Microfluidic Platforms and Alzheimer's Disease

Subjects: Neurosciences
Contributor: Piyush Gupta

The past decade has witnessed a surge in the use of microfluidics technology in neurodegenerative diseases to gradually minimize biomedical research dependence on in vivo models. These platforms have been widely implicated in growing 3D gels that could be further applied in producing a three-dimensional tissue representative of human organs. With the help of these miniaturized devices, the growth of neurons, astrocytes, and microglia have also been facilitated in the form of triculture models.

Keywords: Alzheimer's disease ; microfluidics ; lab-on-chip ; 3D culture ; organ-on-chip

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative condition in which cognition and memory formation decline progressively due to an irreversible loss of neurons in the hippocampus and cortex regions [1]. It is characterized by the extracellular formation of senile plaque mainly constituted by amyloid-beta 42 (Aβ42)peptide and intracellular neurofibrillary tangles (NFTs), composed of hyper-phosphorylated paired helical filaments of the microtubule-associated protein tau (MAPT) [2][3][4]. Apart from Aβ and tau pathology, processes such as impaired synaptic functions, neurotransmission dysfunction, and microglia-mediated inflammation play a key role in AD pathogenesis [5]. Primary symptoms of the disease comprise memory deterioration, apathy, depression, and changes in personality and behavior that finally require full-time medical care [6]. The majority of AD cases present as a late-onset sporadic form (SAD) occurring in individuals aged 65 or older. SAD shows a complex etiology and results from a combination of genetic and environmental influences. To date, the only confirmed genetic risk is represented by the presence of the ε4 allele of Apolipoprotein E (ApoE), the main carrier of cholesterol in the central nervous system (CNS). This variant accelerates the onset of AD by enhancing the Aβ deposition into plaques and reducing its clearance from the cerebral tissue [7]. On the contrary, the rare early-onset forms of AD are familiar with FAD with an autosomal dominant pattern of inheritance in one of the known genes, APP , PSEN1 , and PSEN2 , encoding the Aβ precursor protein (APP), presenilin-1, and presenilin-2, respectively. As all of these are involved in the maturation and processing of APP, mutations in these genes result in increased production or aggregation of Aβ peptides [8]. The 'World Alzheimer Report 2019' shows that AD accounts for more than 70% of the total dementia cases diagnosed worldwide [9][10], therefore an early diagnosis of AD is crucial for disease management [11].

Despite AD prevalence and many years of research, several aspects of its complex etiology remain unexplored [12][13]. Moreover, the current therapeutic strategies are merely symptomatic, attenuating only behavioral symptoms but presenting several side-effects such as confusion, dizziness, depression, constipation, and diarrhea, reported in most medications [14]. Therefore, a more in-depth understanding of the molecular mechanisms underlying AD pathogenesis, revisiting numerous existing concepts, and effective screening for therapies aimed at halting or preventing neurodegeneration in AD is required [15][16]. The lack of suitable experimental models has also presented a bottleneck in understanding the AD pathological mechanism. Moreover, widely accepted notions such as the deposition of Aβ and hyperphosphorylation of microtubular protein tau also lack a direct correlation between the deposition or phosphorylation and the disease progression [17][18].

In recent years, microfluidics is emerging as an economical and versatile platform for biologists to mimic and control the cellular microenvironment in order to model diseases, study cell behavior from single- to multi-cellular organism level, and develop multiple experiments in miniaturized devices suitable for diagnostics, biomedical analysis, pathological studies of neural degeneration and drug developments [19][20]. These devices are popular, especially for their flexibility of design, experimental flexibility, leverage of a sufficient number of controls, handling single cells, controlled co-culture, reduced reagent consumption, reduced contamination risk, and efficient high throughput experimentation.

The past decade has witnessed a surge in the use of microfluidics technology in neurodegenerative diseases to gradually minimize biomedical research dependence on in vivo models [21]. These platforms have been widely implicated in growing 3D gels that could be further applied in producing a three-dimensional tissue representative of human organs. With the help of these miniaturized devices, the growth of neurons, astrocytes, and microglia have also been facilitated in the form of triculture models [22]. This review describes the latest advances in the progress of microfluidics technologies and elaborates various ways through which the domain of microfluidics presents solutions to the management of neurodegenerative disease, with a particular focus on AD. First, we emphasized the applications of microfluidics in the study of disease pathophysiology and the early detection of AD with the help of known biomarkers at a miniaturized level. Subsequently, we examined the impact of microfluidics on accelerating AD research. We then discussed the possible challenges that this field needs to overcome and the directions to be taken before realizing its full-fledged application in the AD field.

