PEER REVIEWED

Compartmentalised Microfluidic Devices for Drug Discovery in the Neurosciences

With an aging global population, neurological disorders are the leading cause in disability-adjusted life-years (the sum of years lived with disability and years of life lost) and the second leading cause of death[1]. Despite the increasing need for new therapeutics in this area, the drug discovery process faces several unique challenges when it comes to addressing the nervous system. Neurological disorders are incredibly complex, and this is reflected in the lack of knowledge of the underlying pathological mechanism for many of these diseases, and in turn to the slow speed at which novel drug targets are identified. Regardless, research in the field remains vibrant, and the emergence of novel research technologies and tools continues to lead to exciting discoveries that hold promise to translate into the development of novel therapeutics.

The emergence and adoption of stem cell reprogramming technology has been an absolute game-changer for neuroscientific research. The ability to differentiate human neurons from healthy or patient sources provides the necessary basis for beginning to develop relevant *in vitro* disease models of neurological disease. However, and as will be discussed further, the ability to generate neuronal populations alone does not faithfully reproduce some critical characteristics of *in vivo* neuronal physiology, especially as it applies to the context of modelling neurological disease. To address some of these shortcomings, several groups have turned to utilising advanced microfabrication techniques to produce a series of innovative compartmentalised microfluidic devices to complement neuronal cultures. The combination of these approaches has led to the emergence of next-generation *in vitro* disease models in neuroscience that are not only valuable in fundamental research settings but are also scalable for high-throughput drug screening.

Physiological Relevance in Neurological Disease Modelling

Physiological relevance is one of the key characteristics to developing a useful cell-based disease model. The chances of successfully identifying a new drug candidate that exhibits efficacy for any given disease is linked to the strength of the assay being used in the drug screening process. In other words, by building physiological relevance early in the discovery process, the goal is to reduce the likelihood that a drug will fail in later (and much more costly) clinical stages of the development process. In the case of neurological diseases, physiological relevance entails generating a model which mimics as many aspects of neuronal physiology as possible such that non-target based phenotypic screens can be performed. Until the advent of iPSC technology to produce human neurons *in vitro*, many cell-based assays used in the early phases of drug discovery were based on immortalised cell lines overexpressing a target of interest. These target-based approaches are problematic for neurological disorders as targets are generally not known. Additionally, such simplified models do not exhibit the most important aspects of neuronal physiology such as cell morphology and excitability which form the basis of neuronal function in both health and disease. Consequently, there is demand for the development of complex, physiologically relevant *in vitro* assays to pursue phenotypic-based drug screening in the context of neurological disorders.

Neuronal Systems are Highly Organised

Generally, induced pluripotent stem cell (iPSC)-derived neuronal cell cultures recapitulate many important characteristics of neurons at the levels of expression profile, cellular morphology, and functionality. However, it is important to recognise that neurons are highly polarised cells, and this polarity is the basis for the formation of highly organised structures *in vivo* that are critical for proper physiological function. Neurons have three distinct cellular compartments: dendrites, somas, and axons. Dendrites and axons serve as the respective functional inputs and outputs of the neuron, while the soma (or neuronal cell body) is mainly responsible to maintain the health of the neuron such that it can perform its transmission function. Given the defined input/output function of the neuron, it is evident that the orientation and physical location of each of these specialised compartments within their environment is critical to establish the directional flow of information throughout the nervous system.

