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Perfused drop microfluidic device for brain slice culture-based drug discovery

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Abstract

Living slices of brain tissue are widely used to model brain processes in vitro. In addition to basic neurophysiology studies, brain slices are also extensively used for pharmacology, toxicology, and drug discovery research. In these experiments, high parallelism and throughput are critical. Capability to conduct long-term electrical recording experiments may also be necessary to address disease processes that require protein synthesis and neural circuit rewiring. We developed a novel perfused drop microfluidic device for use with long term cultures of brain slices (organotypic cultures). Slices of hippocampus were placed into wells cut in polydimethylsiloxane (PDMS) film. Fluid level in the wells was hydrostatically controlled such that a drop was formed around each slice. The drops were continuously perfused with culture medium through microchannels. We found that viable organotypic hippocampal slice cultures could be maintained for at least 9 days in vitro. PDMS microfluidic network could be readily integrated with substrate-printed microelectrodes for parallel electrical recordings of multiple perfused organotypic cultures on a single MEA chip. We expect that this highly scalable perfused drop microfluidic device will facilitate high-throughput drug discovery and toxicology.

Keywords

organotypic; high-throughput; hippocampus; microfluidic; perfusion; MEA

Introduction

Living slices of brain tissue are widely used to study physiology of neurons and neural circuits and to model neurological disorders in vitro (Lynch and Schubert 1980, Sarvey, Burgard et al. 1989, Heinemann and Staley 2014, Butler and Paulsen 2015). A typical experimental setup may include a perfusion system that supplies oxygenated artificial cerebrospinal fluid (aCSF) as well as drugs, inhibitors, or other compounds to the slice. An electrophysiology system may also be included to stimulate and detect activity of neurons

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within the slice. Both perfusion and electrophysiology are highly amenable to miniaturization, and a number of groups reported microfluidic perfusion devices and multiple electrode array chips (MEAs) designed specifically for brain slices. Major goals of microfluidic device development were to precisely control slice microenvironment (Queval, Ghattamaneni et al. 2010), enhance nutrient and oxygen supply (Passeraub, Almeida et al. 2003, Choi, McClain et al. 2007, Rambani, Vukasinovic et al. 2009), or improve experimental access to the slice (Blake, Rodgers et al. 2010) (see (Huang, Williams et al. 2012) for a comprehensive review). MEA development focused on capturing spatiotemporal patterns of neural activity by increasing the number of electrodes per slice (Oka, Shimono et al. 1999, Egert, Heck et al. 2002), recording from neurons deep within the slice (Heuschkel, Fejtl et al. 2002), or improving viability of the slice on electrode array chip (Jahnsen, Kristensen et al. 1999). These efforts may be summarized as applications of microtechnology to improve the fidelity of brain slice experiments.

On the other hand, relatively little attention has been paid to using microtechnology to improve the throughput of brain slice experiments, especially the long-term experiments that require electrical recordings of brain slice cultures for multiple days or weeks. Throughput is particularly important for translational research. Brain slices are extensively used for toxicological and pharmacological profiling, or to carry out candidate compound screens to discover new drugs (Fountain, Ting et al. 1992, Stopps, Allen et al. 2004, Kroker, Rosenbrock et al. 2011, Graef, Wei et al. 2013, Heinemann and Staley 2014). These experiments typically use simple interface or submerged-type perfusion and only one or two electrodes per slice for stimulation or recording. However, a screen of a compound library may require hundreds or thousands of well-controlled experiments with replicates. Multi-slice systems have been developed to allow simultaneous, independent electrophysiological recordings from multiple brain slices (Stopps, Allen et al. 2004, Kroker, Rosenbrock et al. 2011, Graef, Wei et al. 2013). However, these systems require the use of multiple perfusion systems, machined slice chambers and discrete micromanipulator-mounted electrodes, imposing practical limitations on the number of parallel experiments. Slice survival in these multiple-slice systems is limited to only 7 hours, precluding longer term experiments (Kroker, Rosenbrock et al. 2011). However, long term, multiple days- or weeks-long electrical recording experiments may be necessary to address disease processes or endpoints that require protein synthesis, cell proliferation or growth, or neural circuit rewiring.