2. Application of Microfluidics in Neurodegenerative Studies

Convergence of biology with engineering is evident in microfluidic devices used extensively nowadays in different domains of biomedical research contributing to a more powerful tool for drug delivery, point of care devices, and medical diagnostics [23]. Using microfluidics, a multichambered device can be readily prepared and used to grow neurites, glial cells, endothelial cells, and skeletal muscle cells, along with the maintenance of fluid isolation [24]. These devices can recapitulate organ-like structures and provide an opportunity to investigate organogenesis and disease etiology, accelerate drug discovery, screening, and toxicology studies by mimicking pathological conditions [25]. Utilizing hydrostatic pressure and chemical gradient profiles, localized areas of neurons grown in different compartments could be exposed to different kinds of insults applied insoluble form. A vast amount of literature exists highlighting applications of microfluidics in neurodegenerative diseases along with several neurodegenerative-disease-on-a-chip models focusing on AD, Parkinson's disease, and amyotrophic lateral sclerosis [26][27][28][29][30][31]. Furthermore, the microfluidic system has been implicated in the study of regulated cell-cell interactions, elucidating the complexity of intercellular interactions in the neuroinflammation of growing primary brain cells.

It is well known that many brain cells interact with each other under varied conditions to cause neuroinflammation. The microfluidic devices facilitate cell culture, e.g., astrocytes in separate chambers exposed to varied situations. These chambers can be independently regulated and monitored for analyzing morphology, vitality, calcium dynamics, and electrophysiology parameters [32]. It has provided a platform to study neuronal cell death within the brain through simultaneous observation of neuronal connectivity and tau pathology [33]. Unlike 2D culture systems, these 3D cell cultures and microfluidic lab-on-a-chip technologies with in vitro microfluidics systems do not lack the mobility of the cultured cells allowing a better physiological extracellular environment, for examining, neuron-glia interactions minimizing animal morbidity and mortality [34][35][36]. With the help of 3D culture techniques, the discrepancies in the results of in vitro culture systems and animal models in drug discovery can be avoided [37].

The lab-on-chip technologies, with features on a similar physical scale to that of cells, have facilitated the study of complex neural signaling pathways to detect abnormalities, and check whether the application of inhibitors can reverse these without the requirement of animals [38][39]. The microfluidic entities can replicate complicated cell biological processes that control synaptic function, visualize them and manipulate synaptic regions and presynaptic and postsynaptic compartments independently under in vitro conditions, and manipulate synapses and presynaptic and postsynaptic cell bodies independently [40]. Studies show that synapses lose native circuitry and order due to the dissociating of neurons for in vitro studies. The organization of cultured neurons and their connections can be improved and restored by mimicking the natural circuitry in vivo conditions through microfluidic approaches [40]. With the help of microfluidic culture devices, two distinct micro-environments can be established, which may be maintained in fluidic isolation to allow for targeted investigation and treatment.

A compartmented kind of setup to co-culture a wide variety of cells is required to understand the mechanisms of a range of neurodegenerative diseases and model neuromuscular signaling [41][42]. The microfluidic devices fulfill all these requirements and mimic the unique anatomical and cellular interactions of this circuit [42][43]. 3D assay systems have been developed, human brain models allowing the measurement of action potential and velocity, monitoring cell growth, drug discovery, and study of neural–glial interactions and various neurotrophic factors [38][44]. Furthermore, microfluidic neuromuscular co-culture enables innervation by axons crossing from the neuronal to the muscle compartment [45]. The same setup can be used to decipher the impact of genetic alterations on the synaptic function of CNS disorders [46]. Therefore, microfluidics applied widely in various studies of disease, including neurodegeneration. Similarly, its impact on the research and development of AD is overwhelming and promising.

3. Impact of Microfluidic Tools in Alzheimer's Disease Research: Recent Developments

Advancements in microfluidic technology have played a significant role in accelerating the research dedicated to the field of AD, as with other diseases, in terms of both drug discovery, exploring novel drug targets, understanding the pathophysiology, or discovering novel biomarker-based diagnostics. A list of such initiatives has been provided in **Table 1** . Novel AD models, which are more helpful in mimicking the complex features of AD pathology, have started to replace the traditional models. The 3D culture platforms are more suitable for studying AD pathophysiological mechanisms involving cell–cell interactions, controlled flow dynamics, circulating blood cells, and a brain-specific microenvironment. In a study, distinct roles of Aβ on microglial accumulation have been elucidated by quantifying microglial responses in order to gain insights into the pathophysiological role of microglial migration [47].

Table 1. Details of microfluidic devices and their application in the AD research.

