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Microfluidic Brain-on-a-Chip: Perspectives for Mimicking Neural System Disorders

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Abstract

Neurodegenerative diseases (NDDs) include more than 600 types of nervous system disorders in humans that impact tens of millions of people worldwide. Estimates by the World Health Organization (WHO) suggest NDDs will increase by nearly 50% by 2030. Hence, development of advanced models for research on NDDs is needed to explore new therapeutic strategies and explore the pathogenesis of these disorders. Different approaches have been deployed in order to investigate nervous system disorders, including two-and three-dimensional (2D and 3D) cell cultures and animal models. However, these models have limitations, such as lacking cellular tension, fluid shear stress, and compression analysis; thus, studying the biochemical effects of therapeutic molecules on the biophysiological interactions of cells, tissues, and organs is problematic. The microfluidic "organ-on-a-chip" is an inexpensive and rapid analytical technology to create an effective tool for manipulation, monitoring, and assessment of cells, and investigating drug discovery, which enables the culture of various cells in a small amount of fluid (10^{-9} to 10^{-18} L). Thus, these chips have the ability to overcome the mentioned restrictions of 2D and 3D cell cultures, as well as animal models. Stem cells (SCs), particularly neural stem cells (NSCs), induced pluripotent stem cells (iPSCs), and embryonic stem cells (ESCs) have the capability to give rise to various neural system cells. Hence, microfluidic organ-on-a-chip and SCs can be used as potential research tools to study the treatment of central nervous system (CNS) and peripheral nervous system (PNS) disorders. Accordingly, in the present review, we discuss the latest progress in microfluidic brain-on-a-chip as a powerful and advanced technology that can be used in basic studies to investigate normal and abnormal functions of the nervous system.

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Keywords

Nervous system; Brain; Neurodegenerative diseases; Microfluidic brain-on-a-chip; Stem cells

Introduction

Investigation into the central nervous system (CNS) is pivotal to develop novel therapies for neurodegenerative diseases (NDDs). NDDs include more than 600 types of nervous system disorders in humans such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS or Lou Gehrig's), multiple sclerosis (MS), and impact tens of millions of people worldwide of which 15 million suffer from AD [1–4]. According to the World Health Organization (WHO), NDDs will increase by nearly 50% by 2030, and more than twofold by 2050. Therefore, there is an essential necessity to establish effective models of NDDs and efficacious therapeutic strategies to inhibit or slow the progression of these disorders [5, 6]. The United States (US) "Brain Research through Advancing Innovative Neurotechnologies" (BRAIN) initiative has proposed to encourage the development of devices to image and assess the function of the brain, and the European Union (EU) Human Brain Project (HBP) has also tried to construct computational models of the brain. These organizations have allocated more than US $1 billion and EU €1 billion to promote BRAIN and HBP, respectively [7]. Moreover, the Defense Advanced Research Projects Agency (DARPA), National Science Foundation (NSF), and National Institutes of Health (NIH) have contributed to fund the BRAIN initiative to further understand the human brain and it's diseases [8].

The Challenges Faced by Current Researches into Neural System Disorders

There are several models of neural systems that have been used to study the physiological and pathological functions of the CNS in NDDs, including animal models, 2D and 3D cell cultures, and clinical studies. However, investigators have to face different challenges that limit the various models. For example, in randomized clinical trials (RCT), adherence to the Consolidated Standards of Reporting Trials (CONSORT) guidelines requires consideration be given to patient diagnosis and selection process, alongside other ethical issues [9]. Animal models are important to study the function of the CNS and NDDs, although they often cannot be generalized to humans. For instance, rodents have a less complex brain structure and different cognitive functions. Some transgenic animal models of human diseases may not provide the physiopathological features of the human neural system and NDDs. As such, significant differences at the molecular and cellular levels exist between rodents and humans [10]. It has been reported that many clinical trials in humans have failed despite promising results that were achieved in pre-clinical studies on animal models [11, 12].

Classical two-dimensional (2D) monolayer cell cultures have been widely utilized to discover therapeutic molecules, including nano-drug delivery, and to predict their side effects [13–15]. However, these models have a poor ability to assess drug responses in complex diseases, and they cannot fully simulate the physiological condition of tissue

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architecture and the microenvironment as they are altered during the disease (Table 1) [17–19].

Three-dimensional (3D) models comprising bioprinted organ cell cultures, self-organized spherical organoids, and microfabricated organ-on-a-chip systems are used to assess drug delivery, drug discovery, and toxicity in neural systems for therapeutic purposes [20–24]. Organoids are one of the most important 3D culture techniques, which by utilizing self-regenerating SCs enable recapitulating the normal function of organs such as breast, liver, stomach, eye, kidney, lung, gut, pancreas, prostate, and specially the brain, which is the most complex organ in animals and humans [25–27]. Nevertheless, these 3D cell culture models can be very variable structures, and it is hard to predict the exact positions of different neural cells for evaluating the biological activities of neural networks [28, 29]. Furthermore, the genetic and biochemical assessment of the seeded cells in the 3D cell culture models such as the production, entrapment, absorption, secretion, and trans- and intracellular transportation of a vast range of molecules is difficult. In addition, these models are generally unable to account for cellular tension, fluid shear stress, and compression analysis. As well, they are unable to recapitulate the normal microenvironment and tissue architecture including blood supply and circulation of immune cells, and also tissue-tissue interaction between parenchymal cells, connective tissue, and vascular endothelium that are pivotal to organ function in health and disease [30, 31]. A large number of molecules and genes govern cell expansion, tissue regeneration, and organogenesis under the influence of mechanobiological stimuli and epigenetic regulator elements [32, 33]. It is essential to establish a model that recapitulates the in vivo structure of diseases to understand the causes and mechanisms of these illnesses and enables the discovery of new therapeutic strategies for NDDs [34–36]. Although in vivo models have many advantages to understand disease mechanisms, they have some disadvantages like being costly, time consuming, and exhibit uncertain translation of their results to humans. Therefore, in vitro models have attracted a lot of attention due to lower costs, time saving, and the greater simplicity of experiments. Recently, 3D cell culture in vitro models, which use human brain cells, have been developed to study the structure and physiological properties of CNS diseases in greater detail [37]. Recent progress suggests that organoids can be used as a preclinical model of human diseases, including NDDs [38].

As mentioned above, there are several limitations to the current models for research on neural systems. Hence, advanced models are needed to overcome these limitations and improve experimental models. As a result, investigators have put forward human organ-on-a-chip devices to substitute for current models as a safe and applicable strategy for drug discovery (Fig. 1).

Microfluidic Technology

Microfluidic systems can create a powerful microengineered scaffold-free or scaffold-based tool for the manipulation, monitoring, and assessment of cells, and for use in drug discovery with high specificity and resolution [39–46]. This technology was developed in the early 1990s as a very small-scale vehicle, which was usually constructed of microchambers, microchannels, and functionalized microdomains whose dimensions range from tens to hundreds of micrometers, used for culturing various cells in a small 10^{-9} to 10^{-18} L volume

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of fluid, that is constantly under flow conditions to evaluate the function of cells, tissues, and organs (Fig. 2) [39, 44, 47]. Currently, a vast range of microfluidic systems have been fabricated using various materials and methods. These systems are able to be applied in the manufacture of microfluidic brain-on-a-chip devices for brain activities evaluation. Microfluidic chips are usually fabricated by "soft lithography, photolithography, contact printing, laser patterning, and 3D printing" techniques using several materials such as polydimethylsi-loxane (PDMS), polycarbonate (PC), polyetherimide (PEI), silicon, glass, hyaluronic acid, matrigel, collagen, silk protein, agarose, etc. [48, 49]. Although the most commonly used material for microfluidic device is PDMS, there are several other potential candidate materials that can be used for microfluidic devices (Table 2). Microfluidic brain-on-a-chip systems can be manufactured using a combination of soft lithography and photolithography as well as 3D printing technologies. These microfluidic brain-on-a-chip systems have used an optically transparent, highly flexible, nontoxic, and air-permeable polymer called PDMS, which provides the ability for high-resolution optical imaging [44, 47, 51–54]. More detailed discussion of the production methods for various microfluidic brain-on-a-chip systems can be obtained elsewhere [51] and are summarized in Table 3. In comparison with 2D cell cultures and 3D organoids, and also animal models, microfluidic systems consume only a very small amount of samples and materials. They also have the ability to allow detection of cell, tissue, and organ activities in an inexpensive, rapid, highly accurate, and precise analytical method [45, 55, 56]. Moreover, microfluidic brain-on-a-chip systems can potentially improve the drug evaluation process in an in vitro environment. Selected human-derived nerve cells are able to be proliferated, differentiated, and organized in brain-on-a-chip models [57]. Moreover, these platforms are able to create a uniform profile of controlled flow of nutrients, establish individual cellular activity, and provide a platform for monitoring and excitation of neuronal cells. In addition, this technology can allow the study of mechanical, physiological, pharmacological, and biochemical aspects, such as dynamic scaffold alteration and the occurrence of stress seen when nervous diseases undergo progression, and treatments for these diseases are able to be investigated and monitored in real time [37, 58, 59].

The so-called homo chippiens was conceived as a laboratory-on-a-chip (LOC) project to emulate the functions of the entire human body by a collection of different microfluidic organ-on-a-chip systems [60]. The US National Center for Advancing Translational Sciences (NCATS) has also awarded grants to construct synthetic tissues and organs for drug development. The reliability of microfluidic brain-on-a-chip systems can be improved by selecting human-derived nervous cells, and can be more appropriate than conventional animal models used for drug assessment and disease monitoring. The US Food and Drug Administration (FDA) has proposed to encourage this technology to predict results of novel drug testing before they are tested in animal models and in human clinical trials [57, 61–63].

Hence, microfluidic systems have been developed to improve research into NDDs in vitro. In this regard, several studies have been performed based on microfluidic chips for analysis of CNS axon propagation [64], dopaminergic neurons [65, 66], creation of a neurovascular unit-on-a-chip [67], studies on neural stem cells (NSCs) [68], blood–brain barrier (BBB) function [69–74], AD [37, 75], brain tumors [76, 77], and neurotransmitter function (Table 4) [83]. In order to show whether microfluidic brain-on-a-chip devices can overcome these

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restrictions, in the current article, we review novel reports about the applications of microfluidic brain-on-a-chip devices in studies of neural system disorders.

Microfluidic SC-Based Neural Tissue Engineering

SCs have the capability of continuous self-renewal, unrestricted proliferation, and the capacity to differentiate into various different cell types depending on external cues [84, 85]. SCs can be potentially used for drug delivery, cell therapies, tissue remodeling, organ regeneration, and for disease models [86]. This ability is due to transcriptional factors, which are pivotal regulatory molecules governing the maintenance of these cells [87]. NSCs, hiPSCs derived from somatic cells, embryonic stem cells (ESCs), and also embryonic germ cells (EGCs) can all be encouraged to differentiate into astrocytes (ASTs), oligodendrocytes, and neurons (Fig. 3). Hence, the creation of models using SC-based neural systems might facilitate drug discovery and the elucidation of NDD mechanisms [88–93]. The ability to differentiate into different cells makes SCs suitable for autologous cell transplantation [94]. NSCs, ESCs, and hiPSCs have been employed as potential neuro-regenerative cells in neural system including CNS and peripheral nervous system (PNS) in LOC devices [95–98].

