Microfluidic platforms to unravel mysteries of Alzheimer's Disease: How far have we come?

Pragya Prasanna[1*], Shweta Rathee[2], V Rahul[3], M.S. Chandra[1], Niraj Kumar Jha[4], Chiara Villa[5], Saurabh Kumar Jha[4**]

[1]School of Applied Sciences, KK University, Nalanda, Bihar-803115, India

[2]Department of Food Science and Technology, National Institute of Food Technology, Entrepreurship and Management, Sonipat, Haryana-131028, India
[3]Department of Mechanical Engineering, National Institute of Technology, Rourkela-Odisha 769008, India
[4]Department of Biotechnology, Sharda University, Greater Noida, Uttar Pradesh 201310, India
[5]School of Medicine and Surgery, University of Milano-Bicocca, 20900 Monza, Italy

*Corresponding Author
Pragya Prasanna
Assistant Professor
School of Applied Sciences
KK University, Nalanda, Bihar-803115
Ph. No. +91 7991148771
E-mail: pragyaprasanna2019@gmail.com

** Co-Corresponding Author
Dr.Saurabh Kumar Jha
Assistant Professor
Department of Biotechnology
Sharda University
Greater Noida, Uttar Pradesh 201310
Ph. No. +91 7827895545
E-mail: Saurabh.jha@sharda.ac.in, jhasaurabh017@gmail.com
Email addresses: pragyaprasanna36@gmail.com (P. Prasanna), vrahul1803@gmail.com (V. Rahul), mscgoud1234@gmail.com (M.S. Chandra), (niraj.jha@sharda.ac.in) Niraj Kumar Jha, (Saurabh Kumar Jha) Saurabh.jha@sharda.ac.in, chiara.villa@unimib.it (Chiara Villa)

Abstract

Alzheimer's Disease (AD) is a significant health concern worldwide with enormous social and economic impact globally. The gradual deterioration of cognitive functions and irreversible neuronal losses are primary etiological features of the disease. Even after decades of research, most therapeutic options are merely symptomatic, and drugs in clinical practice present numerous side effects. Lack of effective diagnostic techniques prevents the early prognosis of disease, resulting in a gradual deterioration in the quality of life. Furthermore, the mechanism of cognitive impairment and AD pathophysiology is poorly understood. Microfluidics exploits different microscale properties of fluids to mimic environments on microfluidic chip-like devices. These miniature multichambered devices can be used to grow cells and 3D tissues *in vitro*, analyze cell-to-cell communication, decipher the roles of neural cells like microglia, and gain insights into AD pathophysiology. This review focuses on the applications and

impact of microfluidics on AD research. We discuss the technical challenges and possible solutions provided by this new cutting-edge technique to understand disease-associated pathways and mechanisms.

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 both young and older populations [1]. It is characterized by the accumulation and formation of amyloid-beta 42 (Aβ42) and phosphorylated tau, along with excessive activation of glial cells [2–4]. Impaired synaptic functions and insufficient neurotrophin signaling are essential characteristics of AD [5]. Primary symptoms of the disease comprise memory loss, apathy, depression, irritability, etc. [6]. The 'World Alzheimer Report 2019' shows that AD accounts for more than 70% of the total dementia cases diagnosed worldwide [7,8]. Early diagnosis of AD is crucial for disease management as the degeneration of brain tissues is irreversible and may result in the loss of cognitive and memory functions, progressively worsening over time [9].

Despite numerous studies, the etiology, pathophysiology, and mechanisms of cognitive impairments and synaptic dysfunctions are not well characterized [10,11]. Besides, available therapeutic options are merely symptomatic, with several side-effects such as confusion, dizziness, depression, constipation, and diarrhea reported in most medications [12]. Therefore, a more in-depth understanding of the molecular mechanisms underlying AD pathogenesis, revisiting numerous existing concepts, and effective screening for therapies aimed at impeding or preventing neurodegeneration in AD is required [13,14]. The lack of relevant experimental models has also presented a bottleneck in understanding the AD pathological mechanism. Moreover, the widely accepted notion such as the deposition of Aβ and hyperphosphorylation of microtubular protein tau also lacks a direct correlation between the deposition or phosphorylation with the disease progression [15,16].

In recent years, microfluidics is emerging as an economical and versatile platform for biologists to mimic and control cellular microenvironment for the modeling of diseases, study cell behavior from single- to multi-cellular organism level, develop multiple experiments in miniaturized devices suitable for diagnostics, biomedical analysis, pathological studies of neural degeneration and drug developments [17,18]. 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 microfluidic technology widely used in neurodegenerative diseases to gradually minimize biomedical research dependence on the *in vivo* models [19]. These platforms have been plentily implicated in growing 3D gels that could be further applied in producing a three-dimensional tissue representative of the human organs. With the help of these miniatured devices, the growth of neurons, astrocytes, and microglia have also been

facilitated in the form of triculture models [20]. This review describes the latest advances in the progress of microfluidic 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 discussed the possible challenges that this field needs to overcome and directions that need to be taken before realizing its full-fledged application in AD research.

2. Revisiting Alzheimer's Disease: What is known?

(i) History

AD is a neurodegenerative disease that develops through the progressive deterioration of cognitive functions of individuals due to irreversible neuronal losses and brain atrophy, primarily in the cortex and hippocampus regions culminating in a gradual reduction in the quality of life [6,21]. It was first diagnosed by a German psychiatrist and neuropathologist, Alois Alzheimer, in 1906 [14]. However, after 1907, the behavioral and physiological changes in AD and naturally occurring senility and dementia were differentiated [22]. The symptoms like a failure of storage and retrieval of memory, confusion, poor judgment, etc., have been categorized as characteristic features of AD. Other symptoms include language disturbance, agitation, withdrawal, and hallucinations followed by occasional seizures, increased muscle tone, and mutism[1,7,8,10]. Even after decades of research, the social and economic impact of the disease has not decreased yet, and the projections of the World Alzheimer Report 2019 predict more than 150 million cases by 2050 [7,8,10]. With more remarkable advances in science and technology, increased understanding of the disease's pathophysiology and causes has led to improved diagnosis and treatment. However, several aspects of the complex pathophysiology of the disease have remained unexplored [11,23].

(ii) Causes

Several hypotheses have been proposed to define the etiology of AD based on observed clinical, neuropathological features; for example, cholinergic hypothesis, amyloid cascade hypothesis, tau propagation hypothesis, etc. [24]. Some other potential hallmarks of Alzheimer's dementia are mitochondrial dysfunction, calcium deregulation, neurovascular disintegration, neuroinflammation, metal ion dyshomeostasis, defective lymphatic system, etc. [7,25–27]. However, the most well-known and defining features representing AD are amyloid-beta (Aβ) accumulation, phosphorylated tau aggregation, and neuroinflammation [1,28,29]. The amyloid cascade hypothesis postulates that amyloid precursor protein (APP) metabolism and Aβ42 accumulation are the most important triggering factors for AD pathogenesis [30]. This hypothesis holds the accumulation of Aβ peptide responsible for the eventual loss of synapses and neuronal cell death [3,27]. An increasing body of evidence supports toxic Aβ as the primary cause of pathology, which can initiate neuronal dysfunction by inducing granulovacuolar degeneration, astrocytosis, microgliosis, deficient endosomal transport when deposited extracellularly [31].

Tau hypothesis correlates AD pathology with the hyperphosphorylation and intracellular deposition of neurofibrillary tangles of microtubular protein tau [15]. It

further elucidates that the propagation of the pathological form of tau protein from one neuron to another may drive the disease aggressively. Few studies linking both the above hypotheses highlight that aggregation of amyloid plaques leads to the activation of various kinases, causing hyperphosphorylation of the tau protein [16]. The hyperphosphorylated tau proteins polymerize into insoluble neurofibrillary tangles, which might be an essential factor contributing the AD pathogenesis [3,15,16]. The deposition of plaques and neurofibrillary tangles ultimately recruits glial cells aggravating aggressive inflammatory changes via cytokine secretion, e.g., IL-1, IL-6, and TNF-α, etc., leading to neuronal degeneration [32–35]. The neuroinflammation and dysfunction of the blood-brain barrier (BBB) resulting from enhanced permeability and reduced expression of tight junction proteins due to increased production of Aβ, overexpression of MMP-2/-9, and apolipoprotein E (ApoE) independently are also often linked with AD pathogenesis [36–40].

