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Review

Central Nervous System and its Disease Models on a Chip

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Technologies for microfluidics and biological microelectromechanical systems have been rapidly progressing over the past decade, enabling the development of unique microplatforms for *in vitro* human central nervous system (CNS) and related disease models. Most fundamental techniques include manipulation of axons, synapses, and neuronal networks, and different culture conditions are possible, such as compartmental, co-culturing, and 3D. Various CNS disease models, such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), epilepsy, *N*-methyl-D-aspartate receptor (NMDAR) encephalitis, migraine, diffuse axonal injury, and neuronal migration disorders, have been successfully established on microplatforms. In this review, we summarize fundamental technologies and current existing CNS disease models on microplatforms. We also discuss possible future directions, including application of these methods to pathological studies, drug screening, and personalized medicine, with 3D and personalized disease models that could generate more realistic CNS disease models.

In Vitro Models of the CNS

The CNS is highly compartmentalized and layered, containing diverse cell types with plastic connectivity via axon and dendrite outgrowths [1]. Animal-based CNS disease models have been used to study human brain function and related diseases. However, these *in vivo* approaches have various limitations, such as high costs, low-throughput, labor-intensive, and time-consuming processes, and experimental variations. These limitations led neuroscientists to develop simplified and high-throughput *in vitro* CNS disease models. However, the simplicity of *in vitro* tissue models can also lead to biased results and false conclusions. However, these can be reduced by particular technologies that mimic the 3D structure, abundant vasculature, blood–brain barrier (BBB), and cerebrospinal fluid (CSF) of the brain [2]. Organs-on-chips are microengineered platforms that mimic physiological microenvironments and cultured tissues. Until now, the modeled organs-on-chips included the heart, lung, kidney, blood vessels, skin, liver, brain, and pancreas [3,4]. Understanding the mechanisms of CNS functions and causes of diseases also requires systematic platforms capable of mimicking the *in vivo* neuronal environment. Recent progress in microfluidics and microelectromechanical systems (MEMS) has made it possible to develop unique platforms for creating *in vitro* human CNS models that approximate the *in vivo* conditions as far as possible [2]. These technologies have established various *in vitro* CNS disease models of AD, PD, MS, migraine, diffuse axonal injury (DAI), neuronal migration disorders, epilepsy, and NMDAR encephalitis. As shown in Figure 1, the complex *in vivo* environment of the CNS can be established *in vitro* with 2D and 3D neuronal cell cultures on diverse microfluidic platforms, allowing the modeling of critical CNS diseases. The microenvironments and morphology of cells can be controlled by fluidic and patterning technologies. In this review, we identify current fundamental microtechniques that are applicable to the *in vitro* modeling of the CNS and highlight challenges for microplatform-based

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Various microplatforms mimicking *in vivo* microenvironments of central nervous system (CNS) are reviewed.

In vitro CNS disease models, including Alzheimer's disease, Parkinson's disease, and so on, using microplatforms are introduced.

Future directions of *in vitro* CNS disease model based on microplatforms are described, including its application to pathology studies, drug screening, and personalized medicine.

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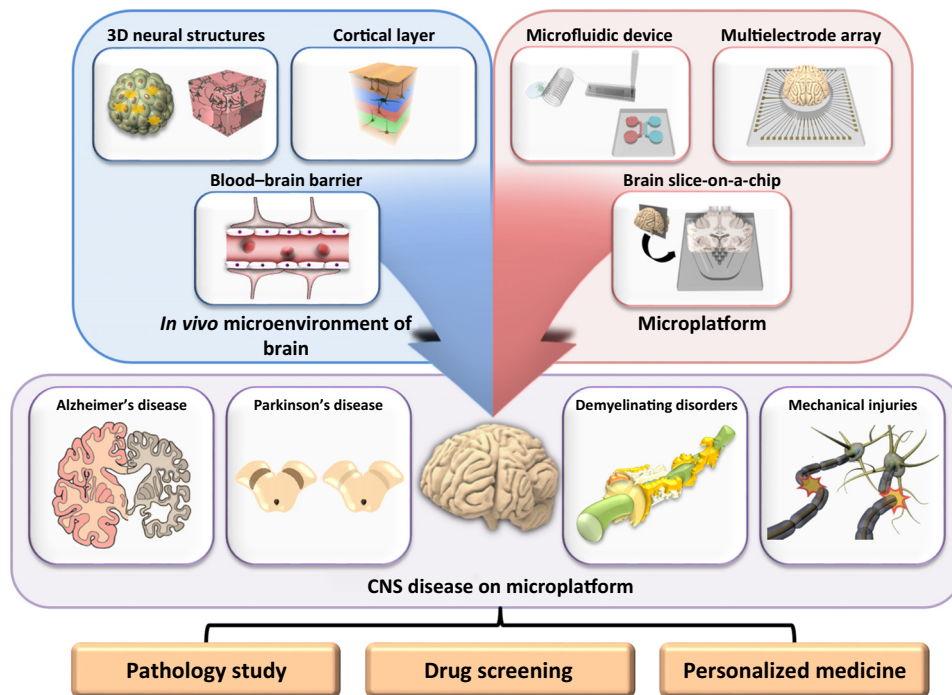
Figure 1. General Conceptions of Central Nervous System (CNS) Disease Models on the Microplatform.

CNS disease models in elucidating underlying mechanisms. Finally, we briefly describe the potential technologies applicable to the CNS and CNS disease models and discuss future perspectives.

Fundamental Techniques Dealing with CNS on Microplatforms

In vitro studies of brain pathophysiology have been performed using controlled cultures of neurons, glial cells, and brain tissues on microplatforms designed to mimic the *in vivo* environment of the CNS as closely as possible. These have several advantages, including flexible control of the microenvironment, single-cell handling, real-time analysis, co-culture, compartmentalized culture, perfusion culture, and long-term culture [5]. Such study models can be categorized as axons, co-cultures of neuronal cells, neuronal networks with directionality, brain slices, and reading neuronal activities via microelectrode arrays (MEAs). Table 1 summarizes the currently applicable techniques for CNS models on microplatforms.

