Harnessing cerebral organoids for Alzheimer's disease research

Harnessing Cerebral Organoids for Alzheimer's Disease research

Adele Bubnys, Li-Huei Tsai

Highlights

- Cerebral organoids derived from Alzheimer's patients develop disease pathology
- Organoids model extracellular matrix dynamics underlying amyloid plaque formation
- Organoids used to study role of APOE4 and other Alzheimer's risk genes
- High throughput drug screens with organoids are under development
- Need to incorporate microglia and vasculature into organoids to improve relevance

Abstract

Alzheimer's Disease (AD) is a devastating neurodegenerative disorder affecting the aging population. Despite much study, there remains an urgent need to identify the root causes of AD, together with potential treatments. Cerebral organoid technology has made it possible to model human neurophysiology and disease with increasing accuracy in patient-derived tissue cultures. Here, we review the most recent advances in modeling AD in organoids and other engineered 3D cell-culture systems. Early studies demonstrated that familial AD patient-derived organoids robustly develop disease pathology. Ongoing work has expanded this focus to investigate the genetic and environmental causes of late-onset sporadic AD, and harness organoids for high-throughput drug screens. Future organoid models will need to incorporate additional cell types and tissues implicated in disease pathogenesis, including microglia and vasculature. We anticipate the continuation of this rapid progress in developing cerebral organoid technology toward facilitating our understanding of and informing treatment strategies for Alzheimer's Disease.

Introduction

The development of human induced pluripotent stem cell (iPS)-derived neurons in the early 2010s opened up opportunities to study the mechanisms of neurodegenerative disease in patient-derived tissue cultures [1–5]. Using these protocols, neurons grown from familial Alzheimer's Disease (AD) patients carrying mutations in key genes such as APP and PSEN1 were shown to recapitulate important AD pathologies, including elevated amyloid-β peptide $A\beta_{42/40}$ ratio, hyperphosphorylated *tau* protein, and electrophysiological hyperactivity [6–14]. These findings launched the application of iPS-derived cultures in high-throughput drug screening for therapeutic agents that alleviate Aβ- and *tau*-induced neurotoxicity [15–17].

It is surprising that Alzheimer's, a disease associated with aging in which even genetic "early-onset" forms only arise when the patient is in their 40s or 50s, can be recapitulated using neuronal cultures that are just a few weeks old without requiring exogenous manipulation to generate an aging phenotype [18,19]. One possible explanation is that, in the absence of other supporting tissues, these two-dimensional monocultures most closely capture cellular processes that arise when the brain's homeostatic mechanisms break down. While iPS-derived

neurons can effectively model Aβ and *tau* pathology associated with AD, the fact that most of the anti-Aβ drugs under development have yet to significantly alleviate cognitive decline in clinical trials suggests that it is time to expand our focus to other potential therapeutic targets [20]. Thus, more recent iPS models of AD seek to harness three-dimensional cerebral organoid cultures, modeling neurodegeneration in a more biologically relevant substrate in order to identify its root causes (summarized in table 1).

Cerebral organoids as an Alzheimer's Disease model

Building upon iPS-derived neuron protocols that generate embryoid body spheroids, in 2014, Lancaster *et al.* reported on the development of a new cerebral organoid culture. By embedding spheroids of iPS cells in Matrigel and subjecting these to small molecule dual-SMAD inhibitors, Lancaster and colleagues were able to drive these cultures to recapitulate cortical development, including migration of radial glia to form stratified cortical layers [21]. Single-cell transcriptomic studies indicate that cerebral organoids consist of diverse neuronal populations approaching the complexity of the pre-natal human brain [22]. Due to their three-dimensional nature, cerebral organoids also capture more aspects of the distinct extracellular microenvironment of the brain, thus facilitating the modeling of amyloid plaque formation.

Cerebral organoids derived from familial AD (fAD) patients display many of the same AD pathologies found in two-dimensional neuronal cultures. As expected from the latter model, organoids bearing APP duplication or PSEN1 mutations have larger and more abundant extracellular amyloid plaques than healthy controls, elevated levels of phosphorylated *tau*, and a higher abundance of endosomes that could be ameliorated by treatment with γ- and β-secretase inhibitors [23]. Other studies have replicated similar patterns of amyloid and *tau* deposition in AD organoids bearing PSEN1 and PSEN2 mutations [24–27].

Unlike two-dimensional neuronal cultures, organoids secrete their own extracellular matrix (ECM) that contains components similar to human brain ECM. Depending on their physical properties, specific ECM components such as proteoglycans, matrix metalloproteases (MMPs), and hyaluronic acid are known to alter amyloid plaque formation [28]. Treatment of forebrain and hippocampus organoids with enzymes such as heparin, heparinase, and hyaluronic acid that specifically break down ECM components attenuated amyloid-induced cytotoxicity and reduced levels of cell-bound $A\beta_{42}$, consistent with their neuroprotective properties, while MMP inhibitor enhanced amyloid-induced cytotoxicity [29]. Another study found that PSEN1 mutant organoids have reduced MMP expression, and that treatment with heparin and heparinase III could reduce amyloid levels and improve cell survival [30]. While these studies hint at the beneficial role that MMPs play in reducing amyloid accumulation and enhancing synaptic plasticity [31,32], excess MMP activation can exacerbate neuronal loss and blood brain barrier breakdown in aging and ischemia [33–35]. Organoids provide opportunities to study ECM dynamics in greater mechanistic detail than conventional two-dimensional cultures and suggest potential therapies to support optimal ECM function.

Cerebral organoids have been shown to recapitulate the same proteomic changes as seen in human postmortem tissue [36] and respond to the same manipulations as established mouse models of tauopathy [37], thereby demonstrating their physiological relevance. Human

iPS-derived organoids present some distinct advantages compared to mouse models. Since mice do not naturally develop AD, most mouse models of neurodegeneration rely either on non-physiological protein overexpression or induction of multiple human fAD mutations [38,39]. While these manipulations reliably induce AD-like pathology, they rely on a preexisting knowledge of the causative factors of AD and preclude the identification of novel risk factors. Because cerebral organoids are derived directly from human AD patients and capture human-specific physiology, they may develop AD pathology without extensive manipulation and represent a more physiological disease state, which makes them an even more appealing disease model.

Cerebral organoids also allow for an unprecedented look at the developmental forces that shape the human brain [40]. Early developmental perturbations may lay the foundation for neurodegeneration later in life. For instance, organoids bearing PSEN1 and APP mutations have a global reduction in 5hmc methylation signal, with differential methylation in genes associated with neurodevelopment and AD risk factors [41]. Such subtle genomic changes could predispose the brain towards certain network pathologies that promote AD in old age [42].

Organoid models of late-onset sporadic AD

Early-onset familial AD constitutes only a small fraction of total AD cases. Thus, a growing area of research is instead examining environmental factors that could induce late-onset sporadic AD (sAD) in the absence of strongly causative genetic factors. Healthy organoids can be induced to develop AD pathology such as elevated $A\beta_{42}$ secretion and $A\beta_{42/40}$ ratio through treatment with the $A\beta_{42}$ inducer aftin-5 [43]. Although such excess amyloid production is associated with neurodegeneration, amyloid fibrils also have antimicrobial activity [44]. The AD pathogen hypothesis posits that viral infection triggers elevated amyloid production, which can result in runaway aggregation that ultimately leads to neurodegeneration [45]. Post-mortem studies of sAD patients have found elevated levels of Herpes simplex virus type 1 (HSV1) DNA associated with amyloid plaques [46,47], suggesting that reactivation of this typically benign virus in the brain may trigger AD onset [48]. Infecting cerebral organoids with even low levels of HSV1 results in formation of amyloid plaques, reactive gliosis, and upregulation of presenilins and pro-inflammatory genes such as TNFα [49]. Antiviral medication attenuated all these pathologies, highlighting the importance of early medical intervention.