We set out to apply microtechnology to increase the throughput of long-term brain slice culture experiments. Techniques for maintaining brain slices in vitro for multiple weeks have been developed over 20 years ago (methods and applications of brain slice cultures are reviewed in (Gähwiler, Capogna et al. 1997)). In an interface method, the slice is placed on a porous membrane, and culture medium is added so that the slice is located at an air-medium interface (Stoppini, Buchs et al. 1991). In a roller-tube method, the slice is attached to a glass cover slip with a blood clot or collagen gel. The slip is then placed into a tube with culture medium that is rotated on a roller drum at a slow speed, bringing the slice in and out of medium (Gähwiler B 1998). Both methods ensure that the slice is directly exposed to atmospheric oxygen (100% of the time with the interface method, and approximately 50% of the time with the roller-tube method) to ensure viability in vitro for multiple weeks. Since slices maintain the cytoarchitecture of the originating brain regions, they are termed

organotypic slice cultures. However, neither interface nor roller-tube methods of producing organotypic cultures are directly compatible with microfluidics. A more recently developed method is based on maintaining a slice in a culture medium-filled well cut in a polydimethylsiloxane (PDMS) film (Berdichevsky, Sabolek et al. 2009). The well is connected with a larger medium reservoir via channels in PDMS film. Glass culture substrate can be easily substituted for a glass-based MEA to enable continuous electrical recording of neural activity in the slice. This device was used to study development of epilepsy after traumatic brain injury (Berdichevsky, Dzhala et al. 2012). A disadvantage of this method is the requirement for a large medium reservoir that takes up valuable MEA area and limits the number of cultures that can be placed on a single MEA chip. Another limitation is the lack of a perfusion system for applying drugs or other compounds to slices.

Here, we report the development of a novel perfused drop technique to enable use of multiple organotypic cultures on a single integrated device. This scalable device is capable of significantly increasing the throughput of long term slice culture experiments. In our design, slices of hippocampus, a brain region involved in learning and memory and in neurological disorders such as epilepsy, are maintained in individual drop confined by PDMS wells. The drops are continuously perfused with fresh culture medium through microchannels. For experiments requiring electrophysiology, PDMS microfluidic network is easily integrated with a planar MEA chip, with a possibility of recording activity in multiple perfused slices simultaneously. The device requires only 3 cm² area per culture, potentially enabling experiments with up to 8 cultures per single 2"×2" MEA chip. A single integrated device may potentially replace an entire system for parallel brain slice perfusion and electrophysiology, and facilitate high-throughput drug discovery and toxicology studies.

Device design and theory

In our design, slice culture is maintained on a glass substrate in a PDMS culture well with lithographically defined microchannel as the medium flow inlet and an open channel as the outlet (Fig. 1). The microchannel is connected to a feeding syringe tube placed at a specific height to provide proper medium flow rate. The outlet is connected to a reservoir where waste medium is removed by a vacuum needle. Culture well is open for optimal oxygen diffusion to the cultures (similar to interface method for organotypic cultures) and to provide culture medium surface tension to push the slices against culture substrate for better tissue attachment.

In the absence of flow, size of the drop in the culture well and fluid level in the reservoir are balanced by surface tension and gravity. Adjustment of fluid height in the reservoir H_1 changes the drop size in the culture well (Fig. 2). Since drop in the culture well is not strictly symmetrical due to the presence of an open outlet channel, we describe it by left contact angle β_l (closest to reservoir) and the right contact angle β_r (closest to inlet), and their average β . We built a simple static model consisting of several force components. In the reservoir, there is pressure due to gravity P_1 , and pressure due to capillary force P_2 ; in culture well, there is also pressure due to gravity P_3 , and pressure due to surface tension P_4 . In the absence of flow,

$$P_2 - P_1 = P_4 - P_3 \quad (1)$$

$$P_1 = \rho g H_1 \quad (2)$$

$$P_2 = 2\gamma \cos \alpha \left(\frac{1}{w} + \frac{1}{h} \right) \quad (3)$$

$$P_3 = \rho g H_2 \quad (4)$$

$$P_4 = \frac{2\gamma \cos \beta}{R} \quad (5)$$

where ρ is the density of fluid, g is gravity acceleration, γ is surface tension of fluid, α is the contact angle in the reservoir, w is the width of the reservoir, h is the length of the reservoir, H_1 is fluid height in the reservoir, H_2 is fluid height in the culture well, R is the radius of the culture well, z_1 is the height of the reservoir, and z_2 is the height of the culture well. H_2 can be described in terms of contact angle β

$$H_2 = z_2 - \frac{R}{\cos \beta} (1 - \sin \beta) \quad (6)$$

$\cos \alpha$ can be described in terms of H_1 :

$$\cos \alpha = - \frac{w(H_1 - z_1)}{\left(\frac{w}{2}\right)^2 + (H_1 - z_1)^2} \quad (7)$$