Control of CNS Cells on Microplatforms

Microfluidic platforms have been developed with suitable spatial control of neurons by physical channels that restrict the movement of cell bodies and generation of chemical gradients. The CNS comprises neurons, astrocytes, and microglia, and these cells support one another (e.g., glial cells support neuronal survival) and communicate with the extracellular matrix (ECM). To mimic the *in vivo* situation of CNS and its diseases, the control of physical and chemical cues and the proper ratio of these different cell types are important. Using cellular responses to surface topology and chemical modification, one can control desired neuronal constructions by the *in vivo* mimicking of ECM. For the control of surface topology, diverse patterning techniques, including microcontact printing, soft- and photolithography, laser ablation, and so forth, could be used [6]. Surface-modification methods that use polylysine, laminin, polyethylene glycol, and albumin have been extensively used for the control of cell adhesion and growth [6]. Controlling the cell ratio is critical to mimic the *in vivo* environment of diverse CNS diseases; however, the
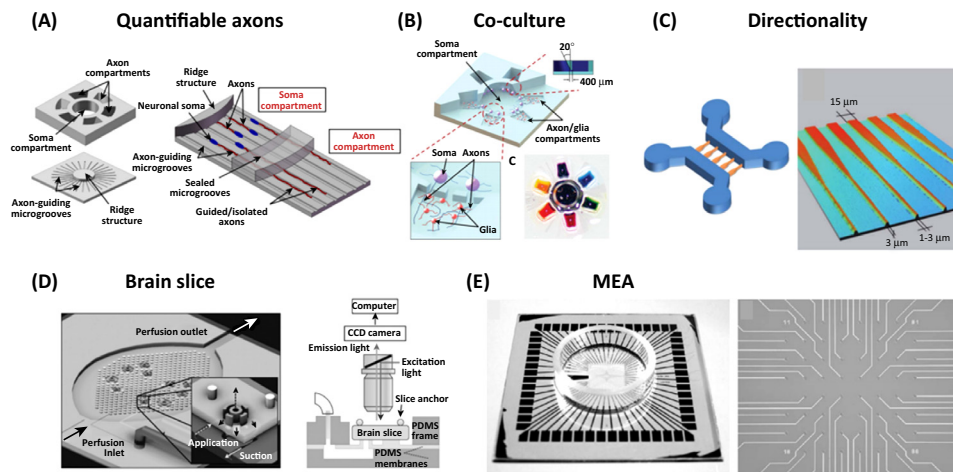
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Table 1. Summary of Applicable Techniques for CNS Models on Microplatforms

Cues	Target	Peculiarity	Refs
Mimic neuronal networks	Axon	Mitochondrial transport	[13]
	Co-culture	Neuron/glia or oligodendrocytes	[21–23]
		Hippocampal CA1/entorhinal cortex	[24]
	Synapse	Synaptic competition	[17]
	Neuronal circuit	Corticostriatal networks	[26]
		Corticothalamic networks coupled to MEAs	[36]
	Brain slice	Focal control of chemicals	[29]
		Functional connections by extending axons through microchannel	[24]
	Cortex layer mimic	Hydrogel	[43]
		Nerve growth factor (NGF)/B27 gradient	[44]
	3D	Concave microwells with osmotic micropump	[48]
		Size-controllable networked neurospheres	[47]
Mimic CNS environments	BBB	Trapping of endothelial cells and co-culture with astrocyte-conditioned media	[56]
		Fluidic shear stress and thin culture membrane	[55]
		Immortalized human brain endothelial cells and fluid shear stress	[54]
		Synthetic microvasculature model of BBB with astrocyte-conditioned media	[57]
	Neurovascular unit	Microporous polycarbonate membrane as vascular channel	[58]
Facilitating inspection and manipulation	Visualization of axons and synapses	Isolated axons without soma or dendrites	[11]
		Multicompartment culture	[10]
		Axonal quantitative analysis	[12]
		Local perfusion chamber for visualizing and manipulating synapses	[15]
		Quantum dot-labeled brain-derived neurotrophic factor	[14]
MEA	mMEA	Neural–electrical and neural–chemical interfaces	[59]
	3D	Layer-by-layer electrode array design to observe 3D neuronal cultures	[60]
	Combined with fluidics	3D microscaffold system	[61]
		Corticothalamic co-cultures in a dual compartment	[38]
		Dual-compartment neurofluidic system with interconnection microchannels	[37]

ratio of each cell type in the CNS is unclear. Generally, neurons and astroglia usually grow to equal densities, mimicking natural conditions, after 1 week in conventional co-culture [7]. However, in the microfluidic co-culture channel, the neuron:glia ratio can be controlled to mimic diverse *in vivo* (normal or abnormal status) ratios and may provide diverse disease models.

Visualized and Quantifiable Axons

The axons of neuronal cells are important for the pathogenesis of neurodegenerative diseases and CNS injuries. Although studies using animal models have provided considerable insights, these models involve multiple parameters and do not allow the real-time monitoring of axon damage or regeneration [8]. Compartmentalized Campenot chambers have been developed for studying axonal behavior *in vitro*, but analyzing the length of randomly grown axons in these

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Figure 2. Fundamental Techniques Dealing with the Central Nervous System (CNS) on Microplatforms. (A) Visualized and quantifiable axons [12]. (B) Co-culturing inside microfluidics [23]. (C) Directionality of neuronal networks [26]. (D) Brain slices on a microfluidic platform [29]. (E) Neuronal activities on microelectrode arrays (MEA) [34].

devices is still a challenge [9]. Microfabrication, microfluidic, and surface-micropatterning techniques were combined to produce a multicompartment neuronal culturing device [10] that facilitated the identification, visualization, and quantification of neurons [11,12], especially axons [13,14] and synapses [15], in an *in vitro* environment. Taylor *et al.* developed a microfluidic device for the compartmental culture of primary CNS neurons that allowed axonal growth and isolation and monitoring of axonal mitochondria [11]. This device is recognized as one of the most useful matrices for neurodegenerative disease research (Figure 2A) [5,8,16–19].

Co-culturing Inside Microfluidics

Co-culture of different CNS cell types in a microfluidic system enables the connection of different chambers and controlled perfusion with one or more fluids. Typical platforms for co-culturing include separated cell culture in two spaces with connections [15,20,21] and combined cell culture in one space [22,23]. They permit an *in vitro* approximation of an *in vivo* extracellular microenvironment and allow us to monitor communication between neuronal cells (Figure 2B). Co-cultures of slices from cortex and hippocampus formed functional connections by extending axons through the microchannels [24], with a controllable microfabricated valve serving as a barrier between the chambers [21]. Perfusion of media from the glia to the neuronal chamber confirmed that glia provided the necessary nutrients for neuronal survival. Synaptic competition and neuronal networks could also be readily investigated [16,17,25]. Furthermore, combined cell culture in one space has usually been used to mimic CNS myelination. The compartmentalized co-culture microplatform enables oligodendrocytes to be placed in the axon and/or glia compartment, and to interact with axons only and not with neuronal somata [22]. Following this, a microsystem mimicking CNS myelination using oligodendrocyte progenitor cells in co-culture with isolated axons was reported [23].

Directionality of Neuronal Networks

All neurons and neuronal networks have directionality that optimizes the speed of processing information in the CNS, and that directionality is critically involved in the degeneration of neuronal networks in the brain. The aforementioned culture models exhibit random neuronal connections, which do not reflect the circuit-to-circuit communication of actual neuronal networks and, therefore, do not truly model the CNS. Notably, fully mature, functional, and properly oriented corticostriatal networks have been reproduced in asymmetric, funnel-shaped microchannels

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(Figure 2C) [26]. This development represents an important step towards reducing the discrepancies between *in vivo* and *in vitro* studies. Although well-defined directional networking of neurons is still challenging, we expect that the rapid progress in microtechnologies will soon enable us to mimic CNS neural circuits as closely as possible.

Brain Slices on Microfluidics

Brain slices are good preparations for obtaining preformed-organized information about the CNS. Microfluidics can provide spatiotemporal control over specific brain slice regions with improved oxygen penetration into the slice [27]. Spatiotemporal information for network signaling can be interpreted by focally perfused chemical stimuli (Figure 2D) [27–29]. Local injection of flow into one portion of a slice [30] and monitoring responses via probes with two [31], four [32], or six [33] hydrodynamically controllable apertures have been demonstrated.

