Modeling Alzheimer's disease using Novel Approach Methodologies

Hector Rosas-Hernandez[1], Katelin Matazel[1], Goodwell Nzou[2], Randy Daughters[2], Andrew N. Shen[1]

[1]Division of Neurotoxicology, National Center for Toxicological Research/FDA. 3900 NCTR Road, Jefferson, AR 72079, USA. [2]Emulate Inc., 27 Drydock Avenue, Boston, MA 02210, USA

Abstract

Alzheimer's disease (AD) pathology is characterized by amyloid plaques containing aggregates of amyloid beta (Aβ) peptides, intracellular neurofibrillary tangles containing hyper-phosphorylated and misfolded tau protein, and by a dysfunctional blood-brain barrier (BBB). Animal models of AD are used in preclinical research to study disease pathology and as tools for drug development. However, these models do not fully recapitulate human pathology or response to drugs or toxicants. New approach methods (NAMs) are currently being used for disease modeling, toxicity testing, and drug development. Such models include patient-derived induced pluripotent stem cells (iPSCs) and microphysiological systems (MPS) or organ-chips. These methods have the potential to more accurately model human response to toxicants and better model human physiology. The aim of the present study was to construct a brain-chip model of AD using iPSCs from a healthy cognitive-normal and an AD patient. iPCS-derived neurons, astrocytes, pericytes and brain-like endothelial cells were cultured in a MPS under physiological conditions, and markers of neurovascular functions and AD pathology were measured. Brain-like microvascular endothelial cells (iBMVECs), pericytes, astrocytes and neurons were successfully differentiated from hiPSCs as demonstrated by the expression of cell-specific markers. In addition, healthy and AD-brain chips were constructed by culturing these cells in an MPS. AD-brain chips presented dysfunctional BBB functions and protein markers of AD pathology were increased in the AD brain-chips compared to healthy brain-chips. In summary, iPSC-derived cells can be coupled with MPS to construct a brain-chip model of AD that recapitulate select characteristics of human pathology. Further experiments need to be conducted to validate this model. Implementation of these NAMs will facilitate internal Agency studies and review of external studies in which these technologies are employed, which will potentially aid in the review process of FDA-regulated products, including treatments for AD.

Introduction

Alzheimer's disease (AD) is the leading cause of dementia and affects millions of people worldwide. The pathophysiology of AD is characterized by the presence of excess free and aggregated amyloid beta (Aβ) peptides and by intracellular neurofibrillary tangles that contain hyperphosphorylated and misfolded tau protein. Recent evidence supports the notion that the brain vasculature is impaired in AD and this impairment may contribute to or exacerbate the underlying pathophysiology of the disease.

Rodent models have been developed to study AD, via insertion of human mutations associated with familial forms of AD. Those rodent models have been invaluable in gaining insight into mechanisms of AD pathology, but few capture the complete pathological features observed in human patients with AD. For this reason, new and innovative models that recapitulate AD pathology are being developed. One such model uses human induced pluripotent stem cells (hiPSCs) derived from patients with AD [11, 12]. Cultured hiPSCs can be differentiated into several cell types, including neurons, astrocytes, pericyte, microglia and brain-like endothelial cells (iBMVECs). These differentiated cells conserve pathological features of the individuals from which they were harvested and can be an extremely valuable tool in the understanding of AD pathology and therapeutic drug development.

In addition to the use of patient-derived hiPSCs, novel in vitro systems are being developed to emulate human physiology by recreating important characteristics of the human body. In particular, microphysiological systems (MPS) or organs-on-chips have been used to culture cells under conditions of blood flow, mechanical forces and cytoarchitecture that resemble those of specific organs, including the brain. Those specific culture conditions enable cells to acquire physiological or pathological functions that cannot be achieved in static 2D cultures. While organ-on-a-chip systems are commercially available, there are no studies that have constructed an AD brain-chip by culturing isogenic neurons, astrocytes, pericytes, microglia and iBVMECs differentiated from hiPSCs obtained from patients with AD. Therefore, the aim of this project is to develop an integrated brain-chip model of AD in which neurons, astrocytes, pericytes, microglia and BiMVECs derived from hiPSCs from patients with AD with the APOE ε4 allele will produce an in vitro model analogous to human neurovascular pathophysiology in AD.

