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Monitoring of Electrophysiological Functions in Brain-on-a-Chip and Brain Organoids

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Though animal models are still the gold standard for fundamental biological studies and drug evaluation for brain diseases, concerns arise from an apparent lack of reflecting the human genetics and pathophysiology. Recently, human avatars such as brain-on-a-chip and brain organoids which are generated in a 3D manner using multiple types of human-originated cells have risen as alternative testing models. Particularly in monitoring the functional neuronal cells that express action potentials in brain-on-a-chip or brain organoids, various methods of measuring their electrophysiological function have been suggested for the study of brain-related disease. Recent methodologies for analyzing the electrophysiology of different types of cells in brain-on-a-chip and brain organoids are summarized in this review. We first emphasize the inherent features of brain-on-a-chip and brain organoids from the perspective of the cell culture environment and accessibility to cells in the deep layer. The applicable monitoring techniques are then overviewed based on these features. Finally, we discuss the unmet needs for electrophysiology monitoring in advanced human brain avatar models.

1. Introduction

For the last few decades, animal models have been indispensable for fundamental biological studies, particularly in brain research and pharmacological studies for neurological diseases.[1] Their utilization stems from an ability to exhibit complex cellular interactions that cannot be replicated by traditional in vitro models. Nonetheless, these models fall short of accurately representing human brain physiology and often result in an unsuccessful translation of promising drug candidates to clinical trials similar to the repeated failures of Alzheimer's disease therapeutics.[2] The translation success rate in drug discovery processes is notoriously low, sometimes under 10%.[3]

Brain-on-a-chip and brain organoids have emerged as innovative human brain model alternatives—avatars of the human brain. While they share a common goal of emulating human brain pathophysiology,[4] they adopt distinct methodologies to recreate the brain's structure and function in vitro.[5] The brain-on-a-chip is often designed to simulate the brain's response to various stimuli.[6] Also, it enables precise control over the types or ratio of the cells and their cellular environment.[7] In contrast, the brain organoids are generated to mimic the organ's dynamic developmental process for an investigation in the intricacies of neural development and function.[8] And it is crucial for the aforementioned models to monitor the neuronal function of the cells for analyzing the changes in

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electrophysiological functions during the transition from normal to disease states.[9] Offering an accurate physiology of convoluted human brain functions or activities, both models can provide critical insights into neuronal function and exhibit the potential for revolutionizing the current drug testing platform. The methods for measuring electrophysiological functions may vary depending on cell-hydrogel ratios, accessibility to deep tissue layer, or other surrounding microenvironments.[10] While the advent of microelectromechanical systems allowed the advancement of devices measuring and monitoring electrophysiological functions in a high spatiotemporal manner, the details of measurement methods were still not fully discussed. Several recent well-written articles have addressed the methods for characterizing electrophysiological analysis in brain-on-a-chip or brain organoids, discussing the challenges faced by both models in measuring electrophysiology separately.[11] Although those two models share common features in terms of electrophysiology, they have distinct structures and microenvironments, requiring a different approach for measuring electrophysiological signals. For example, brain-on-a-chip encounters hurdles in electrophysiology measurement due to the hydrogel matrix employed for 3D culture, whereas brain organoids struggle due to their size and structure.[12]

Therefore, we summarize electrophysiology measurement methods in brain-on-a-chip and brain organoids, with a particular focus on the specificity of each brain model and its unique characteristics regarding the 3D microenvironment. We first overview the difference between brain-on-a-chip and brain organoids in terms of the approaches for mimicking the structural and functional aspects of the human brain. Then, the methodologies for the measurement of electrophysiological functions are summarized with a focus on the platform-specific cell culture environment and deep tissue accessibility. Finally, the unmet needs for current measurement techniques are discussed. It is envisioned that understanding the methodologies for electrophysiology measurement can help the studies of brain connectivity and functional regeneration in brain-on-a-chip and brain organoids.

2. Brain-on-a-Chip and Brain Organoids

2.1. Difference of Brain-on-a-Chip and Brain Organoids

Both brain-on-a-chip and brain organoids were developed to mimic the structure and physiology of the human brain, but those platforms utilize different approaches.[5,13] The brain-on-a-chip was developed in the engineering field as a subcategory of organ-on-a-chip. For the preparation of brain-on-a-chip, a transparent chip made of rubber, plastic, glass, or any combination of them is fabricated as a vessel culturing for cells.[14] Different types of human-derived brain cells are then cultured in two dimensions (2D) or three dimensions (3D).[15] For the 2D cell culture, porous membranes are widely used to build double-layered structures.[16] A molecular transport interface blood-brain barrier between brain blood vessels and tissue cells such as astrocytes and pericytes is a typical example of a multilayered tissue structure mimicked in brain-on-a-chip.[17] In the case of 3D cell culture, brain cells are embedded in the hydrogel and subsequently injected into the chamber or microchannels.[18] After the gelation of the brain cell-laden hydrogel, the endothelial cells can be cultured to the surface of the gelated hydrogel to form a monolayer and act as a blood vessel wall.

Brain organoids were based on stem cell biology.[19] The preparation of brain organoids follows developmental stages.[20] First, stem cells such as induced pluripotent stem cells (iPSCs) or embryonic stem cells are aggregated. Then, the stem cell aggregates are cultured in the media with growth factors and signaling molecules. The types and concentrations of growth factors and signaling molecules are modulated depending on the growth stages. The stem cells proliferate and differentiate in response to the growth factors, signaling molecules, and cell–cell interaction. Ultimately, they form layered and regionally distinct ultrastructures that are not observable in conventional 2D culture models.[21]

The brain-on-a-chip and brain organoids technologies possess distinct features in terms of biomimicry. For example, the brain-on-a-chip has strong potential in recapitulating microenvironmental factors including the mechanical properties of the extracellular matrix (ECM), interstitial fluid flow, and transport of molecules.[22] In contrast, the brain organoid is good for the mimicry of ultrastructures since the generation of organoids follows the developmental stages.[23] In terms of cell density, the brain-on-a-chip has a relatively lower cell-to-ECM ratio since the cells are embedded in the hydrogels. In comparison, the brain organoid is developed from the cell aggregates, and thus the cellular portion is much higher than ECMs. The ECMs are spontaneously deposited by cells or covered when they are embedded in the Matrigel for maturation. For these reasons, the drugs or molecules are not easy to penetrate the deep layer of organoids compared to brain-on-a-chip. The portion of ECM and the accessibility to the deep layer are the key factors when choosing devices and methods for electrophysiology measurement. Here, we introduce the key features of brain-on-a-chip and brain organoids, along with detailed methods for measuring electrophysiology (**Table 1–3**).

