Brain physiome: A concept bridging in vitro 3D brain models and in silico models for predicting drug toxicity in the brain

Yoojin Seo a,1, Seokyoung Bang a,1, Jeongtae Son b, Dongsup Kim b, Yong Jeong b, Pilnam Kim b, Jihun Yang c, Joon-Ho Eom d, Nakwon Choi a,e,f,*, Hong Nam Kim a,e,g,h,*,

a Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul, 02792, Republic of Korea
b Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, 34141, Republic of Korea
c KU-KIST Graduate School of Converging Science and Technology (KIST), Seoul, 02792, Republic of Korea
d NextBio Inc., Seoul, 02841, Republic of Korea
e Medical Device Research Division, National Institute of Food and Drug Safety Evaluation, Cheonggi, 28159, Republic of Korea
f Division of Bio-Medical Science & Technology, KIST School, Korea University of Science and Technology (UST), Seoul, 02841, Republic of Korea
g Division of Bio-Medical Science & Technology, KIST School, Korea University of Science and Technology (UST), Seoul, 02841, Republic of Korea
h School of Mechanical Engineering, Yonsei University, Seoul, 03722, Republic of Korea

A R T I C L E   I N F O

Keywords:
Brain physiome
In vitro 3D platform
Brain organoid
Brain-on-a-chip
In silico model

A B S T R A C T

In the last few decades, adverse reactions to pharmaceuticals have been evaluated using 2D in vitro models and animal models. However, with increasing computational power, and as the key drivers of cellular behavior have been identified, in silico models have emerged. These models are time-efficient and cost-effective, but the prediction of adverse reactions to unknown drugs using these models requires relevant experimental input. Accordingly, the physiome concept has emerged to bridge experimental datasets with in silico models. The brain physiome describes the systemic interactions of its components, which are organized into a multilevel hierarchy. Because of the limitations in obtaining experimental data corresponding to each physiome component from 2D in vitro models and animal models, 3D in vitro brain models, including brain organoids and brain-on-a-chip, have been developed. In this review, we present the concept of the brain physiome and its hierarchical organization, including cell- and tissue-level organizations. We also summarize recently developed 3D in vitro brain models and link them with the elements of the brain physiome as a guideline for dataset collection. The connection between in vitro 3D brain models and in silico modeling will lead to the establishment of cost-effective and time-efficient in silico models for the prediction of the safety of unknown drugs.

1. Introduction

New biopharmaceuticals are constantly being developed for the treatment of severe disorders. However, the prediction of a drug’s biological safety in humans is challenging because, for ethical and economic reasons, human-based trials cannot be used prior to the bona fide clinical trials [1,2]. Recently, in silico models have emerged as an approach to determine drug responses using computational tools. These models are powerful tools for the massive screening of drug candidates in a time-efficient and cost-effective manner. For the development of computational models, large amounts of experimental data, such as those concerning cellular and acellular responses to drug treatment, should be collected. However, because datasets obtained from individual studies pertain to various biological scales, it is difficult to use them directly for in silico modeling.

The physiome is a concept that can be used to organize data distributed on various biological scales. It is an integrated term that describes the functional behavior of a biological system. The elements of the physiome cover a broad range of physiological complexities, including the biochemical, biophysical, and anatomical environments of cells, tissues, and organs [3]. In this regard, the brain physiome is related to the physiological behavior of brain elements, in normal or abnormal states. Consideration of the brain physiome is crucial for determining

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author. Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul, 02792, Republic of Korea.
** Corresponding author. Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul, 02792, Republic of Korea.
E-mail addresses: nakwon.choi@kist.re.kr (N. Choi), hongnam.kim@kist.re.kr (H.N. Kim).
* These authors contributed equally to this study.

https://doi.org/10.1016/j.bioactmat.2021.11.009
Received 9 August 2021; Received in revised form 1 November 2021; Accepted 6 November 2021
Available online 12 November 2021
2452-199X/© 2021 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
drug-mediated responses in the brain tissue. Upon drug administration, drug metabolites transported to the brain can alter cellular behavior and acellular microenvironment therein, in some cases leading to mental or behavioral dysfunction if they are not sufficiently safe for use. Understanding the brain physiome can aid in the analysis of a large dataset because the subcategorized information of the brain physiome can be applied to variables in mathematical models [4].

Traditionally, researchers have evaluated drug toxicity using 2D in vitro and in vivo models [5–7]. 2D in vitro analysis enables high-throughput screening (HTS) of a few tens of thousands of candidates in a short time period, while in vivo models allow the analysis of drug absorption, distribution, metabolism, and excretion (ADME).
Table 1
Pathological brain toxicities on the tissue level and in vivo platforms for their analysis. Representative brain toxicity associated with the side effects of brain-targeted drugs is classified into neurotoxicity, neuroinflammation, and neurodegeneration. Using in vivo 3D brain platforms, such as brain organoid and brain-on-a-chip, the toxicity can be tested and the underlying mechanism of toxicity can be evaluated.

| Toxicity type | Research focus | In vitro platform | Features of in vitro platform | Reference |
|---------------|----------------|-------------------|------------------------------|-----------|
| Neurotoxicity | Neural network | Brain organoid    | - Fused cerebral organoid with other brain region organoid | [36] |
|               |                | Brain-on-a-chip   | - Vascularized brain organoid | [39] |
| Neuroinflammation | Blood-brain barrier | Brain organoid | - Brain spheroid consisting of astrocytes, pericytes, and brain endothelial cells | [41] |
| Neurodegeneration | Myelination | Brain organoid | - Brain organoid consisting of neurons, astrocytes, and oligodendrocytes | [66] |

Abbreviations: CNS, central nervous system; IPSC, induced pluripotent stem cell; PNS, peripheral nervous system.

[8-10]. However, a cell monolayer-based assay does not reflect the physiological microenvironment of the brain tissue. Furthermore, in vivo analysis, the inherent genetic heterogeneity of animal models frequently limits the ability to predict human-specific adverse reactions to drugs. In addition, the 3R principles (replacement, reduction, and refinement) are gaining increasing recognition [11]. The 3R principles refer to (i) replacement of animal experiments with other approaches, (ii) reduction of the number of experimental animals, and (iii) alleviation of pain in experimental animals. Collectively, these principles suggest the need for an alternative to animal experimentation [12].

Of note, the evaluation and prediction of drug responses in brain tissue are more complicated than those in other organs. This is because brain tissue contains more cell types than other organs, and its structural complexity is greater than that of other tissues. Furthermore, the existence of the blood–brain barrier (BBB) limits the transport of small-molecule drugs and biopharmaceuticals to the brain, and hence, drug-associated adverse reactions in the brain tissue are difficult to analyze [13]. Indeed, no brain-targeting biopharmaceuticals have been approved to date, highlighting said difficulties [14].