Neuronal Activities on Microelectrode Arrays

MEAs have been used to explore the electrical activity of neuronal cell networks by making extracellular recordings in real time. MEAs are ideal for investigating long-term neuronal networks and do not limit the number of cells from which recordings can be obtained (Figure 2E) [34,35]. MEAs are sometimes bonded to a dual-compartment system to allow long-term coculture of neuronal cells and recording of spontaneous activity from neuronal networks [36–38]. MEAs have been used to demonstrate the propagation of electrical activities between cortical and thalamic regions [36]. Even though there have been several efforts to develop microfluidic devices for brain slices on a MEA, the long-term culture of brain slices is still challenging due to difficulty with decreased viability and expansiveness [27].

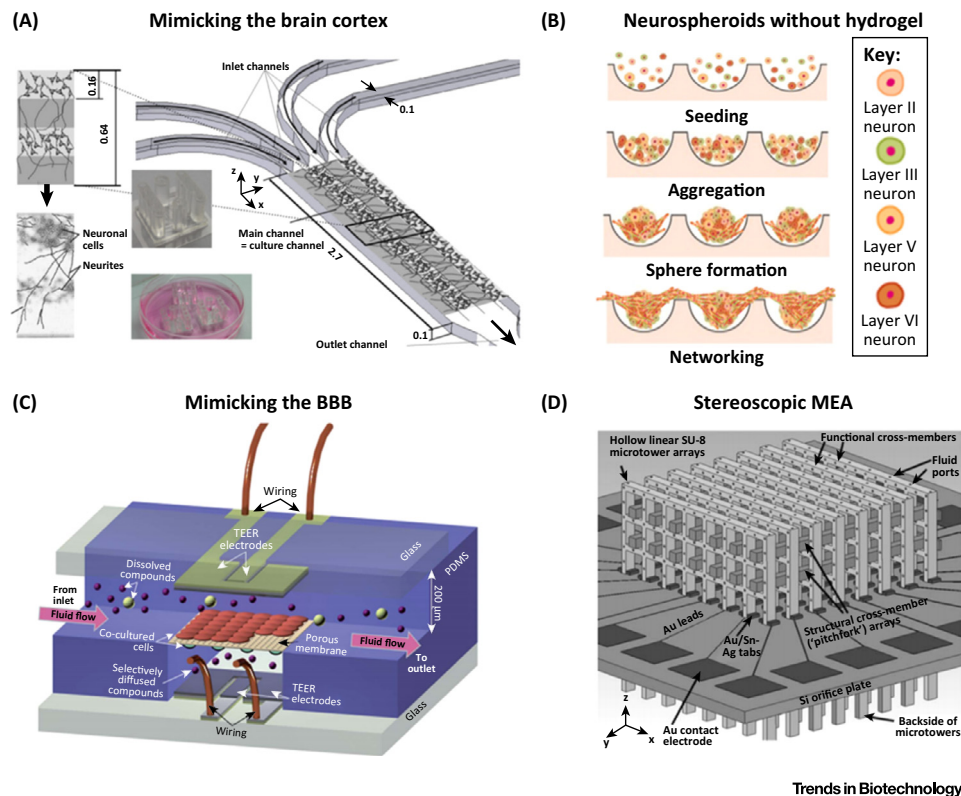### *In Vivo*-Mimicking CNS Models on a Microplatform

Recent progress in microtechnologies has allowed for the construction of a more complicated CNS model that was unattainable with a conventional cell culture system. Here, four microplatform-based models that characterize parts of the CNS are introduced: cortex layer mimics, 3D cultures of neuronal cells, BBB, and stereoscopic MEAs.

Mimicking the Brain Cortex

Construction of a 3D cortex containing all six cortical layers is important for the study of neurodegenerative diseases *in vitro*. For several different cell types, the behavior of cells cultured in a 3D environment is more representative of *in vivo* conditions compared with cells cultured in a monolayer [39]. Given that they more accurately reflect *in vivo* interactions, 3D neural constructs may serve as useful platforms for investigating CNS disease mechanisms [40]. Hydrogel-based microfluidic devices can generate steady, long-term chemical concentration gradients [41,42]. Brain cortex layers can be mimicked (Figure 3A) using neuron-hydrogel layers alternated with neuron-free hydrogel layers [43] and different synaptic densities in the cerebral cortex with linear chemical gradients of nerve growth factor (NGF)/B27 [44].

Neurospheroids without Hydrogels

A recently developed 3D CNS model that does not use hydrogels has attracted considerable attention because it better mimics the *in vivo* environment (Figure 3B). Although hydrogels have been used as scaffolds for 3D cell cultures in many applications, they have some limitations, such as limited nutrient transport and reduced cell viability [45]. Gel-free neurospheroids [46,47], constructed by harvesting cells from all six cortical layers of a rat and seeding them in arrayed concave microwells, were used to analyze the effects of oligomeric amyloid beta (Aβ) on networked neurospheres. These neurospheres were exposed to perfused culture media delivered at a very low flow rate, comparable to that of interstitial flow in the brain, by an osmotic micropump [48]. This system was used to analyze the effect of perfused oligomeric Aβ on networked neurospheres. Jeong *et al.* also developed a concave-well, hemicylindrical-channel-

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Figure 3. *In Vivo*-Mimicking of Central Nervous System (CNS) Models on a Microplatform. (A) Mimicking the brain cortex [43]. (B) Neurospheroids without using hydrogels [47]. (C) Mimicking the blood brain barrier [55]. (D) Stereoscopic microelectrode arrays (MEA) [61].

network applicable to CNS regeneration [49,50]. Takeuchi *et al.* showed that neurospheroids have potential clinical applications, such as neural transplantation therapy. They created a pre-made *in vivo* neural network in a polydimethylsiloxane (PDMS) microchamber array and transplanted it onto host cortical tissue [51].

Mimicking the Blood–Brain Barrier

The physicochemical properties of the BBB are essential for maintaining a CNS microenvironment that supports reliable neuronal activity [52]. Disruption of the BBB has been implicated in multiple neurodegenerative diseases [53]. The smallest BBB model using immortalized human brain endothelial cells had the barrier dysfunction typical of neurodegenerative disease when exposed to fluid shear stress and tumor necrosis factor (TNF)-∝ [54]. A dynamic, thin-membrane *in vitro* BBB model that includes multilayered polymers has been developed [55]. A porous polycarbonate membrane is laid down between two PDMS layers that contain channels and culture chambers to separate the two compartments and allow dynamic culture (Figure 3C). Another BBB model contains a microhole structure for trapping human umbilical vein endothelial cells with astrocyte-conditioned medium [56]. A synthetic microvasculature model of the BBB (SyM-BBB) provides the ability to simultaneously visualize both the vascular and neuronal compartments in real time [57]. This model was further improved by adding neurons [58]. The improved microplatform comprised a vertically stacked neural parenchymal chamber separated by a vascular channel via a microporous polycarbonate membrane. These models are not yet widely applied to CNS disease models, but they could be used specifically for assaying the toxicity and metabolism of CNS drugs and comparing the *in vitro* and *in vivo* outcomes of traumatic injuries to the neurovascular unit.