Cells/Peptide	Flow Control Device	Flow Surface	Active/Passive	Application	Reference(s)
Axon	NA	Glass	P	Study axonal function	[48]
Neural Progenitor Cell	Osmotic micropump	-	A	Study the neurotoxicity of amyloid beta	[49]
Neuron	Osmotic micropump	Glass	A & P	in vitro brain model, high-throughput drug screening	[29]
Brain Cells	Pneumatically-driven pumps	Polysulfone	P	To provide MPSs for in vitro drug discovery	[50]
Aβ42 Peptide	Precision pump	Glass	A	Aβ (1–42) detection	[51]
Aβ Peptide	Syringe	-	A	-	[52]
Axons	N/A	Glass	P	Study impaired axonal deficit	[38]
Axons	N/A	MEA	P	Investigate axonal signals in developmental stage	[53]
Neurites	Syringe	Glass	A	Study durotactic behavior of cells and neurite growth	[44]
Axons	Gravity/Hydrostatic pressure	PCB/Glass	P	Study axonal physiology and modeling CNS injury	[54]
Soma and Axon	N/A	Glass	P	Compartmentalizing the network structure into interconnected sub-populations	[55]
Hippocampal Neuronal/Glia Cells	Pressure gradient	Glass	P	Probing the functional synaptic connectivity between mixed primary hippocampal co-cultures	[46]
Dendrite	N/A	PDMS	NM	Investigate dendrite-to-nucleus signaling	[56]
Oligodendrocyte	N/A	Glass	P	-	[57]
Drg/Mc3t3-E1	N/A	Glass	NM	Mimicking the in vivo scenario to study the interaction between the peripheral nervous system and bone cells	[43]
Nmj	Pipette	Glass	N/A	Study subcellular microenvironments, NMJ formation, maintenance, and disruption	[45]
Axons	Pipette	Glass	P	Perform drug screening assays	[58]

Cells/Peptide	Flow Control Device	Flow Surface	Active/Passive	Application	Reference(s)
Dendrites and Somata	Syringe	Glass	A	Manipulate synaptic regions and presynaptic and postsynaptic compartments in vitro	[40]
Glial Cells/Motor Neurons	N/A	Glass	P	Study interactions with glial cells and other skeletal cells in the chamber	[42]
Astrocyte	N/A	acrylic plate	P	AD triculture model showing beta-amyloid aggregation, phosphorylated tau accumulation, and neuroinflammatory activity	[30]
Tau	N/A	Glass	P	Study effects of tau on mitochondrial transport	[59]
(Aβ) Peptides	N/A	Glass	P	Study effects of local Aβ stress on neuronal sub-compartments and networks	[60]
ADAM10	Syringe	N/A	A	ADAM10 biomarker detection in plasma and cerebrospinal fluid	[61]
Tau	N/A	Glass	P	Quantify AD-derived Tau propagation	[33]
Aβ	N/A	Glass	P	Study roles of Aβ on microglial accumulation	[62]
Aβ	Syringe	Overflow microfluidic networks	A	Study cell-to-cell communication, role of astrocytes derived from cortex and hippocampus on neuronal viability	[32]
Axons	-	Glass	-	Study mechanisms of indirect axonal excitotoxicity	[63]
Neurites	Hydrostatic pressure	Glass and Polystyrene	P	Grow neuronal culture	[28]
Cortical Neurons	Pressure difference	Glass	P	Synthesize experimental models emulating pathological states	[64]
Ren-WT/Ren-AD Cells	N/A	Glass	P	Grow 3D human neural cell culture, screen novel drugs capable of passing through the BBB to reach deeper neural tissues	[34]
Protein	N/A	Glass	P	Detect protein aggregation	[65]
Axons	Hydrostatic pressure	Glass or Polystyrene	P	Study localized axon-glia interaction and signaling	[66]
Axons	N/A	Glass	P	Examine axonal trauma in neuronal networks	[67]
Axons-glia	Hydrostatic pressure	Glass	P	Study axon-glia interactions	[68]
Neurites	Syringe	Glass	A	Investigating chemotaxis of neutrophils	[69]

Abbreviations: MPSs, Micro-physiological systems; DRG, Dorsal root ganglion; NMJ, Neuromuscular junction; MEA, Microelectrode arrays.

The emerging role of exosomes in the detection and study of AD has created the need for large-scale separation of exosomes, which is cumbersome and challenging with traditional techniques like ultra-centrifugation. Microfluidic devices are emerging as an ideal tool for exosome separation and have also started to gain recognition as excellent exosome detection tools [70]. These miniaturized platforms enable quick and cheap processing of nanovesicles even in the small

volumes of liquid samples. Several microfluidic chips based on 3D neuro spheroids have been developed to mimic in vivo brain microenvironment [29]. These kinds of 3D culture-based microfluidic chip provide in vivo microenvironments for high-throughput drug screening and allow the investigation of dendrite-to-nucleus signaling [56]. Synthetic models with AD features such as aggregation of Aβ, and accumulation of phosphorylated tau protein with neuroinflammatory activities have been produced to emulate pathological states. A triculture in vitro model comprising the combination of neurons, astrocytes, and microglia has evolved to address the physiological features and study the durotactic behavior of cells [71]. The human AD triculture model provides an opportunity to learn about microglial recruitment, neurotoxic activities, and astrocytes [71]. A co-culture system with segregated cell bodies, while simultaneously forming myelin sheaths, could also be obtained through a microfluidics approach [57].

