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Engineering microsystems to recapitulate brain physiology on a chip

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The structural and functional organization of the human brain consists of 52 regions with distinct cellular organization. In vitro models for normal and pathological states using isolated brain-region-specific 3D engineered tissues fail to recapitulate information integration and/or transfer that arises from connectivity among neuroanatomical structures. Therefore, development of brain-on-a-chip microsystems must shift to multiple region neuron network designs to be relevant in brain functionality and deficit modeling. However, in vitro formation of multiregional networks on microdevices presents several challenges that we illustrate using a few neurological disorders; and we offer guidance, depending on objectives (HTS, disease modeling, etc.) for rational design of microfluidic systems and better emulation of in vivo conditions.

The need for change

Neurological diseases are a leading cause of disability worldwide, accounting for 10.4% of the global disease burden, as measured by disability-adjusted life years – a metric for the total number of years lost to illness, disability or premature death within a given population [1,2]. This disability presents an immense global socioeconomic burden: for example, mental disorders alone are projected to cause a cumulative economic output loss of at least US$16.3 trillion between the years 2011 and 2030. This estimated economic output loss, owing to mental disorders, compares to the projected loss caused by cardiovascular diseases but is higher than other key diseases such as cancer. Moreover, this loss excludes costs outside healthcare, such as legal costs associated with behavioral deficits [3]. Unfortunately, there is no effective treatment known to reverse progression of neurological diseases and therapeutics that ameliorate their effects have been reported to present elevated risk for adverse side effects. Furthermore, drugs targeting neurological diseases have been reported to fail late in the development process, increasing the financial risk of neuro-drug discovery and, consequently, the cost of medication [4]. It is estimated that the probability of success for new neurotherapeutic agents is a mere 2.85% [5]. Therefore, the drug discovery platform for neurological diseases could utilize superior neurophysiology models to improve the odds for early prediction of efficacy of drugs during the drug development process.

Strategies to overhaul the drug discovery process, especially aiming at increasing prediction efficiency of potential drug targets early in the discovery process, involve utilization of 3D cell-based models, which have been said to post better physiological relevance in comparison to 2D cell-based and biochemical assays [6–8]. 3D cell culture constitutes a means to tune cellular microenvironments to achieve specific tissue size (spatial feature), biochemical composition (biochemical environment) and material stiffness and composition (physical). This results in cell–cell and cell–material interactions and, consequentially, cytoskeletal architecture rearrangements that are otherwise not possible in 2D cultures [9]. The need for finer cellular microenvironment control and recapitulation of organ functionality has inspired a convergence of life and physical sciences to develop novel materials such as hydrogels, scaffolds and other miniaturized devices that can manipulate the microenvironment toward a desired *in vivo* fate. Integration of microfluidic devices in cell culture has aided crafting of complex cell–cell and cell–material interactions, increased precision in control of cellular environmental factors such as flow or shear stress, control of growth factor and toxin gradients, among others. Microfluidic

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devices were first developed in the 1940s for fluid transport studies [10] and electronics [11]. However, stimulus to utilize devices in cell culture was probably inspired by application of microfluidics for biosensing in the 1980s and early 1990s [12]; hundreds of studies have since utilized these devices to control cell culture parameters.

Evolution of microsystem design toward brain-on-a-chip

Initial application of microfluidic devices focused on understanding how microenvironment factors influence neuronal behavior. These studies utilized microsystems to control chemotactic, topographical, haptotactic and electrical microenvironment gradients to understand the effect of biomaterials on neuronal differentiation, cell migration, regeneration, axonal guidance, neurite growth, among others [13]. Since the study by Taylor *et al.* [14], which developed a microfluidic model for axonal regeneration systems, the focus has been shifting to engineering functional microcircuits to mimic physiological or disease phenomena on microfluidic chips. Following this study, more *in vitro* studies have followed suit to develop disease models such as Alzheimer's [15,16], nerve injury [17] and other complex disorders such as schizophrenia [18]. Huh *et al.* [19] later developed a lung biomimetic device by co-culturing human alveolar epithelial and pulmonary microvascular endothelial cells. Their lung-on-a-chip microsystem was reported to recapitulate important physiological responses of a human lung such as: alveoli responses with respect to expansion and contraction of the diaphragm, immune responses to bacterial infection, lung toxicity and inflammation. Moreover, key findings from this study were reproduced using whole mouse lungs, indicating functionality of such systems could have potential to replace whole animal organs in drug screening. The success of this system inspired a wave of attempts to develop systems for recapitulation of other human organs and their functionality on small-scale chips – commonly referred to as organs-on-a-chip – including gut-on-a-chip [20], kidney-on-a-chip [21], liver-on-a-chip [22], cancer-on-a-chip [23] and ALS-on-a-chip [24]. Several brain-on-a-chip designs have been reported as well.