Large-scale genome-wide association studies have helped identify gene variants associated with elevated risk for late-onset AD [50]. Many of these gene variants have a higher incidence in the population than APP or PSEN mutations yet only incur modest risk of disease. About 13.7% of the human population are carriers of the E4 allele of the lipid metabolism gene APOE, which incurs a 3- to 15-fold increased risk for developing AD later in life relative to non-risk E3 carriers [51]. Pathological features associated with AD have been recapitulated in APOE4 neurons and cerebral organoids, including elevated $A\beta_{42}$ and phosphorylated *tau*, synapse loss, and increased apoptosis compared to isogenic APOE3 cultures [52,53]. Notably, this pathology was not confined to neurons, as APOE4 astrocytes and microglia also developed cell-type specific defects in cholesterol processing, amyloid uptake, and inflammation [52]. Other groups working with organoids derived from healthy APOE4 carriers have found that these do not

develop significant Aβ pathology relative to APOE3 organoids, however it is worth noting that additional protective mechanisms may be contributing to the lack of AD pathology in these patients [54]. Much work remains in identifying other risk factors underlying sAD and how these interact with APOE and its downstream pathways.

High-throughput drug-screening applications

Cerebral organoids can be grown rapidly in large quantities relative to animal models. As such, there has been considerable interest in applying cerebral organoid models for high-throughput screening in drug-development applications (figure 1). Organoids only model a subset of neuronal pathologies and lack a blood-brain barrier, yet still serve as an effective tool for assessing potential neurotoxic effects of candidate drugs. These kinds of cultures have been effectively used for high-throughput screening assays of drug neurotoxicity in the presence of APP duplication or PSEN1 mutations [55], and in a prion propagation model of Creutzfeldt-Jakob disease [56].

A major technical hurdle for using organoids in high-throughput drug-screening assays is their inherent variability in size and cellular content [57]. Using a novel acoustofluidic approach, Cai *et al.* generated uniformly sized spheroids containing neurons, astrocytes, and microglia, together with amyloid peptide, and implemented automated time-lapse imaging to longitudinally track AD pathology in these cultures [58]. Others have integrated computational modeling with high-throughput approaches to accelerate drug discovery [59]. Park *et al.* developed a computational model based on databases of known gene interactions to identify several candidate drugs which they applied to sAD patient-derived organoids to significantly attenuate $A\beta_{42}$ and phosphorylated *tau* levels [60]. This powerful approach illustrates how organoids can recapitulate important biological processes, thus accelerating drug development and testing.

Integration of non-neuronal cell types into organoids

Patient-derived cerebral organoids with only a limited subset of cell types have been shown to recapitulate certain AD pathologies such as amyloid plaque deposition and *tau* phosphorylation. Nonetheless, cell types such as astrocytes [61,62], microglia [63,64], and cerebrovasculature [65] also play key roles in neurodegeneration. New protocols for generating these different cell types from iPS cells have emerged, greatly expanding their access for AD studies [66–71] (figure 2).

During early stages of AD, the inflammatory function of microglia initiates clearance of amyloid plaques and other neurotoxic metabolites. However, prolonged microglial inflammation can lead to neurodegeneration. Several groups have used single-cell RNA sequencing to identify a subset of AD-associated microglia with elevated inflammatory function [72–74]. These studies illuminate specific pathways impacted in microglia and suggest mechanisms whereby microglial inflammation impacts neuronal function. Along these, TREM2 mutations are associated with elevated risk for AD [75,76]. As a gene primarily expressed in

microglia, TREM2 activation results in elevated phagocytic function, lipid metabolism, and microglia survival in the brain [77,78]. Using iPS-derived microglia, it was determined that knocking out phospholipase PLCG2 prevents these cells from responding to TREM2 stimulation [79]. These findings suggest that loss of TREM2 function could bias PLCG2 towards activation by the toll-like receptor pathway, thereby driving microglia towards an inflammatory state that contributes to neurodegeneration. On the other hand, acute knockdown of TREM2 in APP/PS1 mice with late stage AD pathology significantly decreased amyloid accumulation and microglia localization around plaques, suggesting that excessive activation of the TREM2 pathway may also contribute to neurodegeneration [80].

In addition to their immune and inflammatory function, microglia promote neuronal development and function [81,82]. Microglia and neuron co-cultures are already being used to uncover novel interactions underlying AD and Schizophrenia [83–85]. Accordingly, incorporating microglia into cerebral organoids would greatly enhance their physiological relevance and aid in uncovering new mechanisms of neurological disease. By tuning their organoid development protocol to promote mesodermal progenitor populations, Ormel *et al.* showed that microglia could develop endogenously within cerebral organoids [86]. However, microglia only made up approximately 1% of the total cells in these organoids, which is much lower than their density *in vivo*. Other studies have aimed to incorporate microglia into more mature organoids at predefined ratios to arrive at a more physiological microglia density [87–90]. Such co-cultures develop enhanced neuronal network maturation and responsiveness to pro-inflammatory stimuli such as LPS and Zika virus, demonstrating their ability to model neuroimmune interactions *in vitro*.

AD and vascular dementia are associated with breakdown of the blood-brain barrier and transcriptional changes to cerebrovascular cell types [91–93]. Although neurons *in vivo* are never more than a few microns from the nearest capillary, cerebral organoids lack a perfusable vasculature. This often results in the formation of a necrotic organoid core due to low nutrient diffusion into deeper layers. Recent developments have been made towards engineering organoids with integrated vasculature [94–97]. In addition to increasing the long-term viability of the culture, these methods open up opportunities to study neurovascular interactions *in vitro* and screen for drugs that cross the blood-brain barrier [98,99]. Organoids enriched for choroid plexus epithelium have also recently been reported, which could facilitate modeling of blood-CSF barrier breakdown in disease [100,101].

Better models of brain barrier tissues could illuminate the interactions between neuroinflammation and the peripheral immune system. Meningeal lymphatics and the glymphatic system play a key role in mediating neuroimmune interactions, which are disrupted during aging and AD [102,103]. Peripheral immune cells rarely infiltrate the brain parenchyma in the healthy brain, however there is a significant population that resides within the meninges that are regularly exposed to brain-derived antigens drained via the dural sinuses. While there are no organoid models reported to date that incorporate a meninx, Worsdorfer *et al.* fused together mesodermal and neuroepithelial organoids, which resulted in vascular infiltration of the neuroepithelial region [104]. Interestingly, they observed $Iba1^+$ cells within the mesodermal portion of the organoid that resemble perivascular macrophages. This type of system could be

adapted to generate cerebral organoids with an integrated meninx, as meningeal epithelial cells arise from the mesodermal lineage.

Engineered 3D cell culture models

It is now possible to adapt iPS-derived co-cultures into highly engineered brain-on-a-chip platforms that may be more tractable than organoids for studies of particular neuroimmune interactions and vascular dynamics [105–111]. Using such technology, Park *et al.* devised a multi-chamber device in which they seeded astrocyte and neuron co-cultures with engineered fAD mutations [112] into the central chamber and microglia into the outer chamber, then quantified microglia migration towards the neuronal compartment secreting pro-inflammatory cytokines CCL2, TNFα, and IFNγ [84]. Generating different cell types separately prior to co-culturing also makes it easier to dissect how disease-causing gene variants function within specific cell types. Blanchard *et al.* developed an *in vitro* cerebrovascular culture with blood-brain barrier properties by co-culturing iPS-derived brain endothelial cells, astrocytes, and mural cells in Matrigel [113]. By conducting experiments with different combinations of isogenic APOE3 and APOE4 cell types, they determined that it is the APOE4 mural cells that drive increased accumulation of amyloid at the vasculature, mainly through defects in NFAT signaling that could be corrected by application of cyclosporine A in 5xFAD mice. This kind of blood-brain barrier co-culture could be adapted into a microfluidic chip format to facilitate the formation of perfusable vasculature [114], as well as combined with cerebral organoids to form an integrated neurovascular unit. The neurovascular unit is highly relevant to AD research, given recent findings that reactive oxygen species play a key role in amyloid-mediated vasoconstriction that ultimately restricts brain oxygenation and exacerbates neuronal loss [115–117].