Considering the above discussion, the emerging in vivo 3D platforms utilizing human cell sources are more attractive than their 2D in vitro and animal models because they can be used to monitor real-time drug effects and predict drug responses, with high similarity to those in humans, and are more reflective of human physiology than other models [15,16]. In general, the emerging in vivo 3D brain models are broadly classified into brain organoids and brain-on-a-chips, depending on whether the 3D cellular structure is formed with or without a scaffold. Each model type is associated with specific advantages in recapitulating the in vivo microenvironment [17-19]. On the one hand, brain organoids mimic the tight cell–cell interactions and region-specific physiology of the brain [20-22]. In contrast, brain-on-a-chip technology encapsulates various types of cells in a miniaturized 3D environment, and thus is able to recapitulate a physiological stimulus in the brain [23-26]. In addition, these emerging in vivo 3D brain models can be used for research at various biological scales. In contrast, the findings of the existing 2D in vitro models cannot be readily translated to the organ level, and those of the in vivo ones cannot be readily translated to the subcellular level [27,28].

In this review, we introduce a novel concept of the brain physiome, which can be used as a translational route to connect the emerging 3D in vivo brain models with in silico ones. For this purpose, we classified the components of the brain physiome into tissue- and cell-level components and summarized the approaches to study adverse drug reactions in the 3D in vivo brain models, with particular focus on the brain physiome. We also present the current limitations and future directions of the physiome concept and 3D in vitro brain models.

Before explaining the brain physiome at the tissue and cell levels, we provide a schematic concept of in vitro 3D platforms that can cover the

Table 2
Brain toxicity parameters on the cellular level, their features, and measurement methods.

| Location          | Cell type | Features                  | Measurement methods                        | Reference |
|-------------------|-----------|---------------------------|-------------------------------------------|-----------|
| Extracellular     | Neuron    | Neurotransmitter          | ELISA                                     | [92]      |
|                   |           | Concentration             | - Microelectrode biosensor                | [93]      |
|                   |           | Dendritic spine density   | - Imaging neurons expressing a fluorescent protein | [100]     |
|                   |           | E/I balance               | - Imaging immunostained neurons (neurofilament, F-actin) | [113]     |
|                   | Endothelial cell | Permeability   | - RT-PCR or western blotting             | [114]     |
|                   |           | Brain-on-a-chip           | - Use of fluorescent molecules with adjustable molecular weight | [116]     |
|                   |           | Neuron                    | - TEER                                    | [117]     |
|                   |           | Ion channel reactivity    | - Imaging immunostained endothelial cells (ZO-1, occludin, claudin) | [129]     |
|                   |           | Receptor                  | - Imaging immunostained neurons (Nav 1.1, CACNA1B, KCN2) | [129]     |
|                   |           | Overexpression or suppression | - Imaging immunostained neurons (GluA1, NMDAR1) | [126]     |
| Intracellular     | Glia      | Glial cell reactivity     | Electrophysiology                         | [130]     |
|                   |           |                          | - Gene expression heat map               | [129]     |
|                   | ALL       | Cell toxicity             | RT-PCR or western blotting               | [134]     |

Abbreviations: CCK, cell-counting kit; E/I, excitation/inhibition; ELISA, enzyme-linked immunosorbent assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; RT-PCR, real-time polymerase chain reaction; TEER, transendothelial electrical resistance.
2. Tissue level

With considerable advances in 3D brain modeling platforms, the side effects and the therapeutic effects of drug candidates for neurodegenerative disorders can be predicted to accurately reflect the responses of human tissue. Below, we explain how in vitro 3D brain models can be used to test representative pathological side effects of drugs at the brain tissue level (i.e., at the level of neural network, BBB, and myelination). We also briefly mention drug testing at each level.

2.1. Neural networks

The brain is organized into multiple functionally heterogeneous regions, and it transmits and processes signals to/from each region to peripheral organs via interconnected neural networks. Adjacent neurons form synapses, with neurotransmitters released by presynaptic neurons and absorbed by postsynaptic ones. These signal transmission events control physiological and mechanical activities [29,30]. Therefore, protection of neural connectivity is crucial for correct signal transmission via neural networks and synapses, and for the maintenance of homeostasis in brain physiology. During chemotherapy, some chemotherapeutic agents exert adverse effects on the nervous system. Therefore, neurotoxicity is usually considered during the development of new drugs in in vitro and in vivo tests. Neurotoxicity is caused by exogenous molecules and endogenous metabolic products, such as nitric oxide (NO). These molecules eventually damage neurons and disrupt neural networks in the nervous system [31]. In this subsection, we focus on in vitro 3D neural network models that can be used for neurotoxicity testing.

The integrity and structural complexity of brain organoids are higher than those of 2D brain cell monolayers [32]. Brain organoids can be classified into whole brain organoids and region-specific brain organoids [33,34]. Lancaster et al. generated whole cerebral organoids to closely implement endogenous development from human pluripotent stem cells (hPSCs) and developed cerebral cortex, ventral telencephalon, choroid plexus, and retinal identities within 1–2 months [35]. Such whole brain organoids are valuable in mimicking the in vivo physiology of the brain but have difficulty analyzing drug responses emerging between different regions precisely because of the heterogeneity in size and region-specificity or—/coverage. However, the whole brain organoids are still useful in investigating the interactions between different brain regions. Alternatively, region-specific brain organoids, recently drawing more interest, possess great potential because they are relatively uniform in size and cellular composition. Single brain organoids do not fully reflect the regional brain identities because the anatomical structures of the native brain are more complicated than those obtained in in vitro brain models. Recently, fused cerebral organoids have been reported, beyond the construction of a single organoid, providing a highly advanced cerebral organoid model and representing the interaction between specific brain regions [36] (Fig. 3A). By fusing differentiated brain organoids, the authors generated a dorsal–ventral axis model exhibiting dorsal and ventral forebrain identities in each region.