NSC-Based Microfluidic Systems—Microfluidic devices have produced a powerful technology for investigation of NSC differentiation. NSCs are known as neural progenitor cells (NPCs) or neural stem/progenitor cells (NSPCs) that are multipotent cells capable of generating both neuronal and glial cells [99, 100]. Glial cells include four main cell types including microglia, ASTs, oligodendrocytes, and their progenitors, NG2-glia [101]. NSC transplantation, which leads to differentiation and expansion of various nervous cells, is pivotal for the restoration of the damaged nervous system and for the treatment of NDDs [102]. In addition, autologous NSCs have also been used to treat non-acute severe traumatic brain injury (TBI) [103]. NSCs express non-specific lineage molecules including stage-specific embryonic antigen (SSEA)-1 and cluster of differentiation (CD)133 (Prominin-1), which can be used to isolate and characterize these cells for research and therapeutic application [104]. Neuronal cells derived from NSCs are able to mature and integrate into the host neural system tissue and recover damage after stroke. Hence, NSCs are therapeutically valuable [105]. Neuronal cells differentiated from NSCs are highly sensitive to physicochemical agents present in their microenvironment, thus these cells must be cultured and evaluated in controlled conditions like those achieved in microfluidic organ-on-a-chip devices [106, 107].

Extracellular matrix (ECM) glycoproteins play important roles in the nervous system and are widely expressed in constituent cells [108, 109]. Early changes in the ECM have been observed in several neurological disorders, including ischemic stroke, MS, AD [109, 110], and schizophrenia [111]. Hence, ECM is important in the study of neural systems and NDDs. In this regard, Wang et al. [68] developed a 3D ECM-based microfluidic system for NSC differentiation and regeneration. NSCs isolated from the fetuses were cultured on a microfluidic device fabricated using PDMS. Then, these cells were investigated under two conditions consisting of conventional static 2D cell culture and a dynamic microfluidic engineered ECM-based 3D model. Their findings showed the dynamic 3D cell culture, using ECM accelerated NSC self-renewal and proliferation, whereas the static 2D cell culture only

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allowed NSC differentiation into the neuron lineage. Spheroid culture on ECM under perfusion allowed NSCs to differentiate into glial cells [68].

Localization and delivery of stem cells to the target site is one of the major challenges in cell transplantation therapies. SCs microencapsulation has several advantages, such as allowing localization of the cells to a single area, and sustaining cell viability through exchange of nutrients and waste products between the surrounding tissue and the encapsulated cells [78, 112]. Murine cortex cells were differentiated to neurospheres from floating cells. These aggregated neurosphere cells were separated by accutase to single NSCs, loaded into alginate–collagen microcapsules, and then they were placed in a control-lable microfluidic device. Ultimately, their results revealed that the 3D-based microfluidic microenvironment had the ability to improve the viability, expansion, and differentiation of NSCs [78].

hiPSC-Based Microfluidic Systems—hiPSCs are a useful cell types for analyzing brain development and NDDs [113]. Neural cells can be derived from hiPSCs and can be used as an important dynamic model to evaluate the molecular, cellular, and structural activities of the human neural system [114–116]. Implementing these cells in drug discovery devices allows the development of novel investigative models for studying treatment of NDDs [117–119]. Wang et al. [79] developed hiPSC-derived 3D brain organoids using an organ-on-a-chip method prepared from PDMS and soft lithography (Table 2). The results demonstrated that the brain organoids possessed the key features of early human brain development, including neural differentiation, regionalization, and cortical organization [79]. These authors [80] also developed a brain organ-on-a-chip as a microfluidic model to assess neuronal dysfunction caused by nicotine. They found that exposure to nicotine enhanced premature neuronal differentiation and the human pluripotent (hPSCs) based microfluidic organ-on-a-chip device could be a model for the nervous system damaged by nicotine [80].

Microfluidic 3D Chip Models for NDDs Studies

NDDs are generally age-dependent disorders, which are characterized by a slow decrease of the number and function of CNS neural cells [120]. AD is the most common type of NDD seen in elderly people [121, 122]. The limited number of relevant animal models for AD is one of the major challenges hindering development of new AD therapies because studies in rodents and non-human primates often do not translate to humans [123]. Hence, new tools to study AD are needed. Park et al. [37] designed a neurospheroid 3D in vitro model based on a microfluidic chip for AD researchers to evaluate the toxicity of β-amyloid on neurospheroids (Figs. 4 and 5). They found that amyloid-β treatment impaired formation of neural networks and significantly decreased the viability of neurospheroids. Indeed, the neural networks became larger and more complex when neurospheroids were cultured in the presence of interstitial flow when compared to those cultured under static conditions. Such difference could be due to cytokines, better access to nutrients and oxygen (O_2), and clearance of metabolic wastes in the flow conditions (Fig. 6) [37].

MacKerron et al. [81] developed a microfluidic platform for the characterization of CNS active compounds. The pharmacological properties of a glutaminergic receptor antagonist could be evaluated using this method [81].

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Motor neuron disease (MND), also called amyotrophic ALS, is a NDD that affects motor neurons with an unknown etiology. This disease has few experimental models for laboratory investigation [124]. Osaki and colleagues [82] studied MND by designing a 3D microfluidic platform containing vascular and neuronal networks. They found that this model enabled study of neurovascular coupling, which is essential to understanding the pathogenesis of NDDs, such as ALS [82].

BBB-on-a-Chip

Blood circulates throughout the body via blood vessels to deliver O_2, nutrients, and hormones to tissues, and to remove CO_2 and metabolic wastes from tissues. Circulating blood in the brain is separated from the parenchyma by a highly selective semipermeable membrane known as the BBB designed to exclude pathogens and toxins from the brain [125]. The BBB is made up of four cell types consisting of pericytes, neurons, ASTs, and brain endothelial cells [126]. The BBB is composed of non-fenestrated endothelial cells with tight intercellular junctions, which regulate the movement of molecules and cells between blood and the CNS [125, 127]. Some special compounds such as O_2, CO_2, and lipid-soluble molecules can generally diffuse freely across the BBB. Essential nutrients like glucose and larger molecules are actively transported across BBB through molecular transporters and receptor-mediated endocytosis, respectively [128]. In addition, harmful agents such as lipophilic agents are effluxed from the endothelial cells by specific membrane efflux pumps like P-glycoprotein (Pgp) [129, 130]. Animal and in vitro models have been used to study the effect of various treatments or drugs on the BBB at cellular, tissue, and systemic levels. Animal models do provide the complexity of the BBB environment for the study of immunology, pharmacodynamics, and pharmacokinetics [131]. However, animal models have less complex brain structures than humans and may not be completely suitable for study of the BBB [96, 132, 133]. The neurovascular unit on-a-chip is an in vitro model, which is reproducible, cost-effective, and time saving, allowing for accelerated evaluation of emerging new drugs [134].

Brown et al. [70] designed a microfluidic device to mimic the BBB function to analyze the influence of a cytokine cocktail and lipopolysaccharide (LPS) on inflammatory reactions. The results revealed that initial exposure to LPS compromised the BBB function and had effects on both physical and metabolic properties of the BBB. Inflammatory cytokines compromised the BBB function as well as LPS [70].

Adriani et al. [69] developed a new 3D microfluidic device containing ASTs, neuron, and human cerebral microvascular endothelial cell (hCMEC/D3) model the permeability properties of the BBB in an in vitro system (Figs. 7 and 8). The immunostaining results showed that endothelial cells had formed a monolayer with intercellular junctions in a monolayer (Fig. 9). In addition, permeability testing revealed that the endothelial cells monolayer acted as a size-selective barrier similar to the BBB in vitro [69, 135].

Wang et al. [74] designed a microfluidic BBB model for in vitro drug permeability assays. The results showed that this model mimicked the physiological BBB function while the measured permeability coefficients for large molecules (caffeine, fluorescein-isothiocyanate (FITC)-dextrans, cimetidine, and doxorubicin) were comparable to those found in in vivo

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models [74]. Indeed, this model formed continuous tight junctions and had a high barrier integrity with values of trans-endothelial electrical resistance (TEER) above 2000 Ω.cm^2 that were similar to in vivo models [74]. Neuroinflammation is known to be involved in the pathophysiology of several neurological and psychiatric disorders and study of neural inflammatory markers can help to discover new treatment options for these disorders [136, 137]. For the study of neural inflammatory processes, Herland et al. [72] engineered a 3D BBB in vitro model using a microfluidic chip containing primary human brain pericytes and microvascular endothelial cells (hBMVECs). Then the cells were stimulated with the inflammatory cytokine tumor necrosis factor alpha (TNF)-α and the cytokine release profile (interleukin (IL)-6 and granulocyte colony-stimulating factor (G-CSF)) was measured. The findings revealed that the chip had a barrier permeability similar to the BBB and the level of inflammatory mediators (IL-6 and G-CSF) was significantly higher in the 3D BBB chip than that for the same cells co-cultured in static trans-well plates [72].

Microfluidic devices are commonly fabricated using PDMS, which has good optical transparency, high flexibility, and high gas permeability that allows fabrication of microtissue models [138]. Sellgren et al. [73] designed a 3D microfluidic BBB model with two PDMS micromolded channels instead of a polyester membrane and found that this model was able to provide optical transparency with suitable physiological fluid shear stress to study the function of the BBB model [73].

Brain Cancer-on-a-Chip

The cellular microenvironment in tumors is heterogeneous and complex with varying levels of vascularization and restricted mass transport that may limit the efficiency of therapeutics [139]. Commonly, cell culture on 2D surfaces is used as an in vitro model to characterize the cell biology of tumors and to evaluate newly developed drugs [140]. However, there is a need for 3D cell culture, which claimed to be more similar to the in vivo situation than 2D cell culture, to study cell-to-cell and cell-to-matrix interactions [139, 141–143]. 3D cell culture have also been used to identify signaling molecules involved in cell–cell and cell–matrix interactions as well as to develop new drugs. Thus, LOC microfluidic technology as a 3D in vitro tissue model could improve the screening of personalized drugs [144, 145].

Glioblastoma multiforme (GBM) is a highly malignant brain tumor and is one of the most challenging types of cancer to treat. Standard treatment regimens for GBM consist of a combination of maximal surgical resection followed by chemotherapy and radiotherapy [146]. The development of advanced models for studying drug delivery to brain tumors could help to discover new treatment options for brain tumors. Fan et al. [76] designed a 3D brain cancer chip to mimic GBM tumors for drug screening. They delivered two different drugs, pitavastatin and irinotecan, to cancerous spheroid cells growing in a PEGDA hydrogel in a PDMS microfluidic device. PEGDA is a hydrophilic long-chain monomer that is suitable as a carrier for drug delivery and biomedical applications. The findings revealed that this 3D brain cancer-on-a-chip was able to generate a useful GBM cancer model for drug screening and drug release assays [76]. Prediction of tumor progression is a challenging issue in brain cancer. Sun and co-workers [77], designed a microfluidic platform for single-cell proteomic analysis of GBM cells. They reported that this microfluidic platform enabled

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accurate prediction of tumor progression. In another study, Altemus et al. [147] developed a microfluidic BBB model to investigate breast tumor metastasis to the brain. The results revealed that the chip had the capability to model the molecular mechanisms of brain metastasis as well as help in the development of new drugs [147].