We can then express the average contact angle β of the culture drop in terms of fluid height H_1 in the reservoir:

$$\frac{2\gamma \cos \beta}{R} - \rho g \left(z_2 - \frac{R}{\cos \beta} (1 - \sin \beta) \right) = - \frac{2\gamma w (H_1 - z_1)}{\left(\frac{w}{2}\right)^2 + (H_1 - z_1)^2} \left(\frac{1}{w} + \frac{1}{h} \right) - \rho g H_1 \quad (8)$$

Solving Navier-Stokes equation gives the velocity profile for a pressure-driven, steady-state flow in a microchannel of rectangular cross section. The flow rate Q can be found by

integrating flow velocity in the inlet channel of width W and height H . For a channel with aspect ratio $a < 1$ ($a = H/W$), flow rate can be approximated by (Tanyeri, Ranka et al. 2011):

$$Q \approx \frac{H^4 \Delta p}{12 \eta L a} (1 - 0.63a) \quad (9)$$

where η is the viscosity of fluid, L is the length of microchannel, and Δp is the pressure difference between two ends of the microchannel, in this case, the gravity pressure due to feeding syringe:

$$\Delta p = \rho g Z \quad (10)$$

Z is the height difference between the fluid level in the feeding syringe and the device.

Experimental

A. Device fabrication

Culture compartments were fabricated from PDMS. Silicon mold masters were prepared by defining a microchannel pattern *via* SU-8 (Microchem) photolithography on a 3" silicon wafer. A pattern of 20 μm height and 50 μm width was fabricated. Liquid PDMS was spin-coated onto the silicon master at 600 rpm for 1 min, and cured at 75 °C for at least 4 h. The resulting 110 μm PDMS film with imprinted microchannels was removed from the silicon wafer. Culture wells of 6 mm diameter and the open channels of 0.5 mm $\times$ 8 mm were cut in the PDMS membrane. Fluid inlets of 0.025 mm diameter were created by puncturing the PDMS membrane with a syringe needle. The length of inlet microchannel L (edge to edge distance between fluid input to the device and culture well) is 6 mm (Fig. 1). PDMS compartments were attached to glass substrate through oxygen plasma bonding. Then 2 mm thick PDMS reservoir (5 mm $\times$ 25 mm inner area) and input tubing stand (5 mm $\times$ 5 mm) were bonded to PDMS membrane. Tubing stand through-hole (0.1 mm diameter) was centered on the punctured fluid inlet. The edge to edge distance between reservoir and culture well is 4 mm. Devices were autoclaved for sterilization. Then each device was placed in a 100 mm petri dish, and incubated in humidified atmosphere at 37 °C overnight, with culture wells coated with poly-D-lysine (PDL, Sigma). Devices were then washed in three changes of sterile distilled water, and incubated for at least 3 hours with culture wells covered with NeurobasalA/B27 medium (Invitrogen) before culturing.

B. Velocity modeling

Flow in the culture well was simulated using COMSOL-Multiphysics. Geometry of the simulated chamber mimicked the ones used for the experiments. A plug flow of 0.01 m/s was applied at the inlet of the microchannel leading to the slice chamber. The velocity was calculated as the preset flow rate of 1 mL/day divide by the cross section area of the microchannel (20 μm $\times$ 50 μm). The fluid surface exposed to air was set as an open boundary and the air phase was neglected, considering the low viscosity of air compared to

water. All the other boundaries were set as no-slip boundaries. Brain tissue in the culture well was represented by a solid cylindroid located at the center with a semi-major axis of 1.2mm, semi-minor axis of 1mm and height of 0.2mm. Fluid parameters corresponding to water were used in the simulation.