These studies claim to reverse the demyelination of axons which can recover the loss of sensory and motor function with the help of co-cultures. The microfluidic devices allow the study of AD-derived tau propagation from neuron to neuron. Application of microfluidic cell culture must be undergone only upon testing the cell lines with the PDMS formulations, checking for leaching of toxic compounds, and examining that the medium composition is well adjusted to suit the device and cells. Microfluidic systems present a reliable method to mimic in vivo fluid conditions of neural tissues by generating gradients to allow the diffusion of two separate fluid phases at the interface [49]. The microfluidic technology facilitates understanding of the mechanism of Aβ under interstitial fluid flow conditions. These kinds of 3D culture-based microfluidic chips provide in vivo microenvironments for high-throughput drug screening [72][73]. These devices have also been used to isolate axons and the cell body to study the targets of excitotoxicity observed in neurodegeneration. In another study, the distal axon is the main target. These models can be widely used for basic mechanistic studies involved in the interaction between neural-glial cells and drug discovery. The microfluidic approach has also been used to grow a 3D human neural cell culture wherein a BBB-like phenotype was developed. The generation of such a phenotype helps in screening novel drugs capable of passing through the BBB to reach deeper neural tissues [34]. This technology facilitates the culturing of cortical neurons in two distinct cell compartments of the same microfluidic device to generate neuronal networks [64]. This setup can bring axonal degeneration in the distal axon chamber without degenerative changes in the untreated somal section [63]. Insults to the selective areas of neurons can be obtained without affecting other neurons by applying hydrostatic pressure [28].

Details of microfluidic devices and their application in the AD research.

4. Challenges in the Application of Microfluidics in the Alzheimer’s Disease Research

Although microfluidics provides a state-of-the-art facility that enables investigations in biomedical research, there are many challenges that need to be addressed before the optimal utilization of this field’s potential. Experts believe that the area of microfluidics research needs to grow further in order to outperform existing laboratory methods and overcome barriers that hinder researchers from adopting microfluidic-based devices as a common research tool.

First of all, the lack of precise fluid handling techniques at such a microscopic level poses great difficulty in attaining the exact quantity of reagents for performing molecular experiments. Though achieved once, it becomes difficult to replicate the experiments with acceptable accuracy. The second major problem is that it is difficult to scale up the experiments under the same experimental conditions with the same volume of reagents. This is because of inability in fluid handling and duplicating culture or reaction conditions. Often cells may respond differently toa change in the substrate of microfluidic devices. Thirdly, the majority of the culture protocols have been optimized on polystyrene culture plates, a significant component in macroscale devices, unlike microfluidic cell culture devices that use PDMS. New production techniques favoring mass production such as microfluidic hot embossing in polystyrene have been found useful in minimizing the risk of translation failure in microfluidic devices, yet PDMS is the most commonly used substrate for fabricating these devices [74].

Any variation in the reagent volume or reaction conditions leads to inaccurate results and protocols. Moreover, a direct comparison with the macroscale experiments become very difficult as a change in the substrate may hinder the transition of the protocols to the microscale levels. Studies indicate that PDMS may absorb or adsorb the biomolecules from the medium, causing biased experimental conditions [75][76]. Absorption and/or adsorption of reagents will alter the reaction volumes, which is another demerit that microfluidic devices currently face. In addition, we do not know whether PDMS, a material known for its transparency and gas permeability, has any impact on cellular behavior. Since it is the material of choice at present, ascertaining its effect on cellular behavior is essential.

The lack of a universal blood substitute or standard culture media that supports all types of tissue is an additional setback. Other drawbacks that must be addressed in the future for the optimal application of microfluidics in Alzheimer's research is its interdisciplinary nature, wherein standardized protocols are generally absent. A combined effort of engineers and molecular biologists is required to fabricate new device designs and carry out biologically relevant experiments [49]. As a range of cell lines are cultured in these devices, generalization in device designs is difficult.