Brain-on-a-chip microsystems

Not to be confused with silicon integrated circuits mimicking neural networks, here brain-on-a-chip refers to a system of living neural tissue on a cell culture microdevice to simulate brain functionality. A notable amount of work has concentrated on models for the blood–brain barrier (BBB) [25–27]. The BBB comprises neurons, astrocytes and microvascular endothelial cells. Endothelial cells tightly restrict the trafficking of molecules, proteins and cell types between the blood and the brain. Astrocytic endfeet ensheathe endothelial cells, providing a link between microvasculature and neurons. Therefore, a key design component has been to establish a co-culture system to model interactions between different cell-type populations and demonstrate basic functionality of the BBB such as formation of tight junctions and control of transport across the barrier. Other 3D culture systems used to understand mechanisms of neurological diseases – such as Alzheimer's disease [28] and Parkinson's disease [29] – have been attempted in microfluidics. Altogether, these systems report physiological outcome, which is facilitated by provision of optimal 3D cellular microenvironments via robust chemical, physical and spatial feature manipulations, that is only possible using microfluidic systems. Despite these advances, the development of effective human-brain tissue-engineered models is still said to be a distant goal [30].

Brain-on-a-chip, easier said than done

The human brain's structural and functional organization is made up of 52 regions with distinct cellular organization (Brodmann regions). Execution of tasks is achieved via interaction of hierarchical networks of excitatory and inhibitory neurons from multiple regions. For example, by generating social recognition memory, the hippocampus acts as a hub for integrating inputs from a larger network that includes the medial prefrontal cortex, anterior cingulate cortex and amygdala [31]. The significance of communication between distributed neuronal populations is also evident in cognition [32] and diseases or disorders like schizophrenia [33,34], depression and anxiety [35] or attention deficit hyperactivity disorder [36], paving the way for network-based analysis over traditional single-region approaches in brain studies [37,38].

A handful of brain tissue microsystems has demonstrated the significance of neural dynamics arising from interregional connectivity for *in vitro* studies. Dauth *et al.* [39] and Kanagasabapathi *et al.* [40] developed brain-on-a-chip systems that arrayed rat primary tissue from prefrontal-cortex-hippocampus-amygdala and cortex-thalamus in compartmented microdevices to form multiple brain region circuits *in vitro*. Networks of multiple brain regions showed different firing patterns compared with when tissue from each of the brain regions was cultured separately. Moreover, these firing patterns were linked to crosstalk between neuronal tissue in different compartments [41] – a key physiological feature otherwise lost in single-brain-region 3D tissue models for normal and pathological brain states. Therefore, brain-on-a-chip microsystems, modeling human brain functionality and deficits, must shift toward design of platforms that array multiple brain-region-specific 3D neuronal subtypes to mimic neurotransmitter circuits in the state being modeled. For example, a network implicated in psychosis and schizophrenia shown in Fig. 1a could be modeled by an *in vitro* network of distinct brain regional subtypes that model aberrations in neurotransmitter systems involved in the disease – demonstrated in Fig. 1b. However, *in vitro* formation of multiregional networks relevant for human brain studies presents several challenges.