C. Organotypic cultures

Hippocampi were dissected from postnatal day 7–8 Sprague-Dawley rat pups (Charles River Laboratories), cut into 350 μm slices on a McIlwain tissue chopper (Mickle Laboratory Eng. Co., Surrey, United Kingdom). Slices were placed in the culture wells of the microfluidic devices, and controls were placed onto PDL coated 6-well tissue culture plates. All cultures were maintained in serum-free NeurobasalA/B27 medium containing 0.5 mM glutaMAX (Invitrogen) and 30 mg/L gentamicin (Invitrogen) and incubated at 37 °C in 5% CO₂. Control slices placed in 6-well culture plate were placed on a rocking platform. Medium was changed twice a week. Slices within microfluidic perfusion devices were gravity-fed from a syringe tube with a loose-fitting cap, and the flow rate was set to approximately 1 ml/day per culture by placing the feeding syringe at a height of 20 cm. Fluid was split by a 1 to 6 mini-manifold (Warner Instruments) to supply 2 triple microfluidic perfusion devices. The fluid level in the feeding syringe was checked daily and replenished every day to maintain same height Z . The feeding tubing was 0.030" ID, 0.065" OD ultra-purity white silicone tubing (McMaster-Carr). Polyethylene tubing with 0.023" ID, 0.038" OD (Warner Instruments) was used as the interconnector of feeding tubing to mini-manifold, and feeding tubing to PDMS tubing stand. Manifolds and tubing were sterilized by autoclaving, except for polyethylene tubing which was sterilized by UV exposure overnight. All animal use protocols were approved by the Institution Animal Care and Use Committee (IACUC) at Lehigh University and were conducted in accordance with the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals.

D. Immunohistochemistry

NeuN antibody is a marker for neurons that specifically recognizes DNA-binding, neuron-specific protein NeuN, which is present in most CNS and PNS neuronal cell types. Cultures were washed in phosphate-buffered saline (PBS), and fixed for 2 h in 4% paraformaldehyde. Cultures were then transferred to 48-well petri dish, and permeabilized in 0.3% Triton X-100 (Sigma-Aldrich) in PBS for 2 h, then blocked with 10% goat serum in PBS for 1 h, followed by incubation for 24 h in 1% NeuN at +4 °C on a shaker. Slices were then washed and mounted for confocal microscopy. Z-stack images were collected on confocal microscope (Zeiss LSM 510 META, Germany) with 5 $\times$ and 40 $\times$ objectives. Z-stack layers were separated by 2 μm , and depth ranged from 40 μm –60 μm . Images were then processed in Fiji (Schindelin, Arganda-Carreras et al. 2012). Neuron numbers were quantified per single field of view of high-magnification images, in the optical slice with maximum number of neurons in CA1 and CA3 areas of control and perfused-drop cultures.

E. Multiple electrode arrays (MEA) fabrication and electrophysiology

Glass slides (Fisher Scientific) were cleaned in Piranha solution. The electrodes pattern was defined photolithographically with negative lift-off process using AZ nLOF 2070 (MicroChem). Titanium (50 nm) and gold (200 nm) were deposited with e-beam evaporator

to make the electrodes. The photoresist was lifted with photoresist stripper AZ 400T (MicroChem) at 80 °C for 3 hours, leaving the electrode pattern on the glass slide. PDMS compartments were assembled to the MEA chip through oxygen plasma bonding, with electrodes centered in culture wells. MEA integrated device was then prepared for slice insertion as described above.

Slices were placed in the culture wells on the MEA chips and maintained in the incubator. Electrical recordings were carried out by connecting the electrode output pads on MEA to a 16-channel extracellular amplifier with high impedance head stage (3600, A-M Systems). Signals were digitized with a multiple-channel digital acquisition board (Measurement Computing). Data were recorded with dClamp software (available from authors upon request), and analyzed in Matlab.

Results and Discussion

A. Static analysis

Devices were evaluated under static (no flow) conditions with bovine serum albumin (BSA) solution (2.5 g/L), which mimics the surface tension of culture medium, and distilled water. We increased the fluid volume in the reservoir to increase the fluid level in the culture well (Fig. 2b). Averages of 3 separate measurements of H_1 , β_l and β_r for each fluid volume were calculated for three different devices and plotted in Fig. 2c. Results were compared to formula (8), evaluated with average dimensions of 3 different devices: $z_1 = 1.7$ mm, $w = 5.8$ mm, $h = 25.8$ mm, $R = 3$ mm, and $z_2 = 110$ μ m, $\rho = 10^3$ kg/m³. Surface tension values of distilled water and BSA solution (2.5g/L) at 37 °C were 70 mN/m and 54 mN/m, respectively (Vargaftik, Volkov et al. 1983, Suttiaprasit, Krisdhasima et al. 1992). Analytical solutions of β dependence on H_1 for BSA-containing solution and water were plotted in Fig. 2c. A good fit with the experimental data for water and BSA solution was observed. Correlation coefficients between experimental and theoretical values were $R^2 = 0.78$ for water, and $R^2 = 0.83$ for BSA solution; $p < 0.001$ for both fluids.