Materials and Methods

Cell lines: iPSCs from a cognitive healthy individual with the ε3/ε3 allele of ApoE were purchased from the Mayo Clinic's Center for Regenerative Medicine; iPSCs from an AD patient with the ε4/ε4 allele of ApoE were purchased from the Coriell Institute for Medical Research. Both hiPSCs lines were cultured and expanded in mTESR1 media, with daily media change to avoid spontaneous differentiation.

iBMVECs differentiation: hiPSCs were differentiated into iBMVECs using a previously described protocol (Qian et al. 2017). Briefly, hiPSCs were directed to a mesoderm lineage by activating the Wnt pathway. Cells were then differentiated into endothelial progenitor cells. Finally, specification of BMVECs was induced by activating the retinoic acid signaling pathway. Cells were characterized by immunocytochemistry (IHC) and western blot using the endothelial markers ZO-1 and occludin.

Pericyte differentiation: Pericytes were differentiated from hiPSCs following a previously described method (Blanchard et al. 2020). hiPSCs were committed to a mesoderm lineage by inhibition of the GSK3 pathway. Pericytes were derived by exposure to PDGFBB and inhibition of the TGF-β pathway. Pericytes were characterized by western blot and IHC using the specific marker, platelet derived growth factor receptor beta (PDGFR-β)

Microglia differentiation: Microglial cells were differentiated following the protocol developed by Brownjohn et al. (2018). hiPSCs were passed into embryoid bodies, which continuously produce primitive macrophage precursors (PMPC) after 3 weeks in culture. PMPCs were differentiated into induced microglia by culturing in media supplemented with IL-34 and GM-CSF for 10 days. Microglia cells were characterized by IHC using the specific marker Iba-1.

Neurons and Astrocyte differentiation: Neurons and astrocytes were differentiated from hiPSCs following the EZ-Sphere method (Ebert et al. 2013). EZ-spheres were maintained in suspension and then dissociated and terminally differentiated into mixed cultures of neurons and astrocytes by culturing in media containing N2, B27 and brain derived neurotrophic factor (BDNF). Western blot and IHC were used to detect neuronal (MAP2) and astrocytic (GFAP) markers.

Brain-Chip: hiPSC-derived brain cells (neurons, astrocytes, pericytes and iBMVECs) were cultured under physiological conditions using the Human Emulation System from Emulate Inc. iBMVECs were cultured on the vascular side of a microfluidic chip (consisting of two channels separated by a porous membrane), while neurons, astrocytes and pericytes were cultured on the opposite side of the membrane (brain channel). Flow rates for both channels was set at 60 µL/h. Chips were cultured for a 5-day period, with daily effluent sampling. Paracellular permeability was evaluated by quantifying the passage of 3 kDa dextran-cascade blue from the vascular to the brain compartment and apparent permeability was calculated from these values.

Production and accumulation of Aβ and tau. Accumulation of Aβ and tau on the brain side of the chips was determined in the media effluent using a commercially available multiplex assay, following the manufacturer's instructions. Levels of Aβ40, Aβ42, total tau and p-tau 181 were quantified.

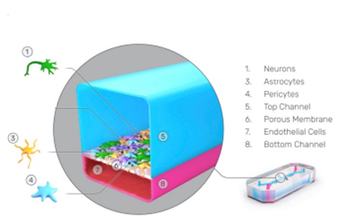

Figure 1. Schematic representation of the brain-chip. iBMVECs are cultured on the bottom (vascular) channel, while iPSC-derived neurons, astrocytes and pericytes are cultured on the top (brain) channel. Image modified from Pediaditakis et al. (2022)