Brain-on-a-chip technology has emerged as an alternative approach to brain organoids. Brain-on-a-chip has strong potential to recapitulate vascularized 3D tissue, which is essential for the prevention of necrosis in thick tissues and for providing physiological stimuli, such as appropriate fluidic conditions [37]. Furthermore, vascularization of the brain tissue is of primary importance because of the BBB structure, which prevents the entry of drugs into the brain [38]. From the cell–cell interaction perspective, the presence of vasculature-like structures results in enhanced functional maturation of organoids [39]. Therefore, a brain model with a functional BBB structure can closely predict the in vivo neurotoxicity of drugs. Integration of brain organoids and brain-on-a-chip has recently been attempted to better mimic the
A few pioneering studies have utilized brain-on-a-chip to evaluate the neurotoxicity of various compounds. Organophosphate-based compounds (OPs), which are toxic substances found in pesticides, represent a major threat to civilian populations [41]. Koo et al. (2018) used a tetra-culture brain-on-a-chip model to show that OPs penetrate the BBB and rapidly inhibit acetylcholinesterase (AChE) activity. In addition, neurons and glia assessed neurotoxicity depending on methylmercury concentration [42] (Fig. 3 D, G) and showed that the toxicity observed in the brain-on-a-chip model was correlated with the available in vivo data. This demonstrates that in vitro 3D brain models can potentially be used for neurotoxicity testing as an alternative to animal model-based experiments.

Various techniques, such as electrical stimulation, optical light stimulation, and chemical stimulation, can stimulate neural networks locally to generate a transmitting action potential (Fig. 4A). The local stimulation technique can analyze signal transmission through neurons/synapses, which is the most critical characteristic of neural circuits. Furthermore, local stimulation can be used to study synaptic plasticity in neural circuits in vitro. Local stimulation in this context means that only a few neurons, or a few areas of neurons, are stimulated instead of the entire area in which neurons are being cultured. Electrical stimulation is achieved by applying an electric potential to the neurons in contact with the electrodes [43] (Fig. 4B-i). Optical light stimulation applies optogenetic techniques to control neurons by irradiating with optical light [44,45] (Fig. 4B-ii). Optogenetics is a technology that expresses photoreceptors on the cell membrane of neurons and can activate or inactivate neuronal activity using light of a specific wavelength [46]. Chemical stimulation involves the administration of a substance that depolarizes neurons. With the aid of microchannels, chemical stimulation can be performed only in specific zones of in vitro neural networks [47] (Fig. 4B-iii).

Long-range signal transmission can be observed and measured using various techniques (e.g., intracellular calcium ion live-imaging, patch-clamp, and multi-electrode array (MEA)) (Fig. 4A). It is essential to measure these signals to evaluate the functional connections of in vitro neural circuits. In a neuron, the intracellular concentration of calcium ions is 100 nM in the resting state, and the concentration of calcium ions increases to 1000 nM when the neurons are activated [48]. Given that the calcium ion concentration changes rapidly, real-time measurements are necessary. Intracellular calcium ion concentration indicators are tools for visualizing the frequency and amplitude of action potentials [49] (Fig. 4C-i). A patch-clamp is a tool developed to record single or multiple ion channel currents, and membrane potential, on the cell membrane. For a patch-clamp measurement, the micropipette containing the electrolyte solution was brought into contact with the cell membrane and a gigaseal was formed by applying negative pressure. Then the current flux through the membrane channels was recorded in...
the electrode [50]. The current measurement method in this state is called the whole-cell patch-clamp, and in this way, the neurons’ action potential can be measured [51] (Fig. 4C-ii). The MEA can identify the interconnections between different neurons in response to electrical stimulation. Using multiple electrodes and complementary metal-oxide-semiconductor (CMOS) technology, massive neural signals can be measured at a very high spatial and temporal resolution. This approach enabled analyzing the connectivity of neural circuits at the cellular level [52] (Fig. 4C-iii).

2.2. BBB

The human brain has a unique vascular structure called the BBB, which is composed of endothelial cells, pericytes surrounding the capillaries, and astrocytes interconnecting the neurons and the capillaries [53]. The BBB controls molecular transport between the blood and the central nervous system (CNS) and restricts access to potentially harmful solutes to the brain [54]. However, under pathological conditions, such as neuroinflammation, the BBB is disrupted, and various molecules penetrate the CNS. Neuroinflammation is caused by multiple factors, including injury, immune system imbalance, or infection. This pathological process includes cytokine release syndrome in humans. Therefore, incorporating a model of the human BBB in in vitro platforms has major consequences for evaluating whether a drug can reach the CNS under normal and pathological conditions.

A tri-co-culture system of brain endothelial cells, astrocytes, and pericytes was used to reproduce BBB properties and function in in vitro BBB models [55,56]. In these models, tight junctions are formed within the organoids with appropriate molecular transporters, and they can be used to model drug penetration across the BBB. Human BBB organoids can be used for rapid (within 1 day) screening of drug permeability across the human BBB by confocal fluorescence microscopy and mass spectrometry imaging [57] (Fig. 3B). In one study, a human BBB chip fabricated using a brain microvascular endothelium derived from human induced pluripotent stem cells (iPSCs) successfully recapitulated the barrier function, preventing antibody transport across the BBB [58] (Fig. 3E). Such BBB models may be used to validate the efficiency of drugs and therapeutic antibody delivery across the human BBB.

Drugs targeting neurons and microglia should be designed considering their interactions with the BBB [59]. Specifically, drug candidates targeting Alzheimer’s disease (AD) and Parkinson’s disease (PD) have to cross the BBB prior to exerting a therapeutic effect on degenerating neurons. To investigate the correlation between BBB dysfunction and AD, Shin et al. developed a human BBB chip composed of brain blood vessels and neurons. This model revealed increased BBB permeability, decreased expression of tight junctions, increased expression of matrix-metalloproteinase-2, increased reactive oxygen species levels, and deposition of β-amyloid (Aβ) peptides in the vascular endothelium in Alzheimer’s disease (AD) [60] (Fig. 3H). Hence, this platform provides opportunities for screening new drugs that target AD and other neurodegenerative diseases.

Furthermore, another CNS barrier is the blood-cerebrospinal fluid (CSF)-barrier (B-CSF-B), which consists of the brain and epithelial barrier, and the choroid plexus (ChP). Similar to the BBB, B-CSF-B prevents free entry of toxic substances or drugs from the blood. While the BBB is formed by brain endothelial cells, astrocytes, and pericytes, B-CSF-B is established by choroid plexus epithelial cells. Pellegrini et al. showed that human ChP organoids with CSF production capability exhibited similar selectivity to small molecules observed in the ChP in vivo. Furthermore, the ChP organoids can predict the central nervous system (CNS) permeability of some small molecules such as bupropionyl, methotrexate, and vincristine [61].

2.3. Myelination

Adverse side effects of drugs can facilitate neurodegeneration, and demyelination is a well-known neurodegenerative pathological process [62,63]. The neural axon is covered with a myelin sheath, which insulates it and supports electrical conduction in neural circuits. Demyelination, a typical adverse drug side effect in neural tissues, refers to the peeling of the myelin sheath covering the axon. When the myelin sheath is damaged, the transmission of nerve signals is disrupted, which
adversely affects body function [64].

