Y. Lin, Y.H. Hsu, M.H. Lin, T.H. Yang, H.M. Chen, Y.C. Chen, H.Y. Hsiao, C.C. Chen, Y. Chern, C. Chang, Neurovascular abnormalities in humans and mice with Huntington's disease, Exp. Neurol. 250 (2013) 20–30.

[138] S. Liebner, M. Corada, T. Bangsow, J. Babbage, A. Taddei, C.J. Czupalla, M. Reis, A. Felici, H. Wolburg, M. Fruttiger, M.M. Taketo, H. von Melchner, K.H. Plate, H. Gerhardt, E. Dejana, Wnt/beta-catenin signaling controls development of the blood-brain barrier, J. Cell Biol. 183 (2008) 409–417.

[139] R. Daneman, D. Agalliu, L. Zhou, F. Kuhnert, C.J. Kuo, B.A. Barres, Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 641–646.

[140] E.J. Benjamin, S.S. Virani, C.W. Callaway, A.M. Chamberlain, A.R. Chang, S. Cheng, S.E. Chiuve, M. Cushman, F.N. Delling, R. Deo, S.D. de Ferranti, J.F. Ferguson, M. Fornage, C. Gillespie, C.R. Isasi, M.C. Jimenez, L.C. Jordan, S.E. Judd, D. Lackland, J.H. Lichtman, L. Lisabeth, S. Liu, C.T. Longenecker, P.L. Lutsey, J.S. Mackey, D.B. Matchar, K. Matsushita, M.E. Mussolino, K. Nasir, M. O'Flaherty, L.P. Palaniappan, A. Pandey, D.K. Pandey, M.J. Reeves, M.D. Ritchey, C.J. Rodriguez, G.A. Roth, W.D. Rosamond, U.K.A. Sampson, G.M. Satou, S.H. Shah, N.L. Spartano, D.L. Tirschwell, C.W. Tsao, J.H. Voeks, J.Z. Willey, J.T. Wilkins, J.H. Wu, H.M. Alger, S.S. Wong, P. Muntner, E. American Heart Association Council on, C. Prevention Statistics, S. Stroke Statistics, Heart disease and stroke statistics-2018 update: A report from the American Heart Association, Circulation 137 (2018) e67–e492.

[141] G.J. Hankey, Stroke, Lancet 389 (2017) 641–654.

[142] G.C. Jickling, D. Liu, B. Stamova, B.P. Ander, X. Zhan, A. Lu, F.R. Sharp, Hemorrhagic transformation after ischemic stroke in animals and humans, J. Cereb. Blood Flow Metab. 34 (2014) 185–199.

[143] B. Niego, R.L. Medcalf, Plasmin-dependent modulation of the blood-brain barrier: a major consideration during tPA-induced thrombolysis? J. Cereb. Blood Flow Metab. 34 (2014) 1283–1296.

[144] O. Adeoye, R. Hornung, P. Khatri, D. Kleindorfer, Recombinant tissue-type plasminogen activator use for ischemic stroke in the United States: a doubling of treatment rates over the course of 5 years, Stroke 42 (2011) 1952–1955.

[145] M. Kanazawa, H. Igarashi, K. Kawamura, T. Takahashi, A. Kakita, H. Takahashi, T. Nakada, M. Nishizawa, T. Shimohata, Inhibition of VEGF signaling pathway attenuates hemorrhage after tPA treatment, J. Cereb. Blood Flow Metab. 31 (2011) 1461–1474.

[146] A. Czlonkowska, A. Kobayashi, M. Skowronska, M. Niewada, I. Sarzynska-Dlugosz, Thrombolysis for stroke in Poland: first 2 years of experience, Int. J. Stroke 1 (2006) 111–112.

[147] M.P. Kahle, G.J. Bix, Successfully climbing the “STAIRs”: surmounting failed translation of experimental ischemic stroke treatments, Stroke Res. Treat. 2012 (2012) 374098.