(iii) Therapeutic Solutions

The gold standard for diagnosing the disease remains clinical neuropathologic assessment of extracellular Aβ plaques and intraneuronal neurofibrillary tangles [41]. Although the treatment is mainly supportive with symptoms managed on an individual basis, some of the therapeutic options approved for AD from the FDA include the cholinesterase inhibitors such as donepezil, rivastigmine, galantamine (reversible AChEIs), and memantine (NMDA inhibitor) [12,42–45]. However, the effectiveness of these drugs is often questioned for their efficacy [46]. Aβ42 peptides and tau proteins found in cerebrospinal fluid as soluble moieties causing synaptic dysfunction upon assembly are considered very sensitive and specific biological markers [4]. Different reliable biomarkers comprising several signaling proteins in blood plasma have also been discovered that can detect Alzheimer's with approximately 90% accuracy even in patients with a mild cognitive impairment which may later progress to AD [47]. A similar study with serum proteins, including A Disintegrin And Metalloprotease 10 (ADAM10), also retained diagnostic accuracy for the early diagnosis of AD [48]. Several blood-based microRNAs (Let-7b and microRNA-206) have also been found to have a strong correlation with cognitive decline and have been used as the predictive biomarkers for AD [49].

### 3. Mysteries of the Alzheimer's disease research

There are long-standing differences in opinion regarding the roles of soluble Aβ fibrils and tau tangles in ameliorating neurotoxicity, inflammation, and AD initiation. The molecular mechanisms and sequence of events in disease progression are still elusive and need detailed omics approaches to elucidate systemwide changes. Due to the overrated role of amyloids in AD pathology, immunization against Aβ was presumed to be an effective strategy, which unfortunately failed to deliver expected outcomes in the clinical trials [29,50]. In subsequent studies, failure to reverse the AD pathology following Aβ42 targeting or delaying plaque formation led researchers to believe that Aβ42 deposition is not the sole reason for AD pathogenesis [51]. Nonetheless, this observation and other findings, like genetic mutations in presenilin-1/-2 and abnormal APP processing in AD, emphasized a significant shift in the focus towards alternative theories [52,53].

The believers of tauopathy also have found challenges in establishing the correlation between the biochemical observations of tau tangles and the clinical progression of the disease in the patients [54]. The specific tau species involved in

neurotoxicity are ambiguous and arduous to decipher by the results obtained in the macroscopic experimental setup [55]. Recent evidence indicates that it is not only the amyloid plaques but also the intermediate amyloidic species and oligomeric assemblies that are neurotoxic and may exaggerate the disease pathology [56,57]. The major drawback experienced in the current laboratory practices is that it is incapable of assessing these deleterious oligomeric assemblies due to the problems associated with its separation from the Aβ. Furthermore, designing a successful Aβ or tau vaccine to target the neuropathologic features of AD requires the knowledge of the most toxic target epitope, which is difficult to achieve in the present experimental apparatus. [50,58]. It is believed that the tau species could be a potential biomarker for AD, but the relevance and clinical success are still far-fetched due to insufficient understanding of mechanisms. These drawbacks call for novel technologies for prospective solutions.

A platform is needed to study neuronal cell death within the brain through simultaneous observation of neuronal connectivity and tau pathology [59]. Additionally, for investigating genetic risk factors like PSEN1 or ApoE, the mechanism of receptor-mediated transport of Aβ and the role of interstitial fluid in regulating the metabolism of Aβ in vitro models need to be determined. However, the 2D culture systems, in general, lack the mobility of the cultured cells. Furthermore, the discrepancies in the results of in vitro culture systems and animal models in drug discovery cannot be underestimated. Therefore, an urgent need to develop an alternative culture system providing such properties could lead to a better understanding for analyzing the more excellent picture and study mechanisms of AD pathogenesis[60]. Moreover, the absence of validated biomarkers, probably due to the inconsistent results produced due to analytical hindrances such as epitope masking and lack of reproducibility, prevents early detection of disease symptoms and poses additional challenges [61,62].

Activation of astrocytes leads to an exacerbated immune response causing neuronal damage and degeneration [63]. Contemporary experimental approaches involve mutant or transgenic animals with disease pathology leading to immense animal mortality [64]. 3D culture techniques and a superior in vitro microfluidics system would provide a more physiological extracellular environment than traditional 2D cultures for examining neuron-glia interactions minimizing animal morbidity and mortality [65,66]. Experts believe that 3D cell cultures and microfluidic lab-on-a-chip technologies can explore these aspects [67]. Thus, though in its nascent form, this technology holds great promise in accelerating the pace of the ongoing research dedicated to AD.   A summary of all the events that aggravate the conditions to Alzheimer's disease are diagrammatically represented in **Figure 1.**
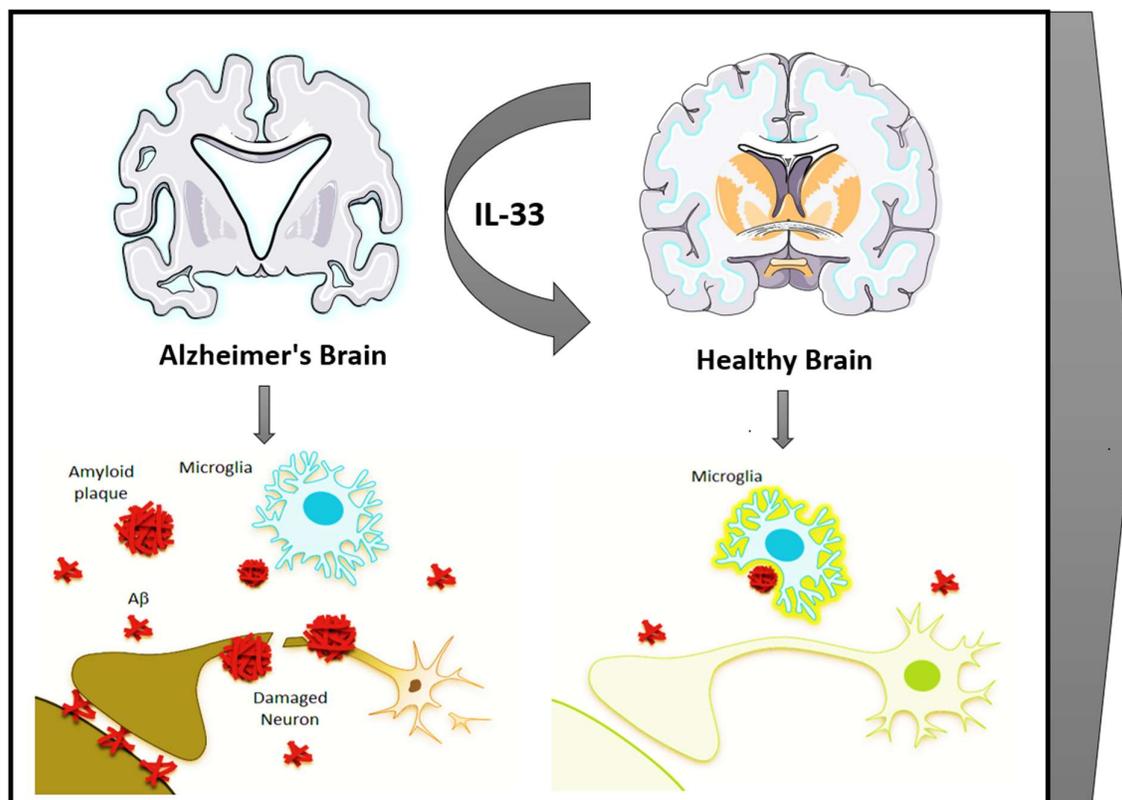
Recently, exosomes have gained considerable attention both as a drug delivery system and a significant biomarker for diagnostics by offering prognostic information [68,69]. These small membrane-bound extracellular vesicles are ubiquitously released from eukaryotic cells to carry and deliver proteins, lipids, and nucleic acids, to the target cells [68]. Though most studies substantiate the benefits of exosomes in the clearance of proteotoxic burden by transferring neuroprotective substances between neural cells, recent findings revealed that exosomes are also involved in the transportation of protein aggregates involved in different neurodegenerative diseases [68,70]. Furthermore, these loaded moieties play a crucial role in AD pathology by spreading Aβ and hyperphosphorylated tau, inducing oxidative/proteotoxic stresses, neuroinflammation, and neuronal loss [69–71]. Since exosomes may prove to be a significant biomarker, better techniques are required to isolate exosomes at a large scale and perform experiments at a co-culture level. However, for the successful implication of these nanovesicles in the domain of exosomes, extensive research is required to ascertain the probable route of administration, safety aspects for clinical application.