References

1. Jha, N.K.; Jha, S.K.; Kar, R.; Nand, P.; Swati, K.; Goswami, V.K. Nuclear factor-kappa β as a therapeutic target for Alzheimer's disease. J. Neurochem. 2019, 150, 113–137.
2. Crimins, J.L.; Pooler, A.; Polydoro, M.; Luebke, J.I.; Spires-Jones, T.L. The Intersection of Amyloid Beta and Tau in Glutamatergic Synaptic Dysfunction and Collapse in Alzheimer's Disease. Ageing Res. Rev. 2013, 12, 757–763.
3. Kumar, P.; Jha, N.K.; Jha, S.K.; Ramani, K.; Ambasta, R.K. Tau phosphorylation, molecular chaperones, and ubiquitin E3 ligase: Clinical relevance in Alzheimer's disease. J. Alzheimers Dis. 2015, 43, 341–461.
4. Lee, J.C.; Kim, S.J.; Hong, S.; Kim, Y.S. Diagnosis of Alzheimer's Disease Utilizing Amyloid and Tau as Fluid Biomarkers. Exp. Mol. Med. 2019, 51, 1–10.
5. Jha, S.K.; Jha, N.K.; Kumar, D.; Sharma, R.; Shrivastava, A.; Ambasta, R.K.; Kumar, P. Stress-Induced Synaptic Dysfunction and Neurotransmitter Release in Alzheimer's Disease: Can Neurotransmitters and Neuromodulators be Potential Therapeutic Targets? J. Alzheimers Dis. 2017, 57, 1017–1039.
6. Jha, N.K.; Jha, S.K.; Kumar, D.; Kejriwal, N.; Sharma, R.; Ambasta, R.K.; Kumar, P. Impact of Insulin Degrading Enzyme and Neprilysin in Alzheimer's Disease Biology: Characterization of Putative Cognates for Therapeutic Applications. J. Alzheimers Dis. 2015, 48, 891–917.
7. Liu, C.-C.; Kanekiuo, T.; Xu, H.; Bu, G. Apolipoprotein E and Alzheimer Disease: Risk, Mechanisms and Therapy. Nat. Rev. Neurol. 2013, 9, 106–118.
8. D'Argenio, V.; Sarnataro, D. New Insights into the Molecular Bases of Familial Alzheimer's Disease. J. Pers. Med. 2020, 10, 26.
9. Lynch, C. World Alzheimer Report 2019: Attitudes to Dementia, a Global Survey. Alzheimers Dement. 2020, 16, e038255.
10. 2021 Alzheimer's Disease Facts and Figures. Alzheimers Dement. 2021, 17, 327–406.
11. International, D. World Alzheimer Report 2020–Design Dignity Dementia: Dementia-Related Design and the Built Environment. Alzheimers Dis. Int. 2020, 1, 106–113.
12. Holtzman, D.M.; Morris, J.C.; Goate, A.M. Alzheimer's Disease: The Challenge of the Second Century. Sci. Transl. Med. 2011, 3, 77.
13. Kocahan, S.; Doğan, Z. Mechanisms of Alzheimer's Disease Pathogenesis and Prevention: The Brain, Neural Pathology, N-Methyl-D-Aspartate Receptors, Tau Protein and Other Risk Factors. Clin. Psychopharmacol. Neurosci. 2017, 15, 1–8.
14. Yiannopoulou, K.G.; Papageorgiou, S.G. Current and Future Treatments for Alzheimer's Disease. Ther. Adv. Neurol. Disord. 2013, 6, 19.
15. Guo, T.; Zhang, D.; Zeng, Y.; Huang, T.Y.; Xu, H.; Zhao, Y. Molecular and Cellular Mechanisms Underlying the Pathogenesis of Alzheimer's Disease. Mol. Neurodegener. 2020, 15, 1–37.
16. Strassnig, M.; Ganguli, M. About a Peculiar Disease of the Cerebral Cortex: Alzheimer's Original Case Revisited. Psychiatry 2005, 2, 30–33.
17. You, Y.; Perkins, A.; Cisternas, P.; Muñoz, B.; Taylor, X.; You, Y.; Garringer, H.J.; Oblak, A.L.; Atwood, B.K.; Vidal, R.; et al. Tau as a Mediator of Neurotoxicity Associated to Cerebral Amyloid Angiopathy. Acta Neuropathol. Commun. 2019, 7, 26.
18. Huang, H.C.; Jiang, Z.F. Accumulated Amyloid-β Peptide and Hyperphosphorylated Tau Protein: Relationship and Links in Alzheimer's Disease. J. Alzheimers Dis. 2009, 16, 15–27.
19. Li, Y.; Li, D.; Zhao, P.; Nandakumar, K.; Wang, L.; Song, Y. Microfluidics-Based Systems in Diagnosis of Alzheimer's Disease and Biomimetic Modeling. Micromachines 2020, 11, 787.
20. Velve-Casquillas, G.; Le Berre, M.; Piel, M.; Tran, P.T. Microfluidic Tools for Cell Biological Research. Nano Today 2010, 5, 28–47.

21. Edmondson, R.; Broglie, J.J.; Adcock, A.F.; Yang, L. Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors. ASSAY Drug Dev. Technol. 2014, 12, 207–218.

22. Lam, D.; Enright, H.A.; Cadena, J.; Peters, S.K.; Sales, A.P.; Osburn, J.J.; Soscia, D.A.; Kulp, K.S.; Wheeler, E.K.; Fischer, N.O. Tissue-specific extracellular matrix accelerates the formation of neural networks and communities in a neuron-glia co-culture on a multi-electrode array. Sci. Rep. 2019, 9, 1–5.

23. Perestrelo, A.R.; Águas, A.C.P.; Rainer, A.; Forte, G. Microfluidic Organ/Body-on-a-Chip Devices at the Convergence of Biology and Microengineering. Sensors 2015, 15, 31142–31170.

24. Haring, A.P.; Sontheimer, H.; Johnson, B.N. Microphysiological Human Brain and Neural Systems-on-a-Chip: Potential Alternatives to Small Animal Models and Emerging Platforms for Drug Discovery and Personalized Medicine. Stem Cell Rev. Rep. 2017, 13, 381–406.