Demetylination is a representative neurodegeneration-promoting process associated with the side effects of drugs. Consequently, in vitro myelination and demetylination models can be used to predict the side effects of drugs. Myelination and demetylination are observed in the CNS and the peripheral nervous system (PNS), depending on whether the myelin sheath is produced by oligodendrocytes or Schwann cells [65].

To date, only the CNS myelination model has been developed for organoids. Because of the lack of protocol optimization, the spontaneous emergence of oligodendrocytes is difficult to control in brain organoids generated using conventional methods. However, with the advent of new differentiation methods, organoids containing neurons, astrocytes, and oligodendrocytes have been developed [66]. Indeed, it has been confirmed that oligodendrocytes in organoids wrap around neuronal axons. In addition, myelin around the axon becomes more compact with culturing, indicating the maturity of myelination. After sufficient myelination in the organoid, demetylination can be simulated by treatment with lysolecithin, a substance known to induce demetylination [67] (Fig. 3C).

Both CNS and PNS models of myelination have been developed using brain-on-a-chip and nerve-on-a-chip respectively. In the CNS model using brain-on-a-chip, optogenetic stimulation and electrical stimulation promoted myelination of oligodendrocytes [68,69] (Fig. 3F). Similarly, in the PNS model using nerve-on-a-chip, optogenetic stimulation promoted myelination [70,71] (Fig. 3I). In addition, robust demetylination by drugs such as lysophosphatidylcholine (LPC), and remyelination by drugs such as benzatropine (Benz) or methylcobalamin (MeCbl), can be reproduced using nerve-on-a-chip [72]. Furthermore, platforms for high-throughput myelination experiments have been developed [73,74] and can be used to test the side effects of various drugs.

Finally, a unique platform has been developed that can simulate myelination and demetylination by oligodendrocytes alone, without neurons. The in vivo process of myelination does not require the affinity of axon signaling. Instead, axon fiber’s diameters play a pivotal role in regulating myelination in vivo [75,76]. The in vivo myelination occurs predominantly around axons with larger (e.g., >2 μm) diameter rather than small diameter. This characteristic was also reproduced with engineered microstructures. In oligodendrocyte culture using an electrospun polystyrene fiber platform, oligodendrocytes wrapped thick fibers (diameter: 2.0–4.0 μm) with a concentrically multilayered sheet structure [77]; on the other hand, no wrapping occurred around thin fibers (diameter: 0.2–2.0 μm). This concentric wrapping morphology is characteristic of in vivo myelination. By applying the characteristics of oligodendrocytes, a conical micropillar array capable of HTS myelination was developed [78]. The conical micropillar had an upper diameter of 2 μm and a lower diameter of 50 μm. Subsequently, oligodendrocytes concentrically wrapped the micropillars and could be imaged readily on a microscope. Because the conical micropillar array can be mass-produced, HTS of myelination-related drugs is possible using this platform.

3. Cellular level

The safety evaluation parameters that are used for predicting the side effects of drugs at the cellular level are discussed below. We have classified them based on the region of interest, in which they can be analyzed, as extracellular, cell membrane, and intracellular regions.

3.1. Extracellular region

In the brain, the extracellular space is the space in the tissue outside of the cellular elements [79]. The extracellular space is filled with interstitial fluid containing various ions, proteins, and non-proteinaceous substances [80]. In this subsection, we focus on the synapse, the structure that plays a vital role in the brain’s function. In the context of extracellular space, the synapse strictly refers to the synaptic cleft [81]; however, we have covered the entire synapse here. We also review neurotransmitters, which are substances that transmit signals in the synaptic cleft, the dendritic spines (the structures that make up the post-synapse), and the excitatory and inhibitory synapses.

The synapse is the structure that transmits signals between neurons. It consists of a presynaptic terminal, a postsynaptic one, and the synaptic cleft [82]. In general, the presynaptic terminal is located at the axon, the postsynaptic terminal is located at the dendritic spine, and the width of the synaptic cleft is 20 nm [83]. When an electrical signal arrives at the presynaptic terminal via the axon, a neurotransmitter is secreted from the presynaptic terminal to the synaptic cleft. When the neurotransmitter passes through the synaptic cleft and reaches the neurotransmitter receptor located at the postsynaptic terminal, it opens a channel in the cellular membrane of the postsynaptic neuron, thus generating an action potential [84].

Various types of neurotransmitters and chemicals transmit signals between neurons in the synaptic cleft. Glutamate is a representative, and abundant, excitatory neurotransmitter [85]. It is present in the extracellular space in the hippocampus at 15–20 μmol/L [86]. A change in the glutamate concentration upon drug administration is considered to be a side effect of the drug because it causes abnormal neuron function or even cell death [87]. For example, the reduction of glutamate concentration by anti-epileptic drugs results in slow information processing [88].

Typical methods for measuring neurotransmitter levels in the extracellular space include enzyme-linked immunosorbent assay (ELISA) and microelectrode biosensor measurements [89,90]. Micro-electrode biosensors can be applied to living tissues and they allow real-time monitoring of neuron function [91]. In brain organoids, ELISA and microelectrode biosensors are both used to measure neurotransmitter concentrations [92,93]. In brain-on-a-chip, cells cultured inside the channel were first collected, and then ELISA was performed [58]. To measure neurotransmitter concentrations, the sensing tip of a conventional microelectrode biosensor is generally inserted into the tissue or culture medium. The electrodes can be integrated within the brain-on-a-chip platform, which enables real-time monitoring of cellular growth and death during the entire culturing period [94]. Furthermore, a brain-on-a-chip designed to expose a hydrogel containing 3D-cultured cells to the external environment has been recently developed [95]. Using this approach, a microelectrode biosensor in the form of a sensing tip can be used, even in a brain-on-a-chip.

Neurotransmitter receptors are located at the postsynaptic terminals. Most postsynaptic terminals are located in the dendrites and form unique dendritic spine structures [96]. Dendritic spine density falls within a specific range for a healthy neuron, and aberrant dendritic spine density induces brain dysfunction [97]. For example, dendritic spine density is increased in autism spectrum disorders (ASD), and reduced in schizophrenia and AD [98]. This is critical, as dendritic spine density can change in response to the administered drug [99]. Dendritic spine density is evaluated by imaging fluorescent protein expressing neurons, or immunostained ones [100,101]. Electron microscopy can also be used to measure the dendritic spine density in brain organoids, although data evaluation is challenging because of the narrow field of view afforded by this technique [102]. Distinguishing dendritic spines of individual neurons using conventional methods that rely on the expression of fluorescent proteins or immunostaining is also challenging because of the high neuron density in brain organoids. Therefore, identifying changes in dendritic spine density in response to drugs in brain organoids is currently difficult. In contrast, in the brain-on-a-chip, neurons are relatively sparse, and dendritic spine density can be measured by fluorescence imaging [103].