[148] S. Page, A. Munsell, A.J. Al-Ahmad, Cerebral hypoxia/ischemia selectively disrupts tight junctions complexes in stem cell-derived human brain microvascular endothelial cells, Fluids Barriers CNS 13 (2016) 16.

[149] O.O. Ogunshola, A. Al-Ahmad, HIF-1 at the blood-brain barrier: a mediator of permeability? High Alt. Med. Biol. 13 (2012) 153–161.

[150] B.J. Kim, O.B. Bee, M.A. McDonagh, M.J. Stebbins, S.P. Palecek, K.S. Doran, E.V. Shusta, Modeling group B Streptococcus and blood-brain barrier interaction by using induced pluripotent stem cell-derived brain endothelial cells, mSphere 2 (2017).

[151] R. Patel, M.A. Hossain, N. German, A.J. Al-Ahmad, Gliotoxin penetrates and impairs the integrity of the human blood-brain barrier in vitro, Mycotoxin Res. 34 (2018) 257 https://link.springer.com/article/10.1007%2Fs12550-018-0320-7#citeas.

[152] S.G. Canfield, M.J. Stebbins, B.S. Morales, S.W. Asai, G.D. Vatine, C.N. Svendsen, S.P. Palecek, E.V. Shusta, An isogenic blood-brain barrier model comprising brain endothelial cells, astrocytes, and neurons derived from human induced pluripotent stem cells, J. Neurochem. 140 (2017) 874–888.

[153] M.W. van der Helm, A.D. van der Meer, J.C. Eijkel, A. van den Berg, L.I. Segerink, Microfluidic organ-on-chip technology for blood-brain barrier research, Tissue Barriers 4 (2016) e1142493.

[154] L.M. Griep, F. Wolbers, B. de Wagenaar, P.M. ter Braak, B.B. Weksler, I.A. Romero, P.O. Couraud, I. Vermes, A.D. van der Meer, A. van den Berg, BBB on chip: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function, Biomed. Microdevices 15 (2013) 145–150.

[155] J.D. Wang, S. Khafagy el, K. Khanafer, S. Takayama, M.E. ElSayed, Organization of endothelial cells, pericytes, and astrocytes into a 3D microfluidic in vitro model of the blood-brain barrier, Mol. Pharm. 13 (2016) 895–906.

[156] G. Adriani, D. Ma, A. Pavesi, R.D. Kamm, E.L. Goh, A 3D neurovascular microfluidic model consisting of neurons, astrocytes and cerebral endothelial cells as a blood-brain barrier, Lab Chip 17 (2017) 448–459.

[157] B.M. Maoz, A. Herland, E.A. FitzGerald, T. Grevesse, C. Vidoudez, A.R. Pacheco, S.P. Sheehy, T.E. Park, S. Dauth, R. Mannix, N. Budnik, K. Shores, A. Cho, J.C. Nawroth, D. Segre, B. Budnik, D.E. Ingber, K.K. Parker, A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells, Nat. Biotechnol. 36 (2018) 865–874.

[158] A. Herland, A.D. van der Meer, E.A. FitzGerald, T.E. Park, J.J. Sleeboom, D.E. Ingber, Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip, PLoS One 11 (2016) e0150360.

[159] Y.I. Wang, H.E. Abaci, M.L. Shuler, Microfluidic blood-brain barrier model provides in vivo-like barrier properties for drug permeability screening, Biotechnol. Bioeng. 114 (2017) 184–194.

[160] S. Sances, R. Ho, G. Vatine, D. West, A. Laperle, A. Meyer, M. Godoy, P.S. Kay, B. Mandefro, S. Hatata, C. Hinojosa, N. Wen, D. Sareen, G.A. Hamilton, C.N. Svendsen, Human iPSC-derived endothelial cells and microengineered organ-chip enhance neuronal development, Stem Cell Rep. 10 (2018) 1222–1236.