First, it requires derivation of neuronal phenotype populations from human pluripotent stem cells (PSCs) to recapitulate regional compositions, arraying them in distinct networks with functional connections between each other. Nevertheless, the optimization of protocols for derivation of specific neuronal phenotypes is still in its nascent stages and/or requires a cocktail of expensive reagents. For example, the efficiency for directing stem cells to a population of dopaminergic neurons has been reported to be as low as 30% [42,43]. Second, prolonged culture is still problematic because of rapid loss of physiological function or low cell viability, therefore efficiency of *in vitro* models is limited when something more than short-term culture is desirable [44]. Recently, studies growing floating 3D brain organoids in a dish achieved timescales of 6–9 months in culture [45,46], yet literature of 3D neuronal tissue grown in microfluidic devices is limited to timescales of ~1 month. Prolonged culture of human PSCs in 3D organoid constructs has been demonstrated to result in superior structural, functional and phenotypic attributes of physiological relevance such as substantial neuronal maturation, spontaneous activity of neuronal networks, formation of dendritic spines and functional synapses, spontaneous derivation of glia, cell diversity and spatial organization of tissue [45–48]. To remain relevant, brain-on-a-chip microsystems need to be designed to support short- and long-term culture applications effectively.

Third, robustness of organ-on-a-chip microsystems is already a key limiting factor for

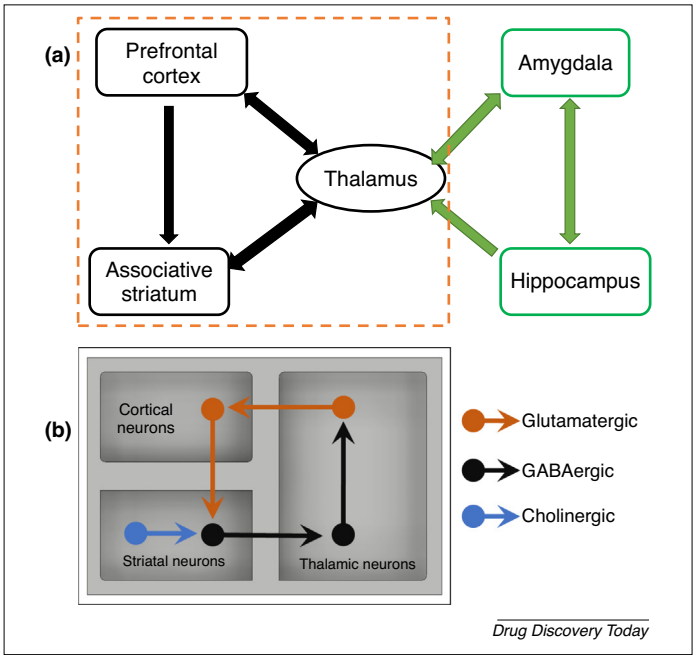

FIGURE 1

Network implicated in psychotic symptoms and schizophrenia. Psychotic symptoms arise from dysfunction in a network comprising multiple brain regions. **(a)** Psychosis requires increased activity in the associative striatum but the primary circuit includes the prefrontal cortex and the thalamus, which project into the associative striatum. Projections from other limbic regions, such as the amygdala and hippocampus, can contribute to sensory perception and emotion. **(b)** A schematic of a conceptual design for a brain-on-a-chip microsystem to model the main circuit for psychosis involving the prefrontal cortex, associative striatum and thalamus.