Furthermore, synapses are classified as excitatory or inhibitory synapses. A signal crossing the excitatory synapse increases the activity of the receiving neuron; conversely, a signal crossing the inhibitory synapse decreases the activity of the receiving neuron [104].
balance between these two types of synapses (excitation/inhibition (E/I) balance) is essential for determining the activity of a neural circuit. When this balance is disrupted, brain activity increases or decreases accordingly [105, 106]. For example, one of the pathologies of ASD is associated with increased E/I balance [107]. The E/I balance can either be analyzed on a single neuron or a neural network scale [108]. At the single neuron level, excitatory and inhibitory synapses can be analyzed in various ways. The type of synapse can be identified using the morphology of individual synapses [109]. Electrophysiological analysis can also compare excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) [110, 111]. In addition to measuring the E/I balance at the single neuron level, which was recently developed, there is a method to count the number of synapses by showing different fluorescence of inhibitory synapses and total synapses through transfection [112]. However, it is difficult to study the E/I balance on a large scale using the methods described above, such as the neural network. The E/I balance analysis of neural networks can be performed using real-time polymerase chain reaction (RT-PCR) and western blotting, using a marker suitable for each synapse type [113]. E/I balance analysis methods for these neural networks facilitate the analysis of the E/I balance before and after drug injection in both brain organoids and brain-on-a-chip.

3.2. Cell membrane

The cell membrane contains various ion channels, receptors, and transmembrane proteins that exchange ions and other molecules between cell compartments [114]. Furthermore, it contains several junctions, such as tight junctions and gap junctions, which help in integrating adherent cells, and in constructing molecular transport barriers.

As mentioned before, BBB permeability is a critical parameter for evaluating BBB toxicity in in vitro studies. The permeability can be evaluated by measuring endothelial cell function and marker expression. To measure endothelial cell function, some model molecules (i.e., albumin and dextran), or drugs are introduced into an engineered blood vessel, and their leakage or transport is monitored [115]. In addition, transendothelial electrical resistance (TEER) is used to measure the tightness of a cellular barrier in in vitro systems [116]. If the endothelial cells are tightly connected, the TEER is low. Tight junction proteins, such as zonula occludens-1 (ZO-1), occludin, and claudin, expressed by endothelial cells, are standard markers of endothelial cell integrity. They are also used as markers of BBB toxicity [117]. The expression of tight junction proteins can be downregulated depending on the concentration of the administered drug. Recently, BBB organoids that reproduce the expression of transporters and drug efflux pumps in vivo have been used to predict BBB function in response to drug concentration [57]. Similar to BBB permeability, B-CSF-B permeability, a parameter of CNS
Because no currently used medication can successfully treat ASD, NMDAR display impaired social interactions and repetitive behaviors. Therefore, some medications for treating autism should target this re
nofluorescence staining of proteins, such as Nav 1.1 (Na
ceptor [125]. In the clinic, individuals harboring genetic variants of
and interaction, and repetitive behavior in early childhood [122]. Although ASD has heterogeneous pathogenesis, various ASD models with Shank gene mutation show NMDAR dysfunction [123,124]. Therefore, some medications for treating autism should target this re
ceptor [125]. In the clinic, individuals harboring genetic variants of NMDAR display impaired social interactions and repetitive behaviors. Because no currently used medication can successfully treat ASD, mechanisms of ASD should be thoroughly analyzed, and candidate drugs that modulate NMDAR function should be identified.

Electrophysiology is a useful tool for monitoring changes in the current in membrane channels and receptors after drug treatment. The expression of ion channels and receptors can be determined by immuno
fluorescence staining of proteins, such as Nav 1.1 (Na+ channel), CACNA1B (Ca2+ channel), KCNC2 (K+ channel), AMPA receptor (GlutA1), and NMDAR1 [126]. In the case of calcium ions, fluctuations in calcium concentrations can be observed under a microscope [127].

3.3. Intracellular region

The central dogma states that within a biological system, DNA is a template for RNA synthesis (for transcription), which in turn acts as a template for protein synthesis (for translation) [128]. Thus, it explains how genetic information is transferred and then expressed as a phenotype. Therefore, abnormalities in gene and protein expression can be determined based on the genetic information.

Advances in molecular technologies have enabled the analysis of the full spectrum of genome sequences in humans, including healthy in
dividuals and patients. Knowledge of patient genomic information is beneficial for understanding disease mechanisms and predicting the therapeutic effects of drugs in idiopathic diseases. For instance, although many genetic background factors cause PD, the LRRK2-G2019S mutation has received much attention. It induces neuronal abnormalities, even in patients with mutated LRRK2 who display a mild phenotype [17]. The progression of this genetic mutation-dependent disease highlights the need for precision medicine.

At the genomic and transcriptomic landscape, whole-genome sequencing can be used to determine the contribution of the genetic background to neuronal abnormalities [129]. RNA-seq is a commonly used approach to bulk and single-cell transcriptome profiling that reveal the high-throughput and systemic mapping of gene expression in brain tissues or individual cell. [130,131].Recent single-cell messenger RNA sequencing (scRNA-seq) initiated a new era of molecular studies that uncover the diversity and cellular identity of brain cell types. By combining many computational techniques used for electrophysiology, these transcriptomic data thus provide a better understanding of functionally distinct brain cell types. Furthermore, single-cell multi-omics, including epigenomics, transcriptomics and proteomics, can also provide informative datasets for computational analysis. [132,133]. At the cytokine or growth factor levels, they are synthesized intracellularly and then secreted. Hence, neuroinflammation can be evaluated based on the secretion of proinflammatory cytokines by cells, as measured by ELISA or cytokine arrays [134]. Furthermore, cytotoxicity of drug treatment can be predicted by using a live/dead assay, a cell counting kit (CCK) assay, or a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphe
nyl-2H-tetrazolium bromide (MTT) assay [135].

4. Preclinical applications combined with imaging technologies and in silico modeling

4.1. Current limitations for collecting in vitro database

For preclinical approaches of in vitro 3D brain models, we need to handle massive amounts of experimental data from thousands of drug candidates (Fig. 5A and C). Because a large number of images are required to produce data, the use of advanced imaging technologies, known as high-content screening (HCS) and HTS (Fig. 5B), is required. HCS is a method for analyzing the adverse reactions of various drugs in living cells at high temporal and spatial resolutions [136]. For HCS, experimental tools and techniques have been utilized to extract large amounts of complex information from tissue-mimetic platforms [137]. To date, organoids and organs-on-a-chip that recreate in vivo molecular physiology are potential candidates for HCS.