The Technical and Biological Challenges of Microfluidic Brain-on-a-Chip Systems

Several technical challenges remain to be overcome in microfluidic brain-on-a-chip systems. One of the remaining major technical challenges is the tendency of drugs and chemicals to undergo non-specific binding to PDMS. As well, PDMS has a structure, which is incompatible with a many organic solvents. Keng et al. [148] developed a compatible type of 2D microscale platform, which was called "electrowetting-on-dielectric" (EWOD). The EWOD device was made of inorganic materials, which were coated with a perfluoropolymer layer. It was manufactured as a typical device including two parallel plates and electrodes that were coated with conductive, dielectric non-wetting layers [148].

Other technical restrictions of microfluidic brain-on-a-chip systems are providing sterile conditions during manufacture, avoiding bubbles, different flow rates between platforms, creating ideal hemoglobin-based oxygenation and nutrient levels, and inclusion of biosensors [149]. Furthermore, the modeling of cell–matrix or cell–cell interactions, cell migration, and 3D cell growth by microfluidic brain-on-a-chip platforms present difficulties. As well, in surface-based microfluidic brain-on-a-chip platforms, cell growth and migration are geometrically restricted in comparison with bulk-based microfluidic brain-on-a-chip systems.

Another important limitation in 3D-based microfluidic brain-on-a-chip platforms is the use of electroactive systems constructed of microelectrode arrays (MEAs) and fluidic channels to simultaneously monitor different microenvironmental factors. More recently, Haehnel et al. [150] utilized a magnetic force method to construct a microelectrode-microfluidic device. This device detected and analyzed many microbiological, chemical, and environmental factors relevant to microfluidic LOC technologies [150].

Most microfluidic systems, with the exception of 3D-printed microfluidic organ-on-a-chip platforms, have not yet used a fully automated technology to create and control their activities [51, 151]. More recently, Kane et al. [152] claimed to have fabricated the first example of an automated microfluidic organ-on-a-chip using SC-derived dopaminergic neurons to evaluate PD. Their automated microfluidic device had the potential ability to allow individual investigation for this neural disease [152]. It is likely that more fully automated biomanufacturing processes for microfluidic brain-on-a-chip systems will be developed in the near future.

However, there are also several biological challenges remaining in brain-on-a-chip systems. The design and creation of these complex systems is not a simple process as the overall size becomes increasingly smaller. In humans and animals, there are complex inter-connections between different organs that exert influences on each other. For instance, the endocrine and immune systems exert an influence on many different organs. In organ-on-a-chip systems, providing these interactions is challenging and complex [153, 154].

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Another important biological and technical challenge is the reconstruction of the entire brain including the simulation of vasculogenesis and angiogenesis in a micro-scale platform. Thus, to better mimic the properties and activities of human brain in vitro, it is essential to create a microvasculature 3D-based microfluidic model of the brain. In comparison with 2D-based models, the endothelial cells taking part in vasculogenesis and angiogenesis in 3D-based ECM microfluidic brain-ob-a-chip systems grow inside ECM with a self-assembled mechanism. Hence, 3D-based ECM microfluidic brain-on-a-chip systems are able to generate a more natural vascular structure of the brain, compared to 2D cell culture.

PDMS-based microfluidic brain-on-a-chip systems have a single layer of microchannels with the diameter ranging from 60 to 200 μm, which allows more precise control of these brain-like microenvironments. Nevertheless, endothelial cells in 3D-based ECM microfluidic brain-on-a-chip systems are adherent to a basement sheet with 40–120 nm thickness using proteins such as collagen IV, laminin, and fibronectin for cell adherence. However, monitoring this small system is an important problem [82, 155, 156].

More recently, studies have shown that creating a 3D microenvironment using scaffolds composed of ECM, hydrogels etc. (Table 3) is almost able to mimic the BBB and neural tissues similar to the human brain [153, 154]. As noted in BBB-on-a-Chip section, Adriani et al. [69] constructed a 3D neurovascular microfluidic BBB system including cerebral endothelial cells, neurons, and ASTs, which mimicked the human BBB [69].

Whole Body-on-a-Chip

In fact, a major challenge of microfluidic brain-on-a-chip technology is the nature of the method, which may be unable to provide mimicry of entire human organs. Many laboratory models lack the ability for pharmacokinetic studies including absorption, distribution, metabolism, and excretion (ADME) [157, 158]. The microfluidic organ-on-a-chip can emulate the biological function of organs such as bone marrow, spleen, gut, brain, and liver. Nevertheless, to predict the effects and toxicity of therapeutic agents, it is necessary to design and manufacture multi-organ-on-a-chip devices using microtechnology. Hence, researchers have designed and generated the "human-on-a-chip" that functions as whole body-on-a-chip. This has been called "homo chippiens." These whole-body models are able to accelerate the pharmacokinetic and pharmacodynamic studies of experimental drugs using the multi-organ-on-a-chip in technology in comparison with microfluidic single-organ-on-chip devices [61, 159–163]. Thus, the whole body-on-a-chip has been designed to emulate multiple different organs such as brain, liver, lung, kidney, adipose tissue, bone marrow, and the heart [164–166].

Conclusions and Future Directions

NDDs affect tens of millions of people worldwide. Therefore, there is an essential need to establish advanced models and develop new therapeutic strategies to inhibit or slow the progression of these disorders. The use of 2D and 3D cell cultures and animal models cannot completely recapitulate the etiology and pathophysiology of NDDs because researchers need to study the biochemical effects of molecules on the microenvironment and architecture of neural tissues. 3D-based ECM microfluidic brain-on-a-chip devices can create a potential

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solution to allow the controlled manipulation, monitoring, and assessment of cells. The enablement of drug discovery on a microscale can facilitate high-throughput screening of large drug libraries. More recently, SC-based microfluidic brain-on-a-chip system have given rise to many neural system structures in the laboratory. Hence, the microfluidic brain-on-a-chip system is a novel and advanced technology, which can be utilized for NDD modeling in order to evaluate both normal and abnormal conditions of the CNS and the PNS for basic medical investigations and can also be used for therapeutic aims in clinical applications and personalized medicine.

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Abbreviations

2D	two-dimensional
3D	three-dimensional
AD	Alzheimer's disease
ADME	adsorption, distribution, metabolism, excretion
ALT	amyotrophic lateral sclerosis
ASTs	astrocytes
BBB	blood–brain barrier
BECs	brain endothelial cells
bFGF	basic fibroblast growth factor
BMECs	brain microvascular endothelial cells
BRAIN	Brain Research through Advancing Innovative Neurotechnologies
CD	cluster of differentiation
CNS	central nervous system
CTIP2	chicken ovalbumin upstream promoter transcription factor-interacting protein 2
DARPA	Defense Advanced Research Projects Agency
DCX	doublecortin
DOX	doxorubicin
ECM	extracellular matrix

ECs	endothelial cells
EGCs	embryonic germ cells
EGFR	epidermal growth factor receptor
EGFR	epidermal growth factor
ESCs	embryonic stem cells
FDA	Food and Drug Administration
FITC	fluorescein isothiocyanate
FOXG1	forkhead box protein G1
GBM	glioblastoma multiforme
G-CSF	granulocyte colony-stimulating factor
GFAP	glial fibrillary acidic protein
hBMVECs	human brain microvascular endothelial cells
HBP	Human Brain Project
HD	Huntington's disease
hiPSCs	human-induced pluripotent stem cells
HUVEC	human umbilical vein endothelial cells
ISL1	insulin gene enhancer protein 1
ITSS	insulin-transferrin–sodium selenite supplement
IL-6	interleukin-6
KROX20	early growth response 2 (egr2)
LOC	laboratory-on-a-chip
LPS	lipopolysaccharide
MS	multiple sclerosis
NCATS	National Center for Advancing Translational Sciences
NDDs	neurodegenerative diseases
NIH	National Institut6es of Health
NPCs	neural progenitor cells
NSCs	neural stem cells
NSF	National Science Foundation

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NG2	neuron glial antigen 2
NSPCs	neural stem/progenitor cells
NVC	neurovascular chip
PD	Parkinson's disease
PAX2/6	paired box gene 2/6
PDMS	polydimethylsiloxane
PEGDA	poly(ethylene) glycol diacrylate
Pgp	P-glycoprotein
PNS	peripheral nervous system
PTEF	polytetrafluoroethylene
PTEN	phosphatase and tensin homolog
RT-PCR	real-time polymerase chain reaction
SCs	stem cells
SCZ	schizophrenia
SEM	scanning electron microscopy
SOX2	sex determining region Y-box 2
SSEA	stage-specific embryonic antigen
TBI	traumatic brain injury
TBR1	T-box brain 1
TEER	trans-endothelial electrical resistance
TNF-α	tumor necrosis factor-alpha
TUJ1	neuron-specific class III beta-tubulin
TUNEL	terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling
ZO-1	zonula occludens-1
GFP	green fluorescent protein
hCMEC/D3	human cerebral microvascular endothelial cell
HUVEC	human umbilical veinendothelial cell

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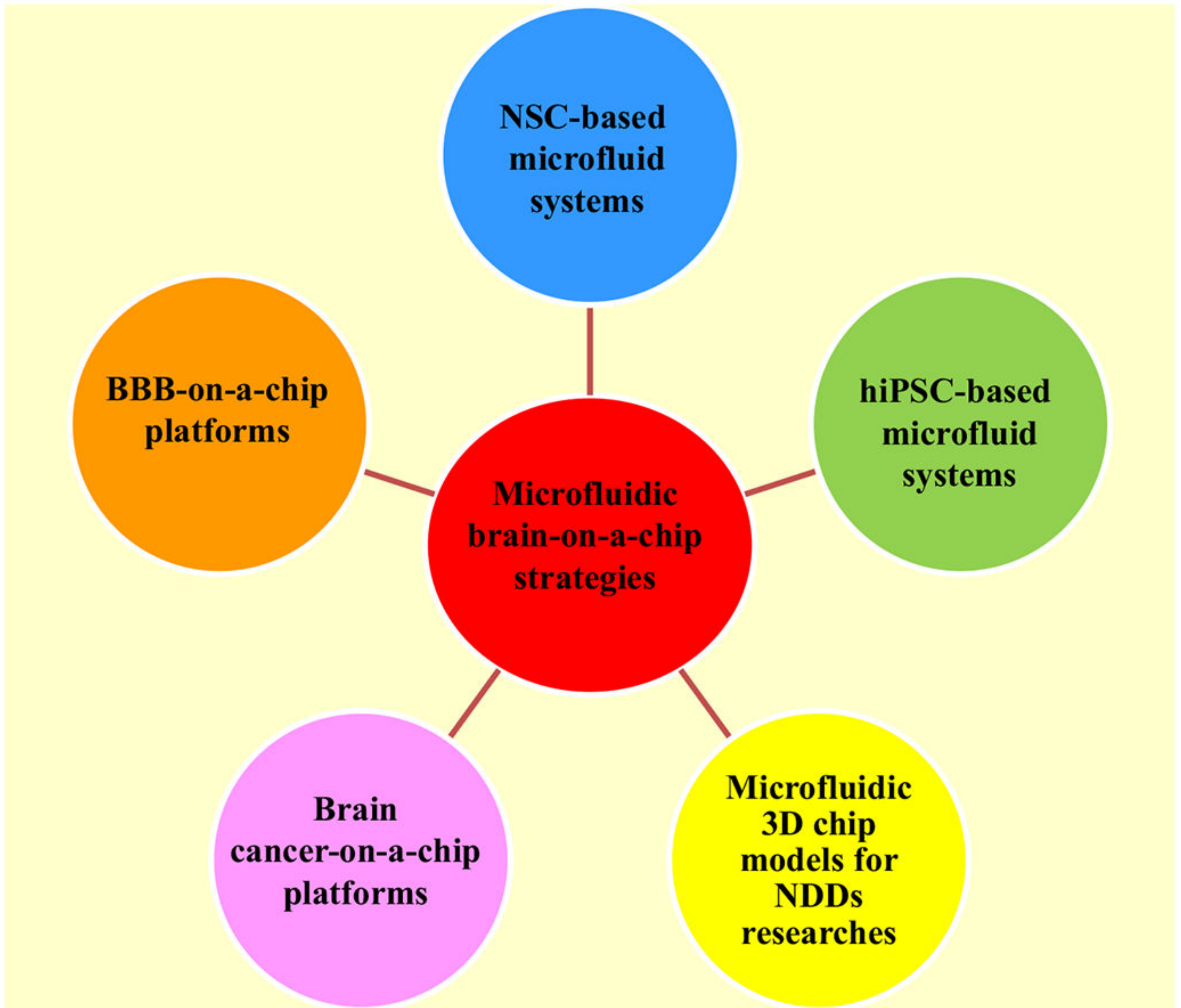

Fig. 1.