3. Electrophysiology Measurement Techniques for 3D in vitro Models

Electrodes of various designs have been engineered to assess electrophysiology in living animals. For example, patch-type graphene electrodes,[24] silicon probes,[25] neuron-like electrodes,[26] and active pixel sensor complementary metal-oxide semiconductor probes,[27] have shown promise in real time in vivo brain electrophysiology monitoring. These devices demonstrated promising capabilities in real-time monitoring of in vivo brain electrophysiology. However, in vitro models have different characteristics compared to in vivo brains in terms of size and accessibility. Given that brain-on-a-chip and brain organoids are smaller than mouse brains, the patch-type electrodes designed for in vivo monitoring are comparatively larger in surface area. In addition, the in vivo brain is readily formed, and thus integration of devices in a noninvasive manner is quite difficult.

Conversely, 3D in vitro models present regions of interest ranging from a few hundred micrometers to a few millimeters, allowing comprehensive access from multiple

Table 1. Characteristics of brain-on-a-chip and brain organoid.

	Brain-on-a-Chip	Brain Organoid
Fundamental discipline	Engineering	Biology
Methodology	Coculture of multiple types of brain-originated cells in a transparent chip	Spontaneous growth and differentiation of stem cell aggregates following developmental process
Key features	Recapitulation of microenvironment including mechanical properties, interstitial flow, vasculature, molecular transport Good at controlling cell–cell interaction	Mimicry of region-specific ultrastructures such as multilayered structure in brain tissue Good at mimicking developmental process-associated diseases of brain tissue
Unmet needs	Formation of layered and aligned structures	Formation of vascular structure and immune systems along with brain organoids

Table 2. Measurement methods of electrophysiology in brain-on-a-chip and 3D brain model.

Electrophysiology device	Structure of platform	Cell type	Findings by using electrophysiology device	References
Conventional glass pipette-type electrophysiology setup	Cells embedded in a 3D collagen matrix within a microfluidic device (aligned)	Primary rat hippocampal neurons and astrocytes	Efficient transfer of electrical signals to another region in the case of aligned neurons Degradation of extracellular matrix using collagenase was required before measurement	Kim[30]
Bendable neural electrode	Cells embedded in 3D collagen matrix (nonaligned)	Primary rat hippocampal neurons and astrocytes	Monitoring of long-range signal transfer and intercellular coupling. Cell-laden hydrogel was seeded	Shin[31]
Film-type neural electrode	Cells embedded in 3D hydrogel matrix (nonaligned)	Human induced pluripotent stem cell-derived neurons and astrocytes	Measurement of spatially distributed electrical signals from 3D cultured cells	Soscia[32]

angles. Accordingly, the monitoring devices developed for these models are scaled-down and designed to precisely capture high-resolution electrophysiological data without the need for invasive techniques, which are often required for a more detailed analysis of neural activity.

3.1. Brain-on-a-Chip

A 3D brain-on-a-chip is meticulously engineered by integrating various neuronal cell types into a hydrogel matrix with precise spatial configuration. This hydrogel-based framework limits the penetration of sharp, needlelike electrodes due to its fibrous, netlike structure, which displaces in response to the electrodes' insertion.[28] Once the hydrogel solidifies, it establishes a firm matrix that resists the deeper insertion of electrodes. Consequently, to effectively capture the electrophysiological dynamics, researchers working with 3D brain-on-a-chip have devised two distinct approaches. One method involves enzymatically degrading the hydrogel matrix to establish direct contact between the cells and electrodes.[29] Another strategy embeds electrodes within the hydrogel at the time of its gelation, which allows for an integrated network and the capacity to measure electrophysiological activity from within the matrix itself. Kim et al. utilized a conventional electrophysiology measurement technique in a 3D neural network within an aligned collagen hydrogel matrix (**Figure 1**A).[30]They demonstrated the strategic pre-deformation of an elastic substrate to induce the alignment of collagen fibrils. By stretching or compressing the cell-laden collagen and allowing the substrates to undergo partial gelation, they facilitated the orientation of collagen fibrils perpendicular or parallel to the axis of applied strain. This alignment was fixed by releasing the strain after a 5-minute gelation period. The embedded neurons, influenced by the topographical cues of the aligned collagen network, exhibited directional neurite outgrowth establishing faster interregional connections than those observed in the nonaligned case. To assess functional connectivity, the conventional glass pipette electrophysiology technique was employed. However, due to the encapsulation of neurons within the collagen matrix, direct access by glass pipettes was impeded. To solve this issue, they degraded the surface of the collagen by treatment with collagenase. Electrical signals were transferred to connected regions in the aligned neural network, but not transferred in the randomly oriented neural network. This finding indicated that electrical signal propagation was efficacious in the aligned networks, underscoring the importance of structural alignment in neural network functionality. However, the degradation of the preexisting matrix, which is essential for establishing contact between cells and electrodes, poses considerable technical difficulties. In response to this challenge, Shin et al. pioneered an alternative strategy by incorporating a shank-type neural electrode within the matrix during the gelation phase (Figure 1B).[31] They developed a 3D multifunctional microelectrode array (MEA) system, characterized by high-density electrode arrays that were enveloped by the neuron-laden matrix.

Table 3. Measurement methods of electrophysiology in brain-related cell clusters.

Electrophysiology device	Cluster type	Findings by using electrophysiology device	References
2D microelectrode array (MEA)	Assembloid (cerebral organoid + motor neuron spheroid)	A model for signal transfer from the brain to the whole body by mimicking the brain−spinal cord connection An increased neural spiking speed on the motor neuron region by caffeine treatment	Son[43]
2D MEA	Cerebral organoid fabricated in microfluidic device	Decreased firing rate after treatment of Δ-9-tetrahydrocannabinol, a major psychoactive compound from marijuana	Ao[44]
2D MEA	Cortical organoid	Maturation-dependent emergence of complex oscillatory waves in cortical organoid	Trujillo[38]
2D MEA	Bioengineered neuronal organoid	Development of complex network bursts over 2 months of culture Recording of short- and long-term potentiation and depression	Zafeiriou[39]
2D MEA	Cerebral organoids generated at the air-liquid interface (ALI)	Monitoring of functional connectivity between specific sites within the ALI organoid	Giandomenico[40]
2D MEA	Cerebral organoids	5 months of monitoring of electrical activities including rapid firing rates and network bursting events	Fair[37]
2D MEA	Human cortical organoids	Monitoring of effects of methadone in the functional interruption of spontaneous activity	Yao[41]
Silicon neural probe	Human brain organoid	Recoding of firing rate in photosensitive brain organoids	Quadrato[49]
Silicon neural probe	Rat-transplanted human cortical organoids	Recording of electrophysiological signals in rat brain-transplanted human cortical organoids	Revah[50]
Microdevice embedded within the 3D neural spheroid	3D neural spheroid made from primary rat cortical neurons	Demonstrated the importance of surface coating materials in successful embedding of microdevice in neural spheroid	Lecomte[48]
Stretchable mesh nanoelectronics	Reassembled brain organoid (hiPSC-derived)	Integrated with brain organoids with capabilities adapted to volume and morphological changes during brain development	Floch[52]
Stretchable mesh microelectronics	Human cortical organoid	Maintained stable electrical impedance under 50% compressive and 50% tensile strain measure stimuli intensity-dependent calcium signals	Li[52]
3D compliant multifunctional mesoscale framework	Engineered assembloids	Monitoring of signals in multiple points of assembloid surfaces	Park[53]
Patch clamp	Dissociated hippocampal-like neurons derived from dorsomedial telencephalic tissue	Validation of maturity of hippocampal-like neurons such as Na-K current and spontaneous excitatory postsynaptic currents	Sakaguchi[45]
Patch clamp	Section of human cortical-spinal cord organoids	Validation of functional connectivity between cortical-spinal cord organoids in sliced assembloid	Andersen[46]
Patch clamp	Sliced assembloid (cortical spheroid and subpallium spheroid)	Validation of the presence of functional synapses in migrated neurons	Birey[47]