25. Zheng, F.; Fu, F.; Cheng, Y.; Wang, C.; Zhao, Y.; Gu, Z. Organ-on-a-Chip Systems: Microengineering to Biomimic Living Systems. Small 2016, 12, 2253–2282.

26. Osaki, T.; Shin, Y.; Sivathanu, V.; Campisi, M.; Kamm, R.D. In vitro Microfluidic Models for Neurodegenerative Disorders. Adv. Healthc. Mater. 2018, 7, 1700489.

27. Kane, K.I.W.; Jarazo, J.; Moreno, E.L.; Fleming, R.M.T.; Schwamborn, J.C. Passive Controlled Flow for Parkinson's Disease Neuronal Cell Culture in 3D Microfluidic Devices. Org. Chip 2020, 2, 100005.

28. Taylor, A.M.; Rhee, S.W.; Tu, C.H.; Cribbs, D.H.; Cotman, C.W.; Jeon, N.L. Microfluidic Multicompartment Device for Neuroscience Research. Langmuir 2003, 19, 1551–1556.

29. Park, J.; Lee, B.K.; Jeong, G.S.; Hyun, J.K.; Lee, C.J.; Lee, S.H. Three-Dimensional Brain-on-a-Chip with an Interstitial Level of Flow and Its Application as an in vitro Model of Alzheimer's Disease. Lab Chip 2015, 15, 141–150.

30. Park, J.; Wetzel, I.; Marriott, I.; Dréau, D.; D'Avanzo, C.; Kim, D.Y.; Tanzi, R.E.; Cho, H. A 3D Human Triculture System Modeling Neurodegeneration and Neuroinflammation in Alzheimer's Disease. Nat. Neurosci. 2018, 21, 941–951.

31. Teixeira, M.I.; Amaral, M.H.; Costa, P.C.; Lopes, C.M.; Lamprou, D.A. Recent Developments in Microfluidic Technologies for Central Nervous System Targeted Studies. Pharmaceutics 2020, 12, 1–37.

32. McDonald, J.C.; Whitesides, G.M. Poly(dimethylsiloxane) as a material for fabricating microfluidic devices. Acc. Chem. Res. 2002, 35, 491–499.

33. Katsikoudi, A.; Ficulle, E.; Cavallini, A.; Sharman, G.; Guyot, A.; Zagnoni, M.; Eastwood, B.J.; Hutton, M.; Bose, S. Quantitative Propagation of Assembled Human Tau from Alzheimer's Disease Brain in Microfluidic Neuronal Cultures. J. Biol. Chem. 2020, 295, 13079–13093.

34. Shin, Y.; Choi, S.H.; Kim, E.; Bylykbashi, E.; Kim, J.A.; Chung, S.; Kim, D.Y.; Kamm, R.D.; Tanzi, R.E. Blood–Brain Barrier Dysfunction in a 3D In vitro Model of Alzheimer's Disease. Adv. Sci. 2019, 6, 1900962.

35. Gupta, N.; Liu, J.R.; Patel, B.; Solomon, D.E.; Vaidya, B.; Gupta, V. Microfluidics-based 3D Cell Culture Models: Utility in Novel Drug Discovery and Delivery Research. Bioeng. Transl. Med. 2016, 1, 63–81.

36. Natarajan, A.; Sethumadhavan, A.; Krishnan, U.M. Toward Building the Neuromuscular Junction: In vitro Models to Study Synaptogenesis and Neurodegeneration. ACS Omega 2019, 4, 12969–12977.

37. Breslin, S.; O'Driscoll, L. Three-dimensional cell culture: The missing link in drug discovery. Drug Discov. Today 2013, 18, 240–249.

38. Poon, W.W.; Blurton-Jones, M.; Tu, C.H.; Feinberg, L.M.; Chabrier, M.A.; Harris, J.W.; Jeon, N.L.; Cotman, C.W. β-Amyloid Impairs Axonal BDNF Retrograde Trafficking. Neurobiol. Aging 2011, 32, 821–833.

39. Jackson, E.L.; Lu, H. Three-Dimensional Models for Studying Development and Disease: Moving on from Organisms to Organs-on-a-Chip and Organoids. Integr. Biol. 2016, 8, 672–683.

40. Taylor, A.M.; Dieterich, D.C.; Ito, H.T.; Kim, S.A.; Schuman, E.M. Microfluidic Local Perfusion Chambers for the Visualization and Manipulation of Synapses. Neuron 2010, 66, 57–68.

41. Ren, Y.; Kunze, A.; Renaud, P. Compartmentalized microfluidics for in vitro Alzheimer's disease studies. Microfluidic and Compartmentalized Platforms for Neurobiological Research. Neuromethods 2015, 103, 197–215.

42. Southam, K.A.; King, A.E.; Blizzard, C.A.; McCormack, G.H.; Dickson, T.C. Microfluidic Primary Culture Model of the Lower Motor Neuron-Neuromuscular Junction Circuit. J. Neurosci. Methods 2013, 218, 164–169.