Schematic illustrations of SCs in a brain-on-a-chip technology for NDDs investigations

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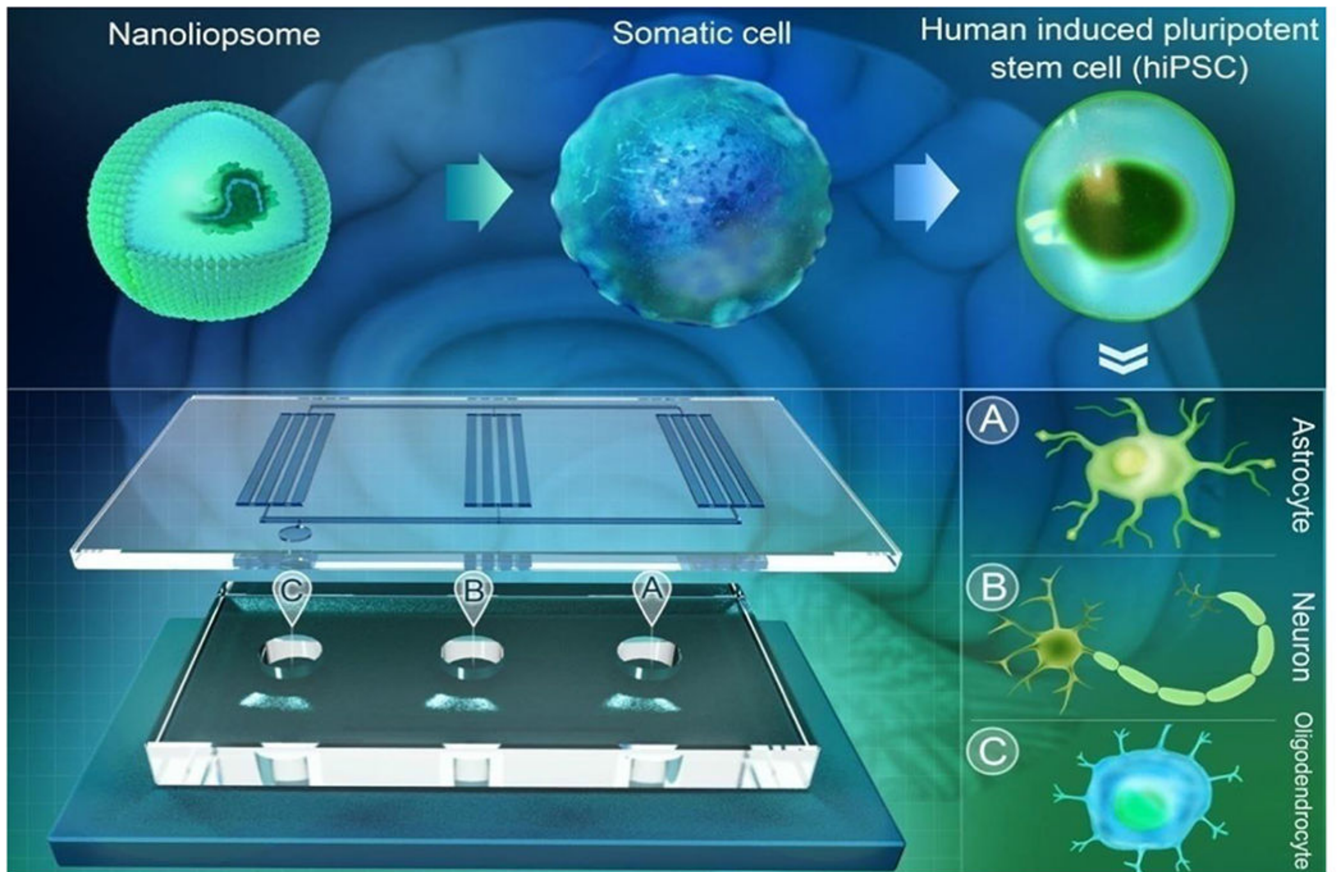

Fig. 2.

Schematic illustration of a microfluidic brain-on-a-chip device. human induced pluripotent stem cells (hiPSC)-based can be produced from adult somatic cells using a nanoliposome-based-clustered regularly interspaced palindromic repeats (CRISPR) system. The hiPSCs can differentiate into many cell types such as (A)) astrocytes (ASTs), (B) neurons, and (C) oligodendrocytes. Co-culture of theses neural cells (A, B, and C) in a microfluidic brain-on-a-chip device can be used to evaluate the molecular, cellular, and structural connections between neural cells such as ASTs, neurons, and oligodendrocytes for NDDs researches

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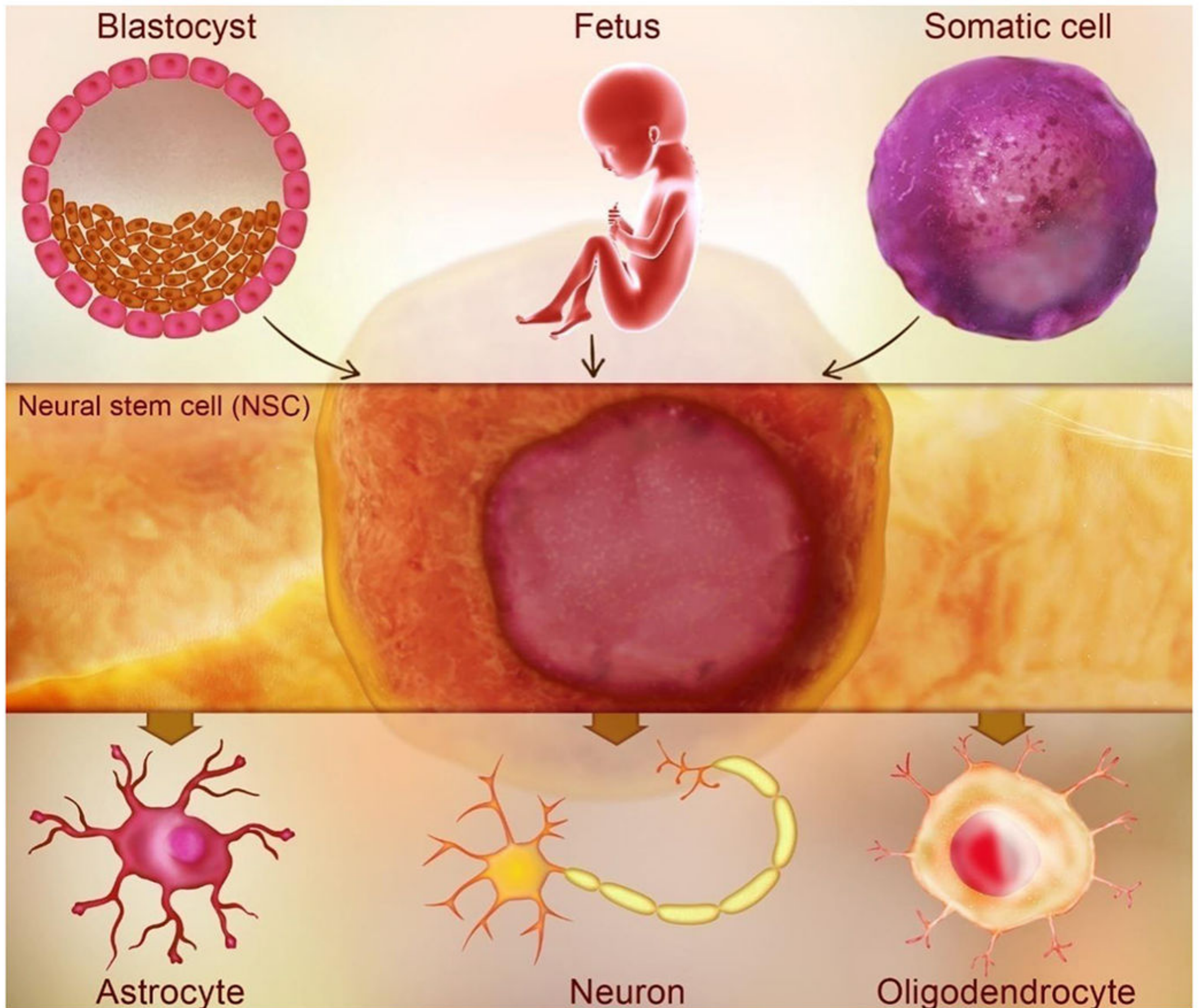

Fig. 3.