While this approach did not yield an aligned neuronal morphology, it enabled the monitoring of signals from multiple cells concurrently. The MEA system's 18 shanks, arranged in a 6 by 3 configuration, were strategically distributed within the 3D neural network, thus allowing for the assessment of functional connectivity among different neural networks. Soscia et al. also demonstrated the functionality of a 3D MEA, which was achieved by mechanically transforming 2D polyimide probe arrays (Figure 1C.[32] These arrays, originally fabricated on glass substrates, were then bent into a vertical orientation. To assess the efficacy of the 3D MEA, a hydrogel composed of collagen was used to embed human-induced pluripotent stem cell (hiPSC)-derived neurons and astrocytes, which were then seeded onto the array. The design featured 256 channels capable of monitoring neuronal activity. Remarkably, it enabled the observation of this activity over a span of 45 days in vitro, suggesting its potential as a reliable platform for noninvasive electrophysiological studies of 3D neural networks in real time. These methodologies are available in 3D cell culture models, and they can be used for the real-time measurement in 3D microphysiological systems.

3.2. Brain Organoids

The simplest form of the 3D cell cluster, spheroids, is a structure that cells spontaneously form into a 3D "sphere" shape primarily

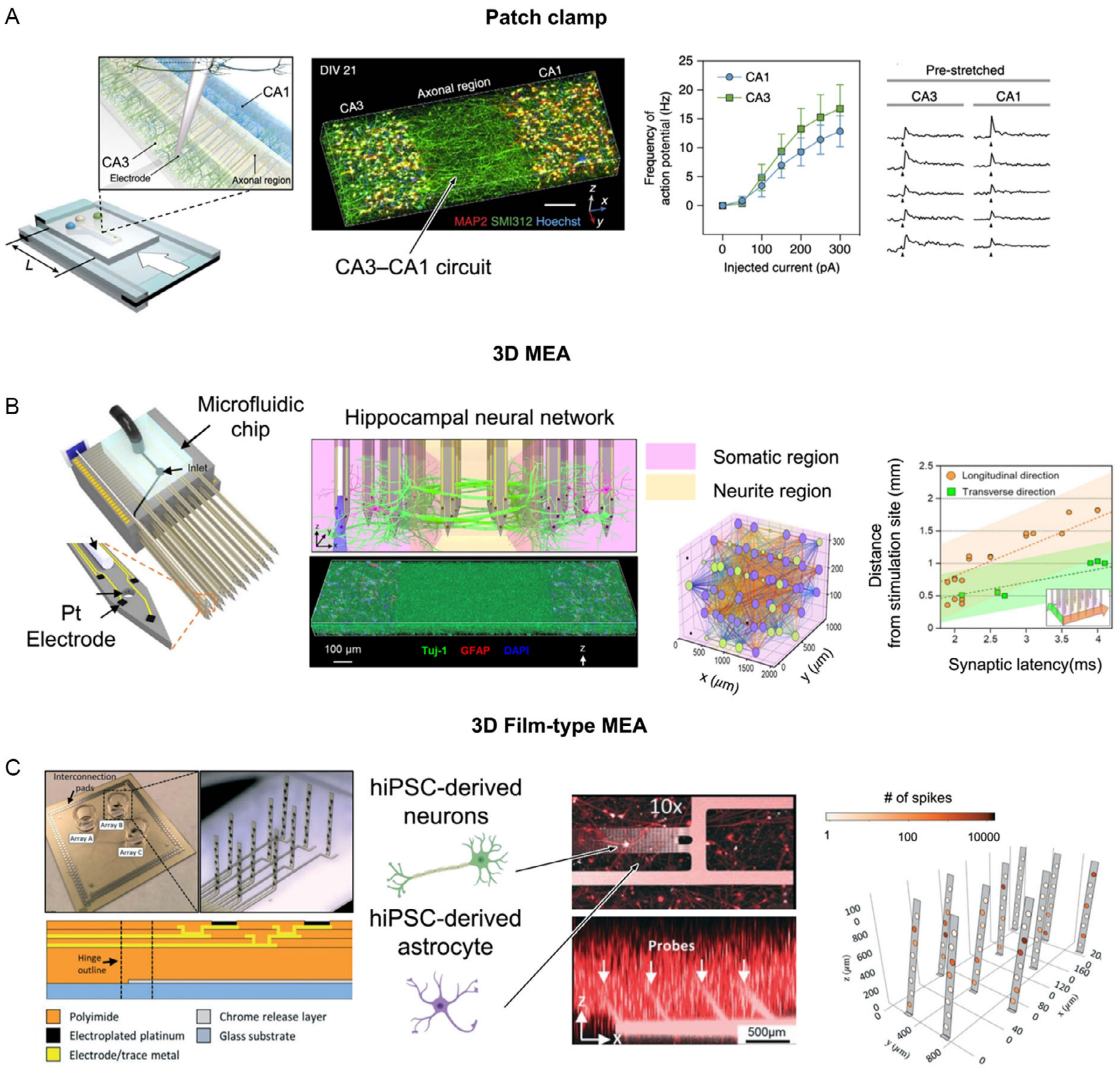

Figure 1. Electrophysiology measurement in brain-on-a-chip. A) Reconstruction of CA3-CA1 hippocampal circuit in an aligned fibrous scaffold, with patch clamp recordings to validate the functional synaptic connections and membrane properties in the 3D construct. Reproduced with permission from Kim et al.[30] (*Nature Communications* 8,1, (2017); Copyright 2018 Springer Nature). B) 3D high-density multifunctional microelectrode array (MEA) with optical and pharmacological interfaces, enabling precise analysis and control of neural circuit dynamics within engineered neural tissues. Reproduced with permission from Shin et al.[31] (*Nature Communications* 12, 1, (2021); Copyright 2021 Springer Nature). C) A thin-film, 3D flexible MEA (3D MEA) for the noninvasive interrogation of electrophysiological activity within a 3D neuronal network and long-term functional monitoring. The 3D neural network was composed of human-induced pluripotent stem cell (hiPSC)-derived neurons and astrocytes. Reproduced with permission from Soscia et al.[32] (*Lab on a Chip* 20, 5, (2020); Copyright 2020 Royal Society of Chemistry).