43. Neto, E.; Alves, C.J.; Sousa, D.M.; Alencastre, I.S.; Lourenço, A.H.; Leitão, L.; Ryu, H.R.; Jeon, N.L.; Fernandes, R.; Aguiar, P.; et al. Sensory Neurons and Osteoblasts: Close Partners in a Microfluidic Platform. Integr. Biol. 2014, 6, 586–595.

44. Sundararaghavan, H.G.; Monteiro, G.A.; Firestein, B.L.; Shreiber, D.I. Neurite Growth in 3D Collagen Gels with Gradients of Mechanical Properties. Biotechnol. Bioeng. 2009, 102, 632–643.

45. Ionescu, A.; Zahavi, E.E.; Gradus, T.; Ben-Yaakov, K.; Perlson, E. Compartmental Microfluidic System for Studying Muscle-Neuron Communication and Neuromuscular Junction Maintenance. Eur. J. Cell Biol. 2016, 95, 69–88.

46. Robertson, G.; Bushell, T.J.; Zagnoni, M. Chemically Induced Synaptic Activity between Mixed Primary Hippocampal Co-Cultures in a Microfluidic System. Integr. Biol. 2014, 6, 636–644.

47. Griciuc, A.; Serrano-Pozo, A.; Parrado, A.R.; Lesinski, A.N.; Asselin, C.N.; Mullin, K.; Hooli, B.; Choi, S.H.; Hyman, B.T.; Tanzi, R.E. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 2013, 78, 631–643.

48. Tsantoulas, C.; Farmer, C.; Machado, P.; Baba, K.; McMahon, S.B.; Raouf, R. Probing Functional Properties of Nociceptive Axons Using a Microfluidic Culture System. PLoS ONE 2013, 8, 80722.

49. Choi, Y.J.; Chae, S.; Kim, J.H.; Barald, K.F.; Park, J.Y.; Lee, S.H. Neurotoxic Amyloid Beta Oligomeric Assemblies Recreated in Microfluidic Platform with Interstitial Level of Slow Flow. Sci. Rep. 2013, 3, 1921.

50. Edington, C.D.; Chen, W.L.K.; Geishecker, E.; Kassis, T.; Soenksen, L.R.; Bhushan, B.M.; Freake, D.; Kirschner, J.; Maass, C.; Tsamandouras, N.; et al. Interconnected Microphysiological Systems for Quantitative Biology and Pharmacology Studies. Sci. Rep. 2018, 8, 4530.

51. Islam, K.; Jang, Y.C.; Chand, R.; Jha, S.K.; Lee, H.H.; Kim, Y.S. Microfluidic Biosensor for β-Amyloid(1-42) Detection Using Cyclic Voltammetry. J. Nanosci. Nanotechnol. 2011, 11, 5657–5662.

52. Lee, J.S.; Park, C.B. Microfluidic Dissociation and Clearance of Alzheimer's β-Amyloid Aggregates. Biomaterials 2010, 31, 6789–6795.

53. Hong, N.; Joo, S.; Nam, Y. Characterization of Axonal Spikes in Cultured Neuronal Networks Using Microelectrode Arrays and Microchannel Devices. IEEE Trans. Biomed. Eng. 2017, 64, 492–498.

54. Lewandowska, M.K.; Bakkum, D.J.; Rompani, S.B.; Hierlemann, A. Recording Large Extracellular Spikes in Microchannels along Many Axonal Sites from Individual Neurons. PLoS ONE 2015, 10.

55. Habibey, R.; Latifi, S.; Mousavi, H.; Pesce, M.; Arab-Tehrany, E.; Blau, A. A Multielectrode Array Microchannel Platform Reveals Both Transient and Slow Changes in Axonal Conduction Velocity. Sci. Rep. 2017, 7, 8558.

56. Cohen, M.S.; Orth, C.B.; Kim, H.J.; Jeon, N.L.; Jaffrey, S.R. Neurotrophin-Mediated Dendrite-to-Nucleus Signaling Revealed by Microfluidic Compartmentalization of Dendrites. Proc. Natl. Acad. Sci. USA 2011, 108, 11246–11251.

57. Yang, I.H.; Gary, D.; Malone, M.; Dria, S.; Houdayer, T.; Belegu, V.; McDonald, J.W.; Thakor, N. Axon Myelination and Electrical Stimulation in a Microfluidic, Compartmentalized Cell Culture Platform. NeuroMolecular Med. 2012, 14, 112–118.

58. Dworak, B.J.; Wheeler, B.C. Novel MEA Platform with PDMS Microtunnels Enables the Detection of Action Potential Propagation from Isolated Axons in Culture. Lab Chip 2009, 9, 404–410.

59. Stoothoff, W.; Jones, P.B.; Spires-Jones, T.L.; Joyner, D.; Chhabra, E.; Bercury, K.; Fan, Z.; Xie, H.; Bacskai, B.; Edd, J.; et al. Differential Effect of Three-Repeat and Four-Repeat Tau on Mitochondrial Axonal Transport. J. Neurochem. 2009, 111, 417–427.