4. Microfluidics: An overview and its biological applications

The interdisciplinary field of microfluidics derived from molecular biology, molecular analysis, and microelectronics emerged in the late 1980s [72]. A timeline of the development of microfluidics from the physical and chemical innovations to its application in biological research has been provided in **Figure 2.** The emergence of this field began after discovering physical techniques like photolithography and soft-lithography, later used for the fabrication of chips, and are still evolving with further technological advancements.   The emergence of fabrication techniques facilitated

the design and fabrication of chip-like 3D structures from solid substrates such as glass, silica, thermoplastics, etc. [72–75]. The first microfluidic devices or chips were made of silicon and glass. Still, due to their brittle nature, low gas permeability, and costly fabrication methods, they have never considered an attractive option in microfluidics. Investigating alternative materials that could be optically transparent, easy to process, flexible, and comparatively cheap resulted in discovering several materials, which were examined to date for making the microfluidic devices **Table 1**.
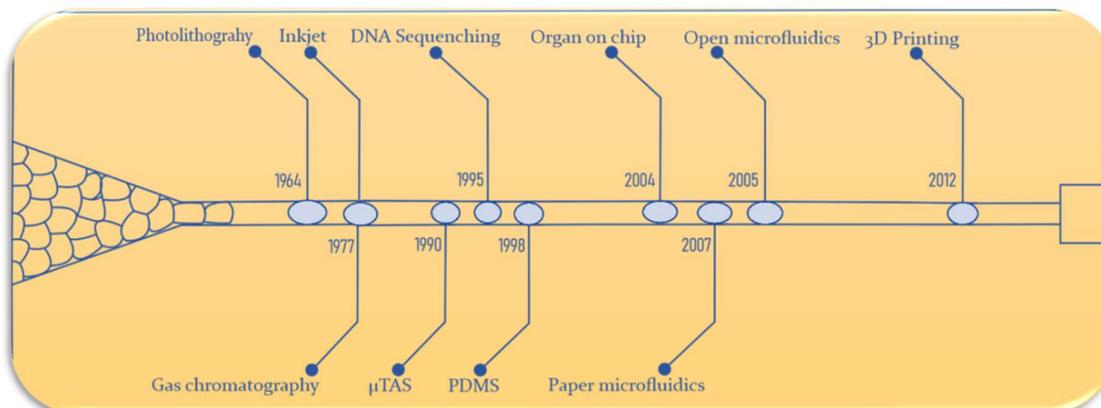
For the materials used in designing microfluidic devices, polydimethylsiloxane (PDMS), an elastomer introduced in the 1990s, is a material of choice for cell co-cultures [75–78]. As PDMS is compatible with cells, the microfluidic devices made of it started to be used for cell biology applications and studies of co-cultures [74,79]. Technology is usually characterized as an engineering subject. Still, the implementation of the proof-of-concept experiments of the domain of microfluidics serves biologists and clinicians to enhance their capabilities in their everyday research. This technology allows the manipulation of small fluid volumes in a fabricated microscale system and has emerged as an excellent tool in modern biology. These microscale, multichambered tiny devices can grow cells and 3D tissues for biology research [18]. It has enabled us to recreate experimental conditions at microscopic levels. It allows manipulation of biological specimens and cells at extraordinary spatiotemporal resolution and reveals the otherwise hidden mechanistic insights leading to a range of biological applications [80]. Properties like rapid sample processing and precise control of fluids in microfluidic technologies have presented an attractive way to replace traditional experimental approaches. The microlitre volumes of reagents mobile in laminar flow matches with the biological microenvironments. Multiple diverse biochemical assays can be performed in a small volume, and the flow control feature at the micron level allows to improve over the traditional macroscale assays. It is widely used in the imaging, bioinformatics, and molecular biology approaches [81,82]. Integration of fluid handling and signal detection features in microfluidics has allowed us to design cheaper yet sensitive point-of-care assay devices for different infectious diseases like Cancer, AIDS, malaria, SARS, dengue, etc. [83–85]. Even paper-based microfluidics as DNA diagnostics are being developed in recent years, which are low-cost, multiplexed diagnostics [86].

Table 1: Properties of materials used in microfluidic chips

Properties	Inorganic Materials	Elastomers	Thermoset	Thermoplastics	Hydrogel	Paper
Examples	Silicon/ Glass	PDMS	Polyester	Polyethylene, Polystyrene Polycarbonate Polyurethane, Teflon	Hyaluronic Acid, Agarose, PEG-DA, Alginate, PMMA And Chitosan	-
Biological Use	Drug Screening, Assays	Assays, Cell Culture	Capillary	Electrophoresis, DNA Sequencing, PCR	Study Cell-Cell and Cell-Matrix Interaction	Diagnostics
Young's Modulus	130-180/50-90	~0.0005	2.0-2.7	1.4-4.1	Low	0.0003-0.0025
Fabrication Technique	Photolithography	Casting, 3D Printing	Casting/ Photopolymerization	Thermomoulding	Casting/Photopolymerization	Photolithography, Printing
Valving	No	Yes	No	No	Yes	Yes
Channel Dimension/ Profile	<100nm/ 3D	<1μm/ 3D	<100nm/ Arbitrary 3D	~100nm/ 3D	~10μm/ 3D	~200μm/ 2D
Thermostability	Very High	Medium	High	Medium - High	Low	Medium
Oxygen Permeability	<0.01	~500	0.03-1	0.05-5	>1	>1
Solvent Compatibility	Very High	Low	High	Medium-High	Low	Medium
Hydrophobicity	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic	Amphiphilic
Surface Charge	Very Stable	Stable	Stable	Stable	-	-
Transparency	No/High	High	High	Medium-High	Low-Medium	Low
Cost	High	Low	High	Low	Medium	Low
Disadvantage	High Cost, Brittle	Protein Adsorption, Permeability, Autofluorescence	Rigid, Poor Conductivity, Non-Recyclable	Low Melting Point, Brittle	Non-Adherent, Low Mechanical Strength	Porous, Sample Consumption
References	[1]	[2][3]	[4]	[5][6]	[7]	[8]

Abbreviations: PEG-DA, Polyethylene Glycol Diacrylate.

Since the 2000s, much organ-on-a-chip technology has been proposed and engineered on the structure and function of tissues and organs 2000 [87]. However, it has evolved rapidly in the past decade due to the advancement in rapid prototyping methods like 3D printing, widely used to produce 3D scaffolds for tissue engineering and devices mimicking a complex microfluidic environment [88]. The first "human-on-

a-chip" cell culture systems to investigate physiological processes and "physiome-on-a-chip" to investigate novel compounds and their side effects on the human body emerged [87,89–91]. The emulation of the pathophysiology of several neurodegenerative diseases in vitro through microfluidic devices has also risen rapidly [92,93]. A comprehensive study of the application of microfluidics in the study of neurodegeneration has been provided in the following sections. A number of microfluidic tools available to date has been shown in **Figure 3.**
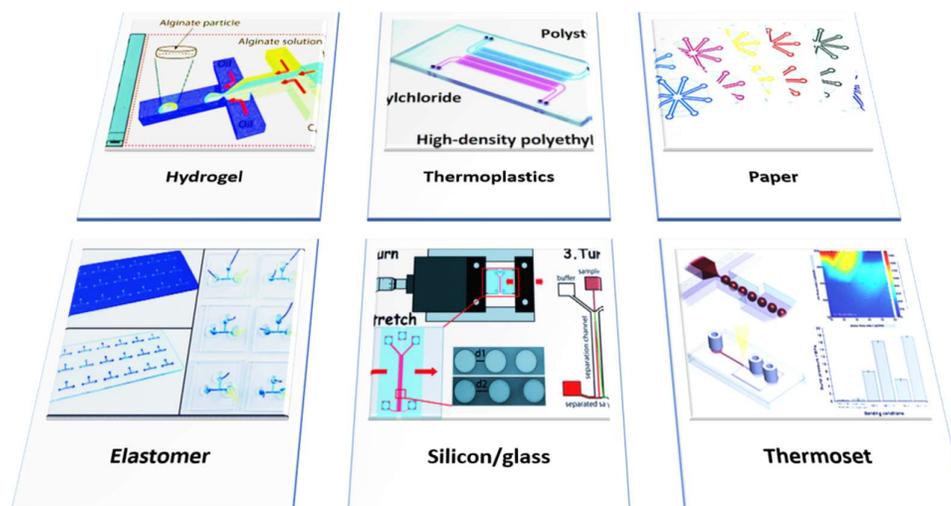
5. Exploring neurodegeneration through microfluidic tools: Need of the hour

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 [94]. Using microfluidic technologies and biomimetic principles, a multichambered device can be readily used to grow neurites, glial cells, endothelial cells, and skeletal muscle cells, along with maintaining fluid isolation [95]. In addition, the 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 [96]. 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 focussing on AD, Parkinson's Disease, and amyotrophic lateral sclerosis [92,93,97–100]. 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. However, studying it in 2D culture conditions is hard to achieve. 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 [101]. To improve our understanding of pathological mechanisms of neurodegenerative diseases, studying the blood-brain barrier's properties and its primary interactions with the central nervous system is essential.