due to stronger cell–cell interactions compared to cell–surface interactions.[33] It closely mimics in vivo environments compared to 2D culture and is often used to create cell clusters such as organoids or assembloids which have a higher complexity of specific organs and tissues.[34] Often containing various types of stem cell-derived progenitor cells in higher levels of tissue complexity, the organoids possess specific functional characteristics similar to the targeted tissues.

The brain organoids that exhibit relevant physiology, function, and developmental processes to that of the human brain are often used for monitoring the electrophysiological function of the human brain.[35] Their ability to recapitulate spontaneous electrical activity in the developing human brain makes them particularly useful for investigating neural activity and exploring potential therapeutic interventions.[36]

MEAs, which are typically composed of electrode arrays that noninvasively capture the electrical activity of neurons across multiple regions or clusters of neurons within a brain organoid, provide insight into the functioning and interconnectivity of neural networks. While the continuous long-term monitoring of neuronal activity in spatiotemporal resolution on a surface can be achieved with the use of the planar MEAs, the 3D MEAs, which comprise multilayered electrodes, can be utilized for capturing the deeper complex electrophysiological dynamics of organoids in a more physiologically relevant context. Fair et al. utilized the MEA platform for long-term electrophysiological properties monitoring of cerebral organoids (COs) with developmentally linked morphological cellular features (**Figure 2**A).[37] They profiled the rapid firing rates and network burst of maturing COs over 5 months using the MEA platform. Trujillo et al. also used the MEA in examining the long-term spontaneous network formation and activity during early brain maturation of the generated human cortical organoids in examining (Figure 2B).[38] The electrical activity of the organoids was evaluated over 10 months. The channel-wise firing rate, burst frequency, and synchrony of the organoids were consistently increased and indicated a continually evolving neural network. The MEA often provides insights into the functional connectivity