Conclusions and Outlook

Cerebral organoids have been shown to model AD-like pathologies to an impressive extent, even though they represent a fairly young developmental age [118,119]. Although much progress has been made in demonstrating the robustness of organoids as an AD model, additional work remains to improve the biological relevance of organoids by incorporating non-neuronal cell types such as microglia and vasculature, and developing high-throughput methods to facilitate drug screening. While limited access to live human brains has hampered our progress in understanding how and when diseases develop, brain organoids provide new opportunities to study the origins of neurological disease. It is clear that human patient-derived organoids have the potential to overcome some of the limitations present in existing animal models, and it will be exciting to see how the field unfolds with respect to studying the interactions between genetic and environmental factors that drive late-onset sporadic AD.

References

1. Morizane A, Doi D, Kikuchi T, Nishimura K, Takahashi J: **Small-molecule inhibitors of**

bone morphogenic protein and activin/nodal signals promote highly efficient neural induction from human pluripotent stem cells. *J Neurosci Res* 2011, doi:10.1002/jnr.22547.

2. Zeng H, Guo M, Martins-Taylor K, Wang X, Zhang Z, Park JW, Zhan S, Kronenberg MS, Lichtler A, Liu H-X, et al.: **Specification of Region-Specific Neurons Including Forebrain Glutamatergic Neurons from Human Induced Pluripotent Stem Cells**. *PLoS One* 2010, **5**:e11853.
3. Shi Y, Kirwan P, Smith J, Robinson HPC, Livesey FJ: **Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses**. *Nat Neurosci* 2012, **15**:477–486.
4. Okada Y, Matsumoto A, Shimazaki T, Enoki R, Koizumi A, Ishii S, Itoyama Y, Sobue G, Okano H: **Spatiotemporal Recapitulation of Central Nervous System Development by Murine Embryonic Stem Cell-Derived Neural Stem/Progenitor Cells**. *Stem Cells* 2008, **26**:3086–3098.
5. Zhang S-C, Wernig M, Duncan ID, Brüstle O, Thomson JA: **In vitro differentiation of transplantable neural precursors from human embryonic stem cells**. *Nat Biotechnol* 2001, **19**:1129–1133.
6. Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T, Yamanaka S, Okano H, Suzuki N: **Modeling familial Alzheimer's disease with induced pluripotent stem cells**. *Hum Mol Genet* 2011, **20**:4530–4539.
7. Armijo E, Gonzalez C, Shahnawaz M, Flores A, Davis B, Soto C: **Increased susceptibility to Aβ toxicity in neuronal cultures derived from familial Alzheimer's disease (PSEN1-A246E) induced pluripotent stem cells**. *Neurosci Lett* 2017, **639**:74–81.
8. Mahairaki V, Ryu J, Peters A, Chang Q, Li T, Park TS, Burridge PW, Talbot CC, Asnaghi L, Martin LJ, et al.: **Induced pluripotent stem cells from familial alzheimer's disease patients differentiate into mature neurons with amyloidogenic properties**. *Stem Cells Dev* 2014, **23**:2996–3010.
9. Koch P, Tamboli IY, Mertens J, Wunderlich P, Ladewig J, Stüber K, Esselmann H, Wiltfang J, Brüstle O, Walter J: **Presenilin-1 L166P mutant human pluripotent stem cell-derived neurons exhibit partial loss of γ-secretase activity in endogenous amyloid-β generation**. *Am J Pathol* 2012, **180**:2404–2416.
10. Sproul AA, Jacob S, Pre D, Kim SH, Nestor MW, Navarro-Sobrino M, Santa-Maria I, Zimmer M, Aubry S, Steele JW, et al.: **Characterization and molecular profiling of PSEN1 familial alzheimer's disease iPSC-Derived neural progenitors**. *PLoS One* 2014, **9**.
11. Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS, et al.: **Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells**. *Nature* 2012, **482**:216–220.
12. Moore S, Evans LDB, Andersson T, Portelius E, Smith J, Dias TB, Saurat N, McGlade A, Kirwan P, Blennow K, et al.: **APP Metabolism Regulates Tau Proteostasis in Human Cerebral Cortex Neurons**. *Cell Rep* 2015, **11**:689–696.
13. Muratore CR, Rice HC, Srikanth P, Callahan DG, Shin T, Benjamin LNP, Walsh DM, Selkoe DJ, Young-Pearse TL: **The familial alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons**. *Hum Mol Genet* 2014, **23**:3523–3536.

14. Shi Y, Kirwan P, Smith J, MacLean G, Orkin SH, Livesey FJ: **A human stem cell model of early Alzheimer's disease pathology in down syndrome**. *Sci Transl Med* 2012, **4**.
15. Xu X, Lei Y, Luo J, Wang J, Zhang S, Yang XJ, Sun M, Nuwaysir E, Fan G, Zhao J, et al.: **Prevention of β-amyloid induced toxicity in human iPS cell-derived neurons by inhibition of Cyclin-dependent kinases and associated cell cycle events**. *Stem Cell Res* 2013, **10**:213–227.
16. Vazin T, Ball KA, Lu H, Park H, Ataeijannati Y, Head-Gordon T, Poo M ming, Schaffer D V.: **Efficient derivation of cortical glutamatergic neurons from human pluripotent stem cells: A model system to study neurotoxicity in Alzheimer's disease**. *Neurobiol Dis* 2014, **62**:62–72.
17. Usenovic M, Niroomand S, Drolet RE, Yao L, Gaspar RC, Hatcher NG, Schachter J, Renger JJ, Parmentier-Batteur S: **Internalized tau oligomers cause neurodegeneration by inducing accumulation of pathogenic tau in human neurons derived from induced pluripotent stem cells**. *J Neurosci* 2015, **35**:14234–14250.
18. Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Tu EY, Mandal PK, Vera E, Shim JW, Kriks S, et al.: **Human iPSC-based modeling of late-onset disease via progerin-induced aging**. *Cell Stem Cell* 2013, **13**:691–705.
19. Mertens J, Reid D, Lau S, Kim Y, Gage FH: **Aging in a dish: iPSC-derived and directly induced neurons for studying brain aging and age-related neurodegenerative diseases**. *Annu Rev Genet* 2018, **52**:271–93.
20. Panza F, Lozupone M, Logroscino G, Imbimbo BP: **A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease**. *Nat Rev Neurol* 2019, **15**:73–88.
21. Lancaster MA, Knoblich JA: **Generation of cerebral organoids from human pluripotent stem cells**. *Nat Protoc* 2014, **9**:2329–2340.

• Lancaster *et al* developed the first cerebral organoid model to recapitulate 3D cortical organization by encapsulating neurosphere cultures in matrigel and following a relatively undirected differentiation protocol.

22. Quadrato G, Nguyen T, Macosko EZ, Sherwood JL, Yang SM, Berger DR, Maria N, Scholvin J, Goldman M, Kinney JP, et al.: **Cell diversity and network dynamics in photosensitive human brain organoids**. *Nature* 2017, **545**:48–53.
23. Raja WK, Mungenast AE, Lin YT, Ko T, Abdurrob F, Seo J, Tsai LH: **Self-organizing 3D human neural tissue derived from induced pluripotent stem cells recapitulate Alzheimer's disease phenotypes**. *PLoS One* 2016, **11**:1–18.

• This was the first study to generate cerebral organoids from fAD patients. The authors demonstrated that these organoids recapitulated key AD pathologies by 90 days of age, including elevated Aβ levels, hyperphosphorylated tau, and enlarged endosomes. Treatment with γ- and β-secretase inhibitors reduced Aβ levels in these organoids.