Schematic illustrations of SCs in a microfluidic brain-on-a-chip for NDD studies. SCs; particularly NSCs, hiPSCs, and ESCs have the capability to give rise to various neural system cells. Hence, the combination of SCs and microfluidic brain on-a-chip is able to be used as a potential strategy for the investigation of NDDs

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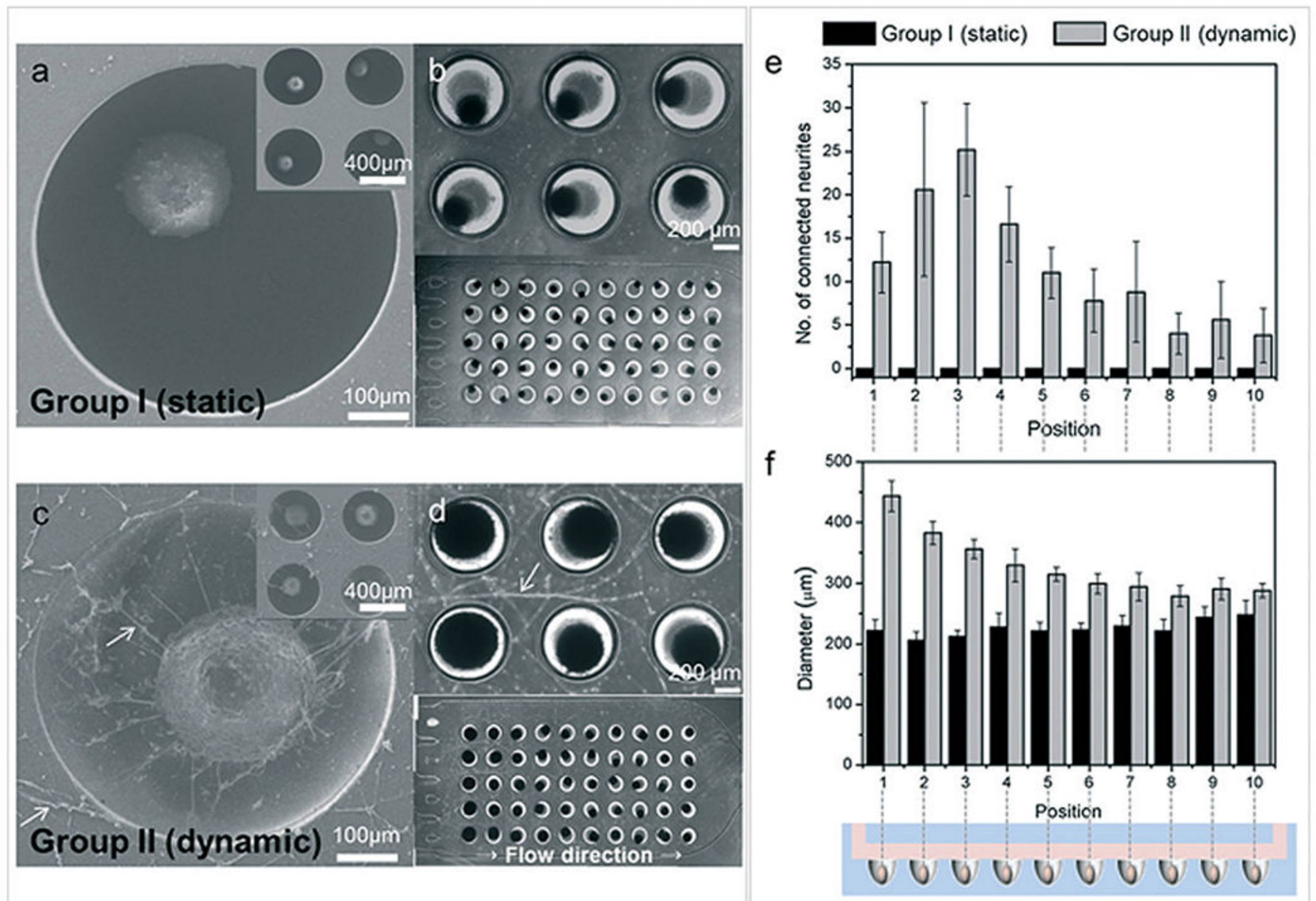

Fig. 4.

Formation of neural networks in a microfluidic device to develop an in vitro model of Alzheimer's disease (AD). Formation of neural networks has a critical role for communication of neurons and brain function. In this study, formation of neural networks was compared in two patterns; group **I** (with static condition) and group **II** (with dynamic condition), (**a** and **c**). Figs 4a and c are scanning electron microscopy (SEM) images of neurospheroids that demonstrates the greater neurite extension in group with the dynamic condition (4a) than the static condition group (4c). Consequently, greater neurite extension in group with dynamic condition leading to the formation of a more robust neural network than the group with the static condition. Figs 4b and d illustrate optical images of the chip with the static and dynamic conditions, respectively, in which demonstrate more active neural network formation in group with dynamic condition (**d**) compare with the static condition (**b**). Figs 4e and f reveals a quantitative analysis of the optical images. As shown in the beneath sections of the figs 4b and d, the chip was divided into ten sections by column that each column contains five microwells from inlet to outlet. For comparative analysis between the groups I and II, the average size of neurospheroids and the total number of neurites that extended from microwells was analyzed in each section. The results demonstrate that no neurites were distinguished from the static group, whereas in the dynamic group, a high number of neurites extending from microwells was detected near the

inlet and declined towards the outlet of the microchip (Fig 4e). Fig 4f have shown that the sizes of neurospheroids in the static group were almost the same throughout the microchip, conversely, neurospheroids were larger near the inlet and become smaller toward the outlet in the dynamic group.Reprinted with permission from ref. [37], Copyright 2015, Royal Society of Chemistry

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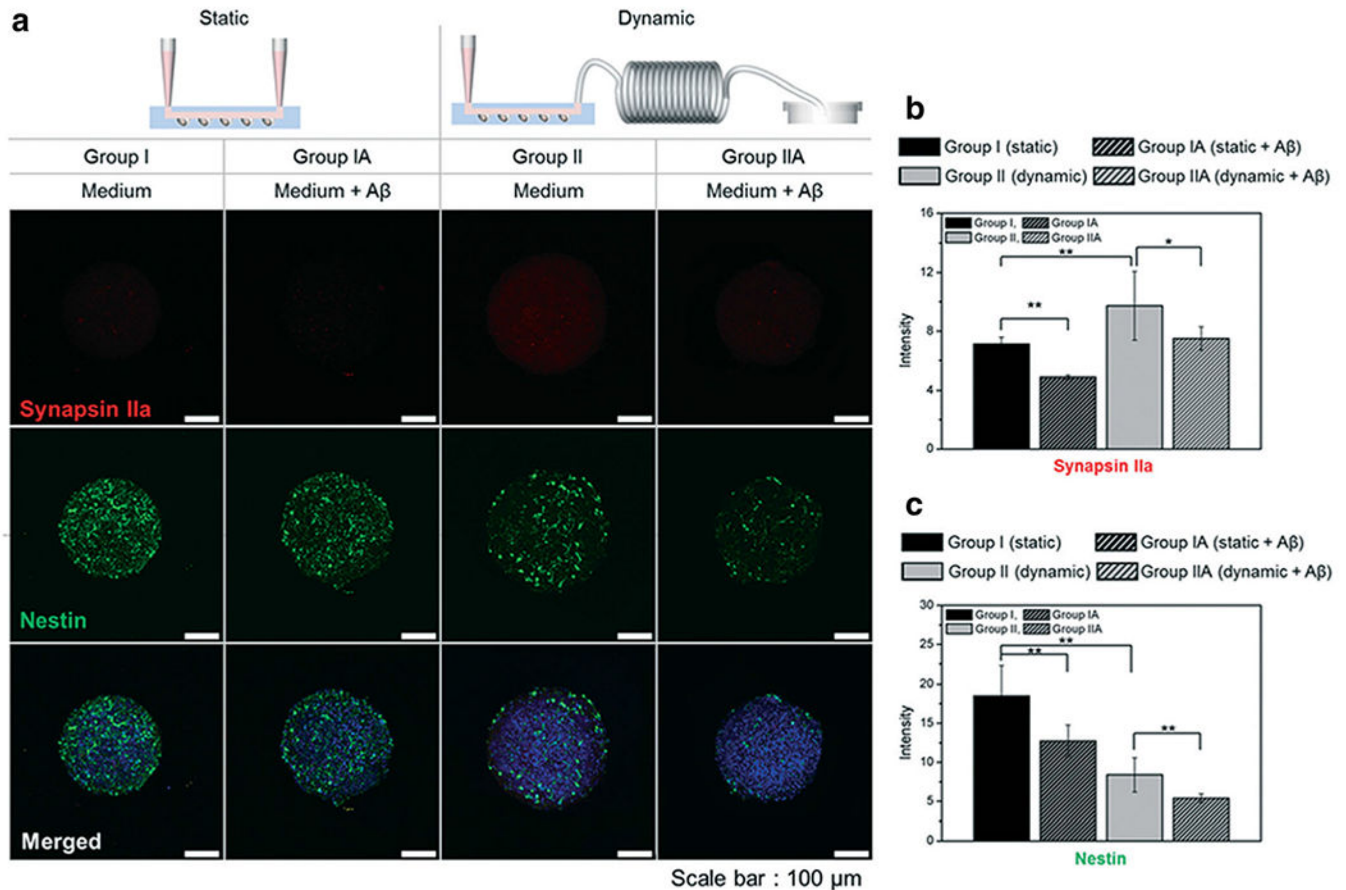

Fig. 5.

Comparative immunostaining of neurospheroids for the synaptic marker between the static and dynamic groups. The neurospheroids were immunostained for the synapsinIIa and nestin. SynapsinIIa was increased in the dynamic model compared with the static model (a) indicating that interstitial flow augments synapse formation that leads to the formation of a complex neural network. Fig 5a also illustrates that the levels of synapsinIIa and nestine were less intense in the groups IA (static, medium + amyloid-β (Aβ)) and IIA dynamic, medium + Aβ), which indicates greater destruction of neural networks in the groups that treated with Aβ. The quantitative analysis of the intensity of synapsinIIa and nestine are illustrates in the Figs 5b and c. Reprinted with permission from ref. [37], Copyright 2015, Royal Society of Chemistry

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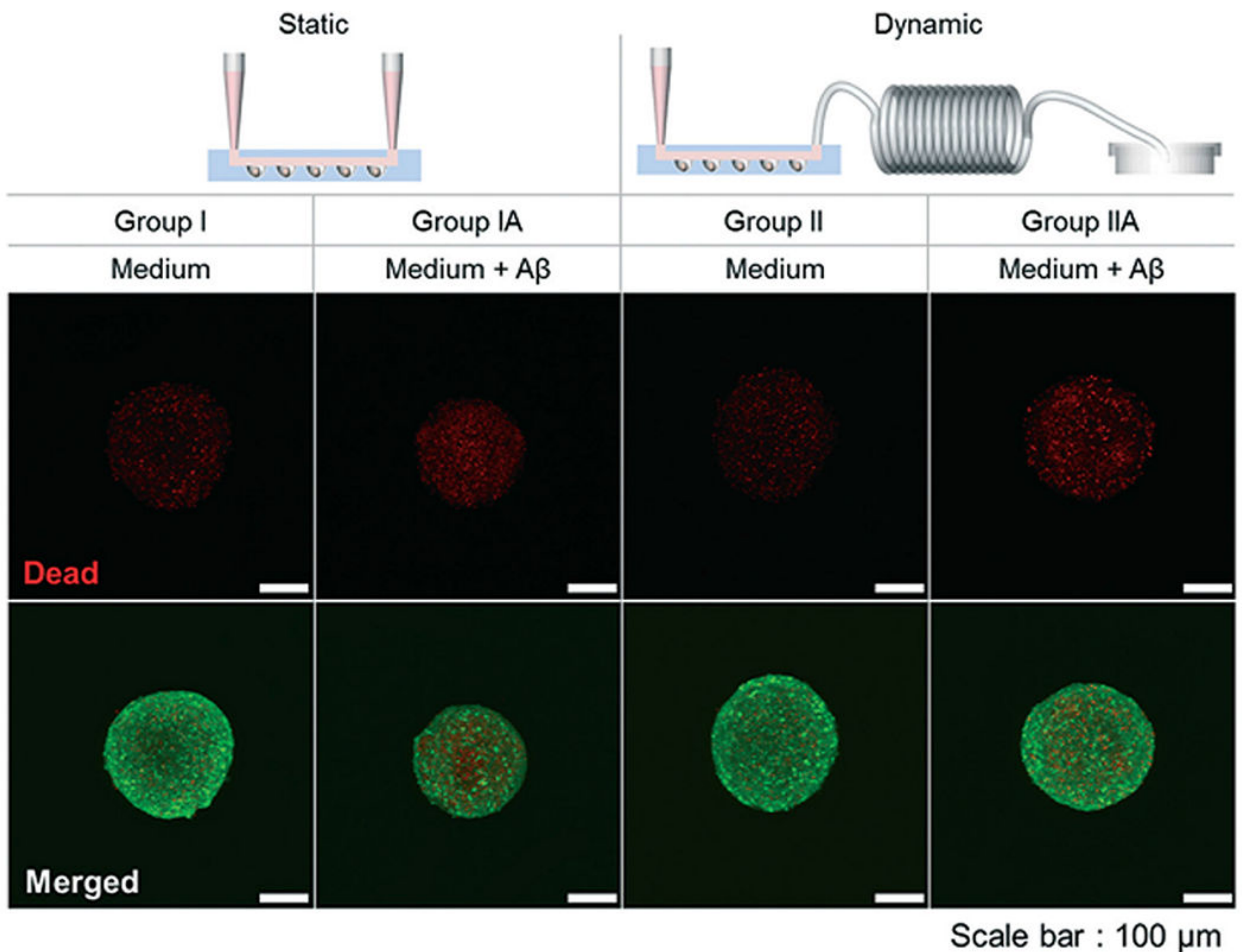