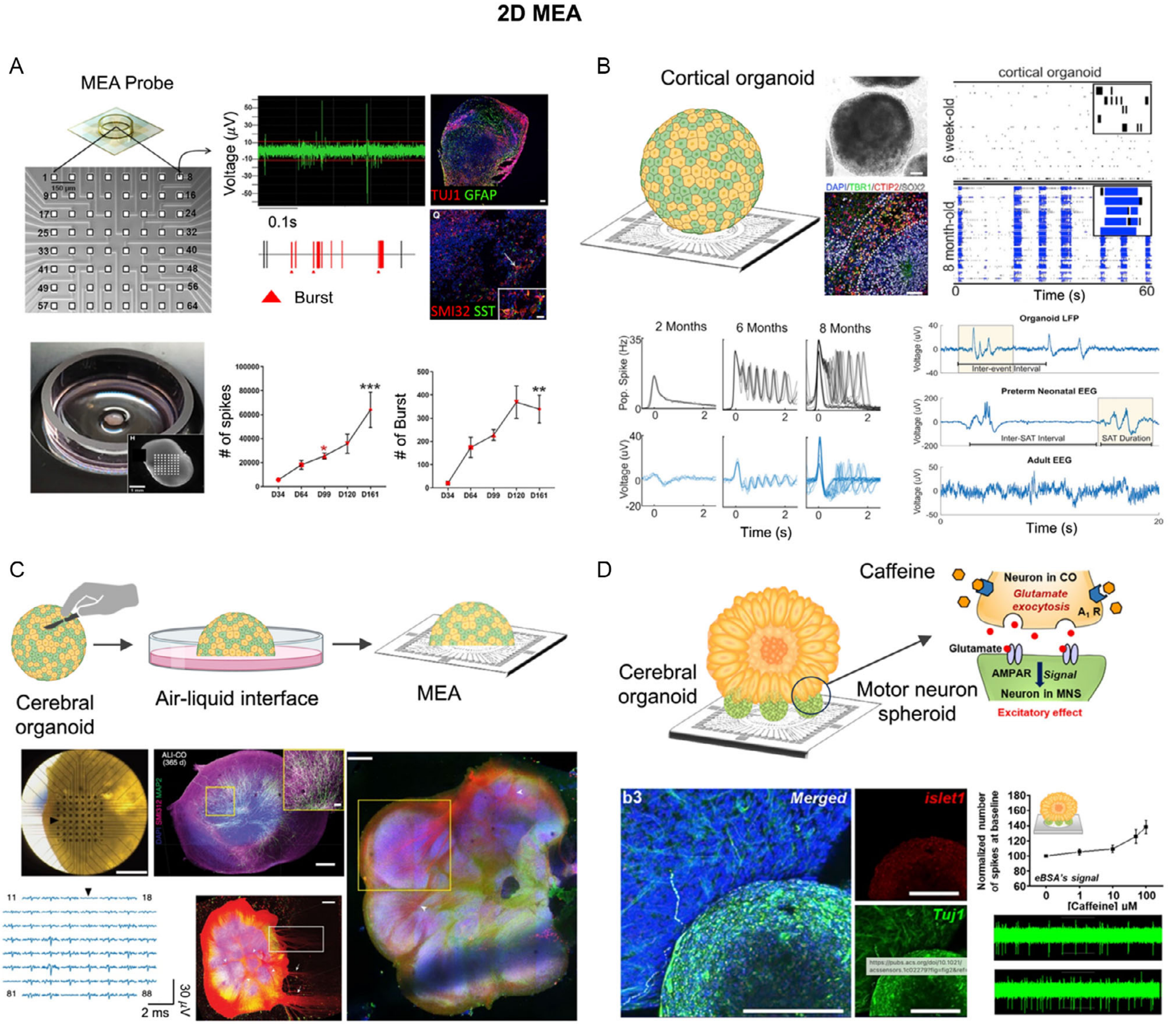

Figure 2. 2D microelectrode array (MEAs) for electrophysiological measurement in neural organoids. A) Dynamic profiling of cerebral organoid maturation using 2D MEA along with morphological and developmental cellular analysis. Reproduced with permission from Fair et al.[37] (*Stem Cell Report*, 15, 4, (2020); Copyright 2020 Cell Press). B) Representative local field potential (LFP) trace from a human cortical organoid showing network events (yellow). Similar events between quiescent periods are seen in a 35-week gestational age preterm neonate electroencephalogram (EEG), contrasting with the continuous activity pattern in adult EEG. SAT: spontaneous activity transient. Reproduced with permission from Trujillo et al.[38] (*Cell Stem Cell*, 25, 4, (2019); Copyright 2020 Cell Press). C) Cerebral organoid culture at the air-liquid interface formed robust axon tract and functional neuronal networks. Reproduced with permission from Giandomenico et al.[40] (*Nature Neuroscience*, 22, 4, (2019); Copyright 2020 Springer Nature). D) Brain–spinal cord assembloid (eBSA) demonstrating functional signal transfer from cerebral organoids to motor neuron spheroids, validated by caffeine-induced neural excitation and monitored via multielectrode array recording. AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. MNS: motor neuron spheroid. Reproduced with permission from Son et al.[43] (*ACS Sensors*, 7, 2, (2022); Copyright 2022 ACS publications).

and synchronization of neuronal networks within brain organoids. Zafeiriou et al. generated bioengineering neuronal organoids (BENOs), which comprise interconnected excitatory and inhibitory neurons with supportive astrocytes and oligodendrocytes.[39] The spatiotemporal neuronal network organization was assessed using MEA to monitor electrical activity and synchronous activity among neurons in BENOs. During the development of BENOs, the number of network bursts gradually increased along with the regional connectivity, mean firing rate, synchronous firing, and formation of complex networks of excitatory and inhibitory neurons in BENOs. Giandomenico et al. adapted air-liquid interface culture to COs and employed MEA for determining specific spatial patterns of the functional connectivity in intracortical and subcortical projecting axon tracts (Figure 2C).[40] Furthermore, the MEA enables the analysis of spontaneous or evoked activity patterns, such as bursts or oscillations, which can reveal the emergent properties of the network and help elucidate the underlying mechanisms. Using the MEA, Yao et al. investigate a reduction in spontaneous action potential and the elimination of the network activity of a human 3D-brain cortical organoid (hCO) upon exposure to methadone.[41] In an effort to mimic early stage of organ development and investigate the interaction among tissue organization, researchers often create assembloids, which can be in a form of organoid-organoid, organoid-spheroid, or spheroid-spheroid.[42] Son et al. engineered a brain–spinal cord assembloid (eBSA) by coculturing COs and motor neuron spheroids (MNSs) (Figure 2D).[43] They observed a successful transfer of the neural stimulation signal from the COs to MNSs in the eBSA during the caffeine treatment using the MEA. In addition, the excitatory effect of caffeine on the COs was transmitted to the MNSs via the release of glutamate from the COs and uncovered a functional neuronal network between them. Ao et al. cultured human COs by incorporating a perusable culture chamber with the systemic air-liquid interface control system.[44] In investigating the effect of prenatal cannabis exposure on early human brain development, the electrical activity of the cultured organoids upon the treatment of Δ-9-tetrahydrocannabinol (THC) was characterized using MEA. The burst activity and mean firing rate of the organoid were dramatically decreased with THC treatment, thus downregulating CB1 expression and reducing the neurite outgrowth and spontaneous neuronal activity.

For the need to investigate a small set or even single ion channels of excitable cells within the brain organoid, the patch clamp device can be utilized. It precisely determines the membrane potential, action potential firing patterns, ion channel kinetics, and synaptic activity/connectivity of the individual neurons within brain organoids. Sakaguchi et al. generated functional hippocampal granule- and pyramidal-like neurons from self-organizing dorsomedial telencephalic tissue using a serum-free floating culture of human embryonic stem cells (hESCs) (**Figure 3**A).[45] With the use of the whole-cell patch clamp, the maturity of the synaptic formation of the generated neurons was validated through the voltage-dependent Na-K current, action potential following injection of depolarizing currents, and spontaneous excitatory postsynaptic currents. Anderson et al. generated a functional 3D cortical-motor assembloid by resembling the hindbrain/spinal cord and human skeletal muscle spheroids for the study of the multisynaptic circuit of the neurons of the descending pathway (Figure 3B).[46] The mono- or di-synaptic connectivity of cortical neurons in human cortical spheroid (hCS) to motor neurons in human spinal spheroid was verified using patch clamp recording. Birey et al. fused the hCS with the human subpallium spheroids (hSS) to indirectly examine the development of the nervous system through the migration of GABAergic (γ-aminobutyric-acid-releasing) neurons from ventral to dorsal forebrain and their integration into the cortical circuit (Figure 3C).[47] The whole-cell patch clamp recording revealed a successful generation of action potential from neurons in hSS in response to depolarization and validated a functional synapse in migrated neurons.

In an effort to monitor the internal functionality and maturity of neurons within the brain organoid, Lecomte et al. proposed the method of embedding a potential monitoring microdevice into neurospheroid without disrupting the general morphology of self-aggregating spheroid (**Figure 4**A).[48] The optimized surface functionalization of the microdevice allowed the normal neuronal activity and synchronous activity of the spheroid. In contrast, neural probes feature a penetrating design, which allows for the direct interface with organoids, facilitating the recording of action potentials from individual neurons within organoids. These probes provide high spatial resolution and the capacity to track the activity of specific neurons over time. Quadrato et al. utilized a silicon neural probe to analyze the functional connectivity and internal signals of matured brain organoids.[49] It allowed the monitoring of the action potential, indicative of excitatory monosynaptic connections, and population firing characteristics of the organoid. By 8 months, the organoid showed spontaneously active neurons, neuronal networks, and photosensitive cells. In addition, Revah et al. transplanted human cortical organoid into the somatosensory cortex of newborn athymic rats (Figure 4B).[50] After the transplantation, the hCO was observed to possess higher maximal firing rates and higher rates of spontaneous excitatory postsynaptic current events using a silicon probe. The use of a silicon probe prevented the entanglement of the electrode with organoids and enabled the precise analysis of continuous neural activity over extended periods at the desired timing.

Stretchable mesh electrodes can also be used for measuring electrophysiology in brain organoids. While it serves as a scaffold to support the growth and development of brain organoids, it examines the functional properties of neurons within the perturbed brain organoids undergoing physiological processes such as tissue deformation. Li et al. fabricated a stretchable mesh electrode system that establishes an intimate in vitro electrical interface with hCOs.[51] The electrical stimulation through the system can elicit intensity-dependent calcium signals without damaging the cells and maintain a stable electrochemical impedance in buffer solution under 50% compressive and 50% tensile strain. Floch et al. created a cyborg brain organoid platform having stretchable mesh electrodes seamlessly distributed across brain organoids (Figure 4C).[52] The platform with capabilities of adapting to volume and morphological changes during brain development allowed a long-term stable monitoring of neural activity throughout the development of brain organoids. It revealed that the increase of action potential amplitude and firing rate suggest changes in neural network connectivity and cellular ion channel expression.