24. Gonzalez C, Armijo E, Bravo-Alegria J, Becerra-Calixto A, Mays CE, Soto C: **Modeling amyloid beta and tau pathology in human cerebral organoids**. *Mol Psychiatry* 2018, **23**:2363–2374.

25. Hernández-Sapiéns MA, Reza-Zaldívar EE, Cevallos RR, Márquez-Aguirre AL, Gazarian K, Canales-Aguirre AA: **A Three-Dimensional Alzheimer's Disease Cell Culture Model Using iPSC-Derived Neurons Carrying A246E Mutation in PSEN1**. *Front Cell Neurosci* 2020, **14**:1–11.
26. Ghatak S, Dolatabadi N, Trudler D, Zhang X, Wu Y, Mohata M, Ambasudhan R, Talantova M, Lipton SA: **Mechanisms of hyperexcitability in alzheimer's disease hiPSC-derived neurons and cerebral organoids vs. Isogenic control**. *Elife* 2019, **8**:1–22.
27. Yin J, Vandongen AM: **Enhanced Neuronal Activity and Asynchronous Calcium Transients Revealed in a 3D Organoid Model of Alzheimer's Disease**. *ACS Biomater Sci Eng* 2021, **7**:254–264.
28. McLaurin J, Yang D-S, Yip CM, Fraser PE: **Review: Modulating Factors in Amyloid-b Fibril Formation**. *J Struct Biol* 2000, **130**:259–270.
29. Bejoy J, Song L, Wang Z, Sang QX, Zhou Y, Li Y: **Neuroprotective Activities of Heparin, Heparinase III, and Hyaluronic Acid on the Aβ42-Treated Forebrain Spheroids Derived from Human Stem Cells**. *ACS Biomater Sci Eng* 2018, **4**:2922–2933.

•The authors treated organoids with amyloid and different extracellular matrix-digesting enzymes and showed that these altered Aβ pathology. This study demonstrates that extracellular matrix dynamics that contribute to neurodegeneration can be modeled in cerebral organoids.

30. Yan Y, Song L, Bejoy J, Zhao J, Kanekiyo T, Bu G, Zhou Y, Li Y: **Modeling neurodegenerative microenvironment using cortical organoids derived from human stem cells**. *Tissue Eng - Part A* 2018, **24**:1125–1137.
31. Fragkouli A, Tsilibary EC, Tzinia AK: **Neuroprotective role of MMP-9 overexpression in the brain of Alzheimer's 5xFAD mice**. *Neurobiol Dis* 2014, **70**:179–189.
32. Kaminari A, Giannakas N, Tzinia A, Tsilibary EC: **Overexpression of matrix metalloproteinase-9 (MMP-9) rescues insulin-mediated impairment in the 5XFAD model of Alzheimer's disease**. *Sci Rep* 2017, **7**:1–12.
33. Romero JR, Vasan RS, Beiser AS, Au R, Benjamin EJ, DeCarli C, Wolf PA, Seshadri S: **Association of matrix metalloproteinases with MRI indices of brain ischemia and aging**. *Neurobiol Aging* 2010, **31**:2128–2135.
34. Kaplan A, Spiller KJ, Towne C, Kanning KC, Choe GT, Geber A, Akay T, Aebischer P, Henderson CE: **Neuronal Matrix Metalloproteinase-9 Is a Determinant of Selective Neurodegeneration**. *Neuron* 2014, **81**:333–348.
35. Brkic M, Balusu S, Libert C, Vandenbroucke RE: **Friends or Foes: Matrix Metalloproteinases and Their Multifaceted Roles in Neurodegenerative Diseases**. *Mediators Inflamm* 2015, **2015**:1–27.
36. Chen M, Lee HK, Moo L, Hanlon E, Stein T, Xia W: **Common proteomic profiles of induced pluripotent stem cell-derived three-dimensional neurons and brain tissue from Alzheimer patients**. *J Proteomics* 2018, **182**:21–33.

•• The authors compared the proteomic profile of AD patient-derived organoids to postmortem tissue and found comparable levels of disruption of proteins involved in

axon growth, mitochondrial function, and oxidative stress. This study demonstrates that organoids recapitulate the *in vivo* proteome.

37. Seo J, Kritskiy O, Ashley Watson L, Barker SJ, Dey D, Raja WK, Lin YT, Ko T, Cho S, Penney J, et al.: **Inhibition of p25/Cdk5 attenuates tauopathy in mouse and iPSC models of frontotemporal dementia**. *J Neurosci* 2017, **37**:9917–9924.
38. Elder GA, Gama Sosa MA, De Gasperi R: **Transgenic mouse models of Alzheimer's disease**. *Mt Sinai J Med* 2010, doi:10.1002/msj.20159.
39. Myers A, McGonigle P: **Overview of Transgenic Mouse Models for Alzheimer's Disease**. *Curr Protoc Neurosci* 2019, doi:10.1002/cpns.81.
40. Di Lullo E, Kriegstein AR: **The use of brain organoids to investigate neural development and disease**. *Nat Rev Neurosci* 2017, **18**:573–584.
41. Kuehner JN, Chen J, Bruggeman EC, Wang F, Li Y, Xu C, McEachin ZT, Li Z, Chen L, Hales CM, et al.: **5-hydroxymethylcytosine is dynamically regulated during forebrain organoid development and aberrantly altered in Alzheimer's disease**. *Cell Rep* 2021, **35**:109042.

• Kuehner *et al* profiled the 5hmc methylation profile of cerebral organoids across several developmental timepoints and found that this was disrupted in fAD organoids. This study suggests that early developmental processes may contribute to neurodegeneration later in life.

42. Busche MA, Konnerth A: **Impairments of neural circuit function in Alzheimer's disease**. *Philos Trans R Soc B Biol Sci* 2016, **371**.
43. Pavoni S, Jarray R, Nassor F, Guyot AC, Cottin S, Rontard J, Mikol J, Mabondzo A, Deslys JP, Yates F: **Small-molecule induction of Aβ-42 peptide production in human cerebral organoids to model Alzheimer's disease associated phenotypes**. *PLoS One* 2018, **13**:1–15.
44. Spitzer P, Condic M, Herrmann M, Oberstein TJ, Scharin-Mehlmann M, Gilbert DF, Friedrich O, Grömer T, Kornhuber J, Lang R, et al.: **Amyloidogenic amyloid-β-peptide variants induce microbial agglutination and exert antimicrobial activity**. *Sci Rep* 2016, doi:10.1038/srep32228.
45. Moir RD, Lathe R, Tanzi RE: **The antimicrobial protection hypothesis of Alzheimer's disease**. *Alzheimer's Dement* 2018, doi:10.1016/j.jalz.2018.06.3040.
46. Jamieson GA, Maitland NJ, Wilcock GK, Craske J, Itzhaki RF: **Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains**. *J Med Virol* 1991, doi:10.1002/jmv.1890330403.
47. Jamieson GA, Maitland NJ, Wilcock GK, Yates CM, Itzhaki RF: **Herpes simplex virus type 1 DNA is present in specific regions of brain from aged people with and without senile dementia of the Alzheimer type**. *J Pathol* 1992, doi:10.1002/path.1711670403.
48. Itzhaki RF, Lathe R: **Herpes Viruses and Senile Dementia: First Population Evidence for a Causal Link**. *J Alzheimer's Dis* 2018, doi:10.3233/JAD-180266.
49. Cairns DM, Rouleau N, Parker RN, Walsh KG, Gehrke L, Kaplan DL: **A 3D human brain–like tissue model of herpes-induced Alzheimer's disease**. *Sci Adv* 2020, **6**:1–14.

••The authors infected neuronal spheroids with herpes simplex virus type 1 and found that they develop AD-like pathology, including formation of amyloid plaques, upregulation of presenilins and pro-inflammatory genes, and reactive gliosis. This study demonstrated a causal link between viral infection and neurodegeneration consistent with the AD pathogen hypothesis.