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Stereoscopic Microelectrode Arrays

Refined MEAs that more closely mimic *in vivo* CNS models have recently been developed. These include multifunctional MEA (mMEA) and 3D MEA. A mMEA with added dopamine-sensing functionality permits both neural-electrical and neural-chemical recordings in a single chip [59]. Two different 3D MEAs were developed for recording electrical activity from more *in vivo*-like brain models. One comprised three repeated PDMS fluidic layers and two microelectrodes, and the other had hollow SU-8 microtowers encased in a PDMS fluid manifold. Both offer multiple electrode contacts and perfusion ports for long-term neuronal cell maintenance. In 3D MEAs, acquisition of true 3D connectivity information is possible (Figure 3D) [60,61].

Neurodegenerative Disease Models

Dysfunction or destruction of neurons and glial cells is the basic underlying concept in CNS diseases. CNS models that represent neurodegenerative diseases usually require long-term culture duration and controllable fluid delivery. Accumulation of abnormal protein aggregates is an important feature of neurodegenerative diseases, as are the mutual influences of healthy and diseased neurons. Heretofore, microplatforms have included compartmented microchannel [12,18,19,62–64], co-culture systems [16,25,65], chemotaxis and gradient-forming systems [66–69], and systems for real-time monitoring of neural activity [68].
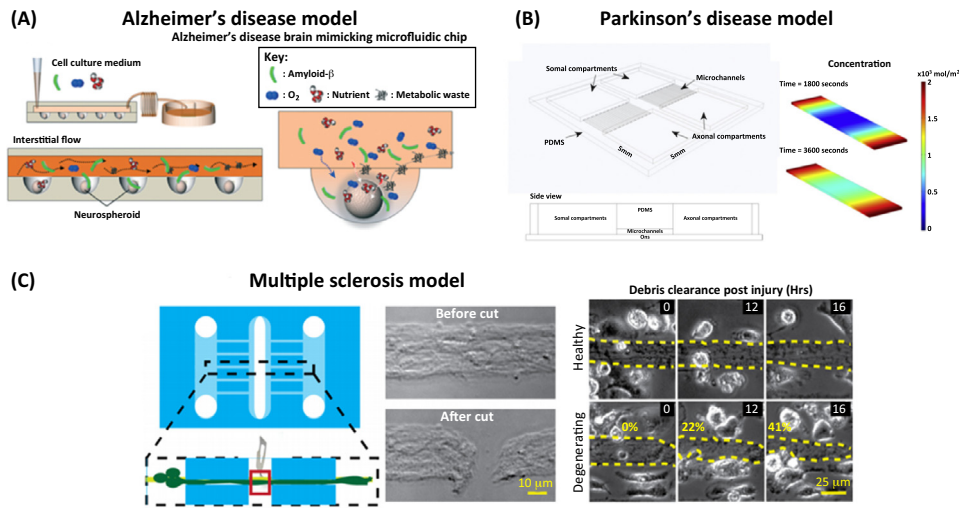
Alzheimer's Disease Models

Extensions of Aβ and tau proteins might contribute to synaptic dysfunction in AD [70]. The aspects of AD modeled in microplatforms include propagation of Aβ or tau protein through neuronal networks and axonal transfer [16,19,62,71,72], Aβ and tau protein toxicity [18,25,67,68], glial cell function [20,65], and the influence of synapses [64].

Aβ accumulation in the CNS may be caused by an imbalance between Aβ production and clearance. Several recent studies investigated the transmission of Aβ using advanced micro-fluidic devices, demonstrating Aβ spreading through neuronal connections (Figure 4A) [62], the effects of Aβ stress on neuronal networks [16], and the chemotaxis of microglia in response to various conditions of Aβ [66]. Deleglise *et al.* reconstructed a directionally oriented cortico-hippocampal network and showed that somatodendritic deposits of Aβ on cortical neurons trigger a rapid cortical presynaptic disconnection before any axonal or somatic cortical degeneration [16]. This represents the early pathway in AD. Aβ assemblies consequently cause synaptic dysfunction by disrupting both neurotransmitter and neurotrophin signaling. Neurons from AD transgenic mice show reduced retrograde axonal transport of BDNF, which is essential for synaptic function, plasticity, and neuronal survival [18].

Neuron-to-cell spread of wild-type tau was visualized in a microfluidic device containing micro-channel chambers [72]. Hyperphosphorylated tau protein may result from an imbalance of tau kinase and phosphatase activities in the affected neurons [73]. Natalia *et al*. reported that the hyperphosphorylation of tau protein and formation of intraneuronal neurofibrillary tangles represent additional neuropathological hallmarks of the AD brain [74]. Using okadaic acid, Kunze *et al.* controlled the generation of two different phosphorylation states between connected neuronal cell compartments in a microfluidic device [66]. Of the six types of tau protein, 3-repeat and 4-repeat tau proteins are considered the main pathological entities. Utilizing a compartmented microfluidic device containing a microchannel, Stoothoff *et al.* found that 3-repeat and 4-repeat tau proteins caused different alterations in retrograde and anterograde transport, with the 3-repeat having a slightly stronger effect on axon transport dynamics [19].

Excessive GABA release from reactive astrocytes is also involved in AD memory decline [75]. Bianco *et al.* compared glial functions in the cortex and hippocampus after exposure to amyloid β fibrils through overflow microfluidic networks [20]. Hippocampal, but not cortical, astrocytes

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Figure 4. Neurodegenerative Disease Models on Microplatforms. (A) Alzheimer's disease model [48]. (B) Parkinson's disease model [13]. (C) Multiple sclerosis model [78].

have a detrimental role on neurons and this can provide one of the proofs of the mechanism of memory decline in AD.

Parkinson's Disease Model

α-Synuclein is the major component of Lewy bodies, the filamentous inclusions characteristic of PD. Severe loss of nigrostriatal dopaminergic fibers is the most consistent and specific lesion in PD [76]. Studies of mitochondrial transport along single axons are difficult with traditional dissociated culture systems. A new microplatform created by Xi Lu *et al.* now makes it possible to monitor the transport of mitochondria along single dopaminergic axons (Figure 4B) [13].

Multiple Sclerosis Model

In neurodegenerative disease, it is important to understand the repair processes as well as the destructive processes. MS is an apt disease for the investigation of repair processes. Microglial cells have a major role in pathogen defense phagocytosis and inflammatory responses [77]. A microfluidic axon–microglia co-culture platform, created by Hosmane *et al.*, demonstrated a Toll/interleukin-1 receptor domain-containing adapter inducing interferon-β (TRIF)-dependent microglial clearance of unmyelinated axon debris (Figure 4C) [78]. Until now, MS treatment has focused only on relapsing-remitting states with limited efficiency. Microplatforms could support the development of real solutions by identifying real-time disease microenvironmental progress.

Migraine

Cortical spreading depression (CSD), thought to be the important mechanism of migraine aura, is a wave of depolarization followed by suppression, usually in the visual cortex. Spatiotemporal information about such phenomena can be better interpreted when chemical stimuli are focally applied and the information passes to the surrounding neurons only via network signaling. Tang *et al*. developed CSD models with a microfluidic device that provided precise focal control of chemical stimuli in brain slices [28,29]. A laminar flow of oxygenated artificial CSF was maintained throughout the dense post array. These experiments demonstrated that CSD was inducible under a range of conditions, including those likely to be encountered in brain injury and during certain awake states, such as migraine. This finding could be used efficiently in disease modeling and drug screening of partial epilepsy as well as migraine.