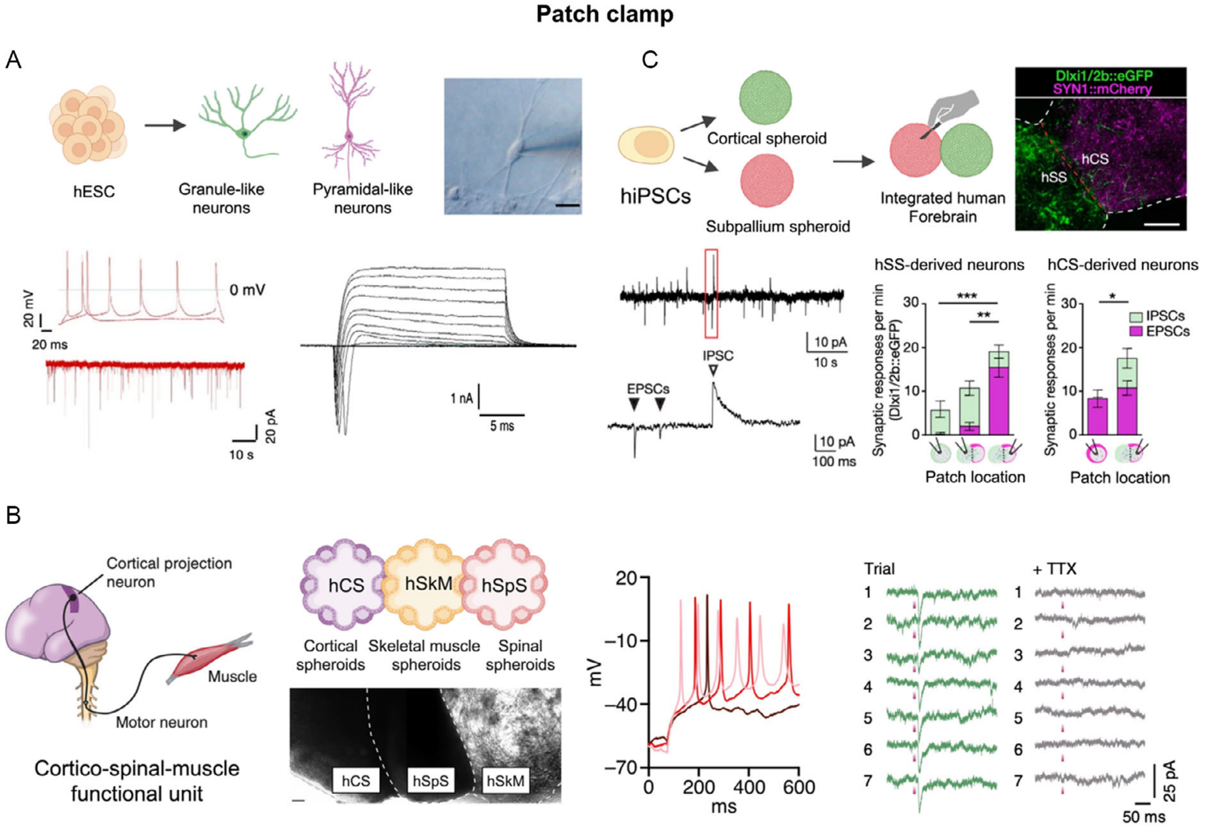

Figure 3. Patch clamp for electrophysiological measurement in neural organoids. A) Functional networks of hippocampal granule and pyramidal-like neurons derived from human embryonic stem cells, demonstrating electrically active network formation. Patch clamp recordings confirmed the functional integrity of these networks. hESC: human embryonic stem cell. Reproduced with permission from Sakaguchi et al.[45] (*Nature Communications*, 6, 1, (2015); Copyright 2015 Springer Nature). B) Assembly of 3D cortico-motor assembloids connecting cerebral cortex, hindbrain/spinal cord organoids, and human skeletal muscle spheroids, with functional connectivity confirmed via patch clamp recordings and robust muscle contraction upon cortical stimulation. TTX: tetrodotoxin. Reproduced with permission from Andersen et al.[46] (*Cell*, 183, 7, (2020); Copyright 2020 Cell Press). C) Formation of 3D forebrain spheroids from human pluripotent stem cells, enabling in vitro modeling of interregional interactions observed in fetal forebrain development. Functional integration was confirmed through patch clamp recordings. hiPSC: human-induced pluripotent stem cell. hCS: human cortical spheroid. hSS: human subpallium spheroid. EPSCs: excitatory postsynaptic currents. IPSCs: inhibitory postsynaptic currents. Reproduced with permission from Birey et al.[47] (*Nature*, 545, 7652, (2017); Copyright 2020 Springer Nature).

The 3D-compliant multifunctional mesoscale framework (MMF) can also be incorporated with MEAs for the recording and stimulation of neuronal activity within brain organoids. This allows researchers to study the electrical properties of neurons, synaptic connectivity, network dynamics, and 3D signal analysis in a controlled manner and provides insights into the functional organization of the organoid's neural circuits without damaging them. Park et al. introduced 3D MMF, multifunctional (electrical, optical, chemical, and thermal) neural interfaces to both spheroids and assembloids (Figure 4D).[53] It enables the observation of the complex 3D spatial propagation of wave spreading and firing and bursting events across the spheroid.

4. Perspective

4.1. Integrative Functional Connectivity

Both brain-on-a-chip and brain organoids hold great potential for elucidating normal physiological processes of the human brain as well as for the generation of reliable disease models. A comprehensive understanding of the electrophysiological properties and functional maturity of brain tissue is essential to unravel the mechanisms underlying human brain development and disorders. As electrophysiological signal measurement technology advances, there is a marked shift from traditional methodologies, such as the patch clamp, to more sophisticated approaches.[54] However, there is still an unmet need for comprehensive methods that can seamlessly investigate and integrate data from the deep tissue to the surface of the brain tissue, thereby providing a full spectrum analysis of brain function. Current techniques often focus either on the surface or deep brain structures but fail to connect these observations into a cohesive model that reflects the intricate nature of brain activity. Developing innovative methods that bridge this gap is crucial for advancing our understanding of the brain's complex dynamics and could significantly enhance our ability to study neurological disorders.