50. Jansen IE, Savage JE, Watanabe K, Bryois J, Williams DM, Steinberg S, Sealock J, Karlsson IK, Hägg S, Athanasiu L, et al.: **Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk**. *Nat Genet* 2019, **51**:404–13.
51. Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G: **Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies**. *Nat Rev Neurol* 2019, doi:10.1038/s41582-019-0228-7.
52. Lin YT, Seo J, Gao F, Feldman HM, Wen HL, Penney J, Cam HP, Gjoneska E, Raja WK, Cheng J, et al.: **APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types**. *Neuron* 2018, **98**:1141-1154.e7.
53. Zhao J, Fu Y, Yamazaki Y, Ren Y, Davis MD, Liu CC, Lu W, Wang X, Chen K, Cherukuri Y, et al.: **APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids**. *Nat Commun* 2020, **11**.
54. Hernández D, Rooney LA, Daniszewski M, Gulluyan L, Liang HH, Cook AL, Hewitt AW, Pébay A: **Culture Variabilities of Human iPSC-Derived Cerebral Organoids Are a Major Issue for the Modelling of Phenotypes Observed in Alzheimer's Disease**. *Stem Cell Rev Reports* 2021, doi:10.1007/s12015-021-10147-5.
55. Jorfi M, D'Avanzo C, Tanzi RE, Kim DY, Irimia D: **Human Neurospheroid Arrays for In Vitro Studies of Alzheimer's Disease**. *Sci Rep* 2018, **8**:1–13.
56. Groveman BR, Ferreira NC, Foliaki ST, Walters RO, Winkler CW, Race B, Hughson AG, Zanusso G, Haigh CL: **Human cerebral organoids as a therapeutic drug screening model for Creutzfeldt–Jakob disease**. *Sci Rep* 2021, **11**:1–9.
57. Yoon SJ, Elahi LS, Pașca AM, Marton RM, Gordon A, Revah O, Miura Y, Walczak EM, Holdgate GM, Fan HC, et al.: **Reliability of human cortical organoid generation**. *Nat Methods* 2019, **16**:75–78.
58. Cai H, Ao Z, Hu L, Moon Y, Wu Z, Lu HC, Kim J, Guo F: **Acoustofluidic assembly of 3D neurospheroids to model Alzheimer's disease**. *Analyst* 2020, **145**:6243–6253.

• The authors developed an acustofluidic assembly method to rapidly generate uniformly sized spheroids containing neurons, microglia, and amyloid protein and monitored the localization of microglia to amyloid aggregates. This assay could be efficiently deployed for high throughput drug screens of amyloid neurotoxicity.

59. Esmail S, Danter WR: **NEUBOrg: Artificially Induced Pluripotent Stem Cell-Derived Brain Organoid to Model and Study Genetics of Alzheimer's Disease Progression**. *Front Aging Neurosci* 2021, **13**:1–13.
60. Park JC, Jang SY, Lee D, Lee J, Kang U, Chang H, Kim HJ, Han SH, Seo J, Choi M, et al.: **A logical network-based drug-screening platform for Alzheimer's disease representing**

pathological features of human brain organoids. *Nat Commun* 2021, **12**:1–13.

••Park *et al* developed a computational model of AD using a database of known gene/protein interactions and used this to identify FDA approved drugs that attenuate AD pathology. They then tested these drugs on sAD patient-derived organoids and found that they successfully attenuated Aβ and tau pathologies. This study demonstrated a powerful new approach integrating the predictive capabilities of computational modeling with rapid experimental validation in cerebral organoids that could greatly accelerate drug discovery.

61. Henstridge CM, Hyman BT, Spires-Jones TL: **Beyond the neuron–cellular interactions early in Alzheimer disease pathogenesis**. *Nat Rev Neurosci* 2019, **20**:94–108.
62. Linnerbauer M, Wheeler MA, Quintana FJ: **Astrocyte Crosstalk in CNS Inflammation**. *Neuron* 2020, doi:10.1016/j.neuron.2020.08.012.
63. Hansen D V, Hanson JE, Sheng M: **Microglia in Alzheimer ' s disease**. *J Cell Biol* 2018, **217**:459–72.
64. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, Walker AJ, Gergits F, Segel M, Nemesh J, et al.: **Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes**. *Immunity* 2019, **50**:253-271.e6.
65. Sweeney MD, Sagare AP, Zlokovic B V.: **Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders**. *Nat Rev Neurol* 2018, **14**:133–150.
66. Abud EM, Ramirez RN, Martinez ES, Healy LM, Nguyen CHH, Newman SA, Yeromin A V., Scarfone VM, Marsh SE, Fimbres C, et al.: **iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases**. *Neuron* 2017, **94**:278-293.e9.
67. Haenseler W, Sansom SN, Buchrieser J, Newey SE, Moore CS, Nicholls FJ, Chintawar S, Schnell C, Antel JP, Allen ND, et al.: **A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response**. *Stem Cell Reports* 2017, doi:10.1016/j.stemcr.2017.05.017.
68. Douvaras P, Wang J, Zimmer M, Hanchuk S, O'Bara M, Sadiq S, Sim F, Goldman J, Fossati V: **Efficient generation of myelinating oligodendrocytes from primary progressive multiple scelrosis patients by induced pluripotent stem cells**. *Stem Cell Reports* 2014, **3**:250–259.
69. Patsch C, Myelan-Challet L, Thoma EC, Urich E, Heckel T, O-Sullivan JF, Grainger SJ, Kapp FG, Sun L, Christensen K, et al.: **Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells**. *Nat Cell Biol* 2015, **17**:994–1006.
70. Qian T, Hernday SE, Bao X, Olson WR, Panzer SE, Shusta E V., Palecek SP: **Directed differentiation of human pluripotent stem cells to blood-brain barrier endothelial cells**. *Sci Adv* 2017, **3**.
71. TCW J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, et al.: **An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells**. *Stem Cell Reports* 2017, **9**:600–614.
72. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, et al.: **A Unique Microglia Type Associated**

with Restricting Development of Alzheimer's Disease. *Cell* 2017, **169**:1276-1290.e17.
73. Mathys H, Adaikkan C, Gao F, Young JZ, Manet E, Hemberg M, De Jager PL, Ransohoff RM, Regev A, Tsai LH: **Temporal Tracking of Microglia Activation in Neurodegeneration at Single-Cell Resolution**. *Cell Rep* 2017, **21**:366–380.
74. Habib N, McCabe C, Medina S, Varshavsky M, Kitsberg D, Dvir-Szternfeld R, Green G, Dionne D, Nguyen L, Marshall JL, et al.: **Disease-associated astrocytes in Alzheimer's disease and aging**. *Nat Neurosci* 2020, doi:10.1038/s41593-020-0624-8.
75. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, Beckers L, O'Loughlin E, Xu Y, Fanek Z, et al.: **The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases**. *Immunity* 2017, **47**:566-581.e9.
76. Mazaheri F, Snaidero N, Kleinberger G, Madore C, Daria A, Werner G, Krasemann S, Capell A, Trumbach D, Wurst W, et al.: **TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury**. *EMBO Rep* 2017, **18**:1176–98.
77. Nugent AA, Lin K, van Lengerich B, Lianoglou S, Przybyla L, Davis SS, Llapashtica C, Wang J, Kim DJ, Xia D, et al.: **TREM2 Regulates Microglial Cholesterol Metabolism upon Chronic Phagocytic Challenge**. *Neuron* 2020, **105**:837-854.e9.
78. Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, Gilfillan S, Krishnan GM, Sudhakar S, Zinselmeyer BH, et al.: **TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model**. *Cell* 2015, **160**:1061–1071.
79. Andreone BJ, Przybyla L, Llapashtica C, Rana A, Davis SS, van Lengerich B, Lin K, Shi J, Mei Y, Astarita G, et al.: **Alzheimer's-associated PLCγ2 is a signaling node required for both TREM2 function and the inflammatory response in human microglia**. *Nat Neurosci* 2020, **23**:927–938.
80. Schoch KM, Ezerskiy LA, Morhaus MM, Bannon RN, Sauerbeck AD, Shabsovich M, Jafar-Nejad P, Rigo F, Miller TM: **Acute Trem2 reduction triggers increased microglial phagocytosis, slowing amyloid deposition in mice**. *Proc Natl Acad Sci U S A* 2021, **118**:1–10.
81. Badimon A, Strasburger HJ, Ayata P, Chen X, Nair A, Ikegami A, Hwang P, Chan AT, Graves SM, Uweru JO, et al.: **Negative feedback control of neuronal activity by microglia**. *Nature* 2020, **586**:417–423.
82. Hughes AN, Appel B: **Microglia phagocytose myelin sheaths to modify developmental myelination**. *Nat Neurosci* 2020, **23**:1055–1066.
83. Park GH, Noh H, Shao Z, Ni P, Qin Y, Liu D, Beaudreault CP, Park JS, Abani CP, Park JM, et al.: **Activated microglia cause metabolic disruptions in developmental cortical interneurons that persist in interneurons from individuals with schizophrenia**. *Nat Neurosci* 2020, **23**:1352–1364.
84. Park J, Wetzel I, Marriott I, Dréau D, D'Avanzo C, Kim DY, Tanzi RE, Cho H: **A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease**. *Nat Neurosci* 2018, **21**:941–951.
85. Guttikonda SR, Sikkema L, Tchieu J, Saurat N, Walsh RM, Harschnitz O, Ciceri G, Sneeboer M, Mazutis L, Setty M, et al.: **Fully defined human pluripotent stem cell-derived microglia and tri-culture system model C3 production in Alzheimer's disease**. *Nat Neurosci* 2021, **24**:343–354.