Moreover, studying brain development and degeneration at the cellular level suffers several limitations due to the inability to isolate cell culture systems, the absence of an organized physiological neuron connection architecture, and so forth. In this regard, microfluidic systems present an irreplaceable tool to simulate the BBB microenvironment, study axonal functions construction of neuronal networks, and develop drug delivery systems through devices such as axonal diodes, minimized wireless devices [20,100,102–105]. Furthermore, the technology has led to the minimization of animal models in the study of neurodegenerative diseases, drastically cutting down labor-intensive efforts, time, and animal mortality. Besides, the discrepancies that arise due to species differences between humans and animal models can also be minimized.

The lab-on-chip technologies with features on a similar physical scale to that of cells facilitated the study of complex neural signaling pathways, detect the abnormalities, and check whether the application of inhibitors can reverse the abnormalities without animals' requirement [106,107]. 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 [77]. Studies show that synapses lose native circuitry and order due to dissociating 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 [77]. 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 [108,109]. The microfluidic devices fulfill all these requirements and mimic the unique anatomical and cellular interactions of this circuit [109,110]. 3D assay systems have been developed for the assays, 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 [106,111]. Furthermore, microfluidic neuromuscular co-culture enables innervation by axons crossing from the neuronal to the muscle compartment [112]. The same setup can be used to decipher the impact of genetic alterations on the synaptic function of CNS disorders [113]. Therefore, microfluidics is tremendously applied in various studies of diseases, including neurodegeneration. Similarly, its impact on the research and development of AD is overwhelming and promising.

6. 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, just like 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 2**. 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 to gain insights into the pathophysiological role of microglial migration   [114,115].

Table 2: Details of microfluidic devices and their application in the AD research

Cells/Peptide	Flow control device	Flow surface	Active/Passive	Application	References
Axon	NA	Glass	P	Study axonal function	[9]
Neural Progenitor Cell	Osmotic micropump	-	A	Study the neurotoxicity of amyloid beta	[10]
Neuron	Osmotic micropump	Glass	A & P	in vitro brain model, high-throughput drug screening	[11]
Brain Cells	pneumatically-driven pumps	Polysulfone	P	To provide MPSs for in vitro drug discovery	[12]
Aβ42 Peptide	Precision pump	Glass	A	β-Amyloid (1-42) detection	[13]
B-Amyloid Peptide	Syringe	-	A	-	[14]
Axons	N/A	Glass	P	Study impaired axonal deficit	[15]
Axons	N/A	MEA	P	Investigate axonal signals in developmental stage.	[16]
Neurites	Syringe	glass	A	Study durotactic behaviour of cells and neurite growth	[17]
Axons	Gravity/ Hydrostatic pressure	PCB/ Glass	P	Study axonal physiology and modeling CNS injury	[18]
Soma And Axon	N/A	Glass	P	Compartmentalizing the network structure into interconnected sub-populations	[19]
Hippocampal Neuronal/Glia Cells	Pressure gradient	Glass	P	Probing the functional synaptic connectivity between mixed primary hippocampal co-cultures	[20]
Dendrite	N/A	PDMS	NM	Investigate dendrite-to-nucleus signaling	[21]
Oligodendrocyte	N/A	Glass	P		[22]
Drg/Mc3t3-E1	N/A	Glass	NM	Mimicking the in vivo scenario to study interaction between the peripheral nervous system and bone cells	[23]
Nmj	Pipette	Glass	N/A	Study subcellular microenvironments; NMJ formation, maintenance and disruption	[24]
Axons	Pipette	Glass	P	Perform drug screening assays	[25]
Dendrites And Somata	Syringe	Glass	A	Manipulate synaptic regions and presynaptic and postsynaptic compartments in vitro.	[26]

Glial Cells/ Motor Neurons	N/A	Glass	P	Study interactions with glial cells and other skeletal cells in chamber	[27]
Astrocyte	N/A	acrylic plate	P	AD triculture model showing beta-amyloid aggregation, phosphorylated tau accumulation, and neuroinflammatory activity.	[28]
Tau	N/A	Glass	P	Study effects of tau on mitochondrial transport	[29]
(Aβ) Peptides	N/A	Glass	P	Study effects of local Aβ stress on neuronal sub compartments and networks	[30]
Adam10	Syringe	N/A	A	ADAM10 biomarker detection in plasma and cerebrospinal fluid	[31]
Tau	N/A	Glass	P	Quantify AD-derived Tau propagation	[32]
Amyloid-B	N/A	Glass	P	Study roles of Aβ on microglial accumulation	[33]
Amyloid-B	Syringe	overflow microfluidic networks	A	Study cell-to-cell communication; Role of astrocytes derived from cortex and hippocampus on neuronal viability.	[34]
Axons		Glass		Study mechanisms of indirect axonal excitotoxicity	[35]
Neurites	Hydrostatic pressure	Glass and Polystyrene	P	Grow neuronal culture	[36]
Cortical Neurons	Pressure difference	Glass	P	Synthesise experimental models emulating pathological states.	[37]
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	[38]
Protein	N/A	Glass	P	Detect protein aggregation	[39]
Axons	Hydrostatic pressure	Glass or Polystyrene	P	Study localized axon-glia interaction and signalling.	[40]
Axons	N/A	Glass	P	Examine axonal trauma in neuronal networks;	[41]
Axons-glia	Hydrostatic pressure	Glass	P	Study axon-glia interactions	[42]
Neurites	Syringe	Glass	A	Investigating chemotaxis of neutrophils	[43]

Abbreviations: MPSs, Microphysiological systems; DRG, dorsal root ganglion; NMJ, Neuromuscular junction; MEA, microelectrode arrays.

Similarly, the effects of axonal trauma on the neuronal networks of primary brain cells and the role of astrocytes were studied on a microfluidic platform [116,117]. The ease, accuracy, and reproducibility of the experiments encouraged a more significant number of studies. Apart from basic research, many disposable biosensors for early detection of AD biomarker ADAM10 and Aβ peptide in the serum have also been developed (limit of detection ~ 0.35 fg/ml) [118,119]. These low-cost diagnostic kits exhibit better accuracy and sensitivity than the well-established enzyme-linked immunosorbent assay test.

The emerging role of exosomes in the detection and study of AD has arisen the need for large-scale separation of exosomes, which is cumbersome and challenging through traditional techniques like ultra-centrifugation. Microfluidic devices are emerging as an ideal tool for exosome separation and are also stared to gain recognition as excellent exosome detection tools [120]. 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 [98]. These kinds of 3D culture-based microfluidic chips provide in vivo microenvironments for high-throughput drug screening and allow the investigation of dendrite-to-nucleus signaling [121]. Synthetic models with AD features such as aggregation of Aβ, accumulation of phosphorylated tau protein with neuroinflammatory activities have been produced to emulate pathological states. A triculture in vitro models comprising the combination of neurons, astrocytes, and microglia have evolved to address the physiological features and study the durotactic behavior of cells [122]. The human AD triculture model provides an opportunity to learn microglial recruitment, neurotoxic activities, and astrocytes [122]. A co-culture system with segregated cell bodies while simultaneously forming myelin sheaths could also be obtained through microfluidics approaches [123].

The 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, check to leach of toxic compounds, and examine that 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 [34]. The microfluidic technology facilitates us to understand 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 [82,87]. These devices have also been used to isolate axons and the cell body to study the targets of excitotoxicity observed in neurodegeneration. In a 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 blood-brain barrier-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 [65]. This technology facilitates us to culture cortical neurons in two distinct cell compartments of the same microfluidic device to generate neuronal networks [124]. This setup can bring axonal degeneration in the distal axon chamber without degenerative changes in the

untreated somal section [125]. Insults to the selective areas of neurons can be obtained without affecting other neurons by applying hydrostatic pressure [97].

7. Challenges in the application of microfluidics in the AD research

Microfluidics provides state of a art facility to facilitate investigations in biomedical research. Samples collected on chips/ microfluidic channels can be analyzed using techniques like PCR, histochemistry, western blots, MS-Spectrometry analyses [80,126]. Despite the advancement in microfluidic solutions, experts in the field say that it still needs to grow more to outperform current methods and overcome barriers that hinder researchers from adopting microfluidic technology to its optimal utilization. For example, limitations such as lack of fluid handling methods prevent attaining a correct quantity of reagent from difficult, leading to change in the scaling processes. This, in turn, results in an inaccurate replication and adaptation of protocols.