In the static model (Fig. 2), we only consider the operation range of $1 \text{ mm} < H_1 < 4 \text{ mm}$ because values of H_1 less than 1mm reduce β below 90 degrees. This may lead to cultures drying out. Fluid confinement by hydrophobic PDMS surface fails for $H_1 > 4$ mm, imposing an upper limit to the range of H_1 . The open channel connecting culture well and reservoir may also contribute to fluid balance. However, considering that its area is less than 10% of the culture well area, its surface tension may be ignored. In Fig. 2c, theoretical curves of water and BSA solution are close to each other signifying that difference in surface tensions between these two fluids does not strongly affect operation of our device. This is also supported by lack of difference between experimental data for water and BSA.

B. Dynamic analysis

Z was set to the same height as in subsequent culture experiments, 20 cm. β_l and β_r were measured while the drop in culture well was perfused with BSA solution, and compared to static values obtained from the same device without perfusion (Fig. 3a,b). The introduction of flow had no significant influence on the geometry of drop in culture well (for β_r , $p =$

0.911; for β_r , $p = 0.760$; $n=3$, paired t test). While fluid flow introduces pressure changes inside the culture well, the highest pressure change is at the fluid inlet (approximately 0.01 Pa, from COMSOL simulation), was less than 0.015% of the pressure in no-flow condition ($P_3 - P_4$ is approximately 70 Pa). Therefore, the shape of the drop in culture well is largely determined by static forces.

We then determined if Eq. (9) accurately models the flow rate in our device. We used the following values: $H = 20 \mu\text{m}$, $a = 0.4$, $Z = 20 \text{ cm}$, and $L = 6 \text{ mm}$. Viscosity of water is $\eta = 0.71 \times 10^{-3} \text{ Pa}\cdot\text{s}$ at 37°C (Korson, Drost-Hansen et al. 1969). Viscosity of BSA solution is approximately the same since low concentration of BSA used in culture medium does not significantly alter fluid viscosity (Yadav, Shire et al. 2011). The calculated flow rate Q was $42 \mu\text{l/h}$, which is approximately 1 ml/day per slice. In culturing experiments approximately 1 ml/day of culture medium was used per slice, which matches with the calculated flow rate.

Increase of flow rate improves the oxygen/nutrient delivery (Hájos and Mody 2009). However, the correspondingly increased fluid velocity can cause larger shear forces that have the potential to damage tissues (Christ, Williamson et al. 2010). In our platform, the flow rate is set at 1 ml/day. For slices cultured in standard 6-well plate, the medium consumption rate is about 0.3 ml/day (Opitz-Araya and Barria 2011). Thus, our perfused drop device is characterized by a comparatively high perfusate exchange rate which may lead to improved culture viability. COMSOL simulation of the streamline and velocity profile $10 \mu\text{m}$ above the substrate (microchannel midpoint) are shown in Fig. 3c, d. A relatively high velocity above $100 \mu\text{m/s}$ only occur near the inlet and outlet. The maximum velocity of 0.018 m/s occurs at the outlet of the microchannel, and decreases rapidly after entering the culture well. As demonstrated in Fig. 3d, velocity is in the range of $5\text{--}20 \mu\text{m/s}$ in the vicinity of cultured slice, indicating that slice experiences relatively little shear stress.

C. Culture viability

H_1 was set to 1.5 mm to make β approximately 100 degrees. This was found to be the optimum drop shape for culture viability, since drops with larger β caused slices to detach, while drops with smaller β caused slices to dry out. The culture viability was evaluated at 5 DIV and 9 DIV by bright field microscope imaging and confocal imaging of NeuN stained organotypic hippocampal cultures (Fig. 4). Compared with control cultures, perfused drop cultures showed similar well maintained cytoarchitecture with clearly defined neural layers. Neuron counts on 9 DIV were not different between control and perfusion slices in CA1 ($p = 0.599$, student t test, $n = 3$ each condition), but perfusion cultures had significantly higher number of neurons than control cultures in CA3 ($p = 0.036$, student t test, $n = 3$ each condition) (Fig. 4c). The results showed that viable cultures could be maintained in microfluidic perfusion system for at least 9 DIV with no reduction in the number of neurons in the hippocampal pyramidal layers. Brightfield and confocal images of a culture on 16 DIV demonstrate well-organized neuronal layers (Fig. 5a, dense bright bands), confirming that cultures remain viable and organotypic after 2 weeks in this platform. Images shown in Fig. 5a are representative of the 5 cultures we maintained until 16 DIV.