86. Ormel PR, Vieira de Sá R, van Bodegraven EJ, Karst H, Harschnitz O, Sneeboer MAM, Johansen LE, van Dijk RE, Scheefhals N, Berdenis van Berlekom A, et al.: **Microglia innately develop within cerebral organoids**. *Nat Commun* 2018, **9**.
87. Abreu CM, Gama L, Krasemann S, Chesnut M, Odwin-Dacosta S, Hogberg HT, Hartung T, Pamies D: **Microglia Increase Inflammatory Responses in iPSC-Derived Human BrainSpheres**. *Front Microbiol* 2018, **9**:1–12.
88. Song L, Yuan X, Jones Z, Vied C, Miao Y, Marzano M, Hua T, Sang QXA, Guan J, Ma T, et al.: **Functionalization of Brain Region-specific Spheroids with Isogenic Microglia-like Cells**. *Sci Rep* 2019, **9**:1–18.

• The authors successfully integrated microglia into dorsal and ventral forebrain patterned spheroids and demonstrated that these successfully upregulated pro-inflammatory factors in response to stimuli.

89. Schmunk G, Kim C, Soliman S, Keefe M, Bogdanoff D, Tejera D, Ziffra R, Shin D, Allen D, Chhun B, et al.: **Human microglia upregulate cytokine signatures and accelerate maturation of neural networks**. 2020, doi:10.1101/2020.03.24.006874.
90. Xu R, Boreland AJ, Li X, Erickson C, Jin M, Atkins C, Pang ZP, Daniels BP, Jiang P: **Developing human pluripotent stem cell-based cerebral organoids with a controllable microglia ratio for modeling brain development and pathology.** *Stem cell reports* 2021, **16**.
91. Iadecola C: **The Pathobiology of Vascular Dementia**. *Neuron* 2013, **80**:844–866.
92. Montagne A, Zhao Z, Zlokovic B V: **Alzheimer ' s disease : A matter of blood – brain barrier dysfunction ? The Journal of Experimental Medicine**. *J Exp Med* 2017, **214**:3151–3169.
93. Yang AC, Vest RT, Kern F, Lee DP, Maat CA, Losada PM, Chen MB, Agam M, Schaum N, Khoury N, et al.: **A human brain vascular atlas reveals diverse cell mediators of Alzheimer's disease risk**. *bioRxiv* 2021,
94. Mansour AA, Gonçalves JT, Bloyd CW, Li H, Fernandes S, Quang D, Johnston S, Parylak SL, Jin X, Gage FH: **An in vivo model of functional and vascularized human brain organoids**. *Nat Biotechnol* 2018, doi:10.1038/nbt.4127.
95. Wimmer RA, Leopoldi A, Aichinger M, Wick N, Hantusch B, Novatchkova M, Taubenschmid J, Hämmerle M, Esk C, Bagley JA, et al.: **Human blood vessel organoids as a model of diabetic vasculopathy**. *Nature* 2019, **565**:505–510.
96. Cakir B, Xiang Y, Tanaka Y, Kural MH, Parent M, Kang Y-J, Chapeton K, Patterson B, Yuan Y, He C-S, et al.: **Engineering of human brain organoids with a functional vascular-like system**. *Nat Methods* 2019, doi:10.1038/s41592-019-0586-5.
97. Nzou G, Wicks RT, Wicks EE, Seale SA, Sane CH, Chen A, Murphy S V., Jackson JD, Atala AJ: **Human cortex spheroid with a functional blood brain barrier for high-throughput neurotoxicity screening and disease modeling**. *Sci Rep* 2018, **8**:1–10.
98. Cho CF, Wolfe JM, Fadzen CM, Calligaris D, Hornburg K, Chiocca EA, Agar NYR, Pentelute BL, Lawler SE: **Blood-brain-barrier spheroids as an in vitro screening platform for brain-penetrating agents**. *Nat Commun* 2017, **8**:1–14.
99. Bergmann S, Lawler SE, Qu Y, Fadzen CM, Wolfe JM, Regan MS, Pentelute BL, Agar NYR,

Cho CF: **Blood–brain-barrier organoids for investigating the permeability of CNS therapeutics**. *Nat Protoc* 2018, **13**:2827–2843.
100. Pellegrini L, Bonfio C, Chadwick J, Begum F, Skehel M, Lancaster MA: **Human CNS barrier-forming organoids with cerebrospinal fluid production**. *Science (80-)* 2020, **5626**.
101. Jacob F, Pather SR, Huang WK, Zhang F, Wong SZH, Zhou H, Cubitt B, Fan W, Chen CZ, Xu M, et al.: **Human Pluripotent Stem Cell-Derived Neural Cells and Brain Organoids Reveal SARS-CoV-2 Neurotropism Predominates in Choroid Plexus Epithelium**. *Cell Stem Cell* 2020, **27**:937-950.e9.
102. Rasmussen MK, Mestre H, Nedergaard M: **The glymphatic pathway in neurological disorders**. *Lancet Neurol* 2018, **17**:1016–1024.
103. Da Mesquita S, Louveau A, Vaccari A, Smirnov I, Cornelison RC, Kingsmore KM, Contarino C, Onengut-Gumuscu S, Farber E, Raper D, et al.: **Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease**. *Nature* 2018, **560**:185–191.
104. Wörsdörfer P, Dalda N, Kern A, Krüger S, Wagner N, Kwok CK, Henke E, Ergün S: **Generation of complex human organoid models including vascular networks by incorporation of mesodermal progenitor cells**. *Sci Rep* 2019, **9**:1–13.
105. Maoz BM, Herland A, Fitzgerald EA, Grevesse T, Vidoudez C, Pacheco AR, Sheehy SP, Park TE, Dauth S, Mannix R, et al.: **A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells**. *Nat Biotechnol* 2018, **36**:865–877.
106. Ahn SI, Sei YJ, Park HJ, Kim J, Ryu Y, Choi JJ, Sung HJ, MacDonald TJ, Levey AI, Kim YT: **Microengineered human blood–brain barrier platform for understanding nanoparticle transport mechanisms**. *Nat Commun* 2020, **11**:1–12.
107. Adriani G, Ma D, Pavesi A, Kamm RD, Goh ELK: **A 3D neurovascular microfluidic model consisting of neurons, astrocytes and cerebral endothelial cells as a blood-brain barrier**. *Lab Chip* 2017, **17**:448–459.
108. Bang S, Lee SR, Ko J, Son K, Tahk D, Ahn J, Im C, Jeon NL: **A Low Permeability Microfluidic Blood-Brain Barrier Platform with Direct Contact between Perfusable Vascular Network and Astrocytes**. *Sci Rep* 2017, **7**:1–10.
109. Seo S, Kim H, Sung JH, Choi N, Lee K, Kim HN: **Microphysiological systems for recapitulating physiology and function of blood-brain barrier**. *Biomaterials* 2020, **232**:119732.
110. Tunesi M, Izzo L, Raimondi I, Albani D, Giordano C: **A miniaturized hydrogel-based in vitro model for dynamic culturing of human cells overexpressing beta-amyloid precursor protein**. *J Tissue Eng* 2020, **11**.
111. Kwak SS, Washicosky KJ, Brand E, von Maydell D, Aronson J, Kim S, Capen DE, Cetinbas M, Sadreyev R, Ning S, et al.: **Amyloid-β42/40 ratio drives tau pathology in 3D human neural cell culture models of Alzheimer's disease**. *Nat Commun* 2020, **11**:1–14.
112. Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, Chen H, Hooli B, Asselin C, Muffat J, et al.: **A three-dimensional human neural cell culture model of Alzheimer's disease**. *Nature* 2014, **515**:274–278.
113. Blanchard JW, Bula M, Davila-Velderrain J, Akay LA, Zhu L, Frank A, Victor MB, Bonner JM, Mathys H, Lin YT, et al.: **Reconstruction of the human blood–brain barrier in vitro reveals a pathogenic mechanism of APOE4 in pericytes**. *Nat Med* 2020, **26**:952–963.