Moreover, the cells may respond differently to the change in the substrate of the culture devices. Another drawback is that 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. Therefore, a direct comparison to the macroscale experiments becomes 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 [127,128]. Furthermore, excessive permeability, technical robustness, and various properties might lead to sample drying and change in osmolarity, posing a considerable obstruction. In addition, we don't 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. A universal blood substitute or standard culture media that supports all types of tissues are other drawbacks that must be addressed in the future for its optimal application. As a range of cell lines are cultured in these devices, a generalization in device designs is difficult. To minimize the risk of translation failure to microfluidic devices, new production techniques such as microfluidic hot embossing in polystyrene have been favored for mass production [129].

Additionally, the interdisciplinary nature of microfluidic cell culture systems, and an increasing number of device designs, standardized protocols are generally absent. A combined effort of engineers and molecular biologists must design and fabricate new devices and carry out biologically relevant experiments [34]. Another demerit of microfluidics is that it produces slow diffusive mixing due to laminar flows, which is a limitation for the system that requires fast homogenization [130]. Although the chips can be well integrated with the in-line sensors and microfluorimetric imaging facilities to control physical parameters such as flow, pressure, temperature, pH and perform real-time monitoring respectively, the acceptance of this organ-on-chip in basic research and drug development require additional features such as feedback control, continuous monitoring, and experimental sample processing. An automated control system is also necessary to expand the domain of users to replace the 2D or 3D culture systems. Despite the success of the 3D triculture AD model, which is undoubtedly advanced over in vitro human AD models, physiologically relevant in vivo studies are still required to confirm its clinical utility [99].

8. Conclusion

Even after a century of extensive research, the field of AD requires more work in the appropriate direction to come up with effective diagnostics and therapeutic cures [10,51,131]. The crucial research problems are challenging to achieve from the current macroscopic laboratory equipment and practices. The research is at a crossroads where rigorous research is required to determine the right direction and correct hypothesis to focus on. Microfluidic systems facilitate us to work on a functional organ at the level of molecular analysis minimizing the complications involved in handling in vivo systems several folds lesser. These devices outperform age-old methodologies through features, such as rapid sample processing, fluid control, the flexibility of designing, controlled co-culture, reduced reagent consumption, low contamination risk, and efficient high throughput experimentation. Undoubtedly, these novel neurotechnological tools are very useful in gaining an in-depth understanding of the brain's functions and discovering novel therapeutic strategies for neurological disorders like AD. However, the extent to which this technology can serve in AD detection and management is still in a nascent phase. This is because the technology has not been developed to recapitulate biological responses to various stimuli such as chemicals or toxins. Although organs-on-chips may lead to identifying biomarkers and validation of lead drug candidates, clinically relevant PK/PD models are required to determine the doses of drugs. In this regard, better scaling approaches to keep an account of fluid flows and volumes of distribution would ensure functional PK/PD models. It is doubtful that organs-on-chips will replace animal testing anytime soon as the organ function and regulatory requirements are highly complex. Nonetheless, these low-cost techniques are up-and-coming and have accelerated the pace of AD research.

Figure Legends
Figure 1: Pathological hallmarks of Alzheimer's disease
Figure 2: Timeline of the progress of microfluidics in biomedical research
Figure 3: Application of different microfluidic devices in the brain research
Tables
Table 1: Properties of materials used in microfluidic chips
Table 2: Details of microfluidic devices and their application in the AD research

Abbreviations

AD	Alzheimer's disease
ADAM10	A Disintegrin And Metalloprotease 10
Apo E	Apolipoprotein E
Aβ	Amyloid-beta
CD33	Complementary determinant 33
IL	Interleukin
MMP	matrix metallopeptidase 9
NMJ	Neuromuscular junction
PCR	Polymerase chain reaction

PDMS   Polydimethylsiloxane

PK/PD   Pharmacokinetic/Pharmacodynamic

TNF-α   Tumor necrosis factor-alpha

TREM2   Triggering receptor expressed on myeloid cells 2

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Author's contribution

Pragya Prasanna: Conceptualization, Supervision, Writing- Original draft preparation, Data curation. Reviewing and Editing; Shweta Rathee: Visualisation, V Rahul: Data curation, Writing- Original draft preparation; Visualisation. M.S. Chandra: Reviewing and Editing, Niraj Kumar Jha: Visualisation, Saurabh Kumar Jha: Reviewing and Editing, Chiara Villa: Reviewing and Editing. All named authors are responsible for the final approval of the version to be submitted and are accountable for all aspects of the study. All authors have read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

References

[1]   De-Paula VJ, Radanovic M, Diniz BS, Forlenza O V. Alzheimer's Disease. Subcell Biochem 2012;65:329–52. https://doi.org/10.1007/978-94-007-5416-4_14.

[2]   Crimins JL, Pooler A, Polydoro M, Luebke JI, Spires-Jones TL. The intersection of amyloid beta and tau in glutamatergic synaptic dysfunction and collapse in Alzheimer's Disease. Ageing Res Rev 2013;12:757–63. https://doi.org/10.1016/j.arr.2013.03.002.

[3]   Rajmohan R, Reddy PH. Amyloid Beta and Phosphorylated Tau Accumulations Cause Abnormalities at Synapses of Alzheimer's disease Neurons. J Alzheimers Dis 2017;57:975. https://doi.org/10.3233/JAD-160612.

[4]   Lee JC, Kim SJ, Hong S, Kim YS. Diagnosis of Alzheimer's Disease utilizing amyloid and tau as fluid biomarkers. Exp Mol Med 2019;51:1–10. https://doi.org/10.1038/s12276-019-0250-2.

[5]   Forner S, Baglietto-Vargas D, Martini AC, Trujillo-Estrada L, LaFerla FM. Synaptic Impairment in Alzheimer's Disease: A Dysregulated Symphony. Trends Neurosci 2017;40:347–57. https://doi.org/10.1016/j.tins.2017.04.002.

[6]   Jahn H. Memory loss in alzheimer's disease. Dialogues Clin Neurosci 2013;15:445–54. https://doi.org/10.31887/dcns.2013.15.4/hjahn.

[7]   Lynch C. World Alzheimer Report 2019: Attitudes to dementia, a global survey. Alzheimer's Dement 2020;16. https://doi.org/10.1002/alz.038255.

[8]   2020 Alzheimer's disease facts and figures. Alzheimer's Dement 2020;16:391–460. https://doi.org/10.1002/alz.12068.

[9]   International D. World Alzheimer Report 2020 – Design Dignity Dementia: dementia-related design and the built environment, Volume 1. 2020.

[10]   Holtzman DM, Morris JC, Goate AM. Alzheimer's Disease: The challenge of the second century. Sci Transl Med 2011;3:77sr1. https://doi.org/10.1126/scitranslmed.3002369.

[11]   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. https://doi.org/10.9758/cpn.2017.15.1.1.

[12]   Yiannopoulou KG, Papageorgiou SG. Current and future treatments for Alzheimer's Disease. Ther Adv Neurol Disord 2013;6:19. https://doi.org/10.1177/1756285612461679.

[13]   Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's Disease. Mol Neurodegener 2020;15. https://doi.org/10.1186/s13024-020-00391-7.

[14]   Strassnig M, Ganguli M. About a peculiar disease of the cerebral cortex: Alzheimer's original case revisited. Psychiatry (Edgmont) 2005;2:30–3.

[15]   You Y, Perkins A, Cisternas P, Muñoz B, Taylor X, You Y, et al. Tau as a mediator of neurotoxicity associated to cerebral amyloid angiopathy. Acta Neuropathol Commun 2019;7:26. https://doi.org/10.1186/s40478-019-0680-z.

[16]   Huang HC, Jiang ZF. Accumulated amyloid-β peptide and hyperphosphorylated tau protein: Relationship and links in Alzheimer's Disease. J Alzheimer's Dis 2009;16:15–27. https://doi.org/10.3233/JAD-2009-0960.

[17]   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. https://doi.org/10.3390/MI11090787.

[18]   Velve-Casquillas G, Le Berre M, Piel M, Tran PT. Microfluidic tools for cell biological research. Nano Today 2010;5:28–47. https://doi.org/10.1016/j.nantod.2009.12.001.

[19]   Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol 2014;12:207–18. https://doi.org/10.1089/adt.2014.573.

[20]   Dworak BJ, Wheeler BC. Novel MEA platform with PDMS microtunnels enables the detection of action potential propagation from isolated axons in culture. Lab Chip 2009;9:404–10. https://doi.org/10.1039/b806689b.

[21]   Alzheimer's Disease Fact Sheet | National Institute on Aging n.d.

[22]   Goedert M, Spillantini MG. A century of Alzheimer's Disease. Science (80-) 2006;314:777–81. https://doi.org/10.1126/science.1132814.

[23]   Deture MA, Dickson DW. The neuropathological diagnosis of Alzheimer's Disease. Mol Neurodegener 2019;14. https://doi.org/10.1186/s13024-019-0333-5.