In contrast, HTS is a fast and economical method of identifying adverse reactions of drugs using laboratory automation [138]. The goal of HTS is to minimize the time and the expenses of screening large numbers of drug libraries [139]. Therefore, to screen the adverse effects of numerous drug libraries in a time-efficient and reliable manner, it is necessary to develop organoids and organs-on-a-chip applicable to HCS and HTS. This development should proceed in two directions: i) development of a system for mass production with low variability and ii) development of a system capable of analyzing numerous inputs in parallel.

4.2. Mass-production and advanced analysis technologies for collecting in vitro database

Mass-production of brain organoids should be focused on reducing sample-to-sample variability. Organoids fabricated using low-attachment plates exhibit uncontrollable size and floating morphology and are unsuitable for reliable drug screening [140]. However, as the method to fabricate a microcavity array in a well-plate was developed, it became possible to manufacture organoids of uniform size, exhibited increased growth speed, reduced time to maturity, and had a fixed location [141,142]. Another advantage of microcavity arrays is that automated systems can replace traditional labor-intensive manual experiments such as cell seeding, medium exchange, drug exposure, and imaging [141,143]. This mass-production system is also being intro
duced in the field of brain organoids [142-144]. Mass-produced BBB organoids maintain the property of showing low permeability to macromolecules [144]. The mass-produced midbrain organoids maintain synchronized neural activity [143]. Mass-produced retinal organoids maintain the expression of photoreceptors [142]. The strategies for mass-production of brain-on-a-chip focus on a simple microfluidic device design combined with traditional labware [73]. This is because conventional labware, such as 96-well plates, is already available for HTS [145]. To this end, it is necessary to change the material used for the production of brain-on-a-chip, from polydimethylsiloxane (PDMS) to polystyrene (PS), and manufacture it by injection-molding fabrication. PS is more suitable for mass production and long-term storage than PDMS in terms of material properties [146].

High-resolution imaging and electrophysiology must be available for the analysis of brain organoids and brain-on-a-chip to allow for the evaluation of drug responses. The limit of the visual depth of conventional confocal microscopy is approximately 1 mm due to light scatt
tering, which is relatively small compared to the diameter of the brain organoid, which is a few millimeters in diameter [147]. Therefore, the internal structure of the brain organoid cannot be identified using conventional imaging techniques. However, a tissue-clearing technique was recently introduced and opened a way to overcome the limitations.
of deep-tissue imaging [148], which allowed for observing the internal structure of the brain organoid with confocal and two-photon microscopy. Furthermore, light-sheet microscopy capable of high-throughput imaging of transparent brain organoids has been developed. Conventional confocal microscopy is not suitable for high-throughput imaging because it has limitations in high-speed imaging due to its point-based illumination and scanning approach. In contrast, light-sheet microscopy illuminates and scans the organoids or 3D tissue construct in a plane-by-plane manner so that the imaging speed dramatically increases, making it suitable for high-throughput imaging [149]. By applying tissue-clearing technology and light-sheet microscopy simultaneously, it is possible to image a whole-brain organoid with single-cell resolution in approximately 15 min [150]. In the case of brain-on-a-chip, fusing this approach with the use of traditional labware is helpful for high-throughput imaging. Incorporation of conventional labware, such as well plates in conjunction with automated microscope systems, in experimental design can accelerate image-based analysis [73].

In electrophysiology, high-throughput analysis can be achieved by transferring the brain organoid onto a commercially available MEA and culturing further [151]. In the case of brain-on-a-chip, commercially available MEAs can be combined with a microfluidic device, enabling high-throughput electrophysiology analysis [152].
4.3. Processing collected big data from in vitro database through in silico modeling

The number of studies based on the brain physiome for in silico modeling is limited, and the standards for data collection based on the brain physiome have not yet been established. The lack of data for the brain physiome hinders the development of effective in silico prediction models. Nonetheless, BBB permeability is the most well-collected database for the brain physiome. BBB permeability databases have been reported in several studies [153,154]. For in silico models predicting BBB permeability, numeric values, such as logBB or logPS, were used to describe BBB permeability. The prediction models were divided into two categories: classification and regression models. Classification models are binary classifiers for BBB permeability, and regression models are used to predict a numeric value representing BBB permeability, such as the logBB value or logPS value. With the advances in machine learning techniques, prediction tools have used different machine learning methods, such as support vector machine (SVM) and random forest [155–157]. Recently, combined machine learning models and consensus models have also been developed to improve prediction performance [158–160].

Most in silico brain physiome models suffer from a lack of data and imbalanced data problems. Even for the BBB permeability models, regression models show limited performance owing to the relatively small datasets, and quantitative models have the problem of imbalanced data [158]. For this reason, most brain physiome studies have focused on the construction of proper datasets. Jiang et al. collected a large number of mouse toxicity datasets to build a chemical neurotoxicity prediction model [161]. To predict in vitro neurotoxicity induced by nanoparticles, Furxhi and Murphy created a dataset containing nanoparticle physicochemical properties, exposure conditions, and in vitro characteristics from experimental neurotoxicity data from multiple studies [162]. Jamal et al. proposed constructing a neurological adverse drug reaction prediction model to arrange the side effects of drugs using biological data from DrugBank, chemical data from PubChem, and phenotypic data from the SIDER database [163]. Oversampling methods such as the synthetic minority oversampling technique (SMOTE) have been used in several studies to handle imbalanced data, which show improvement in model performance [158,159,162,163].

In contrast, a cardiac physiome database is available for predicting the cardiac toxicity of drugs and has already been constructed using in silico tools [4,164,165]. Hence, below, we explain the concept of the brain physiome based on the already established concept of the cardiac physiome, with the aim of extending the hierarchical elements of the brain physiome, to set up the standards for in silico modeling.

Computational modeling and simulation can be used to predict various biological phenomena, such as cardiac electrophysiology, based on experimental data [166,167]. For instance, a comprehensive in vitro proarrhythmia assay (GIPA) represents the relationship between the outcomes of in vitro analysis of drug effects (input data), and those from in vivo studies (output data) [168]. In this manner, assessing drug effects on multiple ion currents involves monitoring the effects on human cardiac myocytes in vitro and human electrocardiogram in vivo, in phase I clinical trials. Similar to cardiac modeling, the effects of brain-targeting drugs may be predicted using the brain physiome by simulated in silico brain modeling. First, drug candidates are selected and their side effects, such as brain toxicity, are examined using in vitro 3D brain models (Fig. 5-B). Then, an in vitro database is constructed to classify the data according to any tendencies, resulting in the construction of big data (Fig. 5-D-II). Next, the selected drug candidates are used as an input, and the predicted drug action and toxicity are used as an output in a drug side-effect database using machine learning (Fig. 5-D-iii and 5D-iv).