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Diffuse Axonal Injury

DAI is characterized by swollen and disconnected axons with multiple spheroids appearing on individual axons [8]. DAI-induced mechanoporation of the plasma membrane can trigger axon blebbing and focal microtubule disruption [79]. Reduction of cell inflammation immediately after DAI may be necessary to improve regeneration and cognitive recovery. The development of appropriate treatments requires accurate models that can simulate an applied injury. Multiple such models were developed on microfluidic platforms, with CNS injury being reproduced by mechanical methods such as stretch [80,81], compression [82], shear force [83], and laser [84].

An axonal stretch-injury model recapitulated immediate microtubule breakage in axons and progressive microtubule loss [80]. A uniaxial strain injury model offers the advantage of making functional connections between two slices and allows real-time and long-term observation of injury responses [81]. A valve-based microfluidic axonal injury microcompression platform also permits observation of axonal deformation before, during, and immediately after focal mechanical injury [82]. All of these models enable observation of neuronal responses to injuries of varying degrees. One recent study monitored intracellular Ca^{2+} levels in cultured astrocytes after application of shear forces [83]. The mechanically induced Ca^{2+} influx commonly associated with neuron models of traumatic brain injury also occurred in astrocytes. Another study attempted to integrate the use of pulsed laser microbeams and microfluidic cultures [84]. The authors showed that laser microbeam dissection within a microfluidic platform produced more precise zones of neuronal injury than was possible with other methods.

Regenerative Experiments in DAI Models

Unlike the peripheral nervous system (PNS), the CNS microenvironment inhibits neuronal regeneration. Although the CNS has microglia, these cells do not aid in debris clearance to the same extent as Schwann cells in the PNS [85]. In the CNS, upregulation and accumulation of compounds such as chondroitin sulfate proteoglycans (CSPGs) [86] and myelin-associated inhibitors [myelin-associated glycoprotein (MAG), Nogo, and oligodendrocyte-myelin glycoprotein (OMgp)] [87] are the major contributors to debris clearance. Recent studies revealed that targeting a particular group of extracellular inhibitory factors is not sufficient to trigger long-distance axon regeneration. Instead of antagonizing a growing list of such factors, tackling a common target that mediates axon growth inhibition offers a more attractive strategy for promoting axon regeneration. In this vein, Hur *et al*. examined the effect of pharmacological inhibition of nonmuscle myosin II (NMII) in a two-compartment microchannel chamber. NMII inhibition leads to the reorganization of both actin and microtubules in the growth cone, allowing rapid axon extension over inhibitory substrates [88].

Neurogenesis Model

Mechanical compression injury can also be adapted to a neuronal migration model. Using a neurosphere culture in a stretchable microfluidic device, Esfandiari *et al*. showed that mechanical compression of neural stem cells could be a factor in accelerating the formation of radial glia [89]. Given that this microsystem has a clear advantage with respect to applying well-controlled and small mechanical forces, such as compressive, stretching, and shear forces, extensive studies of CNS disease models relevant to mechanical forces could be possible in the future.

Epilepsy

Epilepsy is a disorder characterized by excessive synchronized neural activity. Generally, MEAs are more useful for recording large numbers of points compared with conventional extracellular electrode recording techniques, and brain slices are often used as *in vitro* epilepsy models because of the complexity of the underlying propagation properties [90]. Hill *et al*. made a status epilepticus model of hippocampal slices on MEAs and validated the burst activities by using anticonvulsants [71]. To clearly determine seizure onset in frontal lobe epilepsy, Chang *et al*.

studied spatiotemporal changes in epileptiform activity recorded by a MEA in a thalamic-anterior cingulate cortex (ACC) slice. They found that thalamic inputs modulated the duration of ACC seizure activities and the depth of seizure onset locations in brain cortex layers [91]. Recently, an *in vitro* autosomal-dominant nocturnal frontal lobe epilepsy model, using MEA and cells cultured from transgenic mice expressing β2-V287L, helped to determine the role of β2-V287L in synaptic formation [92].

N-Methyl-D-Aspartate Receptor Encephalitis
MEA also provided the basic technology for detecting neuronal activity in the primary rat cortex after exposure *in vitro* to CSF from a patient with NMDAR encephalitis [93]. This could represent a new technique for evaluating the functional consequences of autoimmune encephalitis-related changes in CSF.

Future Directions and Further Applications
To date, most microplatform-based CNS disease model applications have mainly sought to clarify known or suspected mechanisms or verify isolated observations. Moreover, no single device can yet fully recreate the *in vivo* CNS environment. The ultimate goal of microplatform-based CNS disease models is to elucidate specific mechanisms that previously existed only as assumptions and to identify treatment strategies that have not yet been imagined. Thus, it matters that these systems are able to closely mimic the human CNS. In the future, advances in 3D structural microplatforms and personalized CNS disease models will hopefully make it possible to accomplish these goals, with the aid of newer microplatform designs and improved foundation technologies, and in combination with other technologies.

3D Structural Modifications in CNS Disease Models
3D models are more likely to reproduce an *in vivo*-like environment, recapitulating the complexity of directional growth and neuronal connections. Furthermore, since CNS diseases often differentially affect different areas of the brain, models that more closely mimic the compartmentalization of the human brain are needed. A diverse variety of 3D culture substrate materials can be incorporated into microfluidic chips. As previously mentioned, 3D micropatterned hydrogels [44] and 3D networks without hydrogels [48,51] have been successfully investigated. However, since the brain is highly vascularized, reconstructing a genuine brain-like structure is not possible with previous neuronal networks or by mimicking the BBB. A possible solution to this limitation is 3D bioprinting, which is the process of creating 3D functional tissues that mimic organs by using viable cells and supportive hydrogels through several bioprinters, such as inkjet, microextrusion, and laser-assisted bioprinters [94]. Although current studies generally focused on making tissues such as skin, blood vessels, trachea, kidney, and peripheral nerves, only one study adapted it to build neurons and astrocytes in a 3D multilayered collagen gel [95]. However, this study did not include vasculatures, so further studies attempted to create vascular structures of human umbilical vein endothelial cells with rat fibroblasts [96] or human embryonic renal cells [97]. Proper applications of this promising technology to CNS disease models will undoubtedly bring innovative changes.

Inducible Pluripotent Stem Cells on Microplatforms
CNS disease models on microplatforms will soon include stem cell or inducible pluripotent stem cell (iPSC) cultures. A few studies have used human-derived components, such as neural stem cells (NSCs), embryonic stem cells (ESCs) [98–100], and iPSCs. However, of these, iPSCs are the most promising source because of their unique characteristics, such their individualization to each patient and exemption from the ethical problems associated with other embryonic cell sources. The genetic background of the iPSC donor in the disease model can be used in *in vitro* clinical trials. This will overcome the limitations of modeling genetic neurological deficiencies *in vitro*. Individual patient-derived neuronal networks that mimic specific functional units in the brain could be measured by MEA, assisting in diagnosis and treatment decisions.

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Further Applications

The proposed CNS disease model could be used for the discovery of disease mechanisms, drug screening, and personalized medicine, and we ultimately expect that this model will replace animal models. Well-organized CNS disease models on microplatforms might be the answer to overcoming the ethical issues surrounding animal experiments. Although the proposed model is simple, compared with an intact animal model, it can better provide a regulated environment than an animal model can, permitting a clearer analysis of the role of specific molecules in causing disease and as possible targets of therapeutic agents. If a more refined CNS disease model could be realized on a microplatform, it would make drug discovery faster and cheaper compared with conventional processes, profoundly affecting the pharmaceutical industry. Combined with genetics and iPSC techniques, it would be possible to make chip-based personalized diagnoses, checks of disease progress, and treatment, which would represent a major medical contribution. This model could also be used for the screening of food, cosmetics, and other potential environmental toxins.