[24]   Li H, Liu CC, Zheng H, Huang TY. Amyloid, tau, pathogen infection and antimicrobial protection in Alzheimer's Disease -conformist, nonconformist, and realistic prospects for AD pathogenesis. Transl Neurodegener 2018;7:1–16. https://doi.org/10.1186/s40035-018-0139-3.

[25]   De-Paula VJ, Radanovic M, Diniz BS, Forlenza O V. Alzheimer's Disease. Subcell Biochem 2012;65:329–52. https://doi.org/10.1007/978-94-007-5416-4_14.

[26]   Rabbito A, Dulewicz M, Kulczyńska-Przybik A, Mroczko B. Biochemical markers in alzheimer's disease. Int J Mol Sci 2020;21. https://doi.org/10.3390/ijms21061989.

[27] Sardar Sinha M, Ansell-Schultz A, Civitelli L, Hildesjö C, Larsson M, Lannfelt L, et al. Alzheimer's disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. Acta Neuropathol 2018;136:41–56. https://doi.org/10.1007/s00401-018-1868-1.

[28] Murphy MP, LeVine H, III. Alzheimer's disease and the β-Amyloid Peptide. J Alzheimers Dis 2010;19:311. https://doi.org/10.3233/JAD-2010-1221.

[29] Sengupta U, Nilson AN, Kayed R. The Role of Amyloid-β Oligomers in Toxicity, Propagation, and Immunotherapy. EBioMedicine 2016;6:42–9. https://doi.org/10.1016/j.ebiom.2016.03.035.

[30] Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: A genetic perspective. Cell 2005;120:545–55. https://doi.org/10.1016/j.cell.2005.02.008.

[31] Chen XQ, Mobley WC. Exploring the pathogenesis of Alzheimer disease in basal forebrain cholinergic neurons:Converging insights from alternative hypotheses. Front Neurosci 2019;13:1–18. https://doi.org/10.3389/fnins.2019.00446.

[32] Hansen D V, Hanson JE, Sheng M. Microglia in Alzheimer's Disease. J Cell Biol 2018;217:459–72. https://doi.org/10.1083/jcb.201709069.

[33] Mandrekar-Colucci S, Landreth GE. Microglia and Inflammation in Alzheimers Disease. CNS Neurol Disord - Drug Targets 2012;9:156–67. https://doi.org/10.2174/187152710791012071.

[34] Choi YJ, Chae S, Kim JH, Barald KF, Park JY, Lee SH. Neurotoxic amyloid beta oligomeric assemblies recreated in microfluidic platform with interstitial level of slow flow. Sci Rep 2013;3:1–7. https://doi.org/10.1038/srep01921.

[35] Wang W-Y, Tan M-S, Yu J-T, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's Disease. Ann Transl Med 2015;3:136. https://doi.org/10.3978/J.ISSN.2305-5839.2015.03.49.

[36] Yamazaki Y, Kanekiyo T. Blood-brain barrier dysfunction and the pathogenesis of alzheimer's disease. Int J Mol Sci 2017;18. https://doi.org/10.3390/ijms18091965.

[37] Sweeney MD, Sagare AP, Zlokovic B V. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol 2018;14:133–50. https://doi.org/10.1038/nrneurol.2017.188.

[38] Calsolaro V, Edison P. Neuroinflammation in Alzheimer's Disease: Current evidence and future directions. Alzheimer's Dement 2016;12:719–32. https://doi.org/10.1016/j.jalz.2016.02.010.

[39] Rempe RG, Hartz AMS, Bauer B. Matrix metalloproteinases in the brain and blood–brain barrier: Versatile breakers and makers. J Cereb Blood Flow Metab 2016;36:1481. https://doi.org/10.1177/0271678X16655551.

[40] Fernandez CG, Hamby ME, McReynolds ML, Ray WJ. The Role of APOE4 in Disrupting the Homeostatic Functions of Astrocytes and Microglia in Aging and Alzheimer's Disease. Front Aging Neurosci 2019;11. https://doi.org/10.3389/FNAGI.2019.00014.

[41] Bird TD. Genetic Aspects of Alzheimer Disease 2009;10:231–9. https://doi.org/10.1097/GIM.0b013e31816b64dc.Genetic.

[42] Birks JS, Chong LY, Grimley Evans J. Rivastigmine for Alzheimer's Disease. Cochrane Database Syst Rev 2015;2015. https://doi.org/10.1002/14651858.CD001191.pub4.

[43] Woodruff-Pak DS. Galantamine: Effect on nicotinic receptor binding, acetylcholinesterase inhibition, and learning. Proc Natl Acad Sci 2001;98:2089–94. https://doi.org/10.1073/pnas.031584398.

[44] Lilienfeld S. Cholinesterase inhibitors for Alzheimer disease. JAMA 2003;289. https://doi.org/10.1001/jama.289.18.2359-c.

[45] Govind N. Donepezil for dementia due to Alzheimer's Disease. Br J Community Nurs

2020;25:148–9. https://doi.org/10.12968/bjcn.2020.25.3.148.

[46]   Casey DA, Antimisiaris D, Brien JO. Drugs for Alzheimer' s Disease : Are They Effective ? 2010;35:208–11.

[47]   Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. Nat Med 2007;13:1359–62. https://doi.org/10.1038/nm1653.

[48]   O'Bryant SE, Xiao G, Barber R, Reisch J, Hall J, Cullum CM, et al. A blood-based algorithm for the detection of Alzheimer's Disease. Dement Geriatr Cogn Disord 2011;32:55–62. https://doi.org/10.1159/000330750.

[49]   Kenny A, McArdle H, Calero M, Rabano A, Madden SF, Adamson K, et al. Elevated Plasma microRNA-206 Levels Predict Cognitive Decline and Progression to Dementia from Mild Cognitive Impairment. Biomolecules 2019;9:734. https://doi.org/10.3390/biom9110734.

[50]   Wisniewski T, Konietzko U. Amyloid-β immunisation for Alzheimer's Disease. Lancet Neurol 2008;7:805. https://doi.org/10.1016/S1474-4422(08)70170-4.

[51]   Ricciarelli R, Fedele E. The Amyloid Cascade Hypothesis in Alzheimer's Disease: It's Time to Change Our Mind. Curr Neuropharmacol 2017;15. https://doi.org/10.2174/1570159x15666170116143743.

[52]   Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, et al. Probing sporadic and familial Alzheimer's Disease using induced pluripotent stem cells. Nature 2012;482:216–20. https://doi.org/10.1038/nature10821.

[53]   Liu PP, Xie Y, Meng XY, Kang JS. History and progress of hypotheses and clinical trials for alzheimer's disease. Signal Transduct Target Ther 2019;4:1–22. https://doi.org/10.1038/s41392-019-0063-8.

[54]   Takeda S. Tau Propagation as a Diagnostic and Therapeutic Target for Dementia: Potentials and Unanswered Questions. Front Neurosci 2019;13:1274. https://doi.org/10.3389/fnins.2019.01274.

[55]   Ballatore C, Lee VMY, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's Disease and related disorders. Nat Rev Neurosci 2007;8:663–72. https://doi.org/10.1038/nrn2194.

[56]   Marshall KE, Vadukul DM, Dahal L, Theisen A, Fowler MW, Al-Hilaly Y, et al. A critical role for the self-assembly of Amyloid-β1-42 in neurodegeneration. Sci Rep 2016;6:1–13. https://doi.org/10.1038/srep30182.

[57]   Broersen K, Rousseau F, Schymkowitz J. The culprit behind amyloid beta peptide related neurotoxicity in Alzheimer's Disease: oligomer size or conformation? Alzheimers Res Ther 2010;2:12. https://doi.org/10.1186/alzrt36.

[58]   F M, A P. Vaccination against β-Amyloid as a Strategy for the Prevention of Alzheimer's Disease. Biology (Basel) 2020;9. https://doi.org/10.3390/BIOLOGY9120425.

[59]   Katsikoudi A, Ficulle E, Cavallini A, Sharman G, Guyot A, Zagnoni M, et al. Quantitative propagation of assembled human Tau from Alzheimer's disease brain in microfluidic neuronal cultures. J Biol Chem 2020;295:13079–93. https://doi.org/10.1074/jbc.RA120.013325.

[60]   Oxford AE, Stewart ES, Rohn TT. Clinical Trials in Alzheimer's Disease: A Hurdle in the Path of Remedy. Int J Alzheimers Dis 2020;2020. https://doi.org/10.1155/2020/5380346.

[61]   Rabbito A, Dulewicz M, Kulczyńska-Przybik A, Mroczko B. Biochemical markers in alzheimer's disease. Int J Mol Sci 2020;21. https://doi.org/10.3390/ijms21061989.