industrialization of this technology [49,50]. The complexity of microsystems required to model multiple region networks of the brain *in vitro* is likely to require even more robust constructs. Typical footprints of multiorgan microsystem constructs (consisting of interconnected compartmentalized 3D tissue on a single chip) normally span tens of millimeters, and there are peripheral systems integrated to facilitate automation and enable reproducibility – for example sensor systems to monitor biophysical and chemical microenvironments [51] or peristaltic on-chip micropumps dedicated to fluid-flow in specific circuits [52]. Directing stem cells to different neurophenotypes *in vitro* requires addition of specific signaling effectors to cultures. Networks of multiple neuronal subtypes on a single chip can require several peripheral systems, each dedicated to a distinct network, and this is likely to result in substantial footprints. Also, because most publications have reported implementation of peripheral systems in single-chip concepts [53], strategies for automation of multiple organ-on-a-chip replicates in parallel are not well developed. Massive implementation of single-chip concepts for multiregional brain-on-a chip systems is likely to require significant interaction with end-users, thus increasing manual labor and posing difficulty in developing scalable models and/or handling in HTS. Also, in other multiorgan systems, several tissue models are either supplied with the same fluid stream during culture or tissue chips are maintained separately and interconnected later in culture via micro-connectors [54]. By contrast, implementation of multiple-brain-region chips will not be trivial – exchange of soluble phenotype patterning factors between compartmentalized tissue is likely to undermine distinction of neuronal tissue subtypes required to recapitulate networks of different brain regions. Also, because spacing between compartmentalized neuronal subtypes on a brain-on-a-chip network is constrained by a need to establish synaptic connections, fluidic isolation must be maintained, whereas establishing physical connection between networks over a prolonged culture time is required for neuronal maturation. At a microcircuit level, neurons in each brain region are kept under surveillance of glia. Glia occupy ~90% of the human brain (astrocytes ~50%, microglia 12–15% and oligodendrocytes constitute the remainder) and facilitate neuronal functionality by executing a range of tasks, including providing neurotrophic factors, homeostasis of extracellular milieu, recycling and/or removal of neurotransmitters after synaptic transmission; long-range signaling through astrocytic networks has been said to facilitate heterosynaptic metaplasticity, synaptic pruning and conduction of action potentials by ensheathing neurons, among other things. Their dysfunction has been indicated in multiple neurological disorders (including multiple sclerosis, Alzheimer's disease , Bipolar, Schizophrenia and Autism spectrum disorders). Moreover, there are emerging perspectives that glia could be potential drug targets owing to their profound involvement in neurophysiology regulation. Therefore, there is increasing skepticism over the relevance of *in vitro* models that do not incorporate glia. There is excitement from recent 3D brain organoid studies that have reported spontaneous-generation glial phenotypes after pronged culture. Monzel *et al.* [55] reported ~4% astrocytes at day 61 and ~30% of neuronal axons enwrapped by oligodendrocytes; Pasca *et al.* [45] reported up to 7.6% after 76 days in culture which increased to ~20% by day 186. Spontaneous generation of glia resulting from prolonged culture could be limited by short-term culture currently feasible in microdevices. To improve physiological relevance with respect to glial-neuronal composition of tissue, it is imperative that brain-on-a-chip designs consider strategies that will deliberately incorporate or control proportions

of glial phenotypes in tissue. For example, glial cells can be generated in separate modules and introduced into neuronal tissue on a chip via spontaneous migration or other cell-seeding techniques, but addition of glia is likely to aggravate design of multiregional brain circuits on a microfluidic chip with respect to scalability, cost and time, and render them almost unpragmatic for HTS.

Altogether, the need to understand neurophysiology and neuropharmacology from a multivariate perspective is likely to favor use of animal models in brain functionality and disease studies for many years, because the development of practical microsystems to recapitulate human brain physiology on a chip is not likely to be feasible in the near future. Animal models take advantage of innate interregional connectivity, and comparable macro and micro anatomical hierarchies, in intact animal brains. Also, there are behavioral aspects important in brain studies for disease and treatment side-effects, such as social behavior and motor behavior, which can be recreated in animal models but have not even made it to conversation tables for design regarding brain-on-a-chip systems.

Outlook

Although the use of animal brain models seems to provide a more holistic model for human neurological studies, there is increasing scrutiny over their relevance [56]. Data derived from animal models are suspected to come with knowledge gaps owing to genetic differences in the neuronal and glial population and, consequently, neural plasticity differences between humans and animals [57–60]. It is estimated that only one-third of drug research conducted in animals can be translated to human clinical trials [61] and only 8% of the drugs used that enter clinical trials pass Phase I [62]. Other factors include the difficulty in expansion of animal studies, time required to raise animals and recent outcries concerning animal cruelty and ethical appropriateness of using animals in drug testing [63]. It is estimated that up to 100 million animals are used for experimentation worldwide every year [64]. Such weaknesses of animal models are a compelling reason to develop human-cell-based models as an alternative for animals, despite the complexity.