Concluding Remarks

Recent developments in microplatform technology have given rise to new strategies that are expected to generate advances in *in vitro* models of CNS pathophysiology. Here, we introduced basic design principles and various microplatforms for CNS disease models (Table 2). Basic technologies that have commonly been applied to CNS disease models include compartmented microchannel chambers, co-cultures, and MEAs. CNS disease models on microplatforms have great prospects for several reasons. First, it is possible to adjust the variables in the CNS environment within them. Second, they could be the standard tools of measurement for CNS disease progression. Third, continuous and long-term observation can give a better picture about CNS pathophysiology. To date, *in vitro* models still only partially mimic the CNS environment, and we expect that improvements in microtechnologies will enable CNS complexities, such as spatial 3D architecture and physiological stimuli, to be properly modeled. In the near future, we expect new research into microplatform-based CNS disease models to extend to 3D bioprinting and iPSCs on the microplatform (see Outstanding Questions). We further anticipate extension of their applications to the screening of drugs, food, cosmetics, and other toxic materials, the discovery of disease mechanisms, and the diagnosis and treatment of disease.

Outstanding Questions

Can the central nervous system (CNS) be built on a chip?

What is the role of stem cells in developing a 'CNS' model on a chip?

Can the six layers of cortex and their functions be realized on a chip?

Table 2. Summary of CNS Disease Models on Microplatforms

Disease	Pathology	Details	Comments	Device used	Refs
AD	Aβ	Neuronal network/ axonal transfer	Corticohippocampal pathway	Multichamber	[16]
			Transneural transmission	Compartmented microchannel	[62]
			Motility of microglia responding to various types of Aβ in a regulated manner	Microfluidic chemotaxis	[66]
		Aβ toxicity	Quantitatively analyzing effect of oligomeric Aβ on neurons	Microfluidic gradient	[67]
			Aβ oligomers failed TrkB processing	Compartmented microchannel with time lapse	[18]
	Tau	Transfer through synapse	Lentiviral rat model of hippocampal NFD	Microchannel chamber	[72]
			Mitochondrial trafficking in primary neuronal axons	Compartmented microchannel	[19]

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Table 2. (continued)

Disease	Pathology	Details	Comments	Device used	Refs
		Comparison of 3-repeat tau and 4-repeat tau			
		Gradient of okadaic acid in taunopathies	Okadaic acid induces hyperphosphorylation of tau proteins	Co-pathological neural cell culture	[25]
		Hyperphosphorylation	Monitor retrograde neurite degeneration in hippocampal slice	MEA	[68]
	Axonal	Axonal excitotoxicity	Glutamate excitotoxicity	Compartmented microchannel	[63]
	Glia	Role of astrocytes	Hippocampal neuronal damage by soluble mediators of hippocampal astrocytes, which were exposed to Aβ + interleukin (IL)-1β	Overflow microfluidic networks	[20,65]
	Synapse	Synaptoprotective drugs evaluation	NAD$^+$ and Rho-kinase inhibitor Y27632 prevent synaptic disconnection	Compartmented microchannel with a central accessible channel	[64]
PD	Axonal transport	Mitochondrial transport defects of dopaminergic axons	Neurons from transgenic mice	Open compartmented microchannel	[13]
MS	Axonal degeneration	Molecular control of microglial phagocytosis of degenerating axons	TRIF blocks induction of interferon response and inhibits microglial phagocytosis of axon debris	Axon–microglia co-culture microfluidic	[78]
Epilepsy	Status epilepticus	Application of 4-aminopyridine, removal of Mg^{2+} ions	Confirm antiepileptiform drug effects by recording ret hippocampal slice surface	MEA	[71]
	ADNFLE	*In vitro* spontaneous hyperexcitability	Displayed by primary neocortical neurons from transgenic mice (β2-V287L)	MEA	[92]
	ACC epilepsy	Thalamic inputs in thalamic-ACC slice	Repeated stimulation of thalamus inhibited epileptiform activity	MEA	[91]
NMDAR encephalitis	NMDAR binding antibodies	Effects of patient CSF on *in vitro* neuronal network	Served a method of CSF specimen investigation from patients with antibody-negative NMDAR encephalitis	MEA	[93]
Migraine	Cortical spreading depression	A mouse brain slide model	Focal stimulation	Perfusion chamber with fluid injection ports	[28,29]
DAI/traumatic brain injury	Stretch injury	Dynamic stretch injury of axons	Dynamic stretch ruptures axonal microtubules at specific points, triggering depolymerization of microtubules	Controlled air pulses on micropatterned channels	[80]
		Uniaxial strain	Observed in real time and over long time	Two layers of PDMS with microchannels	[81]