[62]   O'Bryant SE, Mielke MM, Rissman RA, Lista S, Vanderstichele H, Zetterberg H, et al. Blood-based biomarkers in Alzheimer disease: Current state of the science and a novel collaborative

paradigm for advancing from discovery to clinic. Alzheimer's Dement 2017;13:45–58. https://doi.org/10.1016/j.jalz.2016.09.014.

[63]   Kwon HS, Koh S-H. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes n.d. https://doi.org/10.1186/s40035-020-00221-2.

[64]   Dawson TM, Golde TE, Lagier-Tourenne C. Animal models of neurodegenerative diseases. Nat Neurosci 2018;21:1370–9. https://doi.org/10.1038/s41593-018-0236-8.

[65]   Shin Y, Choi SH, Kim E, Bylykbashi E, Kim JA, Chung S, et al. Blood–Brain Barrier Dysfunction in a 3D In Vitro Model of Alzheimer's Disease. Adv Sci 2019;6. https://doi.org/10.1002/advs.201900962.

[66]   Gupta N, Liu JR, Patel B, Solomon DE, 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. https://doi.org/10.1002/btm2.10013.

[67]   Natarajan A, Sethumadhavan A, Krishnan UM. Toward building the neuromuscular junction: In vitro models to study synaptogenesis and neurodegeneration. ACS Omega 2019;4:12969–77. https://doi.org/10.1021/acsomega.9b00973.

[68]   Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: Biogenesis, biologic function and clinical potential. Cell Biosci 2019;9. https://doi.org/10.1186/s13578-019-0282-2.

[69]   Yin Q, Ji X, Lv R, Pei JJ, Du Y, Shen C, et al. Targetting exosomes as a new biomarker and therapeutic approach for Alzheimer's Disease. Clin Interv Aging 2020;15:195–205. https://doi.org/10.2147/CIA.S240400.

[70]   Howitt J, Hill AF. Exosomes in the pathology of neurodegenerative diseases. J Biol Chem 2016;291:26589–97. https://doi.org/10.1074/jbc.R116.757955.

[71]   Thompson AG, Gray E, Heman-Ackah SM, Mäger I, Talbot K, El Andaloussi S, et al. Extracellular vesicles in neurodegenerative disease-pathogenesis to biomarkers. Nat Rev Neurol 2016;12:346–57. https://doi.org/10.1038/nrneurol.2016.68.

[72]   Whitesides GM. The origins and the future of microfluidics. Nature 2006;442:368–73. https://doi.org/10.1038/nature05058.

[73]   Xia Y, Whitesides GM. Soft Lithography. Angew Chemie Int Ed 1998;37:550–75. https://doi.org/10.1002/(sici)1521-3773(19980316)37:5<550::aid-anie550>3.0.co;2-g.

[74]   Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. Soft lithography in biology and biochemistry.   Annu   Rev   Biomed   Eng   2001;3:335–73. https://doi.org/10.1146/annurev.bioeng.3.1.335.

[75]   Folch A, Toner M. Cellular micropatterns on biocompatible materials. Biotechnol Prog 1998;14:388–92. https://doi.org/10.1021/bp980037b.

[76]   Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. Rapid prototyping of microfluidic systems   in   poly(dimethylsiloxane).   Anal   Chem   1998;70:4974–84. https://doi.org/10.1021/ac980656z.

[77]   Taylor AM, Dieterich DC, Ito HT, Kim SA, Schuman EM. Microfluidic Local Perfusion Chambers for the Visualization and Manipulation of Synapses. Neuron 2010;66:57–68. https://doi.org/10.1016/j.neuron.2010.03.022.

[78]   Van Midwoud PM, Janse A, Merema MT, Groothuis GMM, Verpoorte E. Comparison of biocompatibility and adsorption properties of different plastics for advanced microfluidic cell and tissue culture models. Anal Chem 2012;84:3938–44. https://doi.org/10.1021/ac300771z.

[79]   Kim SM, Lee SH, Suh KY. Cell research with physically modified microfluidic channels: A review. Lab Chip 2008;8:1015–23. https://doi.org/10.1039/b800835c.

[80]   Sonnen KF, Merten CA. Microfluidics as an Emerging Precision Tool in Developmental

Biology. Dev Cell 2019;48:293–311. https://doi.org/10.1016/j.devcel.2019.01.015.

[81] Duncombe TA, Tentori AM, Herr AE. Microfluidics: Reframing biological enquiry. Nat Rev Mol Cell Biol 2015;16:554–67. https://doi.org/10.1038/nrm4041.

[82] Dittrich PS, Manz A. Lab-on-a-chip: Microfluidics in drug discovery. Nat Rev Drug Discov 2006;5:210–8. https://doi.org/10.1038/nrd1985.

[83] Ying L, Wang Q. Microfluidic chip-based technologies: Emerging platforms for cancer diagnosis. BMC Biotechnol 2013;13:76. https://doi.org/10.1186/1472-6750-13-76.

[84] Chin CD, Laksanasopin T, Cheung YK, Steinmiller D, Linder V, Parsa H, et al. Microfluidics-based diagnostics of infectious diseases in the developing world. Nat Med 2011;17:1015–9. https://doi.org/10.1038/nm.2408.

[85] Tay A, Pavesi A, Yazdi SR, Lim CT, Warkiani ME. Advances in microfluidics in combating infectious diseases. Biotechnol Adv 2016;34:404–21. https://doi.org/10.1016/j.biotechadv.2016.02.002.

[86] Reboud J, Xu G, Garrett A, Adriko M, Yang Z, Tukahebwa EM, et al. Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities. Proc Natl Acad Sci U S A 2019;116:4834–42. https://doi.org/10.1073/pnas.1812296116.

[87] Esch EW, Bahinski A, Huh D. Organs-on-chips at the frontiers of drug discovery. Nat Rev Drug Discov 2015;14:248–60. https://doi.org/10.1038/nrd4539.

[88] Bhattacharjee N, Urrios A, Kang S, Folch A. The upcoming 3D-printing revolution in microfluidics. Lab Chip 2016;16:1720–42. https://doi.org/10.1039/c6lc00163g.

[89] Luni C, Serena E, Elvassore N. Human-on-chip for therapy development and fundamental science. Curr Opin Biotechnol 2014;25:45–50. https://doi.org/10.1016/j.copbio.2013.08.015.

[90] Zhang C, Zhao Z, Abdul Rahim NA, Van Noort D, Yu H. Towards a human-on-chip: Culturing multiple cell types on a chip with compartmentalized microenvironments. Lab Chip 2009;9:3185–92. https://doi.org/10.1039/b915147h.

[91] Baudoin R, Corlu A, Griscom L, Legallais C, Leclerc E. Trends in the development of microfluidic cell biochips for in vitro hepatotoxicity. Toxicol Vitr 2007;21:535–44. https://doi.org/10.1016/j.tiv.2006.11.004.

[92] Osaki T, Shin Y, Sivathanu V, Campisi M, Kamm RD. In Vitro Microfluidic Models for Neurodegenerative Disorders. Adv Healthc Mater 2018;7:1700489. https://doi.org/10.1002/adhm.201700489.

[93] Kane KIW, Jarazo J, Moreno EL, Fleming RMT, Schwamborn JC. Passive controlled flow for Parkinson's disease neuronal cell culture in 3D microfluidic devices. Organs-on-a-Chip 2020;2:100005. https://doi.org/10.1016/j.ooc.2020.100005.

[94] Perestrelo AR, Águas ACP, Rainer A, Forte G. Microfluidic organ/body-on-a-chip devices at the convergence of biology and microengineering. Sensors (Switzerland) 2015;15:31142–70. https://doi.org/10.3390/s151229848.

[95] Haring AP, Sontheimer H, Johnson BN. 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 Reports 2017;13:381–406. https://doi.org/10.1007/s12015-017-9738-0.

[96] Zheng F, Fu F, Cheng Y, Wang C, Zhao Y, Gu Z. Organ-on-a-Chip Systems: Microengineering to Biomimic Living Systems. Small 2016;12:2253–82. https://doi.org/10.1002/smll.201503208.

[97] Taylor AM, Rhee SW, Tu CH, Cribbs DH, Cotman CW, Jeon NL. Microfluidic multicompartment device for neuroscience research. Langmuir 2003;19:1551–6. https://doi.org/10.1021/la026417v.

[98]   Park J, Lee BK, Jeong GS, Hyun JK, Lee CJ, Lee SH. 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;15:141–50. https://doi.org/10.1039/c4lc00962b.

[99]   Park J, Wetzel I, Marriott I, Dréau D, D'Avanzo C, Kim DY, et al. A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's Disease. Nat Neurosci 2018;21:941–51. https://doi.org/10.1038/s41593-018-0175-4.