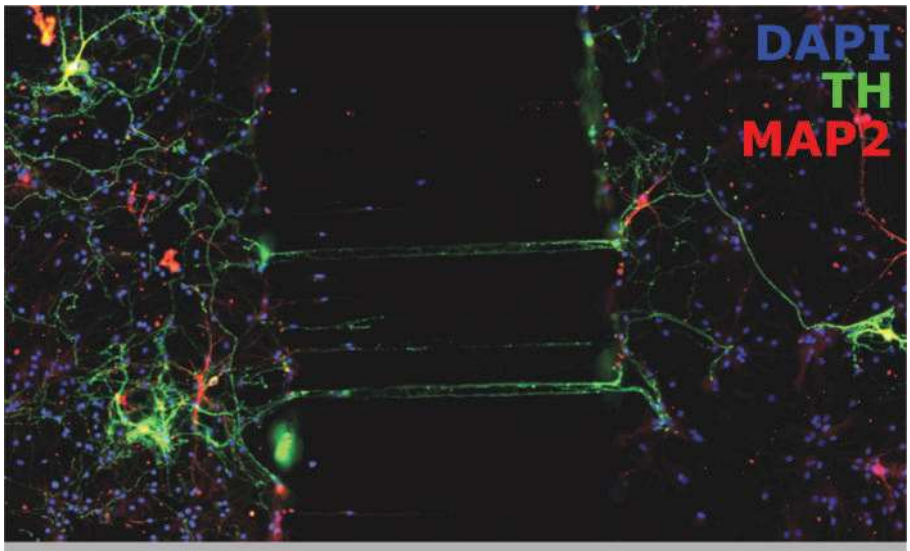
For example, in the human neuromuscular system, the lower motor neurons are responsible for relaying motor information from the central nervous system to skeletal muscle effectors. The lower motor neuron somas are located in the ventral horn of the spinal cord where they project axons that form synapses on skeletal muscle located some distance away (in some cases this distance can be as much as one metre). *In vivo*, it is the axonal outputs of the motor neurons that innervate their muscle target, while their dendrites and soma do not come into physical contact with the muscle tissue. During development, this neuronal orientation and positioning are established by a complex guidance system that is generally based on the release of chemical cues in a spatially and temporally defined manner. In this way, neuronal inputs and outputs are correctly guided to their specific target and location.

In the absence of environmental cues, as is the case when neurons are seeded in standard laboratory culture vessels, neurons will project dendrites and axons in random directions. Axonal projections will readily form connections or synapses with neighbouring neurons in a random fashion and may even synapse onto themselves (autapses). This neuronal "rat's nest" is simply not representative of the organisation and polarity of neurons *in vivo*, greatly reducing the value of such systems as physiological models of the nervous system. To address this, neuroscientists have turned to utilising microfabricated and microfluidic devices in conjunction with neuronal cultures. In their most basic forms, such devices contain a series of microchannels (or micro-tunnels) that act as structural guides for projecting axons, leading them away from their somas into an adjacent compartment. In this way, compartmentalised microfluidic devices can successfully mimic the polarisation and

organisation of neurons observed *in vivo*. As discussed further below, these simple, passive tools open a wealth of possibility when it comes to developing complex, physiologically relevant models of the nervous system.

How Compartmentalisation is Achieved Using Microfluidics

In basic neuronal applications, microfluidic devices contain two adjacent chambers that are separated by a series of microchannels. These microchannels are sufficiently small such that fluid flowing through them is strictly laminar, where the direction of flow depends on forces provided by the fluid level in each of the adjacent chambers. Fluidic isolation of one chamber from the other becomes possible by simply adjusting the relative fluid levels in each of these chambers. Despite their small size, the microchannels are still large enough to permit axons to pass through them, and neurons seeded in one chamber will project their axons through the microchannels into the adjacent chamber (Figure 1). This effectively segregates axonal and somatic components of the neuron, and these can be subsequently accessed, treated, and analysed individually.



Mesencephalic cultures were established on a glia feeder layer in both chambers of the OMEGA device. After 21 DIV, the culture was fixed and labelled with DAPI (blue), anti-tyrosine hydroxylase antibody (TH; green; dopaminergic neuron marker), and anti-MAP2 antibody (red).

Image courtesy of Samuel Burke-Nanni and Dr. Louis-Éric Trudeau, Université de Montreal