5. Summary and outlook

Although newly developed biopharmaceuticals are effective against severe disorders, the analysis of their safety is challenging. Analyzing adverse reactions to drugs targeting the brain is even more challenging because of the structural complexity of the brain and the inability to monitor deep tissue. To understand the complex characteristics of the brain, it is necessary to introduce the concept of the physiome. Considering the genetic heterogeneity of the physiome of the animal brain and that of the human brain, an in vitro brain model based on human cells that can recapitulate the brain physiome is required. Brain organoids and brain-on-a-chips are suitable solutions for these unmet needs in the field. Furthermore, to predict and minimize adverse reactions to novel drugs, it is necessary to introduce in silico tools that rely on computational resources to analyze the beneficial or adverse effects of chemicals or drugs [169,170]. To establish reliable in silico models, it is necessary to bridge the data obtained from in vitro brain models, such as brain organoids and brain-on-a-chips, with an in silico database. The standardization of input and output is also important for the evaluation and prediction of the side effects of newly developed drugs in a systemic manner. Once that is established, we anticipate that the number of in vivo experiments needed to predict the safety of biopharmaceuticals will be minimized (Fig. 6).

Author contributions

S. Bang, Y. Seo, N. Choi, and H. N. Kim conceived the entire review and wrote the manuscript. Y. Seo and S. Bang surveyed all the literature and categorized reports into the hierarchical levels of the brain physiome. J.-H. Eom, H. N. Kim, P. Kim, and Y. Jeong conceptualized the scenario of the brain physiome-based prediction and evaluation of drug toxicity using in silico and in vitro models, respectively. J. Son and D. Kim reviewed reports related explicitly to in silico models and wrote the manuscript. All the authors reviewed the manuscript, provided constructive corrections and feedback toward strengthening the rationales of this review, and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We appreciate Prof. Se-Bum Paik for the discussion on the brain physiome concept. This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant nos. 2020R1A1A3A01098991 and 2020R1A1A3A01099935), by the National Research Foundation of Korea (NRF) funded by the Korean Government (MSIT) (grant nos. 2021R1A2B5B02086828, 2018MS3C7A1056896, and 2020M3E5D907974412), and by a grant (grant no. 20172MFD5S196) funded by the Ministry of Food and Drug Safety. The funder did not play any role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

References

[1] L.E. Crowell, A.E. Lu, K.R. Love, A. Stockdale, S.M. Timmick, D. Wu, Y. Wang, W. Doherty, A. Bonnyman, N. Vecchizziello, C. Goodwine, L. Bradbury, J. R. Brady, J.J. Clark, N.A. Colant, A. Cvetkovic, N.C. Dalvie, D.N. Liu, Y.J. Liu, C. A. Mascalzehn, C.B. Matthews, N.J. Mozdzierz, K.A. Shah, S.L. Wu, W. S. Hancock, R.D. Braatz, S.M. Cramer, J.C. Love, On-demand manufacturing of clinical-quality biopharmaceuticals, Nat. Biotechnol. 36 (10) (2018) 988–995.
[2] G. Walsh, Biopharmaceutical benchmarks 2018, Nat. Biotechnol. 36 (12) (2018) 1136–1145.
[3] P.J. Hunter, T.K. Borg, Integration from proteins to organs: the physiome project, Nat. Rev. Mol. Cell Biol. 4 (3) (2003) 237–243.
enhanced Blood-Brain Barrier Chip recapitulates human barrier function and shuffling of drugs and antibodies, Nat. Commun. 10 (1) (2019) 2621.

M.A. García-Cabanas, Y.J. John, H. Barbas, B. Zikopoulos, Distinction of neurons, gli and endothelial cells in the cerebral cortex: an algorithm based on cytological features, Front. Neuroanat. 10 (2016),

Y. Shin, S.H. Choi, E. Kim, E. Byukbhash, J.A. Kim, Chung, D.Y. Kim, R. Dam, R.E. Tanzi, Blood-brain barrier function in a 3D in vitro model of Alzheimer's disease, Adv. Sci. 6 (20) (2019).

L. Pellegrini, C. Bonfil, J. Chadwick, F. Begum, M. Skelel, M.A. Lancaster, Human CNS barriers-forming organs with cerebrospinal fluid production, Science 369 (6500) (2020).

E. Kemanetzoglou, E. Andreadou, CNS demyelination with Tnf-a blockers, Curr. Neurol. Neurosci. Rep. 17 (4) (2017) 36.

H. Walther, Development of thermite’s sign during cisplatin chemotherapy: possible drug-induced toxicity causing spinal cord demyelination, Cancer 60 (9) (1987) 2170-2172.

R.J. Franklin, Remyelination in the CNS: from biology to therapy, Nat. Rev. Neurosci. 9 (1) (2008) 839-855.

K.A. Nave, H.B. Werner, Myelination of the nervous system: mechanisms and functions, Annu. Rev. Cell Dev. Biol. 30 (2014) 503-533.

M. Madhavan, N.S. Nevin, H.E. Shick, E. Garrison, C. Clark, M. Karl, B.L. Clayton, D.C. Factor, K.C. Allan, L. Barbar, Induction of myelinating oligodendrocytes in human cortical spheres, Nat. Methods 15 (2018) 700-706.

R.M. Marton, Y. Miura, S.A. Sloan, Q. Li, O. Revah, R.J. Levy, J.R. Huguenard, S. M. Franklin, Remyelination in the CNS: from biology to therapy, Nat. Rev. Neurosci. 14 (3) (2013) 159-171.

B.L. Clayton, D.C. Factor, K.C. Allan, L. Barbar, Induction of myelinating oligodendrocytes in human three-dimensional neural cultures, Nat. Neurosci. 22 (3) (2019) 484-491.

H.U. Lee, L. Blasski, D.T. Alegro, D.T. Bong, N.V. Thakor, A.H. Ali, J.S. Ho, J. Yang, Subcellular electrical stimulation of neurons enhances the myelination by axons of oligodendrocytes, PLoS One 12 (7) (2017) e0179642.

H.U. Lee, S. Nag, A.B. Tsur, J. Lim, H.Y. Yang, Subcellular optogenetic stimulation for activity-dependent myelination of axons in a novel microfluidic compartmentalized platform, ACS Chem. Neurosci. 7 (10) (2016) 1317-1324.