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References

1. McNamara, J. (2012) *Brain Facts: A Primer on the Brain and Nervous System.* (7th edn), Society for Neuroscience
2. van der Meer, A.D. and van den Berg, A. (2012) Organs-on-chips: breaking the in vitro impasse. *Integr. Biol. (Camb.)* 4, 461–470
3. Lee, S.A. *et al.* (2013) Spheroid-based three-dimensional liver-on-a-chip to investigate hepatocyte-hepatic stellate cell interactions and flow effects. *Lab Chip* 13, 3529–3537
4. Huh, D. *et al.* (2010) Reconstituting organ-level lung functions on a chip. *Science* 328, 1662–1668
5. Whitesides, G.M. (2006) The origins and the future of microfluidics. *Nature* 442, 368–373
6. Wheeler, B.C. and Brewer, G.J. (2010) Designing neural networks in culture: experiments are described for controlled growth, of nerve cells taken from rats, in predesigned geometrical patterns on laboratory culture dishes. *Proc. IEEE Inst. Electr. Electron. Eng.* 98, 398–406
7. Nam, Y. *et al.* (2007) Development of astroglial cells in patterned neuronal cultures. *J. Biomater. Sci. Polym. Ed.* 18, 1091–1100
8. Siddique, R. and Thakor, N. (2014) Investigation of nerve injury through microfluidic devices. *J. R. Soc. Interface* 11, 20130676
9. Campenot, R.B. (1977) Local control of neurite development by nerve growth factor. *Proc. Natl. Acad. Sci. U.S.A.* 74, 4516–4519
10. Park, J.W. *et al.* (2006) Microfluidic culture platform for neuroscience research. *Nat. Protoc.* 1, 2128–2136
11. Taylor, A.M. *et al.* (2005) A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat. Methods* 2, 599–605
12. Park, J. *et al.* (2014) A microchip for quantitative analysis of CNS axon growth under localized biomolecular treatments. *J. Neurosci. Methods* 221, 166–174
13. Lu, X. *et al.* (2012) A microdevice platform for visualizing mitochondrial transport in aligned dopaminergic axons. *J. Neurosci. Methods* 209, 35–39
14. Zhao, X. *et al.* (2014) Real-time imaging of axonal transport of quantum dot-labeled BDNF in primary neurons. *J. Vis. Exp.* 51899
15. Taylor, A.M. *et al.* (2010) Microfluidic local perfusion chambers for the visualization and manipulation of synapses. *Neuron* 66, 57–68
16. Deleglise, B. *et al.* (2014) β-amyloid induces a dying-back process and remote trans-synaptic alterations in a microfluidic–based reconstructed neuronal network. *Acta Neuropathol. Commun.* 2, 145
17. Coquinco, A. *et al.* (2014) A microfluidic based in vitro model of synaptic competition. *Mol. Cell. Neurosci.* 60, 43–52
18. Poon, W.W. *et al.* (2011) beta-Amyloid impairs axonal BDNF retrograde trafficking. *Neurobiol. Aging* 32, 821–833
19. Stoothoff, W. *et al.* (2009) Differential effect of three-repeat and four-repeat tau on mitochondrial axonal transport. *J. Neurochem.* 111, 417–427
20. Bianco, F. *et al.* (2012) Overflow microfluidic networks: application to the biochemical analysis of brain cell interactions in complex neuroinflammatory scenarios. *Anal. Chem.* 84, 9833–9840
21. Majumdar, D. *et al.* (2011) Co-culture of neurons and glia in a novel microfluidic platform. *J. Neurosci. Methods* 196, 38–44
22. Park, J. *et al.* (2009) Microfluidic compartmentalized co-culture platform for CNS axon myelination research. *Biomed. Microdevices* 11, 1145–1153
23. Park, J. *et al.* (2012) Multi-compartment neuron-glia co-culture platform for localized CNS axon-glia interaction study. *Lab Chip* 12, 3296–3304
24. Berdichevsky, Y. *et al.* (2010) Building and manipulating neural pathways with microfluidics. *Lab Chip* 10, 999–1004
25. Kunze, A. *et al.* (2011) Co-pathological connected primary neurons in a microfluidic device for Alzheimer studies. *Biotechnol. Bioeng.* 108, 2241–2245
26. Peyrin, J.M. *et al.* (2011) Axon diodes for the reconstruction of oriented neuronal networks in microfluidic chambers. *Lab Chip* 11, 3663–3673
27. Huang, Y. *et al.* (2012) Brain slice on a chip: opportunities and challenges of applying microfluidic technology to intact tissues. *Lab Chip* 12, 2103–2117
28. Tang, Y.T. *et al.* (2011) Development and characterization of a microfluidic chamber incorporating fluid ports with active suction for localized chemical stimulation of brain slices. *Lab Chip* 11, 2247–2254
29. Tang, Y.T. *et al.* (2014) Minimum conditions for the induction of cortical spreading depression in brain slices. *J. Neurophysiol.* 112, 2572–2579
30. Blake, A.J. *et al.* (2007) Multilayer PDMS microfluidic chamber for controlling brain slice microenvironment. *Lab Chip* 7, 842–849
31. Christ, K.V. and Turner, K.T. (2011) Design of hydrodynamically confined microfluidics: controlling flow envelope and pressure. *Lab Chip* 11, 1491–1501
32. Qasaimeh, M.A. *et al.* (2011) Microfluidic quadrupole and floating concentration gradient. *Nat. Commun.* 2, 464
33. Queval, A. *et al.* (2010) Chamber and microfluidic probe for microperfusion of organotypic brain slices. *Lab Chip* 10, 326–334
34. Hofmann, F. and Bading, H. (2006) Long term recordings with microelectrode arrays: studies of transcription-dependent neuronal plasticity and axonal regeneration. *J. Physiol. Paris* 99, 125–132
35. Gross, G.W. *et al.* (1997) Neural network cultures on multielectrode chips: self-organization of electrically active networks and their uses in neurotoxicology and neuropharmacology. *Eur. J. Cell Biol.* 74, 36
36. Kanagasabapathi, T.T. *et al.* (2012) Functional connectivity and dynamics of cortical-thalamic networks co-cultured in a dual compartment device. *J. Neural Eng.* 9, 036010
37. Kanagasabapathi, T.T. *et al.* (2011) Dual-compartment neurofluidic system for electrophysiological measurements in physically segregated and functionally connected neuronal cell culture. *Front. Neuroeng.* 4, 13
38. Kanagasabapathi, T.T. *et al.* (2013) Selective pharmacological manipulation of cortical-thalamic co-cultures in a dual-compartment device. *J. Neurosci. Methods* 214, 1–8
39. Schindler, M. *et al.* (2006) Living in three dimensions: 3D nano-structured environments for cell culture and regenerative medicine. *Cell Biochem. Biophys.* 45, 215–227
40. Irons, H.R. *et al.* (2008) Three-dimensional neural constructs: a novel platform for neurophysiological investigation. *J. Neural Eng.* 5, 333–341
41. Wong, A.P. *et al.* (2008) Partitioning microfluidic channels with hydrogel to construct tunable 3-D cellular microenvironments. *Biomaterials* 29, 1853–1861
42. Cheng, S.Y. *et al.* (2007) A hydrogel-based microfluidic device for the studies of directed cell migration. *Lab Chip* 7, 763–769
43. Kunze, A. *et al.* (2011) Micropatterning neural cell cultures in 3D with a multi-layered scaffold. *Biomaterials* 32, 2088–2098
44. Kunze, A. *et al.* (2011) Synergistic NGF/B27 gradients position synapses heterogeneously in 3D micropatterned neural cultures. *PLoS ONE* 6, e26187
45. Li, X.J. *et al.* (2012) Microfluidic 3D cell culture: potential application for tissue-based bioassays. *Bioanalysis* 4, 1509–1525
46. Choi, Y.Y. *et al.* (2010) Controlled-size embryoid body formation in concave microwell arrays. *Biomaterials* 31, 4296–4303

47. Choi, Y.J. *et al.* (2013) Size-controllable networked neurospheres as a 3D neuronal tissue model for Alzheimer's disease studies. *Biomaterials* 34, 2938–2946

48. Park, J. *et al.* (2015) 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* 15, 141–150

49. Jeong, G. (2014) Networked neuro-spheres formed by topological attractants for engineering of 3-dimensional nervous system. *TERM* 11, 297–303

50. Jeong, G.S. *et al.* (2015) Networked neural spheroid by neuro–bundle mimicking nervous system created by topology effect. *Mol. Brain* 8, 17

51. Kato-Negishi, M. *et al.* (2010) A neurospheroid network-stamping method for neural transplantation to the brain. *Biomaterials* 31, 8939–8945

52. Abbott, N.J. *et al.* (2006) Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* 7, 41–53

53. Palmer, A.M. (2011) The role of the blood brain barrier in neuro-degenerative disorders and their treatment. *J. Alzheimers Dis.* 24, 643–656

54. Griep, L.M. *et al.* (2013) BBB on chip: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function. *Biomed. Microdevices* 15, 145–150

55. Booth, R. and Kim, H. (2012) Characterization of a microfluidic in vitro model of the blood-brain barrier (muBBB). *Lab Chip* 12, 1784–1792

56. Yeon, J.H. *et al.* (2012) Reliable permeability assay system in a microfluidic device mimicking cerebral vasculatures. *Biomed. Microdevices* 14, 1141–1148