Figure 1

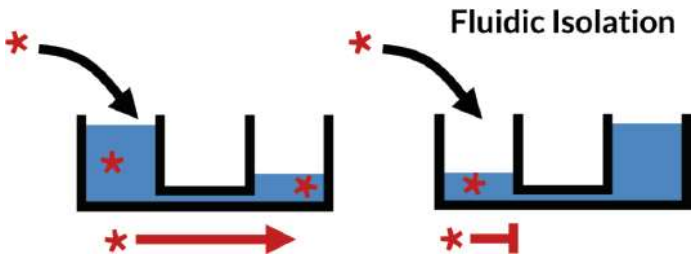


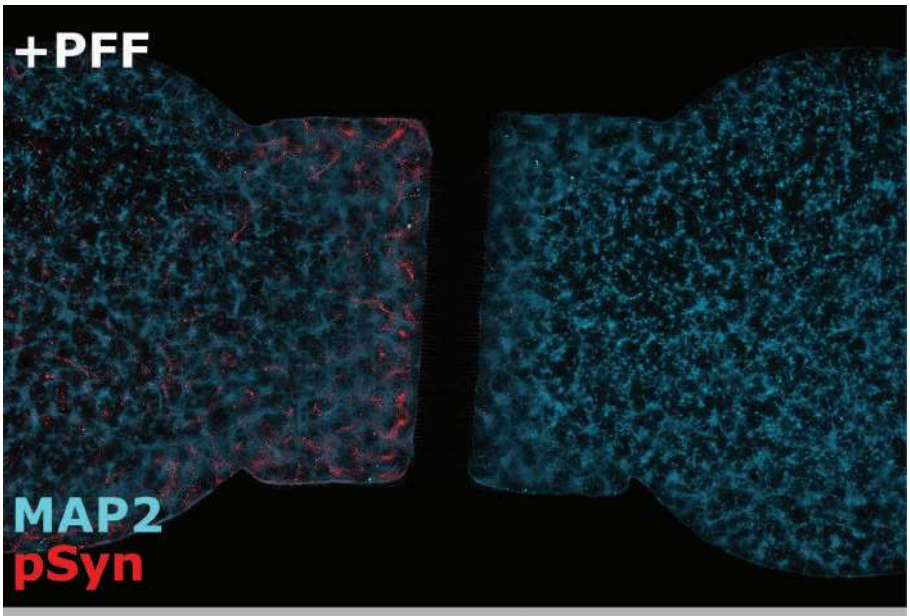
Figure 2

The ability to fluidically isolate an individual chamber allows molecules to be applied to one component of the neurons, without exposing the components in the adjacent chamber (Figure 2). This unique feature of compartmentalised microfluidic devices is particularly advantageous in the development of powerful disease models of neurodegeneration. For example, a neuronal compartmentalisation model is well suited for studying neuronal uptake, intraneuronal transport, and propagation of toxic or misfolded proteins such as tau, α-synuclein pre-formed fibrils (PFF), amyloid-ß or prion PrP (Figure 3) where compartmentalisation greatly simplifies the interpretation of data. Fluidic isolation is also useful in co-culture applications where adjacent chambers contain distinct cell types. For example, motor neurons located in one chamber will project axons into an adjacent chamber containing skeletal muscle. In this case, skeletal muscle media is not ideal for the neurons to differentiate, so fluidic isolation of the neuronal chamber from the adjacent muscle chamber prevents mixing of the media.

Advanced Neurological Disease Models Made Possible

Most microfabricated and microfluidic devices are made from polydimethylsiloxane (PDMS), a flexible, non-toxic polymer with excellent optical properties. These microfabricated PDMS structures are typically bonded to a glass bottom that serves to form the surface for seeding and culturing cells. There is a wealth of information available that includes a huge variety of diverse microfabrication techniques and methodology based on using PDMS. Combined with its low cost, availability, and well-described biocompatibility, it is no wonder that PDMS has become the material of choice when it comes to fabricating microfluidic devices for cell culture purposes. With few limitations, PDMS-based microfluidic devices are available in a plethora of designs and configurations to accommodate a defined experimental purpose.

The versatility of employing PDMS-based compartmentalised microfluidic devices opens the possibility for the development of even more complex models with the goal of increasing physiological relevance (Figure 4). For example, the newest microfluidic devices have designs to house self-assembled 3D tissues (such as spheroids and organoids) or tissue explants that can be configured to interface through microchannels with more classical monolayer (2D) cultures. In other applications, additional chambers and microfluidic interfaces can be added or reconfigured to create multi-compartment models. The addition of a third compartment can add an extra layer of complexity (and interpretational confidence) to neuronal propagation assays. Since these devices can incorporate cultures from both neuronal and non-neuronal sources, compartmentalised devices are ideal platforms to model sensory and motor systems. Examples include the co-culture of motor neurons with skeletal muscle to model neuromuscular degeneration (e.g.,



Primary neuronal cultures were established in both chambers of the OMEGA microfluidic device. Neurons project their axons across the microchannels in both directions. As the left chamber is kept fluidically isolated from the right chamber, unlabeled α-synuclein PFFs are added to the left chamber only. After 10 DIV, cultures were fixed and immunolabeled against MAP2 (blue) and pSyn (red).