•Blanchard *et al* developed an iPS-derived cerebrovascular co-culture that recapitulates key blood-brain barrier properties and showed that APOE4 mural cells promote Aβ deposition at the vasculature via defects in calcineurin-NFAT signaling. This study demonstrates how iPS-derived co-cultures can be harnessed for detailed mechanistic dissections of the causal role that disease risk genes play in specific cell types.

114. Campisi M, Shin Y, Osaki T, Hajal C, Chiono V, Kamm RD: **3D self-organized microvascular model of the human blood-brain barrier with endothelial cells, pericytes and astrocytes**. *Biomaterials* 2018, **180**:117–129.
115. Nortley R, Korte N, Izquierdo P, Hirunpattarasilp C, Mishra A, Jaunmuktane Z, Kyrargyri V, Pfeiffer T, Khennouf L, Madry C, et al.: **Amyloid β oligomers constrict human capillaries in alzheimer's disease via signaling to pericytes**. *Science (80-)* 2019, **365**.
116. Kisler K, Nelson A, Montagne A, Zlokovic B: **Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease**. *Nat Rev Neurosci* 2017, **18**:419–34.
117. Lendahl U, Nilsson P, Betsholtz C: **Emerging links between cerebrovascular and neurodegenerative diseases—a special role for pericytes**. *EMBO Rep* 2019, **20**:1–18.
118. Trujillo CA, Gao R, Negraes PD, Gu J, Buchanan J, Preissl S, Wang A, Wu W, Haddad GG, Chaim IA, et al.: **Complex Oscillatory Waves Emerging from Cortical Organoids Model Early Human Brain Network Development**. *Cell Stem Cell* 2019, **25**:558-569.e7.
119. Gordon A, Yoon SJ, Tran SS, Makinson CD, Park JY, Andersen J, Valencia AM, Horvath S, Xiao X, Huguenard JR, et al.: **Long-term maturation of human cortical organoids matches key early postnatal transitions**. *Nat Neurosci* 2021, **24**:331–342.

Table 1: summary of cerebral organoid models of Alzheimer's Disease

publication	Cell culture type	AD model	Additional cell types	pathology
Choi *et al* (2014)	3D neuron cell culture in matrigel	APP, PSEN1 mutation	no	Aβ plaques, p-tau, tau filaments
Raja *et al* (2016)	Cerebral organoid	APP, PSEN1 mutation	no	Aβ accumulation, p-tau, enlarged endosomes
Seo *et al* (2017)	Cerebral organoid	P301L tau mutation	no	tau accumulation, rescue with p35 knock-in
Bejoy *et al* (2018)	Cerebral organoid	WT, incubated with Aβ	no	cell bound $A\beta_{42}$, LDH levels, neuronal loss
Chen *et al* (2018)	Dorsal forebrain spheroid	sAD patients	no	axon growth, mitochondrial function, oxidative stress
Gonzalez *et al* (2018)	Cerebral organoid	fAD, Down syndrome, Creutzfeldt-Jacob disease patients	no	amyloid plaques, neurofibrillary tangles
Jorfi *et al* (2018)	Neuronal spheroid	APP, PSEN1 mutation	no	Aβ accumulation, p-tau
Lin *et al* (2018)	cerebral organoid, iPS astrocytes, microglia, neurons	APOE4	microglia, astrocytes	Aβ accumulation and uptake, synaptic deficits, lipid accumulation, inflammation
Park *et al* (2018)	3D neuron/ microglia co-culture in matrigel	APP overexpression, PSEN1 mutation	microglia	Aβ accumulation, p-tau, inflammation
Pavoni *et al* (2018)	Cerebral organoid	aftin-5 induction	no	$A\beta_{42}$ secretion
Yan *et al* (2018)	Forebrain spheroid	APP mutation	no	$A\beta_{42}$ secretion
Ghatak *et al* (2019)	Cerebral organoid	APP, PSEN1 mutation	no	neuronal hyperexcitability, decreased neurite length, altered synaptic density
Blanchard *et al* (2020)	3D cerebrovasculature	APOE4	Endothelial cells, mural cells, astrocytes	Aβ accumulation
Cai *et al* (2020)	Neuronal spheroid	WT, incubated with Aβ	Neurons, microglia	Aβ toxicity
Cairns *et al* (2020)	Neuronal spheroid	WT, infected with HSV1	no	Aβ plaques, PSEN1/2 upregulation, inflammatory genes, reactive gliosis
Hernández-Sapiéns *et al* (2020)	Neuronal spheroid	PSEN1 mutation	no	Aβ oligomer production
Kwak *et al* (2020)	3D neuron cell culture in matrigel	APP, PSEN1 mutation	no	$A\beta_{40/42}$ ratio, tau accumulation
Tunesi *et al* (2020)	3D neuron culture in microfluidic device	APP mutation	no	$A\beta_{42}$ secretion
Zhao *et al* (2020)	Cerebral organoid	APOE4	no	Aβ accumulation, pTau, synapse loss, apoptosis
Hernandes *et al* (2021)	Cerebral organoid	APOE4	no	APOE levels, $A\beta_{40/42}$ ratio, p-tau
Kuehner *et al* (2021)	Forebrain spheroid	fAD patients: APP, PSEN1 mutation	no	5hmc methylation
Park *et al* (2021)	Cerebral organoid	sAD patients, APOE4	no	$A\beta_{40/42}$ ratio, tau accumulation, p-tau, calcium transients
Yin *et al* (2021)	Cerebral organoid	PSEN2 mutation	no	$A\beta_{40/42}$ ratio, neuronal network activity, apoptosis