60. Deleglise, B.; Magnifico, S.; Duplus, E.; Vaur, P.; Soubeyre, V.; Belle, M.; Vignes, M.; Viovy, J.L.; Jacotot, E.; Peyrin, J.M.; et al. β-Amyloid Induces a Dying-Back Process and Remote Trans-Synaptic Alterations in a Microfluidic-Based Reconstructed Neuronal Network. Acta Neuropathol. Commun. 2014, 2, 145.

61. De Oliveira, T.R.; Erbereli, C.R.; Manzine, P.R.; Magalhães, T.N.C.; Balthazar, M.L.F.; Cominetti, M.R.; Faria, R.C. Early Diagnosis of Alzheimer's Disease in Blood Using a Disposable Electrochemical Microfluidic Platform. ACS Sens. 2020, 5, 1010–1019.

62. Cho, H.; Hashimoto, T.; Wong, E.; Hori, Y.; Wood, L.B.; Zhao, L.; Haigis, K.M.; Hyman, B.T.; Irimia, D. Microfluidic Chemotaxis Platform for Differentiating the Roles of Soluble and Bound Amyloid-β on Microglial Accumulation. Sci. Rep. 2013, 3, 1823.

63. Hosie, K.A.; King, A.E.; Blizzard, C.A.; Vickers, J.C.; Dickson, T.C. Chronic Excitotoxin-Induced Axon Degeneration in a Compartmented Neuronal Culture Model. ASN Neuro 2012, 4, 47–57.

64. Kunze, A.; Meissner, R.; Brando, S.; Renaud, P. Co-Pathological Connected Primary Neurons in a Microfluidic Device for Alzheimer Studies. Biotechnol. Bioeng. 2011, 108, 2241–2245.

65. Choi, I.; Huh, Y.S.; Erickson, D. Size-Selective Concentration and Label-Free Characterization of Protein Aggregates Using a Raman Active Nanofluidic Device. Lab Chip 2011, 11, 632–638.

66. Park, J.; Koito, H.; Li, J.; Han, A. Microfluidic Compartmentalized Co-Culture Platform for CNS Axon Myelination Research. Biomed. Microdevices 2009, 11, 1145–1153.

67. Deleglise, B.; Lassus, B.; Soubeyre, V.; Alleaume-Butaux, A.; Hjorth, J.J.; Vignes, M.; Schneider, B.; Brugg, B.; Viovy, J.L.; Peyrin, J.M. Synapto-Protective Drugs Evaluation in Reconstructed Neuronal Network. PLoS ONE 2013, 8, e71103.
68. Hosmane, S.; Yang, I.H.; Ruffin, A.; Thakor, N.; Venkatesan, A. Circular Compartmentalized Microfluidic Platform: Study of Axon-Glia Interactions. Lab Chip 2010, 10, 741–747.
69. Taylor, A.M.; Rhee, S.W.; Jeon, N.L. Microfluidic Chambers for Cell Migration and Neuroscience Research. Methods Mol. Biol. 2006, 321, 167–177.
70. Yang, F.; Liao, X.; Tian, Y.; Li, G. Exosome Separation Using Microfluidic Systems: Size-Based, Immunoaffinity-Based and Dynamic Methodologies. Biotechnol. J. 2017, 12, 12.
71. Jorfi, M.; D'Avanzo, C.; Kim, D.Y.; Irimia, D. Three-Dimensional Models of the Human Brain Development and Diseases. Adv. Healthc. Mater. 2018, 7, 1002.
72. Dittrich, P.S.; Manz, A. Lab-on-a-Chip: Microfluidics in Drug Discovery. Nat. Rev. Drug Discov. 2006, 5, 210–218.
73. Esch, E.W.; Bahinski, A.; Huh, D. Organs-on-Chips at the Frontiers of Drug Discovery. Nat. Rev. Drug Discov. 2015, 14, 248–260.
74. Goral, V.; Hsieh, Y.-C.; Petzold, O.; Faris, R.; Yuen, P.K. Hot Embossing of Plastic Microfluidic Devices Using Poly(Dimethylsiloxane) Molds. J. Micromech. Microeng. 2011, 21, 017002.
75. Chumbimuni-Torres, K.Y.; Coronado, R.E.; Mfuh, A.M.; Castro-Guerrero, C.; Silva, M.F.; Negrete, G.R.; Bizios, R.; Garcia, C.D. Adsorption of Proteins to Thin-Films of PDMS and Its Effect on the Adhesion of Human Endothelial Cells. RSC Adv. 2011, 1, 706–714.
76. Gomez-Sjoberg, R.; Leyrat, A.; Houseman, B.; Shokat, K.; Quake, S. Biocompatibility and Reduced Drug Absorption of Sol-Gel-Treated Poly(Dimethyl Siloxane) for Microfluidic Cell Culture Applications. Anal. Chem. 2010, 82, 8954–8960.