Results and Discussion

Characterization of hiPSC-derived brain cells

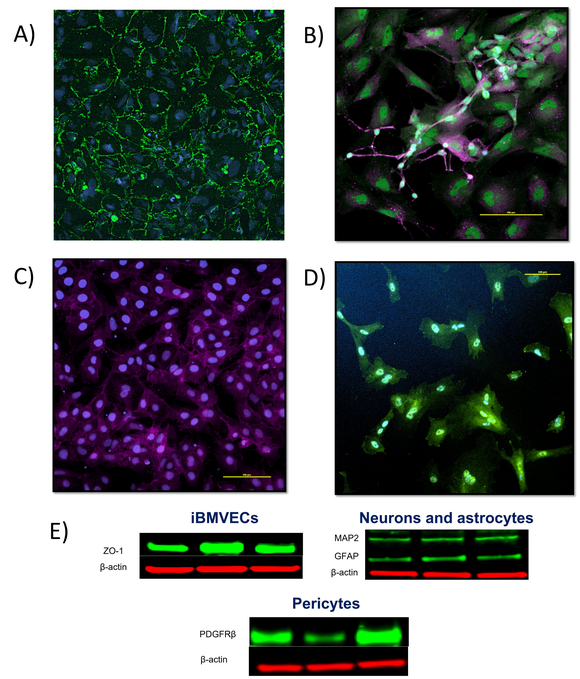

Figure 2. Characterization of hiPSC-derived brain cells. Cellular differentiation was characterized by IHC for cellular specific markers in A) iBMVECs, (ZO-1,green), B) neurons (MAP2, magenta) and astrocytes (GFAP, green), C) pericytes (PDGFRβ, magenta), D) microglia (Iba-1, green). Images were acquired at 20X (A-C) and 40X (D) magnification using an inverted confocal microscope (Nikon). E) Cellular differentiation was confirmed by WB using the same markers as in IHC.

Brain-chip construction

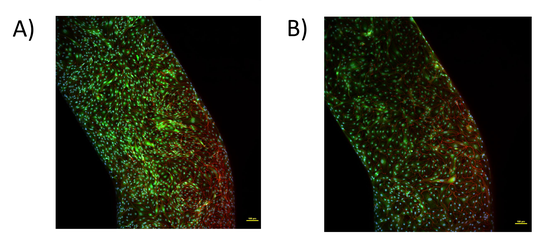

Figure 3. iBMVECs cultures on the brain-chip. iBMVECs are cultured on the bottom (vascular) channel of the brain chip. Control A) and AD B) brain-chips were immunostained for the tight junction protein ZO-1 (red) and the membrane-bound transporter P-glycoprotein (green). Images were acquired using an inverted confocal microscope (Nikon) at 10X magnification.

AD brain-chips present impaired barrier properties

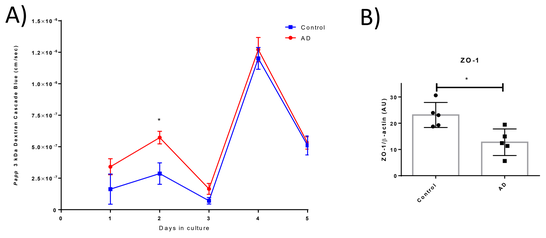

Figure 4. Analysis of barrier function on brain-chips. Control and AD brain-chips were maintained in culture under flow conditions for 5 days. A) Paracellular permeability using a 3 kDa dextran was assessed daily. B) Expression of the tight junction protein ZO-1 on the vascular channel of the brain-chips was determined by western blot at day 5. Preliminary results. Do not cite. N=6 chips/condition. *p<0.05.

Amyloid beta and tau production by brain-chips

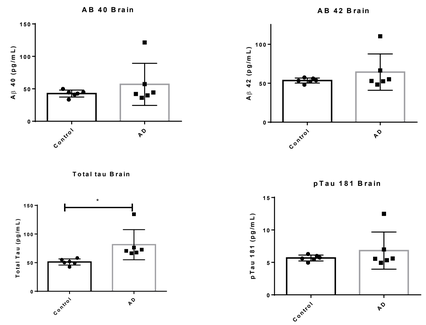

Figure 5. Quantification of Aβ and tau production. Control and AD brain-chips were maintained in culture under flow conditions for 5 days. Effluent from the brain channel was taken at day 5 of culture after 24-hour accumulation. Levels of Aβ40, Aβ42, total tau and phosphorylated tau (Ser181) were determined using a multiplex assay. Preliminary results. Do not cite. N=6 chips/condition. *p<0.05.

Conclusion

New approach methodologies, including hiPSCs-derived cells and microphysiological systems can be coupled to produce models that closely mimic human physiology and pathology.

We have successfully differentiated iBMVECs, neurons, astrocytes, pericytes and microglia from hiPSCs derived from a healthy donor and AD patient. Preliminary data indicates that AD brain-chips present an altered barrier function and produce increased levels of proteins implicated in AD pathology. A more extensive characterization of the recapitulation of AD pathology in the brain-chips is currently underway.

References

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