S. Hyung, S.R. Lee, Y.J. Kim, S. Bang, D. Tak, J.C. Park, J.K.F. Suh, N.L. Jeon, Optogenetic modulation of macrophage outgrowth and myelination of motor neurons in a three-dimensional motor neuron-Schwann cell coculture model on a microfluidic biochip, Biotechnol. Bioproc. Eng. 116 (10) (2019) 2425-2438.

K. Jung, H.N. Kim, N.L. Jeon, S. Hyung, Comparison of the efficacy of optogenetic stimulation of glial axons in myelination, ACS Chem. Neurosci. 11 (4) (2020) 4280–4288.

S. Hyung, S.R. Lee, J. Kim, Y. Kim, S. Kim, H.N. Kim, N.L. Jeon, A 3D disease and regeneration model of peripheral nervous system-on-a-chip, Sci Adv 7 (5) (2021).

S.R. Lee, S. Hyung, V. Dierckx, Y. Lee, J. Ko, J. Jung, Models of neural circuit, blood-brain barrier, and myelination on a microfluidic 96 well plate, Biofabrication 11 (3) (2019), 035013.

A.D. Sharma, L. McCoy, E. Jacobos, H. Willey, J.Q. Behn, H. Nguyen, B. Bolen, J. L. Carley, M.J. Moore, Engineering a 3D functional human peripheral nerve in vitro using the Nerve-on-a-Chip platform, Rep. Eng. (1) (2019) 1–12.

R.L. Fiedre, Control of myelination by axon caliber. (With a model of the control mechanism), J. Comp. Neurol. 144 (2) (1972) 233-252.

J.T. Vovodics, Target size regulates calibre and myelination of sympathetic axons, Nature 342 (6248) (1989) 430-433.

S. Lee, M.K. Leach, S.A. Redmond, S.Y.C. Chong, S.H. Mellon, S.J. Tuck, Z. Feng, J.M. Cohen, K. Chung, A. Chang, M. Lee, A. Fu, L. Gao, J. Lim, J. Yu, J. Kim, N.L. Jeon, B. Park, D. L. Wang, Brain barrier function in a 3D in vitro model of Alzheimer’s disease phenotypes, PLoS One 11 (9) (2016) 0161969.

J.J. Burmeister, P. Fomeruel, M. Palmer, B.K. Day, P. Hueltl, G.A. Gerhardt, Improved ceramic-based multisite microelectrode for rapid measurements of local cerebral blood flow in animal models, Neurosci. Methods 369 (2020) 102731.

H. Hettetadi, S.N. Mojab, M. Ranjan, J. Shams, H. Sabraei, H. Hedayati, F. Asefi, Aquous extract of saffron (Crocus sativus) increases brain dopamine and glutamate concentrations in rats, J. Behav. Brain. Sci. 13 (3) 0396.03.

N. Dale, S. Hatz, F. Tian, E. Llabert, Listening to the brain: microwire biosensors for neurochemicals, Trends Biotechnol. 23 (8) (2005) 420–428.

B. Nasr, R. Chatterton, J.Y.H.M. Yong, P. Jamshidi, G.M. D’Abaco, A.R. Bjorksten, G. Kavehi, G. Chana, M. Dottori, E. Skafidas, Self-organized nano-structured modified microelectrode for sensitive electrical recording in vivo, Detection in stem cells-derived brain organoids, Biosensors 8 (1) (2018) 14.

W.K. Raja, A.E. Mungun, Y.T. Lin, T. Ko, F. Abdurrah, J. Seo, L.H. Tsai, Self-organizing 3D human neurons on the same chip from induced pluripotent stem cells: Remeptalize Alzheimer’s disease phenotype, PLoS One 11 (9) (2016) e0161969.

A. Welin, K. Slotwinski, J. Kieninger, I. Moser, G. Jobs, M. Wego, R. Ehret, G. A. Urban, Cell culture monitoring for drug screening and cancer research: a transparent, microfluidic, multi-sensor microsystem, Lab Chip 14 (1) (2014) 138-146.

D. Tak, S. Bang, S. Hyung, J. Lim, J. Yu, J. Kim, N.L. Jeon, H.N. Kim, Self-detachable UV-curable polymers for open-access microfluidic platforms, Lab Chip 20 (22) (2020) 4215-4224.

Esther A. Nimchinsky, a, Bernardo L. Sabatini, K. Svoboda, Structure and function of dendritic spines, Annu. Rev. Physiol. 64 (1) (2002) 313–353.

D.H. Bhatt, S. Zhang, W.-B. Gan, Dendritic spine dynamics, Annu. Rev. Physiol. 71 (1) (2009) 29-51.

P. Penez, M.E. Cahill, K.A. Jones, J.E. VanLeeuwen, K.M. Woolfrey, Dendritic spine pathology in neuronal dystrophies, Nat. Neurosci. 14 (3) (2011) 285–292.

R.R. Mahmmoud, S. Sase, Y.D. Aher, A. Sane, M. Gröger, M. Mokhtar, H. Hüger, G. Lubec, Spatial and working memory is linked to spine density and mushroom spines, PloS One 10 (10) (2015) e0139739.

V.S. Sohal, J.L. Rubenstein, Excitation-inhibition balance as a framework for neuronal functions, Annu. Rev. Physiol. 64 (1) (2002) 313-353.

J.R.B. Atluri, S.P. Kanthikeel, P.V.B. Reddy, A. Yndart, M.P.N. Hair, Human synaptic plasticity gene expression profile and dendritic spine density changes in HIV-affected human CNS cells: role in HIV-associated neurocognitive disorders (HAND), PLoS One 8 (4) (2013) e61399.

Z. Huang, H.G. Khaled, M. Kirschmann, S.M.H. Gobes, R.H.R. Hahnloser, Human dendritic spine dynamics, Annu. Rev. Physiol. 75 (2013) 171-200.

T. Patriarchi, S. Amabile, E. Frullanti, E. Landucci, C. Lo Rizzo, F. Ariani, Excitation of rat prefrontal cortical neurons by optogenetic stimulation for activity-dependent myelination of axons in a novel microfluidic regeneration model of peripheral nervous system-on-a-chip, Sci Adv 7 (5) (2021).

V.S.R. Atluri, S.P. Kanthikeel, P.V.B. Reddy, A. Yndart, M.P.N. Nair, Human dendritic spine dynamics, Annu. Rev. Physiol. 75 (2013) 171-200.

H. Lodish, A. Berk, S.L. Zipursky, D. Baltimore, J. Darnell, Molecular Cell Biology, 5th edition, New York, USA, (2000) 917.

Z. Huang, H.G. Khaled, M. Kirschmann, S.M.H. Gobes, R.H.R. Hahnloser, Human dendritic spine dynamics, Annu. Rev. Physiol. 75 (2013) 171-200.