[100]   Teixeira MI, Amaral MH, Costa PC, Lopes CM, Lamprou DA. Recent developments in microfluidic technologies for central nervous system targeted studies. Pharmaceutics 2020;12:1–37. https://doi.org/10.3390/pharmaceutics12060542.

[101]   Bianco F, Tonna N, Lovchik RD, Mastrangelo R, Morini R, Ruiz A, et al. Overflow microfluidic networks: Application to the biochemical analysis of brain cell interactions in complex neuroinflammatory scenarios. Anal Chem 2012;84:9833–40. https://doi.org/10.1021/ac302094z.

[102]   Peyrin JM, Deleglise B, Saias L, Vignes M, Gougis P, Magnifico S, et al. Axon diodes for the reconstruction of oriented neuronal networks in microfluidic chambers. Lab Chip 2011;11:3663–73. https://doi.org/10.1039/c1lc20014c.

[103]   Gutruf P, Rogers JA. Implantable, wireless device platforms for neuroscience research. Curr Opin Neurobiol 2018;50:42–9. https://doi.org/10.1016/j.conb.2017.12.007.

[104]   Tsantoulas C, Farmer C, Machado P, Baba K, McMahon SB, Raouf R. Probing functional properties of nociceptive axons using a microfluidic culture system. PLoS One 2013;8:80722. https://doi.org/10.1371/journal.pone.0080722.

[105]   Choi YJ, Chae S, Kim JH, Barald KF, Park JY, Lee SH. Neurotoxic amyloid beta oligomeric assemblies recreated in microfluidic platform with interstitial level of slow flow. Sci Rep 2013;3:1–7. https://doi.org/10.1038/srep01921.

5.

[106]   Poon WW, Blurton-Jones M, Tu CH, Feinberg LM, Chabrier MA, Harris JW, et al. β-Amyloid impairs axonal BDNF retrograde trafficking. Neurobiol Aging 2011;32:821–33. https://doi.org/10.1016/j.neurobiolaging.2009.05.012.

[107]   Jackson EL, Lu H. Three-dimensional models for studying development and Disease: Moving on from organisms to organs-on-a-chip and organoids. Integr Biol (United Kingdom) 2016;8:672–83. https://doi.org/10.1039/c6ib00039h.

[108]   Ren Y, Kunze A, Renaud P. Compartmentalized microfluidics for in vitro Alzheimer's disease studies. Microfluid. Compart. Platforms Neurobiol. Res., Springer New York; 2015, p. 197–215. https://doi.org/10.1007/978-1-4939-2510-0_12.

[109]   Southam KA, King AE, Blizzard CA, McCormack GH, Dickson TC. Microfluidic primary culture model of the lower motor neuron-neuromuscular junction circuit. J Neurosci Methods 2013;218:164–9. https://doi.org/10.1016/j.jneumeth.2013.06.002.

[110]   Neto E, Alves CJ, Sousa DM, Alencastre IS, Lourenço AH, Leitão L, et al. Sensory neurons and osteoblasts: Close partners in a microfluidic platform. Integr Biol (United Kingdom) 2014;6:586–95. https://doi.org/10.1039/c4ib00035h.

[111]   Sundararaghavan HG, Monteiro GA, Firestein BL, Shreiber DI. Neurite growth in 3D collagen gels with gradients of mechanical properties. Biotechnol Bioeng 2009;102:632–43. https://doi.org/10.1002/bit.22074.

[112]   Ionescu A, Zahavi EE, 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. https://doi.org/10.1016/j.ejcb.2015.11.004.

[113]   Robertson G, Bushell TJ, Zagnoni M. Chemically induced synaptic activity between mixed

primary hippocampal co-cultures in a microfluidic system. Integr Biol (United Kingdom) 2014;6:636–44. https://doi.org/10.1039/c3ib40221e.

[114] Choi I, Huh YS, Erickson D. Size-selective concentration and label-free characterization of protein aggregates using a Raman active nanofluidic device. Lab Chip 2011;11:632–8. https://doi.org/10.1039/c0lc00383b.

[115] Cho H, Hashimoto T, Wong E, Hori Y, Wood LB, Zhao L, et al. Microfluidic chemotaxis platform for differentiating the roles of soluble and bound amyloid-β on microglial accumulation. Sci Rep 2013;3. https://doi.org/10.1038/srep01823.

[116] Bianco F, Tonna N, Lovchik RD, Mastrangelo R, Morini R, Ruiz A, et al. Overflow microfluidic networks: Application to the biochemical analysis of brain cell interactions in complex neuroinflammatory scenarios. Anal Chem 2012;84:9833–40. https://doi.org/10.1021/ac302094z.

[117] Deleglise B, Lassus B, Soubeyre V, Alleaume-Butaux A, Hjorth JJ, Vignes M, et al. Synapto-Protective Drugs Evaluation in Reconstructed Neuronal Network. PLoS One 2013;8. https://doi.org/10.1371/journal.pone.0071103.

[118] De Oliveira TR, Erbereli CR, Manzine PR, Magalhães TNC, Balthazar MLF, Cominetti MR, et al. Early Diagnosis of Alzheimer's Disease in Blood Using a Disposable Electrochemical Microfluidic Platform. ACS Sensors 2020;5:1010–9. https://doi.org/10.1021/acssensors.9b02463.

[119] Islam K, Jang YC, Chand R, Jha SK, Lee HH, Kim YS. Microfluidic biosensor for β-amyloid(1-42) detection using cyclic voltammetry. J. Nanosci. Nanotechnol., vol. 11, J Nanosci Nanotechnol; 2011, p. 5657–62. https://doi.org/10.1166/jnn.2011.4498.

[120] Yang F, Liao X, Tian Y, Li G. Exosome separation using microfluidic systems: size-based, immunoaffinity-based and dynamic methodologies. Biotechnol J 2017;12. https://doi.org/10.1002/biot.201600699.

[121] Cohen MS, Orth CB, Kim HJ, Jeon NL, Jaffrey SR. Neurotrophin-mediated dendrite-to-nucleus signaling revealed by microfluidic compartmentalization of dendrites. Proc Natl Acad Sci U S A 2011;108:11246–51. https://doi.org/10.1073/pnas.1012401108.

[122] Jorfi M, D'Avanzo C, Kim DY, Irimia D. Three-Dimensional Models of the Human Brain Development and Diseases. Adv Healthc Mater 2018;7. https://doi.org/10.1002/adhm.201700723.

[123] Yang IH, Gary D, Malone M, Dria S, Houdayer T, Belegu V, et al. Axon myelination and electrical stimulation in a microfluidic, compartmentalized cell culture platform. NeuroMolecular Med 2012;14:112–8. https://doi.org/10.1007/s12017-012-8170-5.

[124] Kunze A, Meissner R, Brando S, Renaud P. Co-pathological connected primary neurons in a microfluidic device for Alzheimer studies. Biotechnol Bioeng 2011;108:2241–5. https://doi.org/10.1002/bit.23128.

[125] Hosie KA, King AE, Blizzard CA, Vickers JC, Dickson TC. Chronic excitotoxin-induced axon degeneration in a compartmented neuronal culture model. ASN Neuro 2012;4:47–57. https://doi.org/10.1042/AN20110031.

[126] Wang X, Yi L, Mukhitov N, Schrell AM, Dhumpa R, Roper MG. Microfluidics-to-mass spectrometry: A review of coupling methods and applications. J Chromatogr A 2015;1382:98–116. https://doi.org/10.1016/j.chroma.2014.10.039.

[127] Chumbimuni-Torres KY, Coronado RE, Mfuh AM, Castro-Guerrero C, Silva MF, Negrete GR, et al. Adsorption of proteins to thin-films of PDMS and its effect on the adhesion of human endothelial cells. RSC Adv 2011;1:706–14. https://doi.org/10.1039/c1ra00198a.

[128] 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. https://doi.org/10.1021/ac101870s.

[129] Goral V, Hsieh Y-C, Petzold O, Faris R, Yuen PK. Hot embossing of plastic microfluidic devices using poly (dimethylsiloxane) molds. J Micromechanics Microengineering - J MICROMECHANIC MICROENGINEER 2011;21. https://doi.org/10.1088/0960-1317/21/1/017002.

[130] Ward K, Fan ZH. Mixing in microfluidic devices and enhancement methods. J Micromechanics Microengineering 2015;25. https://doi.org/10.1088/0960-1317/25/9/094001.

[131] Oxford AE, Stewart ES, Rohn TT. Clinical Trials in Alzheimer's Disease: A Hurdle in the Path of Remedy. Int J Alzheimers Dis 2020;2020. https://doi.org/10.1155/2020/5380346.