Fig. 6.
Comparative immunofluorescence imaging of neurospheroids stained with thioflavin S (green) and immunostained against neural marker β-III tubulin (red). The fluorescence intensity of neurospheroids that stained with thioflavin S (green) was increased after treatment with AB. In contrast, lower intensity of β-III tubulin was detected in the groups that treated with amyloid-β (section a, groups IA and IIA). The quantitative analysis of the intensity of immunofluorescence images are illustrates in the Figs 6b and c. Reprinted with permission from ref. [37], Copyright 2015, Royal Society of Chemistry

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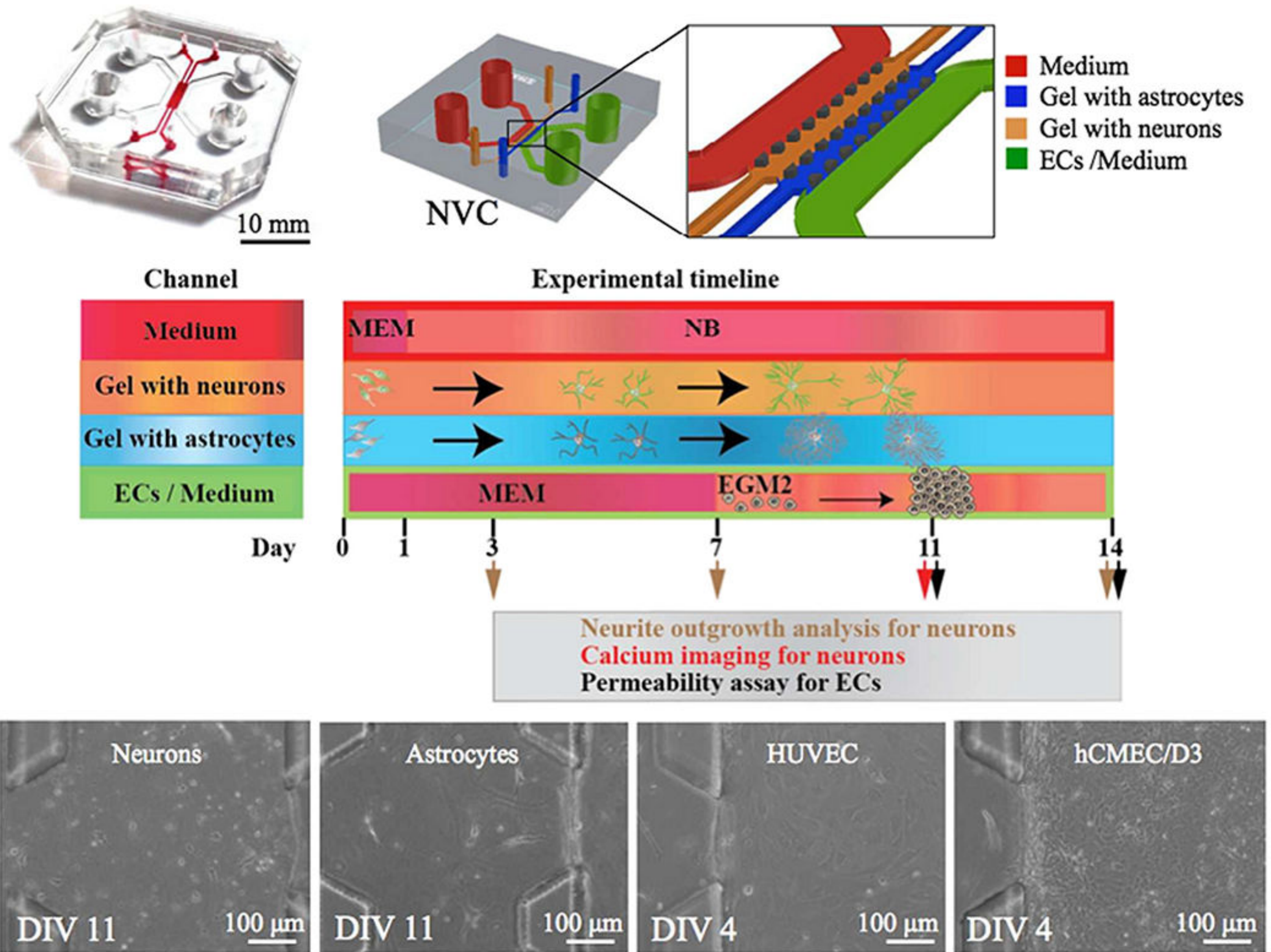

Fig. 7.

A schematic illustration of 3D model of neurovascular system in a microfluidic device.(a) a designed model of a microfluidic device stained with a food dye (left) and a schematic design of the 3D neurovascular chip (NVC) with focus on their channels: ASTs and neurons are cultures in two central hydrogel channels (blue and orange channels, respectively), endothelial cells (ECs) and the media are hosted in two side channels (green and red, respectively). (b) experiment timeline. (c) Phase contrast imaging of the growth and development of primary neurons, primary ASTs, and ECs (HUVEC and hCMEC/D3) in their respective microfluidic channels over time. Reprinted with permission from ref. [69], Copyright 2017, Royal Society of Chemistry

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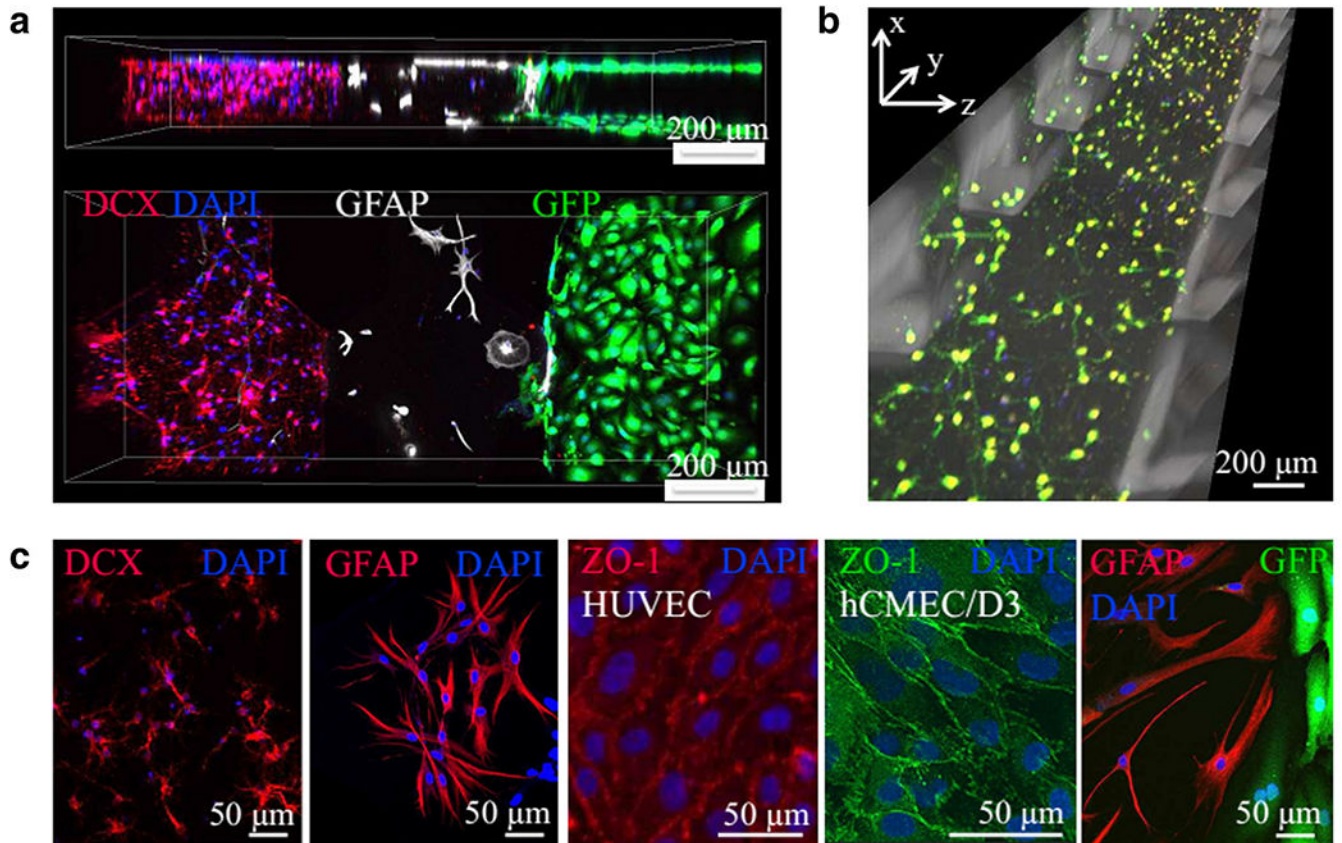

Fig. 8.