The integration of neural probe technologies with 3D MEAs represents a synergistic approach that has the potential to

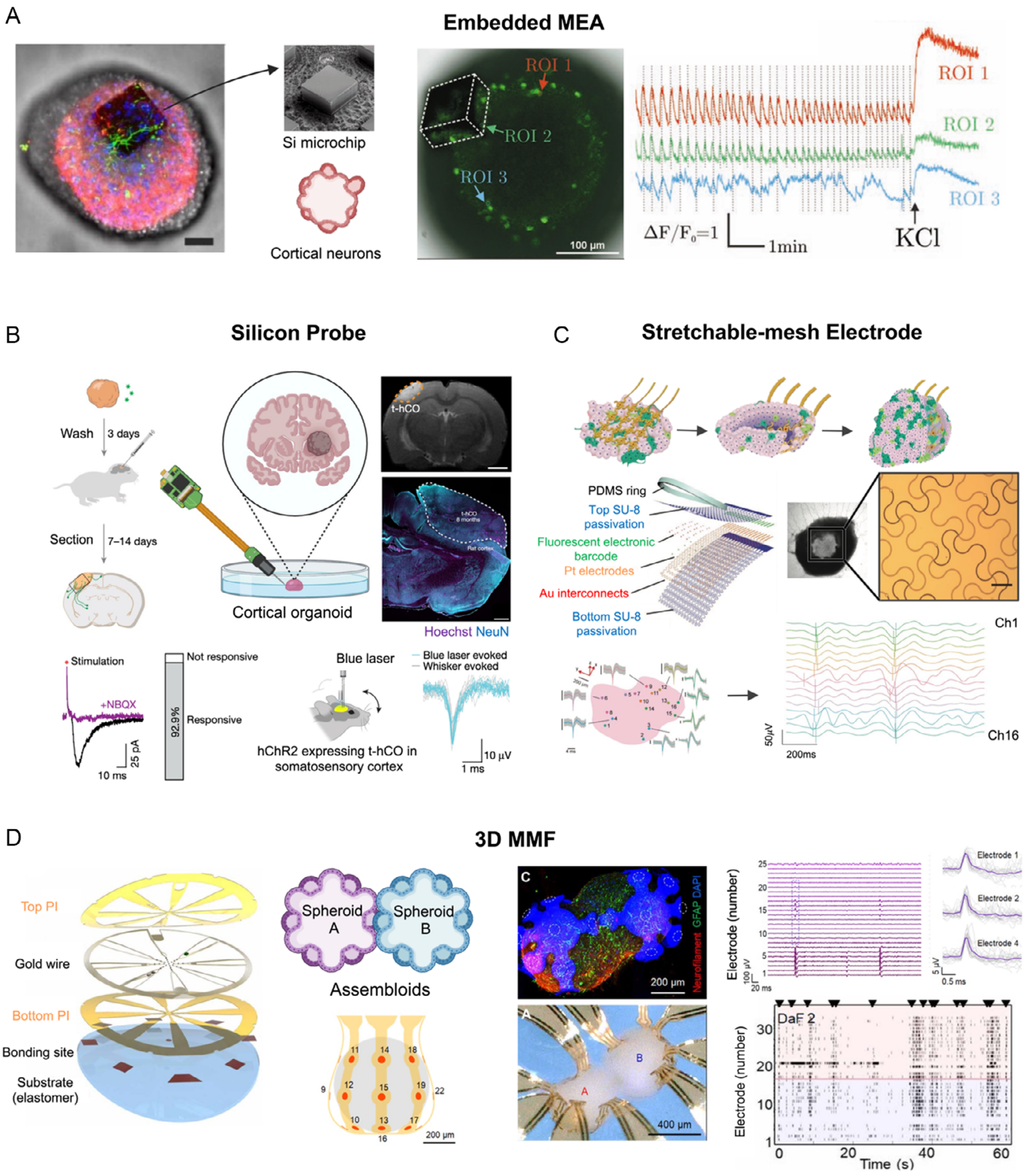

Figure 4. Various techniques for electrophysiological measurements in brain organoids. A) Hybrid neural spheroids formed by integrating silicon sham microchips with primary cortical cells. Built-in bioelectronic sensors were used to record neural activity in 3D models. Reproduced with permission from Lecomte et al.[48] (*Advanced Biosystems*, 4, 11, (2020); Copyright 2020 Wiley). B) Maturation and integration of human stem cell-derived cortical organoids transplanted into the somatosensory cortex of newborn athymic rats. Silicon probes enable detailed tracing of complex morphological and functional properties at the circuit level. Current traces from transplanted human cortical organoids (t-hCO) neurons following electrical stimulation with (purple) and without (black) NBQX, along with raw voltage traces during blue laser stimulation. NBQX: 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist NBQX inhibits the actions of glutamate at AMPARs. Reproduced with permission from Revah et al.[50] (*Nature*, 610, 7931, (2022); Copyright 2022 Springer Nature). C) Cyborg brain organoid platform with stretchable mesh nanoelectronics for stable 3D bioelectrical interfaces. It enables continuous recording and captures the emergence of single-cell action potentials during organoid development. Reproduced with permission from Le Floch et al.[52] (*Advanced Materials*, 34, 11 (2022); Copyright 2022 Wiley). D) Microfabricated 3D frameworks as multifunctional neural interfaces to cortical spheroids. It enables detailed studies of coordinated bursting events and formation processes in basic neuroscience research. Reproduced with permission from Park et al.[53] (*Science Advances*, 7, 12, (2021); Copyright 2021American Association for the Advancement of Science).

leverage the strengths of both systems, enabling researchers to investigate electrophysiological processes in a more comprehensive manner.[55] Neural probes, with their ability to penetrate organoids and record internal activity, offer precise measurements of specific neurons, generating high-resolution data from deep brain structures. However, they may provide a limited view of the broader network dynamics. In contrast, the multilayer electrode configurations are excellent at capturing complex interactions across extensive neural networks, offering a comprehensive overview of electrical activity on the brain organoid surface.[56] By combining the subsurface insight provided by neural probes with the wide-field network perspective provided by 3D MEAs, researchers can achieve a more holistic understanding of brain activity, from the single-neuron spike to the broader neural network. This integration could provide the much-needed link between the surface-level and deep tissue analyses, offering insights into the complex network dynamics of brain activity from micro to macro scales.

In addition to the synergy of neural probe technologies with 3D MEAs, the development of assembloids created by fusing different types of brain organoids offers novel insights into the interactions and connectivity between distinct brain regions. This method enhances our understanding of how separate brain areas communicate and function cohesively, offering a more integrated and physiologically relevant context for exploring the brain's complexities. Utilizing MEAs allows researchers to monitor electrical activity within these assembloids, capturing local neuronal communications within one region as well as long-range synaptic transmissions between fused organoids representing different brain areas.[57] This capability to measure electrophysiological signals underscores the potential of assembloids to provide unprecedented insights into neural circuits, particularly how long-range synaptic connections contribute to higher-order functions such as cognition and behavior. It allows for a closer approximation to the neural dynamics of the actual human brain and offers a more relevant in vitro model for studying complex neurological diseases and disorders, as well as testing the efficacy and safety of neuroactive drugs. Thus, assembloids, combined with sophisticated measurement technologies, represent valuable models for advancing our understanding of the connectivity and function of the human brain.