D. Electrophysiology

Chronic electrical monitoring was conducted from 3 DIV to 16 DIV. Raster plots were constructed from electrical data with interictal and ictal activities pseudocolored based on spike frequency (Fig. 5b). Seizures were defined as paroxysmal events (with significantly larger amplitudes than unit and multi-unit neural activity) that occurred for at least 10 seconds with event frequency of at least 2 Hz, and colored red in the raster plot. Neural activity was observed in all cultures, including unit and multiunit activity, population spikes, as well as epileptiform ictal and interictal-like activity, with examples shown in Fig. 5c. The results showed that organotypic cultures go through a latent period with little population activity before developing electrographic seizures (Fig. 5d, $n = 5$). These activity patterns and epilepsy progression were similar to previous observations in organotypic hippocampal cultures maintained with traditional methods (Dyhrfeld-Johnsen, Berdichevsky et al. 2010, Berdichevsky, Dzhalala et al. 2012, Berdichevsky, Dryer et al. 2013, Lillis, Wang et al. 2015).

Organotypic brain slice cultures are used to model long-term processes that occur in the brain, including neural circuit development, rewiring after injury, neurogenesis, and development of epilepsy (Gähwiler, Capogna et al. 1997, Bausch and McNamara 2000, De Simoni, Griesinger et al. 2003, Radojevic and Kapfhammer 2004, Uesaka, Hirai et al. 2005, Berdichevsky, Dzhalala et al. 2012, Berdichevsky, Dryer et al. 2013). Evaluation of changes in neural activity that may occur in organotypic cultures over days or weeks may contribute to understanding of neural development and disease. However, previously reported methods of performing chronic electrical recordings in this culture system are characterized by low throughput or high expense. This is primarily due to the requirements posed by standard methods of organotypic culture. Interface method requires a porous culture substrate, while roller-tube method requires embedding of the slice in a blood clot or collagen gel in a sealed tube on a roller drum. In this work, we developed a microfluidics-based method that is capable of maintaining multiple organotypic cultures on a single stationary, planar glass or plastic substrate. Our method is compatible with planar MEA technology, and will enable high-throughput, low-cost chronic electrical recordings of neural activity in organotypic cultures.

Our morphological and electrophysiological findings show that perfused drop method enables organotypic cultures that are as viable as cultures maintained with roller-tube or interface methods. Perfused drop method enables integration of organotypic cultures with MEA fabricated on any PDMS-bondable substrate such as glass, without the need for specialty perforated MEA (Jahnsen, Kristensen et al. 1999), thus potentially reducing the costs of organotypic culture-based drug discovery or toxicology screens. Our device is also capable of maintaining multiple cultures on a single MEA chip. Traditional methods of maintaining organotypic cultures require large area of culture substrate to hold the culture medium. In a 6 well plate, each slice takes up to $\sim 10 \text{ cm}^2$, while actual area of a hippocampal slice is only $\sim 0.07 \text{ cm}^2$. In our triple device (Fig. 1d), each slice takes up only 3 cm^2 if reservoir area is taken into account. The reduction in required area is achieved by moving the medium storage off the device. Since more than three culture wells can share one reservoir, the amount of area required per cultured slice will become smaller with more slices per device. More cultures can be added by simply patterning more wells and

microchannels in the PDMS film without an increase in the cost of the device. Thus, our perfused drop device is inherently scalable, and may replace entire systems (Stopps, Allen et al. 2004, Kroker, Rosenbrock et al. 2011, Graef, Wei et al. 2013) with a single integrated microfluidic-MEA chip. Another advantage of our platform is the transparent and planar design that enables convenient optical microscopy.

Conclusions

We developed a novel perfused drop technique that enables use of multiple organotypic cultures in a single microfluidic device. This device is easily integrated with a planar MEA to conduct parallel electrophysiological experiments on multiple brain slice cultures simultaneously, on a single chip. We expect that the development of this highly scalable device will pave the way for high-throughput neurological drug discovery and toxicology.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.