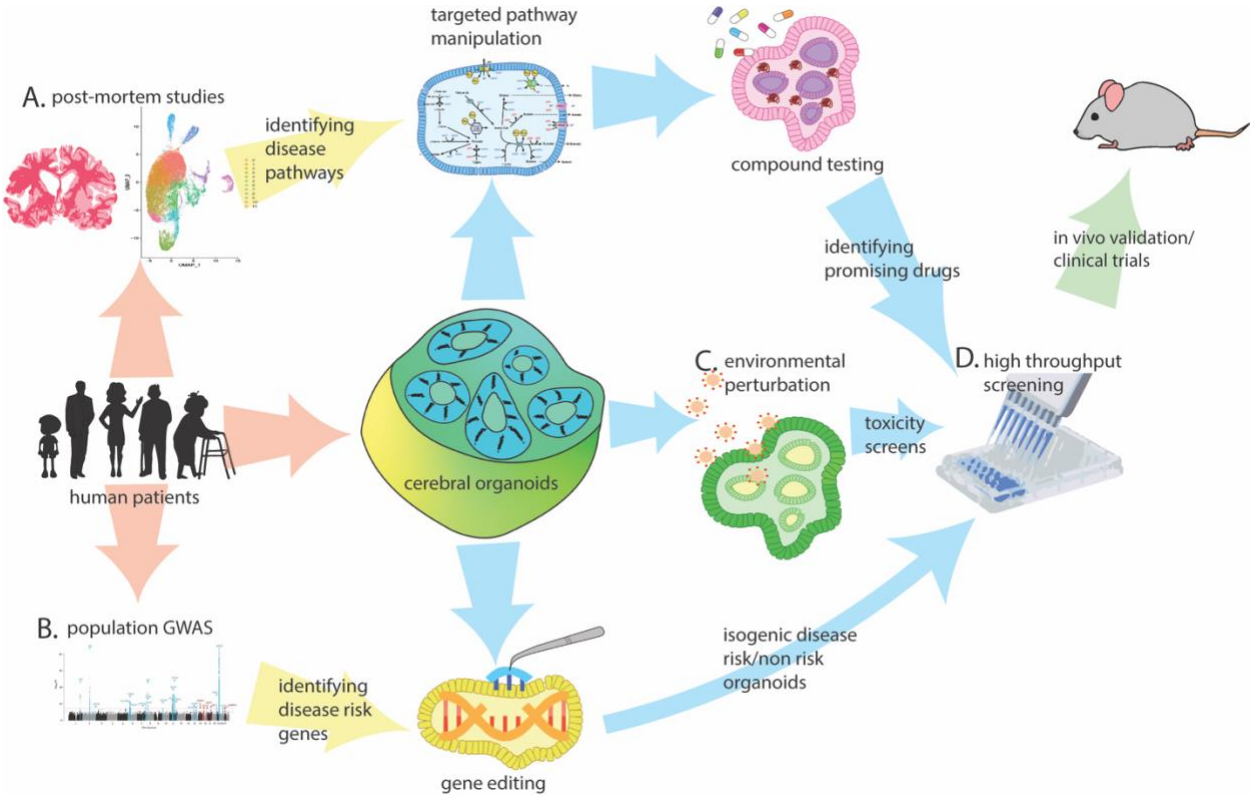

Figure 1: Harnessing cerebral organoids for drug discovery

Cerebral organoids provide unprecedented access to physiologically relevant tissue derived directly from human patients, which opens up opportunities for drug discovery. They can be used to (A) experimentally validate results from postmortem human single cell RNAseq studies that identify specific molecular pathways affected in disease, (B) probe the effects of disease risk gene variants identified in population-wide GWAS studies by using gene editing to generate isogenic cell lines with and without the disease risk gene variant of interest, and (C) screen for different kinds of environmental perturbations that can promote disease in otherwise healthy patients. (D) Integrating insights from these approaches together with improved methods for high throughput screening can yield promising drug candidates for further validation using mouse models and full clinical trials.

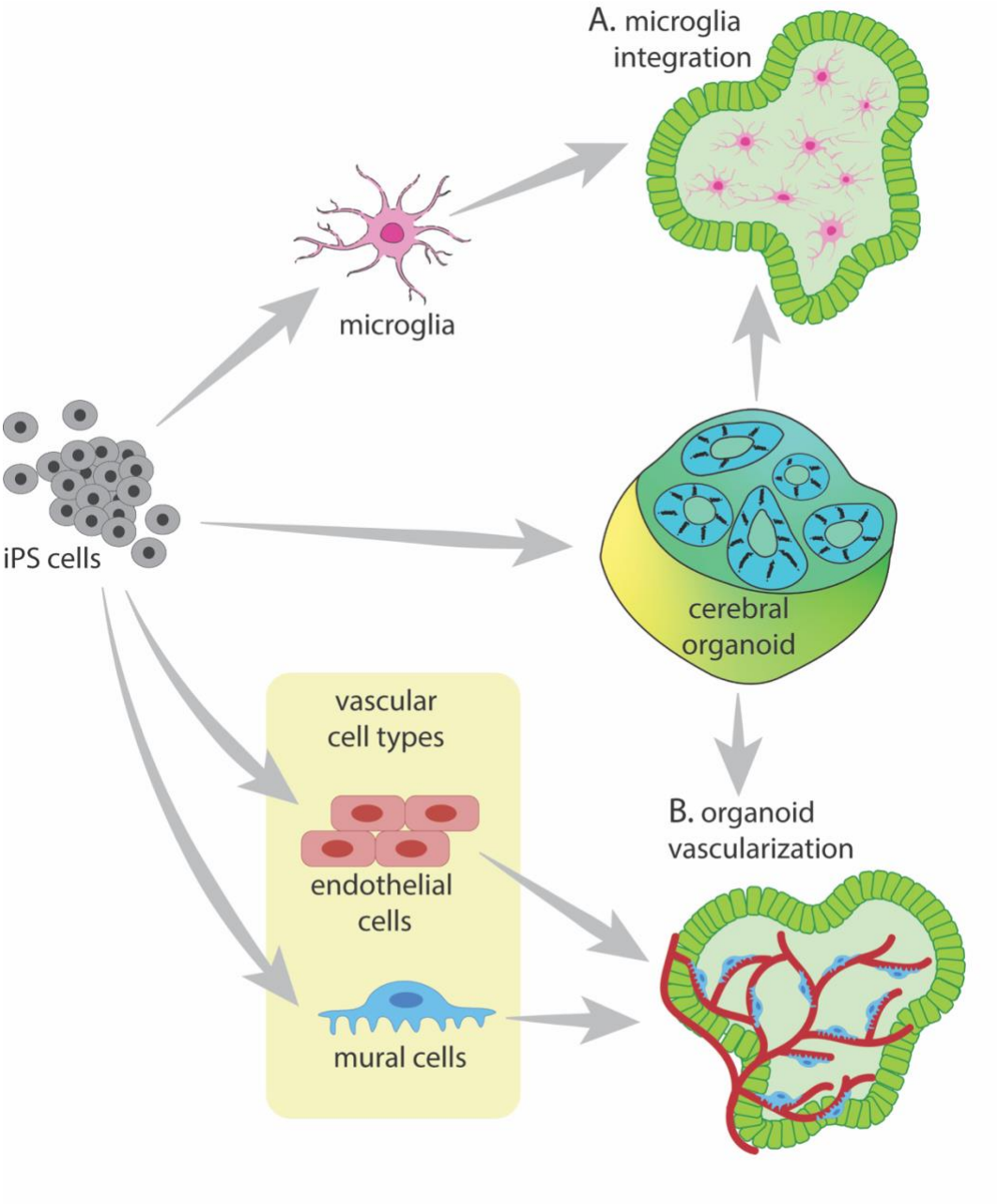

Figure 2: Incorporating non-neuronal cell types into organoids

The physiological relevance of cerebral organoids may be greatly improved by incorporating additional cell types that do not develop endogenously within the organoid using existing protocols. (A) iPS-derived microglia can be used to model neuroimmune interactions in organoids which underlie healthy brain function and neurodegeneration. (B) Incorporating cerebrovascular endothelial cells and mural cells promotes organoid vascularization, which is beneficial for cell viability and allows for studies of neurovascular interactions and blood-brain barrier function.