Image courtesy of Drs. Lenka Schorova and Simon Wing, McGill University Health Center

Figure 3

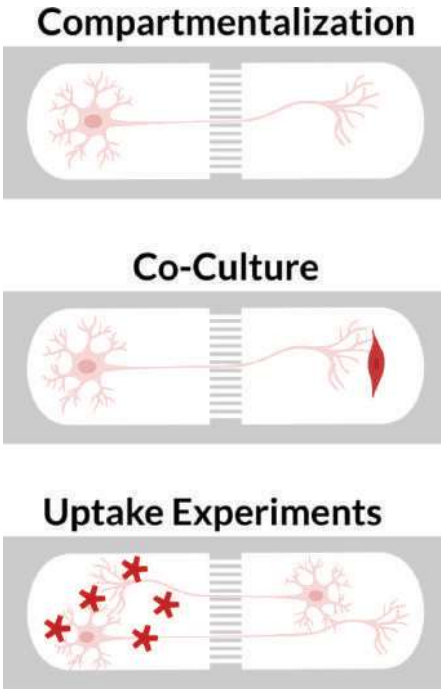


Figure 4

ALS or SBMA) or sensory neurons with skin fibroblasts to model pain. For even more complex multi-co-culture models, a triple compartment device can house motor neurons, Schwann cells and skeletal muscle in adjacent chambers.

Adapting Devices for Drug Screening

One of the earliest phases of the drug discovery process is the use of cell-based *in vitro* assays in high-throughput drug screens to identify promising drug candidates from large chemical libraries. The large number of molecules to be screened necessitates the use of automated machinery and robotics for cell culture seeding, maintenance and processing. For many phenotypic screens, imaging data is commonly collected using high content imaging platforms, and subsequently analysed using well-defined parameters. Importantly, these systems are nearly all designed around a standardised consumables format, the familiar 96-well microplate format.

Although neuronal microfluidic and compartmentalisation devices have existed for quite some time, their use in high-throughput drug screening has been limited. This is largely due to an inherent design flaw in most compartmentalised devices that stems directly from limitations in the way they are traditionally microfabricated. While these conventional devices are still extremely useful in an academic or fundamental research setting where individual scientists experiment with them in an artisanal manner, they are incompatible with automated or robotic systems used to carry out large-scale drug screening. Additionally, many conventional microfluidic devices require users to go through a series of time-consuming pre-assembly steps (e.g., plasma bonding to glass and sterilisation) prior to their use in culture, again making these completely impractical for implementation in a high-throughput setting.

Recently, advances in microfabrication methods have overcome these design limitations and have led to the development of a series of compartmentalised micro-fluidic devices that are fully high-throughput screening-compatible (Figure 5). These new-generation devices are catered specifically to the requirements and needs of the pharmaceutical and safety pharmacology industries, and are designed in industry-standard ANSI/SLAS microplate formats for seamless compatibility with existing infrastructure. Furthermore, these new devices are available pre-bonded to a glass, pre-coated and sterile, further streamlining

Figure 5

their implementation in large drug screening programmes.

Moving Forward

Microfluidic technologies are simple tools that provide necessary sophistication to enhance the physiological relevance of neurological disease models. Microfluidic systems were largely incompatible with high-throughput drug screening, precluding their implementation in the pharmaceutical industry. With recent advances in microfabrication methods, the combination of microfluidics and stem cell-derived neuronal culture technologies form a solid basis for generating powerful *in vitro* models of neurological disease.

Innovative solutions to scientific problems are often developed in response to the evolving needs of researchers looking to push the boundaries of science. By continuing to communicate new ideas and current limitations openly, academic, and industrial labs can work efficiently to create better tools and methods to move forward. In line with this, the continuous effort to improve upon current model systems for drug screening and safety testing by utilising appropriate new technologies, such as microfabricated cell culture devices for neuronal applications, have the potential to dramatically accelerate and facilitate the discovery of promising new therapeutics for neurological disease.

REFERENCE

1. GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 18(5): 459–480. (2019)

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Mark is a co-founder and chief scientific officer of eNUVIO. After obtaining his PhD in pharmacology at McGill University, he continued his training at the Montreal Neurological Institute, where he worked to develop new stem cell-derived models of neurodegenerative disease suitable for drug screening purposes. At eNUVIO, he works closely with R&D to design, develop and commercialise novel microfabricated and microfluidic devices to address the specific needs of life science researchers.

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