4.2. Strategies for Electrophysiological Data Management

Advances in electrophysiological techniques have led to the generation of extensive datasets from many electrodes, presenting significant challenges in management, processing, and interpretation. Electrophysiological datasets are often massive, sometimes encompassing hundreds of gigabytes, and demand efficient systems for handling. Thus, the preprocessing stage is pivotal, setting the stage for converting raw data into a format suitable for detailed analysis. Essential preprocessing steps include filtering out irrelevant signals, reducing noise to enhance signal quality, and categorizing neuronal signals. These steps are crucial to ensure that later analyses, like the identification of action potentials and network oscillations, are based on data of the highest fidelity.

Machine learning algorithms have been widely used in managing and analyzing large-scale datasets.[58] As researchers navigate the complexities of these large, refined datasets, machine learning algorithms stand as essential tools for extracting patterns[59] and classifying.[60] Supervised learning is employed to train models to recognize and classify the pattern of neural activity and predict outcomes.[61] In parallel, unsupervised learning is adept at clustering within unlabeled datasets to reveal intrinsic patterns.[62] Moreover, the outstanding feature extraction capabilities of deep learning have encouraged its application in the field of neuroscience.[63] Deep-learning technologies have shown remarkable success in analyzing visual images[64] such as neuroimaging, while specialized neural networks such as recurrent neural networks[65] and their variations of long–short-term memory[66] have been shown excellent in processing time-dependent data that characterizes electrophysiological signals, taking into account the temporal correlations among input variables.[67] The advantage of these models lies in their ability to learn from the data directly, often achieving superior performance as the amount of data increases.[68] Optimal design and integration of aforementioned tools would effectively manage a broad range of electrophysiological data and nurture us with deeper insights into neural function and developmental mechanism of brain-related disease.

Another data strategy is building a comprehensive data atlas. A data atlas refers to an organized repository that integrates diverse datasets into a unified framework.[69] It archives the electrophysiological data obtained from various experiments in standardized formats.[70] It would categorize the information based on different parameters, such as neuronal types, structural brain regions, electrophysiological activity, and genetic and proteomic information while also correlating them with metadata such as experimental conditions and methodology used.[71] By providing standardized, high-quality datasets for training and testing models, atlases ensure robust and reliable machine learning applications. Ultimately, the data atlas contributes to advancing the field by enabling the integration of complex datasets, which is crucial for unraveling the underlying mechanisms of neural function and for identifying potential targets for therapeutic intervention in neurological disorders.

4.3. Innovated Fabrication Techniques

The traditional fabrication techniques of bioelectronic devices heavily relied on photolithography and electron-beam (e-beam) lithography.[72] As the technology evolved, more complex and sophisticated fabrication techniques have been recently introduced.[73] For instance, inkjet printing of functional materials has become widely adopted due to its advantages in low-cost, rapid prototyping, and large-area fabrication.[74] Inkjet-printed gold MEAs have successfully recorded neuronal action potentials.[75] Aerosol jet-printed high-aspect ratio microneedle electrode arrays were also introduced to conduct electrophysiological recordings of iPSC-derived COs.[76] Moreover, a multi-length-scale electrode consisting of an array of 3D-printed silver micropillars covered with graphene nanoflakes has succeeded in the detection of dopamine at a detection limit of 500 attomoles.[77]

Meanwhile, advances in additive manufacturing have accelerated the field known as 3D bioprinting by enabling the direct printing of bioelectronic devices and scaffolds, sometimes even those embedded with cells. This cutting-edge approach involves the precise layer-by-layer deposition of biomaterials such as conductive polymers, hydrogels, and bio-inks to create a 3D biological framework. For instance, 3D nanoprinted micro-scaffolds have been proven to better mimic the complex topology of the human brain's structure and function. In this artificial nerve unit, action potentials and spontaneous excitatory postsynaptic currents of human iPSC-derived neurons were successfully monitored, highlighting the potential of direct scaffold printing for neural circuits.[78] Similarly, a 3D bioprinted PEDOT (poly (3,4-ethylenedioxythiophene)-containing conductive hydrogel significantly improved the differentiation of neural stem cells in vitro.[79] Consequently, the conductive biomimetic scaffold dramatically promoted the recovery of hindlimb motor function in a rat spinal cord injury model.

Moreover, 3D bioprinting has revolutionized regenerative medicine and tissue engineering by enabling the creation of intricate tissue models with unprecedented accuracy and complexity.[80] Human cortical-striatal brain tissue can be generated through the precise printing of neuronal progenitor cells and subsequent differentiation into various neural subtypes.[81] This tissue exhibited functional neural network connectivity within and between tissues, as confirmed by neurite growth and synapse formation. Additionally, it has become possible to directly create desired tissue through bioprinting various cell types. Precisely printed induced pluripotent stem cell (iPSC)-derived spinal neuronal progenitor cells (sNPCs) and oligodendrocyte progenitor cells within biocompatible scaffolds induce differentiation of sNPCs and the formation of functional neuronal networks.[82] This approach effectively demonstrates the potential of direct printing of multiple cell types for regenerative applications, particularly in central nervous system tissue damage.

As described, the technological shift toward advanced fabrication methods marks a significant milestone in the development of bioelectronic devices as well as tissue engineering, enabling more precise and versatile applications in biological research, medical diagnostics, and the potential for personalized medicine.

5. Conclusion

Despite the inherent limitations of the in vitro culture of brain-on-a-chip and brain organoid due to the absence of the dynamic nature of continuously alternating neurons, inadequate diversity of cell types, and hardships in establishing a proper synaptic connection, neuronal circuits or higher-order brain function such as cognition or memory, the emergence of brain-on-a-chip and brain organoid technologies, which serve as advanced models for human brain function and disease, neuroscience research is still advancing into new frontiers. Each platform possesses unique characteristics. Brain-on-a-chip offers precise control over the microenvironment, while brain organoids bring the complexity of organ-like structures. Therefore, it is important to carefully select the appropriate platforms and corresponding electrophysiological measurement methods to obtain accurate and meaningful results while leveraging individual strengths. Moving forward, the integration of brain-on-a-chip and brain organoid models with precision measurement techniques will undoubtedly catalyze breakthroughs in personalized medical approaches and revolutionize the treatment of neurological disorders.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared

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