Immunocytochemical staining of initial neurons, primary ASTs, and ECs in 3D neurovascular microfluidic model. (a) The side and top views of the three types of cells in the 3D microfluidic devices: neuronsidentified by doublecortin (DCX) (red), ASTs are positive for GFAP (white), and HUVEC are GFP labeled (green). (b) a 3D view of the neuron in the gel regions. (c) Representative images from the left to right showing immature neurons identified by DCX, AST susing GFAP which exhibited star-shaped morphology, HUVEC and hCMEC/D3 express ingzonula occuldens-1 (ZO-1)(which is a tight junction protein), and GFAP positive ASTs (red) residing to GFP-marked ECs (green) in the NVC. Reprinted with permission from ref. [69], Copyright 2017, Royal Society of Chemistry

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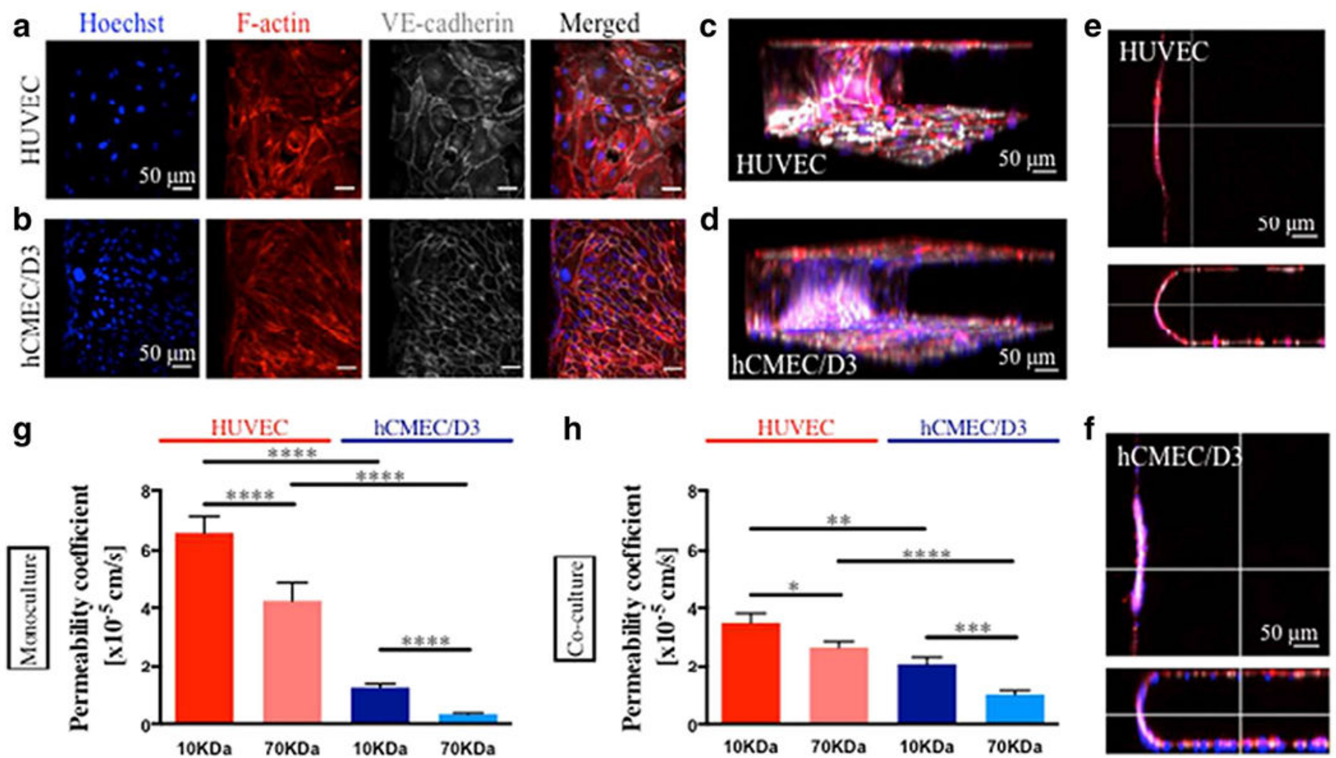

Fig. 9.

The characterization of the endothelial barrier in neurovascular microfluidic 3D model. Monolayers of HUVEC and (a) hCMEC/D3 (b) were stained with Hoechst for nucleus, rhodamine phalloidin for F-actin, and monoclonal antibody and NucBlue against VE-cadherin. Images c,d and e,f represent the 3D visualization and sections of the endothelial walls for HUVEC and hCMEC/D3, respectively. Permeability coefficients of 10kDa and 70kDa dextrans in monoculture (with endothelial cells only) and triple co-culture of HUVEC and hCMEC/D3 are calculated in the Graph g and h, respectively 7 days and 4 days in vitro as the permeability time point were chosen for hCMEC/D3 and HUVEC, respectively. Reprinted with permission from ref. [69], Copyright 2017, Royal Society of Chemistry

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Table 1

Some advantages and disadvantages of 2D and 3D cell cultures

Models	Advantages	Disadvantages	Reference
2D cell cultures	► Economical ►Well established and fast for primary assessment ► Easy and convenient for the analysis setup ► High-throughput capacity: feasible for mass screening	► Stiff, plastic substrate ► Lacking cell-cell and cell-matrix interaction ►Unable to mimic in vivo microenvironment ► Homogenous drug distribution ►Unable to assess the tissue penetration ability and bystander killing efficiency of drugs, or the drug resistance of cells ► High requirement for design–in vitro–in vivo–redesign cycle: overall high cost	(https://www.creative-biolabs.com/adc/2d-vs-3d.htm) [16]
3D cell cultures	► Substrates mimic the natural extracellular matrix ► Mimic the in vivo microenvironment, as well as cell–cell and cell–matrix interactions ► Enable the assessment of tissue penetration ability and bystander killing efficiency of drugs, as well as the drug resistance of cells ► Low design-in vitro-in vivo-redesign requirement: reduce overall cost for the pre-clinical in vivo test	► More expensive and laborious for the establishment of culture models and analyzing experiments ► Relatively low throughput capacity	(https://www.creative-biolabs.com/adc/2d-vs-3d.htm) [16]

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Table 2

Potential materials used in microfluidics

Material	Relevant property	Proposed application
Collagen or chitosan	Biocompatibility, versatile control of structure and chemistry	Bio-sensing, film assembly
Silkworm (*Bombyx mori*)	Biocompatibility, mechanically robust, flexibility, high mechanical modulus, and toughness	Fabrication of microfluidic channel
Agarose hydrogel	Lox cytotoxicity, biodegradability, mechanical stability at low solid fractions	Cell culture, sensors, and actuators
Teflon	Ease of fabrication with maximum chemical resistance	High-precision assay, super clean tools, valves, and pumps fabrication
Acrylonitrile butadiene styrene (ABS)	High resolution, excellent surface finish	Making of the master mold, microfluidics interface (MI), pathogen detection, biological assay
Photocurable resin/polymer	Very high resolution with small features	Biology observation of cell growth
ABS, polycarbonate, polyphenylsulfone, elastomers	Cheap material, ease of support removal	Pathogen detection of bacteria and viruses
Polyamide	Fast build speed, multi-material printing, very durable and high-temperature stable material	Making of the master mold
Hydrogels	Swelling and contraction, act as sensors and actuators	Self-regulating valves, microlens arrays, drug release systems, binding of antigens and proteins and glucose. Flow sensors pH regulators, flooding cooling devices
Polyurethane-methacrylate (PUMA)	Economical to manufacture, biocompatible, non-toxic, strong electro-osmotic mobility	High-aspect-ratio microstructures
Polyethylene glycols (PEGs)	Relatively inexpensive, available in a wide variety of molecular weights, biocompatible, negligible cytotoxicity	Microfluidic valves, channel cover to improve the microfluidic lifetime
Polyhydroxyalkanoates (PHAs)	Biocompatibility, tunable biodegradability	Microfilm barrier for vapor and oxygen
Gelatin methacrylate (gel-MA)	Photopolymerizable, porous membrane	Mechanistic vascular and valvular biology cell support matrix
Polylactic acid (PLA) and polyglycolic acid (PGA)	Tunable biodegradation	Porous scaffold for cell culture with better adhesion
Poly(polyol sebacate) (PPS)	Biocompatibility, design adaptability, mechanical compliance, low cytotoxicity, degradability	3D microfluidic system, microbioreactor
Poly(ethylene glycol) diacrylate (PEGDA) and gelatin methacryloyl (GelMA)	Biocompatibility, neovascularization potential, multi-material fabrication capability at a high spatial resolution	Tissue engineering, regenerative medicine, and bio-sensing
Poly(methyl methacrylate)	Favorable mechanical and thermal resistance, chemical compatibility	Genomic analysis
Styrene ethylene butylene styrene (SEBS)	Biocompatibility, rheological characteristics	Fabrication of complex and more sophisticated microfluidic networks (pFNs)
SEBS	Electrical surface properties, stable and relatively high zeta potential magnitude	Microdevices for electrokinetic applications
SEBS	Reduced drug absorption, optical transmittance, mechanical performance	Cell culture

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Table 3

Manufacturing approaches for mcrofluidic brain-on-a-chip

Manufacturing	The most used materials	Advantages	Disadvantages
Photolithography	Silicon	► Highly precise designs with computer-aided manufacturing (CAM)	► During the platform fabrication, the manufacturing procedure does not support simultaneous integration with biology because such steps like material removal and photoresist curing typically require extreme pH, high temperature, and exposure to radiation
Soft lithography	PDMS	► Highly precise designs due to the use of photolithography for master creation and compatibility with computer-aided design (CAD)resources	► The disadvantages of photolithography are applied to soft lithography because this method requires photolithography for master creation. Hence, the manufacturing procedure does not support simultaneous integration with biology
Contact printing	PDMS	►Similar advantages to soft lithography (e.g., compatibility with CAD and precise designs) ► Compatibility with a wide range of analytes ► Creation of periodic functionalized microdomains across macroscopic length scales	► This method can only be used to deposit small molecules on the thin material layers ► There are some challenges for repeated contact printing on the same substrate because the mechanical contact step is usually manual
Laser patterning	PEGDA, agarose, silicon	► Unique advantages for directing cell growth in silicon hydrogel NSCs due to the ability to spatially control 3D hydrogel chemistry	► Limits material availability because in this method only hydrogels and biomolecules functionalized with photoreactive groups can be used
3D Printing	Alginate, agarose, Polycaprolactone (PCL), gellan gum	► This method has been used in tissue engineering ► 3D Printing is a digital model and printer path gellan gum information can be derived from medical imaging data ► This method is a CAM process that affords repeatability and robustness in multi-layer and multi-material assembly ► A diverse material set such as thermosets, thermoplastics, composites, hydrogels, and solutions can be used in certain types of 3D printing such as micro-extrusion printing	► Because 3D printing is a serial processing technique, throughput can be limited for large parts that contain intricate path geometries