57. Prabhakarpandian, B. *et al.* (2013) SyM-BBB: a microfluidic blood brain barrier model. *Lab Chip* 13, 1093–1101

58. Achyuta, A.K. *et al.* (2013) A modular approach to create a neurovascular unit-on-a-chip. *Lab Chip* 13, 542–553

59. Chuang, M.C. *et al.* (2013) Multifunctional microelectrode array (mMEA) chip for neural-electrical and neural-chemical interfaces: characterization of comb interdigitated electrode towards dopamine detection. *Biosens. Bioelectron.* 41, 602–607

60. Musick, K. *et al.* (2009) Three-dimensional micro-electrode array for recording dissociated neuronal cultures. *Lab Chip* 9, 2036–2042

61. Rowe, L. *et al.* (2007) Active 3-D microscaffold system with fluid perfusion for culturing in vitro neuronal networks. *Lab Chip* 7, 475–482

62. Song, H.L. *et al.* (2014) beta-Amyloid is transmitted via neuronal connections along axonal membranes. *Ann. Neurol.* 75, 88–97

63. Hosie, K.A. *et al.* (2012) Chronic excitotoxin-induced axon degeneration in a compartmented neuronal culture model. *ASN Neuro* 4, e00076

64. Deleglise, B. *et al.* (2013) Synapto-protective drugs evaluation in reconstructed neuronal network. *PLoS ONE* 8, e71103

65. Hai, A. *et al.* (2010) In-cell recordings by extracellular micro-electrodes. *Nat. Methods* 7, 200–202

66. Cho, H. *et al.* (2013) Microfluidic chemotaxis platform for differentiating the roles of soluble and bound amyloid-beta on microglial accumulation. *Sci. Rep.* 3, 1823

67. Choi, Y.J. *et al.* (2013) Neurotoxic amyloid beta oligomeric assemblies recreated in microfluidic platform with interstitial level of slow flow. *Sci. Rep.* 3, 1921

68. Jahnke, H.G. *et al.* (2012) Impedance spectroscopy based measurement system for quantitative and label-free real-time monitoring of tauopathy in hippocampal slice cultures. *Biosens. Bioelectron.* 32, 250–258

69. Diao, J. *et al.* (2006) A three-channel microfluidic device for generating static linear gradients and its application to the quantitative analysis of bacterial chemotaxis. *Lab Chip* 6, 381–388

70. Grundke-Iqbal, I. *et al.* (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U.S.A.* 83, 4913–4917

71. Hill, A.J. *et al.* (2010) Development of multi-electrode array screening for anticonvulsants in acute rat brain slices. *J. Neurosci. Methods* 185, 246–256

72. Dujardin, S. *et al.* (2014) Neuron-to-neuron wild-type Tau protein transfer through a trans-synaptic mechanism: relevance to sporadic tauopathies. *Acta. Neuropathol. Commun.* 30, 14

73. Gong, C.X. *et al.* (2000) Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain - implications for neurofibrillary degeneration in Alzheimer's disease. *J. Biol. Chem.* 275, 5535–5544

74. Crespo–Biel, N. *et al.* (2012) Protein tau: prime cause of synaptic and neuronal degeneration in Alzheimer's disease. *Int. J. Alzheimers Dis.* 2012, 251426

75. Jo, S. *et al.* (2014) GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's disease. *Nat. Med.* 20, 886–896

76. Lees, A.J. (2009) The Parkinson chimera. *Neurology* 72, S2–S11

77. Napoli, I. and Neumann, H. (2009) Microglial clearance function in health and disease. *Neuroscience* 158, 1030–1038

78. Hosmane, S. *et al.* (2012) Toll/interleukin-1 receptor domain-containing adapter inducing interferon-beta mediates microglial phagocytosis of degenerating axons. *J. Neurosci.* 32, 7745–7757

79. Kilinc, D. *et al.* (2008) Mechanically-induced membrane poration causes axonal beading and localized cytoskeletal damage. *Exp. Neurol.* 212, 422–430

80. Tang-Schomer, M.D. *et al.* (2010) Mechanical breaking of microtubules in axons during dynamic stretch injury underlies delayed elasticity, microtubule disassembly, and axon degeneration. *FASEB J.* 24, 1401–1410

81. Dolle, J.P. *et al.* (2013) An organotypic uniaxial strain model using microfluidics. *Lab Chip* 13, 432–442

82. Hosmane, S. *et al.* (2011) Valve-based microfluidic compression platform: single axon injury and regrowth. *Lab Chip* 11, 3888–3895

83. Maneshi, M.M. *et al.* (2014) A threshold shear force for calcium influx in an astrocyte model of traumatic brain injury. *J. Neurotrauma* 32, 1020–1029

84. Hellman, A.N. *et al.* (2010) Examination of axonal injury and regeneration in micropatterned neuronal culture using pulsed laser microbeam dissection. *Lab Chip* 10, 2083–2092

85. Kim, B.C. *et al.* (2012) Osteoblastic/cementoblastic and neural differentiation of dental stem cells and their applications to tissue engineering and regenerative medicine. *Tissue Eng. Part B: Rev.* 18, 235–244

86. Jones, L.L. *et al.* (2003) The chondroitin sulfate proteoglycans neurocan, brevican, phosphacan, and versican are differentially regulated following spinal cord injury. *Exp. Neurol.* 182, 399–411

87. Domeniconi, M. and Filbin, M.T. (2005) Overcoming inhibitors in myelin to promote axonal regeneration. *J. Neurol. Sci.* 233, 43–47

88. Hur, E.M. *et al.* (2011) Engineering neuronal growth cones to promote axon regeneration over inhibitory molecules. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5057–5062

89. Esfandiari, L. *et al.* (2012) Initial studies of mechanical compression on neurogenesis with neonatal neural stem cells. *Nanomedicine* 8, 415–418

90. Trevelyan, A.J. *et al.* (2007) Feedforward inhibition contributes to the control of epileptiform propagation speed. *J. Neurosci.* 27, 3383–3387

91. Chang, W.P. *et al.* (2011) Spatiotemporal organization and thalamic modulation of seizures in the mouse medial thalamic-anterior cingulate slice. *Epilepsia* 52, 2344–2355

92. Gullo, F. *et al.* (2014) Multi-electrode array study of neuronal cultures expressing nicotinic beta2-V287L subunits, linked to autosomal dominant nocturnal frontal lobe epilepsy. An in vitro model of spontaneous epilepsy. *Front. Neural Circuits* 8, 87

93. Jantzen, S.U. *et al.* (2013) In vitro neuronal network activity in NMDA receptor encephalitis. *BMC Neurosci.* 14, 17

94. Murphy, S.V. and Atala, A. (2014) 3D bioprinting of tissues and organs. *Nat. Biotechnol.* 32, 773–785

95. Lee, W. *et al.* (2009) Three-dimensional bioprinting of rat embryonic neural cells. *Neuroreport* 20, 798–803

96. Kolesky, D.B. *et al.* (2014) 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv. Mater.* 26, 3124–3130

97. Miller, J.S. *et al.* (2012) Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat. Mater.* 11, 768–774

98. Chung, B.G. *et al.* (2005) Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab Chip* 5, 401–406

99. Yla-Outinen, L. *et al.* (2010) Human cell-based micro electrode array platform for studying neurotoxicity. *Front. Neuroeng.* 3, 111

100. Park, J.Y. *et al.* (2009) Differentiation of neural progenitor cells in a microfluidic chip-generated cytokine gradient. *Stem Cells* 27, 2646–2654