Initial strides toward design of multiple-brain-region circuits on a chip using human 3D cell-based models could benefit from strategies previously employed by multiregion brain-on-a-chip studies, which arrayed freshly extracted animal brain tissue to form robust networks *in vitro* (highlighted in the previous section) [39–41]. The concept could be translated to human brain tissue models by using hybrid systems where 3D neuronal regional subtypes are separately derived on a massive scale in organoid constructs, and later assembled into networks of multiregion organoids in compartmented microdevices. Besides prolonged culture, brain organoid studies using human induced pluripotent stem cells (iPSCs) have had a lot of success in derivation of 3D tissues of distinct neuronal subtypes to model various brain regions (e.g., cerebral cortex [48], forebrain, hypothalamus and midbrain [65], hippocampus [66], etc.). Therefore, hybrid systems can circumvent challenges associated with developing effective brain-on-a-chip models – such as short-term culture limitations, complexity associated with need to maintain fluidic isolation in maturing neuronal tissue modeling distinct brain regional subtypes on a chip and design strategies to incorporation of glial phenotypes in tissue – and potentially accelerate evaluation of physiological relevance of 3D human-derived tissue in multiregion circuit models for brain states. Also, it is important to note that, if proportions of glia in neuronal tissue are important in normal and diseased brain states, the numbers reported by organoid studies fall way short. Microfluidics could provide a framework in which a spontaneously generated glial population could be boosted by addition of cells via perfusion systems or other controlled cell-seeding techniques compatible with 3D culture microfluids. Complementary efforts to improve time in culture and make brain-on-a-chip microsystems accessible for HTS involves design of microdevices that lend themselves to automation (on a single-chip basis and multiple chips in parallel) with respect to cell seeding, liquid handling, microscopy, biochemical analysis and electrophysiology. Automation of brain-on-a-chip systems on a massive scale will minimize user interaction, making the technology convenient for fewer technical users, although minimizing tissue disruption during culture is likely to increase timescales achieved in microdevices.

It is evident that application of brain-on-a-chip microsystems in industrial-scale combinatorial cell-based assays in drug discovery could be problematic for some years but a good place to start assessing their worth might be in low-scale applications such as personalized medicine. Improved understanding of genetics and its role in disease progression is opening up possibilities for individualized therapeutic strategies [67]. It is now apparent that individual genetic variations can drive differences in clinical responses to treatments [68]. The average drug efficiency for patients receiving major treatments lies between 25% and 68% [69] and there was a significant number of adverse, genetic-related drug reactions that led to hospitalization [70]. The future of personalized medicine for neurological diseases will benefit from advances in stem cell technologies [44], and certainly from development of complex *in vitro* platforms such as 3D nerve tissue culture chips. Techniques to reverse-engineer iPSCs from somatic cells are already providing opportunities to derive patient-specific stem cells and convert them to *in vitro* tissue in patients with genetic neurological deficits such as muscular atrophy, dystonia, Leopard's disease [71–74] and Familial dysautonomia [75]. Integration of microdevices with stem cell technology for personalized medicine has the potential to create *in vitro* disease models with very high accuracy and could facilitate overcoming challenges of achieving long-term culture and loss of functionality tissue, by providing complete microenvironments that mimic *in vivo* conditions with higher precision.

Concluding remarks

Although the development of brain-on-a-chip technology is still in its infancy – and certainly far from completion – implications of success of this technology can be drawn based on lessons from other successful biomimetic devices. Organ-on-a-chip microsystems provide better drug screening platforms in comparison with conventional cell culture techniques by several orders of magnitude [23]. These observations have led us to speculate that their application in brain modeling has the physiological potential to replace animals in drug screening. The concept of isolation of functionally connected regions *in vitro* is analogous to lesion model animal modes but, compared with animal lesion models, such brain-on-a-chip microdevices are likely to provide a means to model behavior in a reductive manner. Animal lesions and knockout models have been suspected to be accompanied by compensatory mechanisms facilitated by neural plasticity, which could confound results [76]. Utilizing human rather than animal cells to model neurophysiological phenomena could improve current neurological disease drug development platforms by providing a means for early prediction of drug efficacy in drug development stages. The overall effect is likely to be a reduction of drug development times, associated drug research costs and consequential market cost. Availability of cheaper drugs is likely to improve affordability of mental healthcare, enhancing global health and wellbeing.

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