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Table 4

A summary of studies on microfluidic systems in NDDs

Subject	Study aims	Experimental design	Main findings	Conclusion	Reference
NSC-based microfluidic system	► Development of a 3D ECM-based microfluidic system for NSC differentiation and neural tissue regeneration	►NSCs isolated from the cortex of Sprague-Dawley rats ► The microfluidic device fabricated by a PDMS microfluidic chip and two PDMS-coated glass slides as the cover ► Five types of NSC microenvironments, including 2D cellular monolayer culture, 2D cellular monolayer culture on the ECM, dispersed cells in the ECM, 3D spheroid aggregates, and 3D spheroids cultured in the ECM, were constructed within an integrated microfluidic chip ► The effect of perfusion and static culture on NSCs was evaluated ► The NSC viability, self-renewal, proliferation, and differentiation into neurons, astrocytes, or oligodendrocytes were evaluated	► The NSCs in the microfluidic device established good viability ► The 3D culture in the ECM accelerated NSC self-renewal and proliferation ► The static 2D culture allowed NSC differentiation into the neuron lineage ► The spheroid culture on the ECM under perfusion and the monolayer culture in static state allowed NSCs differentiation into glial cells	► This microfluidic device could provide a NSC-based model for NDD therapy	[68]
	► Development of a reproducible encapsulation of NSCs and dental pulp stem cells (DPSCs) using microcapsules	► Spinal cord tissues, DPSCs and NSCs were isolated from C57BL/6 and the cortex of E14 C57BL/6 mice, respectively C57BL/6 mice, respectively ►NSCs and DPSCs were individually encapsulated within alginate-collagen microcapsules ► Microcapsules containing undifferentiated NSCs, undifferentiated DPSCs, and neuronal pre-differentiated DPSCs transplanted in ex vivo spinal cord injury (SCI) model	► Both DPSCs and NSCs maintained their multipotency and survived in the microcapsules ► In the SCI model, the microcapsules efficiently maintained the transplanted DPSCs and NSCs at the site of implantation ► After transplantation, the cells survived and differentiated to a neural lineage	► This microfluidic device provides an effective method to study SCs behavior ► This technique provides a useful method for the encapsulation of DPSCs and NSCs within alginate-collagen microcapsules	[78]
hiPSC-based microfluidic system	► Assessment of a new technology to develop a hiPSC-derived 3D brain organoids with organs-on-a-chip method	► The brain organoid-on-a-chip device was made of PDMS using soft lithography procedure ► Sodium fluorescein permeation assay was performed to validate drug distribution on chip ► Embryoid bodies (EBs) were generated via 3D culture of the hiPSCs that derived from skin fibroblasts, then perfused into hydrogel channel of the chip ► To evaluate this brain organ-on-a-chip model, immunohistochemical staining using pluripotent markers of hiPSCs (TUJ1, PAX6, PAX2, SOX2, Nestin, CTIP2, TBR1, CD133, and ISL1) were performed ► TUNEL assay and real-time PCR were conducted to evaluate apoptosis of cells within EBs and the expressions of brain regional markers (PAX2, PAX6and ISL1), cortical layer markers (CTIP2 and TBR1), and neural markers (SOX2, TUJ1, and Nestin), respectively	► The brain organoids under perfused culture demonstrated the key features of early human brain development, including neural differentiation, regionalization, and cortical organization ► The brain organoids displayed an upregulation of cortical layer markers (CTIP2 and TBR1)	► This technology created a robust and simple brain organoid-on-a-chip that can be used for studies of NDDs	[79]
	► Development of a brain organ-on-a-chip as to study of prenatal nicotine exposure	► The microfluidic chip was constructed of PDMS using soft lithography procedure ► Embryoid bodies mast be deleted and added only (EB) were generated via 3D culture of the hiPSCs derived from skin fibroblasts of a healthy individual, then perfused into hydrogel channels of the chip and exposed to different doses of nicotine ► Immunohistochemical staining, TUNEL assay, and real-time PCR were performed to validate the efficacy of this chip	► Gene expression showed a high expression of PAX2, PAX6, and ISL1 markers ► The PAX2 or ISL1 positive cells were located either together or separately in PAX6 sites ► TUNEL assay revealed nearly 40% cell death within organoids in a static culture condition	► This technology provides a model for NDDs under environmental exposure ► These findings showed that nicotine exposure impairs neurogenesis in early fetal brain development	[80]

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Subject	Study aims	Experimental design	Main findings	Conclusion	Reference
			► Exposure to nicotine enhanced premature neuronal differentiation with upregulation of TUJ1 marker ► Impairment of brain regionalization and cortical development were found in the nicotine-exposed organoids		
Microfluidic 3D chip models for NDD studies	► Design a neurospheroids 3D microfluidic chip mimics the brain microenvironment to study NDDs ► Investigate the influence of flow on neurospheroid size, neural differentiation, and neural network formation ► Evaluation of the toxicity of β-amyloid on neurospheroids	► Prenatal cortical neurons were isolated from the rat embryos, seeded in chip according to different conditions: group I, static conditions (neurospheroids cultured without flow); group IA, static conditions with amyloid-β treatment; group II, dynamic conditions (neurospheroids cultured with flow); group IIA, dynamic conditions with amyloid-β treatment ► Cell viability assay, immunocytochemistry, and scanning electron microscopy (SEM) were evaluated for validation of this chip	► In comparison with static conditions, neurospheroids with flow condition were more robust, larger, and formed complex neural networks, suggested a pivotal effect of flow on oxygen, cytokine transport, and removal of metabolic wastes In comparison with static conditions, amyloid-β treatment significantly decreased the viability of neurospheroids and impaired neural networks more than the flow condition	► This neurospheroid 3D microfluidic chip can be used as an in vitro model for NDDs studies	[37]
	► Development of a microfluidic platform for the characterization of CNS active compounds based on calcium imaging readouts	► Microfluidic devices were fabricated using soft-lithography. ► Primary hippocampal cultures were obtained from the Sprague-Dawley rat pups and cultured on the microfluidic devices ► Immunocytochemistry and calcium imaging were conducted	► Using this microfluidic device, pharmacological investigations on glutamatergic receptor antagonist can be performed.	► This microfluidic platform can be used to study CNS active compounds.	[81]
	► Engineering a 3D microfluidic platform of vascular and neuronal networks to study motor neuron disease (MND).	► Microfluidic devices were made of PDMS using soft lithography ► Co-culture of human ES-derived MN spheroids and endothelial cells were performed in microfluidic devices ► immunostaining of Tuj1, vascular endothelial (VE)-cadherin, HB9, and islet1 and enzyme-linked immunosorbent assay (ELISA) to measure growth factor concentrations (BMP-2 and BDNF) were conducted ► Ca^{2+} oscillation imaging, perfusability of vascular networks, and vessel permeability coefficient were evaluated as well	► Co-culture with endothelial cells enhances neuronal connectivity and neurite elongation as measured by Ca^{2+} oscillation ► Bi-directional signaling was detected in the neural networks that affected vascular network formation	► This in vitro model enables investigations of neurovascular coupling, which is essential to understanding the pathogenesis of NDDs, including ALS	[82]
Blood–brain barrier-on-a--chip	► Development of a microfluidic device that models BBB function to analyze the influence of LPS and cytokine cocktail of inflammatory reactions	► Neurovascular unit microfluidic was fabricated in a two-chamber system; the device was coated with laminin and constructed of PDMS ► Human brain-derived microvascular endothelial cells introduced into the lower chamber and the pericytes and astrocytes were loaded into the upper chamber ► LPS and cytokine cocktail were delivered to neural cells in the vascular chamber ► Different assays, including live/dead evaluation, transendothelial electrical resistance (TEER), FITC-dextran diffusion, tight junction staining, and cytokine measurement were performed to examine the effects of inflammation on BBB function	► The initial exposure to LPS compromised the BBB function, reduced presence of tight junctions, and increased diffusion ► Cytokine cocktail compromised the BBB function as well	► This microfluidic device provides a suitable BBB model that can be used to study the effects of inflammatory stimulation on BBB function	[70]
	► Design a 3D microfluidic neurovascular chip (NVC) to study BBB function	► Astrocytes were prepared from the primary cortical neurons of rat embryos ► Human umbilical vein endothelial cell (HUVEC) human cerebral microvascular endothelial cell (hCMEC/D3) cultured on collagen coated cell culture dishes	► The results have shown distinct cell type–specific morphological and functional analysis of neurons ► Monolayer cells with size-selective permeability were formed from human	3D microfluidic NVC can be a useful device for neurovascular studies	[69]

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Subject	Study aims	Experimental design	Main findings	Conclusion	Reference
		► The microfluidic device was made of PDMS using soft lithography procedure ► Endothelial cells, neurons, and astrocytes were seeded into the NVC ► Different assays, including immunocytochemistry and confocal imaging, calcium imaging, permeability assay, quantification of permeability coefficient, and neurite analysis, were conducted to evaluate validation of this microfluidic neurovascular chip	cerebral endothelial cells similar to existing in vitro BBB models		
	► Development of a microfluidic BBB model for in vitro drug permeability assays	► Brain microvascular endothelial cells (BMECs) were derived from hiPSCs and co-cultured with primary rat astrocytes on the two sides of a porous membrane on a pumpless microfluidic platform ► The microfluidic device was designed according to the human brain blood residence time mimicking in vivo-like BBB model ► Permeability capacity of the BBB model was conducted after the trans-endothelial electrical resistance (TEER) aiming to evaluate drug permeability of large molecules fluorescein isothiocyanate (FITC)-dextrans	► The results revealed that this microfluidic BBB model formed continuous tight junction and a suitable TEER resistance ► The permeability level were comparable to in vivo values	► This microfluidic BBB model mimics physiological BBB function and can be applied as a screening tool for of drug assessments	[74]
	► Engineering a microfluidic 3D BBB chip and study of inflammatory reactions	► Human brain microvascular endothelial cells (hBMVECs), human brain pericytes, and human astrocytes were used ► The microfluidic device was made of PDMS using soft lithography procedure ► The human ASTs were inserted into the gel and incubated for 18 h. Then, the pericytes and hBMVECs were seeded to form cylindrical lumen using these two cell types ► Permeability assay was performed by the permeability coefficient of small molecular (3 kDa) fluorescent dextran ► Transwell cell culture was conducted on 24-well Transwell coated with rat-tail collagen I; then, the inserts were inverted and pericytes or astrocytes were seeded. After incubation, the inserts were placed in 24-well plates and seeded with hBMVEC. TEER values were measured after 120 h of culture ► Inflammatory stimulation of microfluidic chips was performed with TNF-α and the cytokine release profile was assayed ► Immunocytochemistry staining was conducted using rabbit anti-glial fibrillary acidic protein (GFAP), mouse VE-cadherin, mouse anti-PECAM, mouse anti-zonula occludens-1 (ZO-1), rabbit anti-alpha-smooth muscle actin (SMA), and mouse anti-collagen IV	► The chip exhibited barrier permeability similar to BBB ► Different secretion profiles of interleukin-6 (IL-6) and granulocyte colony-stimulating factor (G-CSF) were observed after inflammatory trigger with TNF-α ► The level of inflammatory reactions was significantly higher in the 3D BBB chip than the same cells that co-cultured in static Transwell plates	► This microfluidic 3D BBB chip can be use to study human neurovascular function and inflammatory reactions in vitro.	[72]
	► Design of a microfluidic BBB model which enable to provide both optical transparency and physiological shear stress.	► The device were by designed by using sandwiching of an optically transparent nanoporous membrane between two PDMS micromolded channels instead of polyester membrane. ► ASTs were encapsulated into collagen hydrogels and inserted into basolateral compartment of the device. The brain ECs were grown on the surface of separating transparent membrane in the cell culture area of a microfluidic to form a ECs layer. ► Immunofluorescence staining for claudin-5 and phalloidin staining for actin were performed.	► The results demonstrated that the model was capable to simulate the physiological fluid shear stress and have optical transparency to study the function of the BBB model	► This BBB model can used to study of BBB function with optical transparency and physiological shear stress	[73]
Brain cancer-on-a--chip	► Development of a 3D brain cancer chip for drug screening	► The chip composed of polymerizable poly (ethylene) glycol diacrylate (PEGDA) hydrogel and made of PDMS ► Glioblastoma multiforme (GBM) cells were cultured in the chip and treated with pitavastatin and irinotecan	► The finding demonstrated that this brain cancer-on-a-chip is able to generate a potent GBM cancer model to evaluate the efficacy, screening, and release of drugs	► This chip is a suitable model of brain cancer for drug screening	[76]

Subject	Study aims	Experimental design	Main findings	Conclusion	Reference
	► Design of a microfluidic platform for single-cell proteomic analysis of cancer cells	► Human glioblastoma cell line and brain tumor cells isolated from patients to analyze the clinical application of the microfluidic system ► A PDMS-based microfluidic attached to poly-L-lysine was designed ► The microfluidic chip was validated by immunohistochemistry and confirmed the striking intertumoral and intratumoral heterogeneity characteristic of glioblastoma	► Their results and bioinformatics analysis revealed that the microfluidic data enable to quantify molecular signatures and predict tumor progression	► This microfluidic platform enables the study of single-cell proteomic analysis of cancer cells	[77]

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